PSYCHONEUROENDOCRINOLOGY OF PSYCHOSIS DISORDERS

EDITED BY: Grazia Rutigliano, Mary V. Seeman and Boris Chaumette
PUBLISHED IN: Frontiers in Psychiatry

frontiers Research Topics

frontiers

ISSN 1664-8714
ISBN 978-2-88966-324-8
DOI 10.3389/978-2-88966-324-8

About Frontiers

Frontiers is more than just an open-access publisher of scholarly articles: it is a pioneering approach to the world of academia, radically improving the way scholarly research is managed. The grand vision of Frontiers is a world where all people have an equal opportunity to seek, share and generate knowledge. Frontiers provides immediate and permanent online open access to all its publications, but this alone is not enough to realize our grand goals.

Frontiers Journal Series

The Frontiers Journal Series is a multi-tier and interdisciplinary set of open-access, online journals, promising a paradigm shift from the current review, selection and dissemination processes in academic publishing. All Frontiers journals are driven by researchers for researchers; therefore, they constitute a service to the scholarly community. At the same time, the Frontiers Journal Series operates on a revolutionary invention, the tiered publishing system, initially addressing specific communities of scholars, and gradually climbing up to broader public understanding, thus serving the interests of the lay society, too.

Dedication to Quality

Each Frontiers article is a landmark of the highest quality, thanks to genuinely collaborative interactions between authors and review editors, who include some of the world's best academicians. Research must be certified by peers before entering a stream of knowledge that may eventually reach the public - and shape society; therefore, Frontiers only applies the most rigorous and unbiased reviews.
Frontiers revolutionizes research publishing by freely delivering the most outstanding research, evaluated with no bias from both the academic and social point of view. By applying the most advanced information technologies, Frontiers is catapulting scholarly publishing into a new generation.

What are Frontiers Research Topics?

Frontiers Research Topics are very popular trademarks of the Frontiers Journals Series: they are collections of at least ten articles, all centered on a particular subject. With their unique mix of varied contributions from Original Research to Review Articles, Frontiers Research Topics unify the most influential researchers, the latest key findings and historical advances in a hot research area! Find out more on how to host your own Frontiers Research Topic or contribute to one as an author by contacting the Frontiers Editorial Office: researchtopics@frontiersin.org

PSYCHONEUROENDOCRINOLOGY OF PSYCHOSIS DISORDERS

Topic Editors:
Grazia Rutigliano, University of Pisa, Italy
Mary V. Seeman, University of Toronto, Canada
Boris Chaumette, INSERM U1266 Institut de Psychiatrie et Neurosciences de Paris, France

Citation: Rutigliano, G., Seeman, M. V., Chaumette, B., eds. (2021). Psychoneuroendocrinology of Psychosis Disorders. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-88966-324-8

Table of Contents

frontiers in Psychiatry

EDITORIAL
published: 09 November 2020
doi: 10.3389/fpsyt.2020.607590

Editorial: Psychoneuroendocrinology of Psychosis Disorders

Grazia Rutigliano[1]*, *Boris Chaumette*[2,3] and *Mary V. Seeman*[4]

[1] Department of Pathology, University of Pisa, Pisa, Italy, [2] Université de Paris, Institut de Psychiatrie et Neurosciences de Paris (IPNP), INSERM U1266, GHU Paris Psychiatrie et Neurosciences, Paris, France, [3] Department of Psychiatry, McGill University, Montréal, QC, Canada, [4] Department of Psychiatry, University of Toronto, Toronto, ON, Canada

Keywords: neuroendocrine system, immune-inflammatory system, hypothalamus, cortisol, insulin resistance, stress, schizophrenia, schizoaffective disorder

Editorial on the Research Topic

Psychoneuroendocrinology of Psychosis Disorders

The pathophysiology of psychotic disorders is complex and imperfectly understood. Clinical evidence and animal research link psychotic disorders to multiple systems beyond neuropsychology, in particular the neuroendocrinological and immune-inflammatory systems. That a relationship exists between hormones and the brain has been observed in clinical practice for several centuries. Already back in 1891, Emil Kraepelin hypothesized an association between hormones and dementia praecox, the name he gave to the disease now known as schizophrenia (1). A further insight into this association came from Hoskins (2), who analyzed post-mortem tissues and attempted to treat schizophrenia with glandular extracts (2). The discovery in the 1970s of hypothalamic hormones represented a fundamental milestone in this understanding, which reached a peak when the pituitary gland entered the stage in the 1980s. The notion that neurotransmitters regulate pituitary hormone release via hypothalamic hormones is illustrated in the catchphrase, "the pituitary is the window to the brain" (3).

At present, more than a hundred hormones have been identified, all with complex and partially undetermined actions. Pituitary, thyroid, adrenal, and gonadal dysfunctions are all known to result, on occasion, in psychotic symptoms. Hormones can be briefly involved in the pathophysiology of psychotic disorders through short-term activation that temporarily modifies neuronal function [e.g., adrenocorticotropic hormone (ACTH) release after a stressful stimulus], or through early organizing effects that result in long-lasting structural change (e.g., altered formation of hypothalamic nuclei during fetal life).

Hormones interact broadly with key neurotransmitters implicated in the development of psychosis, namely dopamine, serotonin, glutamate and GABA, the downstream effect being striatal dopamine over-reactivity (4), the most well-established pathophysiological mechanism underlying psychosis. Abnormalities in the hypothalamus-pituitary-adrenal axis (HPA) and hypothalamus-pituitary-thyroid axis (HPT), for instance, are consistently found in people suffering from psychotic disorders, including during early phases of the disorder. In this Special Issue, we feature an analysis of the functional status of the HPA and HPT in a naturalistic population of 486 inpatients with schizophrenia and 154 healthy controls in Shanghai (Zhu et al.). This research team found that hormonal levels varied according to diagnosis of schizophrenia, disease stage (e.g., first-episode vs. recurrent) and gender. Also, a weak association was observed between disease severity and cortisol level. Duval et al. used an integrative experimental approach to investigate HPA and HPT reactivity in response to appropriate stimulating/inhibiting drugs in subjects diagnosed with schizophrenia, schizoaffective or bipolar disorder and healthy controls. Although, due to the small sample size, this remains a preliminary finding, the results showed pathophysiological

OPEN ACCESS

Edited and reviewed by:
Ingrid Melle,
University of Oslo, Norway

***Correspondence:**
Grazia Rutigliano
grazia.rutigliano.gr@gmail.com

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *17 September 2020*
Accepted: *16 October 2020*
Published: *09 November 2020*

Citation:
Rutigliano G, Chaumette B and Seeman MV (2020) Editorial: Psychoneuroendocrinology of Psychosis Disorders. Front. Psychiatry 11:607590. doi: 10.3389/fpsyt.2020.607590

differences between schizophrenia and depression in schizoaffective and bipolar disorder, which were tentatively explained by distinct dopamine and noradrenaline abnormalities.

Hormonal balance is known to be impacted by environmental triggers. According to the neural diathesis-stress model, abnormalities in the HPA result in an exaggerated response to psychosocial stressors, which, in turn, can impact the dopaminergic and glutamatergic systems. This could explain the role of psychosocial stressors as so-called second-hit risk factors in the neurodevelopment trajectories that culminate in psychosis. The issue of the triangular relationship among stress, HPA and psychosis is addressed in three articles of the present Research Topic. Cullen et al. conducted a meta-analysis to study the concordance between naturally-occurring psychosocial stressors and cortisol levels in subjects with psychosis or at ultra-high risk (UHR) for psychosis and in healthy subjects. The authors did not observe any significant differences in cortisol responses to stressors between subjects in the psychosis spectrum and healthy subjects. Their results contradict the classical view that HPA abnormalities in psychosis are caused by increased exposure or increased sensitivity to stress, instead suggesting that cortisol alterations are epiphenomena of global physiological dysregulation. In the same vein, applying a Mendelian randomization model to a longitudinal cohort of 133 UHR subjects, Iftimovici et al. showed that, given the same cortisol levels, conversion to psychosis is more likely in female subjects with relatively high levels of expression of the glucocorticoid receptor NR3C1. It appears, therefore, that hormonal levels are only one piece in a more complex puzzle that involves genetic, epigenetic, and other physiological regulators. In the last two decades, the microbiome has emerged as an important signaling system that modulates the effects of stress exposure on brain development through cross-talk with glucocorticoid hormones and neurotrophins, as beautifully reviewed by Hoffman et al.. One highly stressful experience is admission to the emergency department as a result of an acute mental crisis. Potvin et al. report an increase in peripheral endogenous cannabinoid levels, such as anandamide and oleoylethanolamide, in 107 patients with schizophrenia/schizoaffective disorder experiencing a mental crisis. An increase in cannabinoid level was positively associated with depressive symptoms, possibly mediated by dysregulation of the HPA axis.

Psychotic disorders usually appear shortly after puberty and present differently in male and female subjects. In addition, psychotic symptoms appear to be exacerbated in women during periods of estrogen withdrawal, such as post-partum and para-menopause. Clinical observations such as these have fuelled research into the role of gonadal hormones in the pathogenesis of psychotic disorders. Animal research has shown that gonadal hormones regulate neurodevelopment and plasticity, and they interact with neurotransmitter systems, including the dopaminergic system. Brzezinski-Sinai and Brzezinski have summarized the vast body of knowledge linking gonadal hormone fluctuations and the course of psychotic disorders in women. Their mini-review offers practical suggestions for optimizing psychosis management throughout various stages of female reproductive life. Aside from physiological fluctuations, the gonadal hormone-gonadotropin releasing hormone (GnRH) feedback system can be impaired in pathological conditions, such as the polycystic ovary syndrome (PCOS). PCOS is frequently co-morbid with psychosis and remains a condition that is often recognized late and treated late. Doretto et al. review pathophysiological hypotheses about PCOS and its co-occurrence with psychosis and address the effects of antipsychotic drugs on this condition. The authors emphasize that antipsychotic-induced weight gain, metabolic disturbances and hyperprolactinaemia worsen PCOS symptoms and are much easier to prevent than to treat once they occur. Exogenous hormones interfere with the production of natural hormones so that contraceptives, hormone replacement therapies, and hormonal treatments for infertility may all indirectly impact proneness to psychosis. The systematic review by González-Rodríguez et al. explores the effects on psychopathology of fertility treatments that induce hypoestrogenism. They can exacerbate depressive and psychotic symptoms and act as triggers of mood and psychotic disorders. Despite several anecdotal reports of psychotic symptoms induced or worsened by fertility treatments, proper trials are still lacking. The authors review nine trials, all conducted in non-clinical populations, that focus on mood profile and advocate for future investigations with participants experiencing psychotic disorders.

Patients with schizophrenia-spectrum disorders are known to have a reduced life expectancy, mostly accounted for by cardiovascular comorbidities. It has been proposed that psychotic disorders and cardiometabolic conditions may share genetic liability as well as neuroendocrinological pathogenicity. Investigating the overlap has been difficult because most studies conducted in patients with schizophrenia are confounded by long-term use of antipsychotics as well as by the effects of chronicity—e.g., social isolation, sedentary habits, poor diet, and substance abuse. Studies assessing patients with recent-onset psychosis are in a better position to yield useful information. In this Research Topic, three studies have focused on this topic. Petruzzelli et al. retrospectively analyse clinical records of a precious, albeit small, sample of drug-naïve children and adolescents admitted to their ward with a diagnosis of schizophrenia-spectrum or affective-spectrum disorders. Young patients with a diagnosis of schizophrenia-spectrum disorder had high fasting glucose, fasting insulin, and insulin resistance index HOMA-IR, indicating a potentially higher risk of diabetes mellitus type 2, when compared to patients with affective-spectrum disorders. However, in a sample of 60 patients with recent-onset psychosis and 50 healthy controls, Montalvo et al. did not detect any significant differences in glucose parameters. Nevertheless, they describe an inverse association between glycated hemoglobin (HbA1c)—a marker of long-term exposure to high glucose levels—and several hippocampus- and prefrontal cortex-dependent cognitive domains. Finally, Lis et al. found significant alterations in leptin levels, glucose metabolism, and lipid profile in patients with a first episode of psychosis when compared to healthy controls, indicating an impairment of the

adipo-insular axis in early psychosis. Also in this study, the authors observed a negative correlation between a metabolic parameter—leptin in this case—and cognitive performance. No alteration in the adipo-insular axis was detected in unaffected offspring of schizophrenia patients, failing to support the hypothesis of a shared familial liability between psychotic and metabolic disorders. Future studies with larger sample sizes are needed to confirm these findings.

Psychoneuroimmunology is a branch of psychoneuroendocrinology that studies the interrelationships between the immune-inflammatory system and the central nervous system. The role of the immune system in the etiopathogenesis of psychotic disorders is supported by genetic data that identify several genes located in the major histocompatibility complex and involved in antimicrobial defense as susceptibility genes for schizophrenia. Furthermore, there is an epidemiological connection between maternal infections during pregnancy and psychotic disorders. Aguilar-Valles et al. provide a review of the literature that encompasses both clinical evidence and animal models and explores the effect of maternal inflammatory activation on the development of the dopaminergic neurotransmission system.

Our Research Topic highlights the key role of neuroendocrinological and immune-inflammatory factors in the development, maintenance, and outcome of psychotic disorders. The papers summarized here clearly show that body and mind are one, and that a holistic approach is vital for the effective clinical management of patients with psychosis.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

GR received a grant from the University of Pisa, PRA 2020-21. BC received a grant from the Fondation Bettencourt Schueller.

REFERENCES

1. Kendler KS, Jablensky A. Kraepelin's concept of psychiatric illness. *Psychol Med.* (2011) 41:1119–26. doi: 10.1017/S0033291710001509
2. Hoskins RG. Endocrine factors in dementia precox. *N Engl J Med.* (1929) 200:361.
3. Fink G. Chapter 5-Neural control of the anterior lobe of the pituitary gland (pars distalis). In: Fink G, Donald WP, Levine JE, editors. *Handbook of Neuroendocrinology*. San Diego, CA: Academic Press (2012). p. 97–137.
4. Seeman P. All roads to schizophrenia lead to dopamine supersensitivity and elevated dopamine D2High receptors. *CNS Neurosci Ther.* (2011) 17:118–32. doi: 10.1111/j.1755-5949.2010.00162.x

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 27 February 2020
doi: 10.3389/fpsyt.2020.00065

Functional Status of Hypothalamic–Pituitary–Thyroid and Hypothalamic–Pituitary–Adrenal Axes in Hospitalized Schizophrenics in Shanghai

Yuncheng Zhu[1,2†], Haifeng Ji[2†], Lily Tao[3], Qing Cai[3], Fang Wang[4], Weidong Ji[2], Guohai Li[5] and Yiru Fang[1,6,7*]*

[1] Clinical Research Center & Division of Mood Disorders, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, Shanghai, China, [2] Division of Psychiatry, Shanghai Changning Mental Health Center, Affiliated Greenland Hospital of Bio-X Institute, Shanghai Jiao Tong University, Shanghai, China, [3] Key Laboratory of Brain Functional Genomics (MOE & STCSM), Shanghai Changning-ECNU Mental Health Center, Institute of Cognitive Neuroscience, School of Psychology and Cognitive Science, East China Normal University, Shanghai, China, [4] Division of Psychiatry, Shanghai Yangpu Mental Health Center, Shanghai, China, [5] Zhenjiang Mental Health Center, Zhenjiang, China, [6] CAS Center for Excellence in Brain Science and Intelligence Technology, Shanghai, China, [7] Shanghai Key Laboratory of Psychotic Disorders, Shanghai, China

OPEN ACCESS

Edited by:
Grazia Rutigliano,
University of Pisa, Italy

Reviewed by:
Javier Labad,
Corporació Sanitària Parc Taulí, Spain
Anilkumar Pillai,
Georgia Regents Medical Center, United States

***Correspondence:**
Yiru Fang
yirufang@aliyun.com
Fang Wang
wangfangpsychiatry@163.com

[†]These authors have contributed equally to this work

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: 22 October 2019
Accepted: 24 January 2020
Published: 27 February 2020

Citation:
Zhu Y, Ji H, Tao L, Cai Q, Wang F, Ji W, Li G and Fang Y (2020) Functional Status of Hypothalamic–Pituitary–Thyroid and Hypothalamic–Pituitary–Adrenal Axes in Hospitalized Schizophrenics in Shanghai. Front. Psychiatry 11:65. doi: 10.3389/fpsyt.2020.00065

Objective: Neuroendocrine dysfunction is related to the pathogenesis of mental disorders, but conclusions from clinical research lack consistency. We aimed to investigate the neuroendocrinal pathophysiology and its correlation with clinical symptoms in patients with schizophrenia.

Methods: The present cross-sectional study included 486 inpatients with schizophrenia admitted at a psychiatric hospital in Shanghai within one year, and 154 healthy controls (HC) matched on age and gender. The serum hemoconcentrations of thyroid-stimulating hormone (TSH), total triiodothyronine (TT3), total thyroxine (TT4), free triiodothyronine (FT3), free thyroxine (FT4), adrenocorticotrophic hormone (ACTH), and cortisol (COR) were measured *via* electrochemical luminescence immunoassay. Pathophysiological conversions of neuroendocrine were then associated with gender, age, age at onset, antipsychotic treatment using hierarchical multiple linear regression.

Results: When compared to HC, the schizophrenia group showed elevated ACTH and COR levels and decreased TT3 and TT4 levels (p's < 0.05). First-episode patients showed lower TSH and higher FT3 and FT4 (p's < 0.05) compared to recurrent patients. Female patients showed higher TSH and lower TT3, FT3, and ACTH levels (p's < 0.05) compared to males. We observed the area under the curve (AUC) of the predictive model to distinguish between schizophrenia and HC to be 0.737 among total samples and between first-episode and recurrent schizophrenia to be 0.890 among subgroups.

Conclusions: Decreased TT3 and TT4 and elevated ACTH and COR levels appear to be associated with schizophrenia symptoms. The chronic recurrent trait of schizophrenia

may cause long-term effects on FT3 and FT4 while changes in thyroid, and adrenal function as a result of mental disorder varied with gender. The pathophysiological parameters provide fair to good accuracy of these models.

Keywords: schizophrenia, thyroid function tests, pituitary–adrenal function tests, neuroendocrine system, chronic stress

BACKGROUND

Neuroendocrine dysfunction is related to the pathogenesis of mental disease. To date, however, research findings lack evidence of clinical consistency. For example, many psychoneuroendocrinological studies have focused on treatment-naïve patients (1). Because antipsychotics need to be maintained for as long as is necessary, follow-up psychoneuroendocrinological changes are short of longitudinal evidence.

Hypothalamic–pituitary–thyroid axis (HPTA) function is significantly associated with the onset of schizophrenia, by affecting emotion regulation and cognitive functioning (2). The hypothalamic–pituitary–adrenal gland axis (HPAA) also plays an important role in the relationship between psychological stress and neural activity (3).

The neuroendocrine functions of HPTA and HPAA are involved in the regulation of moods, emotions, and cognitive behaviors in acute or chronic stress. Lowering pituitary volume in schizophrenia is most likely by enhancing stress regulation and lowering the distress due to psychotic symptoms (4). For example, clinically significant fluctuations in thyroid hormones were found after surgery for Cushing's syndrome (5). These findings suggest that there may be multiaxial changes in pituitary function associated with the appearance of psychiatric symptoms. Most neuroendocrine studies of schizophrenia focused on antipsychotics, course of disease, symptoms, and other influencing factors as research objectives. Thyroid and adrenal function in hospitalized patients with schizophrenia may be associated with factors of disease, acute or hospitalized phase, gender, and recurrence. Women are more prone to endocrine dyscrasia. Many, however, underestimate gender differences, which affect the generalizability of results (6–8). It is important to include these variables in analyses involving schizophrenia since they are likely to be involved in the pathogenesis of psychosis.

Aside from clear-cut neuroendocrine disorders of the pituitary axes (*e.g.*, hyperthyroidism), which are shown to be high risk factors of psychiatric symptoms (9), subclinical neuroendocrinal hyper- or hypofunctions are also commonly seen in the course of diagnosis and treatment of schizophrenia (10, 11). Due to the side effects of antipsychotic drugs, cardiac deceases, diabetes or smoking, neuroendocrine diseases have a higher comorbidity in patients with schizophrenia (12), particularly disorders in HPTA and HPAA, which are closely related to the occurrence and development of schizophrenia (13, 14).

Patients with schizophrenia may self-regulate their hormone levels in the HPTA and HPAA under chronic stress exposure (15). For example, free triiodothyronine (FT3) level is positively associated with cognitive function (16), while cortisol (COR) responses to stress (17). In terms of thyroid hormonal components, conjugated thyroxine is the storage and transportation form of the hormone, while FT4 is the active component. Total thyroxine (TT4) is composed of T4 and free thyroxine (FT4), with only 0.02% of the circulating hormone being FT4 (18). T4 undergoes extra-thyroidal conversion to T3, which is three to four times more active than T4 (19). Hence, compared to T3, T4 is a more moderate adjustment for overall thyroid function. The main adrenal function, as we know, activates to boost metabolism and to increase excitability of the central and peripheral nervous systems (20). Moreover, type II iodothyronine 5′ deiodinases catalyzes the conversion of T4 to T3 (21), and chronic physiological COR increase may result in a decline in T3 by inhibiting this enzyme in schizophrenia (22).

Many studies have found sex differences in HPAA in patients with early psychosis or schizophrenia. Patients with recent onset of psychosis were observed to have a significant sex difference, with a blunted COR response to awakening in men but not in women (23), while a significantly lower dehydroepiandrosterone sulfate (DHEA-S) was found in male patients (24). COR-to-DHEA-S (C/D) ratio might predict health levels of patients (25). In the domain of executive function, COR predicted poor performance on the cognitive functioning in male schizophrenics (26). On the other hand, Labad J et al. (27) suggest that there are sex differences in the relationship between HPAA measures and cognitive abilities in early psychosis. So, there are different interpretations of HPAA by gender due to first episode or recurrent schizophrenia. Furthermore, we have noticed that the most common diseases of the neuroendocrine systems present with obvious gender differences (28) (**Table 1**).

In short, we noticed that there was not too much on the topic and that few studies had included both first episode and chronic schizophrenics focusing on both of hemoconcentrations of HPTA and HPAA hormones. We collected them among patients with schizophrenia and healthy controls (HC). There have been numerous studies conducted over the past years that

TABLE 1 | Gender ratios in common hypothalamic–pituitary–glandular axial disorders.

Disease	Gender ratio (Male : Female)
Hyperthyroidism	1:4–6
Hypothyroidism	1:4
Cushing disease	1:3
Addison's disease	1:2–3

Abbreviations: HPTA, hypothalamic–pituitary–thyroid axis; HPAA, hypothalamic–pituitary–adrenal gland axis; HPGA, hypothalamic–pituitary–gonad axis; COR, cortisol; HC, healthy controls; PANSS, Positive and Negative Syndrome Scale; TSH, thyroid-stimulating hormone; TT3, total triiodothyronine; FT3, free triiodothyronine; TT4, total thyroxine; FT4, thyroid hormone; ACTH, adrenocorticotrophic hormone.

have examined HPAA and HPTA in samples of patients with schizophrenia and psychosis. Findings from these studies have been examined in meta-analyses and systematic reviews (29–31). However, many of these markers have not been examined in the same samples. We excluded potential endocrine diseases related with these two axes before analysis, and we hypothesized that schizophrenia with different episodes of disease or gender difference may have an effect on neuroendocrine function.

PARTICIPANTS AND METHOD

Participants

The study was approved by the Institutional Ethical Committee for clinical research of Shanghai Changning Mental Health Center, Shanghai, China. Informed consent was provided according to the *Declaration of Helsinki*. We recruited 486 patients with schizophrenia and 154 healthy individuals matched on age, gender, and ethnicity (Han).

Patients were recruited from Shanghai Changning Mental Health Center, an in-patient psychiatric ward, from 01 March 2017 to 28 February 2018. There were approximately 590,000 permanent residents in the Changning district of Shanghai (32). Therefore, we expected that the psychiatric demographics was representative of that of the city of Shanghai.

The diagnosis of schizophrenia without comorbid diagnosis was ascertained according to the DSM-IV criteria. The mental examination was conducted by three-level ward round including at least one chief physician. Patient inclusion criteria were: Chinese Han ethnicity, aged 18 to 65 years, at stable phase (no more acutely symptomatic reoccurrence at the time of investigation under more than a year of stable medication adherence) or first-episode patients. Exclusion criteria were: More than one antipsychotic medication for the acute psychosis treatment in a year, first-diagnosed but not antipsychotic naïve (use of levothyroxine, antithyroid, glucocorticoid, bromocriptine, testosterone, estrogen, progestin, oral contraceptive, amiodarone, lithium, psychoactive substance or other medicines related to neuroendocrine diseases within a year); use of stimulants or inhibitors (microsomal drug metabolizing enzyme inducers or inhibitors, diuretics, or any affecting thyroid or adrenal function shown in medicine instruction); pregnant or in *postpartum* period; history of central nervous system disease; history of thyroid, adrenal, or gonad diseases tested using B-ultrasonography or immunoserology; any neuroendocrine diseases confirmed by the neuroendocrine test; Pittsburgh sleep quality index (PSQI) (33) scores >7. We defined that the first-episode patients were not on antipsychotic medication, while the recurrent patients took only one medication for at least one year.

Blood samples of the HC group were collected from medical examination items of the general population in Shanghai, recorded in the medical examination center of Tongren Hospital, affiliated to Shanghai Jiaotong University School of Medicine. The healthy individuals were voluntarily recruited by advertisement to participate in the study with no psychiatric history, which were excluded by a self-made questionnaire. The Mini-International Neuropsychiatric Interview (M.I.N.I) (34) and the PSQI were then used for screening any psychotic disorder of the HCs (see **Figure 1** for a flow diagram of sample selection).

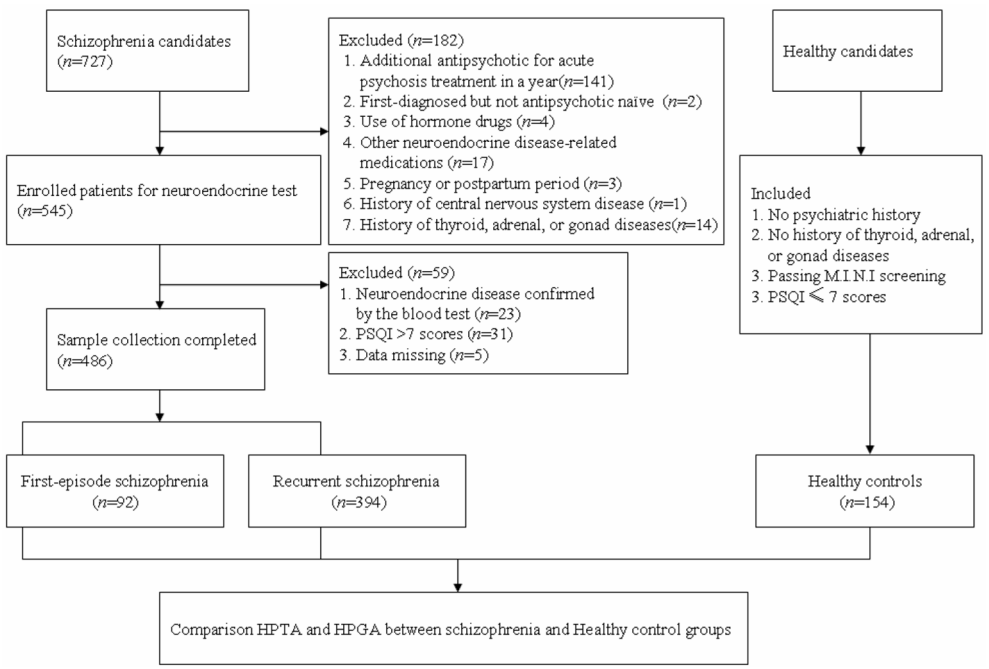

FIGURE 1 | Flowchart of screening process and data classification.

Measures

Positive and Negative Syndrome Scale

The Chinese Mandarin version of the Positive and Negative Syndrome Scale (PANSS) (35) has been shown to be a reliable and valid instrument for the assessment of the severity of psychopathology in hospitalized patients with schizophrenia. The scale consists of 30 items, each rated using a 7-point scale. We recorded patients' total PANSS, positive symptoms, negative symptoms, and general psychopathology scores as variables.

Hemoconcentration of Hormones in HPAA and HPTA

The hormones tested include serum concentrations of thyroid-stimulating hormone (TSH), TT3, FT3, TT4, FT4, adrenocorticotrophic hormone (ACTH), and COR. We collected venous blood of the patients who must be under inpatient sleep management with good sleep rhythm, as well as healthy individuals, between 6:00 a.m. and 8:00 a.m. Blood samples were taken before breakfast to minimize the effects of circadian variation. A total of 5 ml blood was collected by a single venipuncture into yellow plain tubes (with coagulants and separation gel). After standing at room temperature for 30 min, the blood sample was centrifuged for 15 min at 1,800 g. The serum was carefully aliquoted into 2-ml screw-top microtubes for subsequent storage. Two aliquots were collected from each study individual and one for standby application. Each microtube was labeled with a coded identification label and stored at −80°C (36). The panel of 640 sera was used to measure hormone concentrations over two widely available commercial automated analyzer systems with standard procedure: Roche Cobas e601 and Modular e170 automatic electrochemiluminescence immunoassay system (ECLIA) for TSH, T3, T4, and COR and ACTH, respectively (37, 38). The hormonal assay was performed in the Lanwei Clinical Testing Laboratory, Shanghai, China. We used the reference intervals of these hormone concentrations for comparison according to the People's Republic of health industry standards: TSH 95% CI (range, 0.27–4.20 mIU/L), TT3 (range, 1.3–3.1 nmol/L), FT3 (range, 2.8–7.1 pmol/L), TT4 (range, 66–181 nmol/L), FT4 (range, 12–22 pmol/L), ACTH (range, 7.2–63.3 ng/L) and COR (range, 171~536 nmol/L).

Statistical Analysis

Given the relatively large sample size (640 data points for HPTA and HPAA), sample size calculation was omitted. All statistical computations were performed using SPSS 17.0. Data were represented as mean (± SD). Comparisons of the candidate values between patients with schizophrenia and HCs were performed *via* independent sample Student's *t*-test or one-way ANOVA for normally distributed data, and Wilcoxon *W*-Test or Mann-Whitney *U*-test for skewed distribution data, as appropriate. Analysis of covariance (ANCOVA) was conducted for comparison of hormones in these two axes (age, BMI, drug dose as covariables), as appropriate. Chlorpromazine (CPZ)-equivalent dose was conversed for analyzing risk of antipsychotic treatment in recurrent schizophrenics (39). The differences between groups were analyzed by *post hoc* with Bonferroni correction (40). A hierarchical multiple linear regression analysis for each PANSS subscore was conducted by including HTA axis hormones (TSH, FT4, and FT3) and HPA axis hormones (ACTH, COR) and main covariates (gender, age, age at onset, and CPZ-equivalent dose). All statistical analyses were defined as two-tailed p value, significance level of 5% (α = 0.05). After normal transformation where necessary, the nonnormal distribution data were conducted with statistical disposal for *Cohen's d* or η^2. Effect sizes were provided by OR, *Cohen's d* or η^2.

RESULTS

Comparison Between Schizophrenia and HC Groups

Table 2 presents the statistics for the participant groups. The median of illness duration and age at onset was 9 years and 26 years old, respectively. There was no difference in age between the two groups ($p > 0.05$). Age and BMI were considered as covariables to measure the difference in these two axes between groups, and there were no group × age or group × BMI interactions (p's > 0.05). The schizophrenia group showed significantly higher BMI, FT4, ACTH, and COR levels and significantly lower TT3 and TT4 levels compared to HC (p's < 0.05).

Although there were no significant differences between the two groups in TSH or FT3 levels (p's > 0.05), higher TSH and lower FT3 levels of female patients were observed compared to female HC (p's < 0.05), while higher FT3 of male patients was observed compared to male HC (p's < 0.05). The female schizophrenia group showed higher FT4, and the male schizophrenia group showed higher ACTH compared to each isosexual HC (p's < 0.05).

Mean (± SD) for the normal distribution data and median (Q1, Q3) for the skewed distribution data; BMI, body mass index; TSH, thyroid-stimulating hormone; TT3, total triiodothyronine; FT3, free triiodothyronine; TT4, total thyroxine; FT4, free thyroxine; ACTH, adrenocorticotrophic hormone and COR, cortisol.

We constructed a hierarchical multivariable prediction model using the TSH, FT3, FT4, ACTH, COR, gender (woman = 0, man = 1), and age for diagnosis of schizophrenia. The receiver operating characteristic (ROC) curve showed the fair accuracy of this model, yielding an area under the curve (AUC) of 0.737 (95% CI, 0.699–0.774), and the best cut-off value (Youden index) was 0.463.

Table 3 presents the number of participants with abnormal endocrine concentrations between groups for HPTA and HPAA hormone levels outside of the upper or lower limit of the normal range. The only significant differences were TSH, TT3, FT4, ACTH, and COR levels between female groups, and TT3 and COR between male groups (p's < 0.05). However, there was no difference in FT4 or ACTH level between the male subgroup as well as FT4 between female's (p's > 0.05).

TABLE 2 | Quantitative comparison between schizophrenia and HC groups in the gender subgroup.

	Schizophrenics (*n* = 486, male/female = 292/194)	Healthy controls (*n* = 154, male/female = 93/61)	*Z/t/F*	*P*	*Cohen's d*
Age (y)	39.3 ± 12.6	37.3 ± 8.1	0.687	0.492	0.064
Male	39.0 ± 12.9	38.4 ± 7.2	0.660	0.510	0.079
Female	39.8 ± 12.2	35.7 ± 9.3	1.819	0.069	0.267
BMI (kg/m^2)	23.6 ± 4.0	22.3 ± 3.2	4.169	<0.001	0.386
Male	23.9 ± 4.0	22.7 ± 3.5	2.594	0.010	0.309
Female	23.2 ± 3.9	21.8 ± 2.6	3.416	0.001	0.501
TSH (mIU/L)	2,09 ± 1.48	1.86 ± 0.86	0.387	0.699	0.036
Male	1.85 ± 1.37	1.88 ± 0.81	1.870	0.061	0.223
Female	2.46 ± 1.58	1.83 ± 0.93	2.874	0.004	0.422
TT3 (nmol/L)	1.58 ± 0.30	1.88 ± 0.51	75.332	<0.001	0.637
Male	1.64 ± 0.30	1.92 ± 0.55	37.091	<0.001	0.560
Female	1.49 ± 0.27	1.81 ± 0.43	50.181	<0.001	0.802
FT3 (pmol/L)	4.48 ± 0.79	4.43 ± 0.97	0.914	0.339	0.062
Male	4.66 ± 0.78	4.38 ± 0.98	7.968	0.005	0.297
Female	4.21 ± 0.74	4.49 ± 0.94	7.351	0.007	0.309
TT4 (nmol/L)	103.2 ± 21.2	121.2 ± 20,2	9.148	<0.001	0.846
Male	102.2 ± 20.8	122.5 ± 20.8	7.693	<0.001	0.916
Female	104.9 ± 21.9	120.2 ± 19.5	5.024	<0.001	0.738
FT4 (pmol/L)	18.1 ± 3.4	17.3 ± 3.0	2.850	0.004	0.264
Male	18.2 ± 3.4	17.5 ± 3.0	1.812	0.070	0.216
Female	18.0 ± 3.5	17.0 ± 3.1	2.345	0.019	0.344
ACTH (ng/L)	34.3 ± 23.4	23.1 ± 9.3	5.418	<0.001	0.501
Male	39.4 ± 25.6	23.7 ± 11.3	6.213	<0.001	0.740
Female	26.8 ± 17.0	22.2 ± 4.81	0.707	0.480	0.104
COR (nmol/L)	490.2 ± 214.3	397.1 ± 75.7	38.984	<0.001	0.912
Male	500.6 ± 199.0	373.4 ± 84.3	32.637	<0.001	1.041
Female	474.7 ± 235.1	382.8 ± 60.3	3.726	<0.001	0.727

TABLE 3 | Qualitative comparison between schizophrenia and HC groups outside of upper or lower limit of the normal range in the gender subgroups.

	Abnormal value (*n*%)		*χ2* value	*p*	*OR*	*95% CI for OR*	
	Schizophrenics (*n* = 486)	Healthy controls (*n* = 154)				Lower	Upper
TSH	45(9.3%)	3(1.9%)	9.011	0.003	5.128	1.572	16.67
Male	19(6.5%)	3(3.2%)	1.409	0.235	2.075	0.604	7.246
Female	26(13.4%)	0(0%)	/	0.001[a]	/	/	/
TT3	95(19.5%)	7(4.5%)	19.645	<0.001	5.102	2.315	11.24
Male	44(15.1%)	6(6.5%)	4.635	0.031	2.551	1.059	6.250
Female	51(26.3%)	1(1.6%)	17.369	<0.001	21.277	2.890	166.7
FT3	6(1.2%)	6(3.9%)	3.172	0.075	0.308	0.098	0.970
Male	2(0.7%)	3(3.2%)	/	0.093[a]	0.205	0.034	1.258
Female	4(2.1%)	3(4.9%)	0.550	0.458	0.407	0.089	1.873
TT4	6(1.2%)	0(0%)	/	0.344[a]	/	/	/
Male	2(0.7%)	0(0%)	/	1.000[a]	/	/	/
Female	4(5.1%)	0(0%)	/	0.575	/	/	/
FT4	74(15.2%)	13(8.4%)	4.583	0.032	1.949	1.048	3.323
Male	38(13.0%)	7(7.5%)	2.057	0.151	1.880	0.792	4.274
Female	36(18.6%)	6(9.8%)	2.565	0.109	2.088	0.835	5.236
ACTH	70(14.4%)	6(3.9%)	12.337	<0.001	4.149	1.767	9.709
Male	38(13.0%)	6(6.5%)	3.001	0.083	2.151	0.887	5.319
Female	32(16.5%)	0(0%)	/	<0.001[a]	/	/	/
COR	236(48.6%)	6(3.9%)	99.208	<0.001	23.256	10.101	52.63
Male	138(47.3%)	6(6.5%)	50.171	<0.001	12.987`	5.495	30.30
Female	98(50.5%)	0(0%)	/	<0.001[a]	/	/	/

[a]*p was calculated by Fisher's exact test.*

Comparison Between First-Episode and Recurrent Schizophrenia Subgroups and HCs

The median ages of the recurrent schizophrenia group and the HC group were 15 and 13 years older, respectively, than that of the first-episode schizophrenia group ($p < 0.001$). The BMI showed no change between the first-episode schizophrenia and the recurrent schizophrenia subgroups ($p = 0.001$). The median CPZ-equivalent dose of the recurrent schizophrenia group was 328.3 (mg/day). Total PANSS score and its sub-scales showed no differences (p's > 0.05).

Age and BMI were considered as covariables to measure the difference in these two axes between groups, and there were no group × age or group × BMI interactions (p's > 0.05). The subgroups of schizophrenia showed significantly higher ACTH and COR levels and significantly lower TT3 and TT4 levels compared to HC (p's < 0.05), but there was no difference in those levels between subgroups of schizophrenia (p's > 0.05). The first-episode and HC groups showed significantly lower TSH level than that in the recurrent group ($p < 0.05$) while the first-episode group showed significantly higher FT3 and FT4 levels compared to recurrent schizophrenia and HC (p's < 0.05). See **Table 4**.

We constructed a hierarchical multivariable prediction model using the TSH, FT3, FT4, ACTH, COR, gender, and age as diagnostic predictor of first-episode and recurrent schizophrenia. The ROC curve shows the good accuracy of this model, yielding an AUC of 0.890 (95% CI, 0.855–0.924), and the best cut-off value (Youden index) was 0.700.

Comparison Between Genders in the Schizophrenia Group

Of the 486 patients, 292 cases (60%) were males. There was no difference in demographic characteristics between the female and male patients with schizophrenia, including age, illness duration, first-episode age, BMI, and CPZ-equivalent dose (p's > 0.05). Age, BMI, and CPZ-equivalent dose were chosen as covariables to measure the difference in these two axes between groups. There were sex × age and sex × BMI interactions (p's < 0.05), but not sex × CPZ-equivalent dose interaction ($p > 0.05$). Positive symptom score and TSH level in the female schizophrenia group were significantly higher (p's < 0.05), while negative symptom score, TT3, FT3, and ACTH levels were significantly lower than those in the male schizophrenia group (p's < 0.05). See **Table 5**.

Correlations Between Clinical Features and HPTA and HPAA Hormone Levels in the Schizophrenia Group

The hierarchical multiple linear regression models of the three regression analyses (PANSS positive, PANSS negative, PANSS general) are shown in **Table 6**. These equations were constructed by X_1 = TSH, X_2 = FT3, X_3 = FT4, X_4 = ACTH, X_5 = COR, X_6 = gender, X_7 = age, X_8 = age at onset, X_9 =BMI, X_{10} = CPZ-equivalent dose (p's < 0.05). The regression equations were finally observed as follows:

$$\begin{aligned}\text{Logit(Positive)} = {} & 10.987 - 0.031X_1 + 0.075X_2 + 0.200X_3 \\ & + 0.003X_4 + 0.003X_5 - 1.620X_6 + 0.077X_7 \\ & - 0.014X_8 - 0.097X_9 - 0.003X_{10};\end{aligned}$$

$$\begin{aligned}\text{Logit(Negative)} = {} & 20.639 - 0.061X_1 - 1.472X_2 + 0.143X_3 \\ & + 0.014X_4 + 0.001X_5 + 2.224X_6 + 0.004X_7 \\ & - 0.033X_8 - 0.027X_9 + 0.002X_{10};\end{aligned}$$

$$\begin{aligned}\text{Logit(General)} = {} & 35.158 - 0.132X_1 + 0.051X_2 - 0.015X_3 \\ & - 0.004X_4 + 0.004X_5 + 0.711X_6 + 0.037X_7 \\ & - 0.008X_8 - 0.025X_9 + 0.001X_{10}\,.\end{aligned}$$

In the subscales, the score of positive symptoms was positively correlated with FT4 level, woman, and age (p's < 0.05). The negative symptom score was negatively correlated with FT3 level

TABLE 4 | Comparison between first-episode and recurrent schizophrenia subgroups and HCs.

	Schizophrenics		Healthy controls (n = 154)	*Z/t/F*	*p*	*Corhen's d/η²*	*Post Hoc*[a]
	First-episode (n = 92)	Recurrent (n = 394)					
Age (y)	27.2 ± 7.0	42.1 ± 12.0	37.3 ± 8.1	156.670	<0.001	0.330	1 < 3 < 2
BMI (kg/m^2)	23.6 ± 4.1	23.6 ± 4.0	22.3 ± 3.2	6.924	0.001	0.021	1,2 < 3
Total PANSS	85.7 ± 9.8	85.5 ± 11.8	/	0.153	0.878	0.018	/
PANSS positive	19.2 ± 5.2	19.6 ± 7.0	/	0.445	0.656	0.052	/
PANSS negative	17.5 ± 5.2	17.8 ± 5.1	/	0.357	0.721	0.041	/
PANSS general	37.7 ± 4.7	37.6 ± 5.4	/	0.222	0.825	0.026	/
TSH (mIU/L)	1.68 ± 0.95	2.19 ± 1.57	1.86 ± 0.86	5.826	0.054	0.021	1,3 < 2
TT3 (nmol/L)	1.61 ± 0.31	1.57 ± 0.29	1.88 ± 0.51	38.016	<0.001	0.112	1,2 < 3
FT3 (pmol/L)	4.67 ± 0.85	4.44 ± 0.78	4.43 ± 0.97	3.239	0.040	0.010	2,3 < 1
TT4 (nmol/L)	106.7 ± 20.7	102.4 ± 21.3	121.6 ± 20.2	85.993	<0.001	0.127	1,2 < 3
FT4 (pmol/L)	19.4 ± 3.7	17.8 ± 3.3	17.3 ± 3.0	16.943	<0.001	0.036	2,3 < 1
ACTH (ng/L)	33.2 ± 21.9	34.6 ± 23.7	23.1 ± 9.3	29.536	<0.001	0.051	3 < 1,2
COR (nmol/L)	512.7 ± 173.7	485.0 ± 222.6	397.1 ± 75.7	21.083	<0.001	0.063	3 < 1,2

[a]*Bonferroni correction for multiple comparisons was applied, and the result was p < 0.05; BMI, body mass index; PANSS, Positive and Negative Syndrome Scale; TSH, thyroid-stimulating hormone; TT3, total triiodothyronine; FT3, free triiodothyronine; TT4, total thyroxine; FT4, free thyroxine; ACTH, adrenocorticotrophic hormone and COR, cortisol.*

TABLE 5 | Comparison between genders in the schizophrenia group.

	Schizophrenics		Z/t	P	Corhen's d
	Female (n = 194)	Male (n = 292)			
Age (y)	39.8 ± 12.1	39.0 ± 12.9	0.532	0.595	–
Course (y)	13.2 ± 12.7	11.0 ± 10.6	1.743	0.081	–
Age at onset (y)	26.6 ± 8.2	28.0 ± 8.5	1.881	0.060	–
Body Mass Index (kg/m^2)	23.2 ± 3.9	23.9 ± 4.0	1.752	0.080	–
CPZ-equivalent dose (mg/day)[a]	325.0 ± 143.9	332.0 ± 152.0	0.463	0.644	–
Total PANSS	85.2 ± 11.3	85.8 ± 11.6	0.590	0.555	0.055
Positive symptoms	20.5 ± 5.5	18.9 ± 7.3	2.603	0.010	0.241
Negative symptoms	16.7 ± 4.6	18.4 ± 5.4	3.775	0.000	0.350
General psychopathology	37.2 ± 5.1	37.9 ± 5.3	1.555	0.121	0.144
TSH (mIU/L)	2.45 ± 1.58	1.85 ± 1.37	5.113	<0.001	0.488
TT3 (nmol/L)	1.49 ± 0.27	1.64 ± 0.30	11.394	<0.001	0.503
FT3 (pmol/L)	4.21 ± 0.74	4.66 ± 0.78	13.474	<0.001	0.587
TT4 (nmol/L)	104.8 ± 21.9	102.2 ± 20.8	1.503	0.133	0.132
FT4 (pmol/L)	18.0 ± 3.5	18.2 ± 3.4	0.584	0.559	0.083
ACTH (ng/L)	26.8 ± 17.0	39.4 ± 25.6	5.994	<0.001	0.588
COR (nmol/L)	474.7 ± 235.1	500.6 ± 199.0	1.758	0.079	0.132

PANSS, Positive and Negative Syndrome Scale; TSH, thyroid-stimulating hormone; TT3, total triiodothyronine; FT3, free triiodothyronine; TT4, total thyroxine; FT4, free thyroxine; ACTH, adrenocorticotrophic hormone and COR, cortisol; CPZ, chlorpromazine; a, derived from the antipsychotic treatment in the recurrent schizophrenia subgroup (n = 394, male/female = 222/172).

($p < 0.05$) and positively correlated with FT4 level and man ($p < 0.05$). The general psychopathology score was positively correlated with COR levels ($p < 0.05$). The r values of the three models were 0.239, 0.290, and 0.194, respectively.

DISCUSSION

At first, we analyzed the overall level of these hormones in **Table 2** and the probability of abnormal value in **Table 3** between schizophrenia and HC groups. Age and BMI were considered as confounders, so we tried to eliminate them using the ANCOVA for normally distributed data. While the differences were statistically significant, the clinical significance of these differences remains questionable since the mean values were within the normal laboratory reference range. Therefore, it might help to compare if the rates of abnormal values were greater/lesser in the schizophrenia group using the laboratory cut-off values. Results showed that qualitative values of TSH were affected by gender, whereas the other converted qualitative values for HPTA hormones were consistent with their quantitative data. The Fisher's exact test was applied because of the small expected sample size of abnormal TT4 values. For HPAA, ACTH qualitative data of female patients differed from the corresponding quantitative data, whereas for male patients, they were consistent. This may be due to the skewed distribution of ACTH, in which the most elevated hormone levels were still within the normal range (41). The other converted qualitative data were consistent with quantitative results.

TABLE 6 | Hierarchical multiple linear regression analysis of HPTA and HPAA hormone levels for each PANSS subscore.

Model	β^a	S.E.	β^b	t	p
PANSS positive					
Constant	10.987	3.211		3.422	0.001
TSH	–0.031	0.214	–0.007	–0.143	0.886
FT3	0.075	0.418	0.009	0.178	0.858
FT4	0.200	0.096	0.102	2.087	0.037
ACTH	0.003	0.015	0.010	0.195	0.845
Cortisol	0.003	0.002	0.082	1.625	0.105
Gender	–1.620	0.687	–0.118	–2.358	0.019
Age	0.077	0.029	0.148	2.671	0.008
Age at onset	–0.014	0.042	–0.018	–0.333	0.739
BMI	–0.097	0.082	0.055	1.191	0.234
CPZ-equivalent dose	–0.003	0.002	–0.077	–1.599	0.110
PANSS negative					
Constant	20.639	2.425		8.509	<0.001
TSH	–0.061	0.162	–0.018	–0.375	0.708
FT3	–1.472	0.316	–0.227	–4.660	<0.001
FT4	0.143	0.072	0.095	1.979	0.048
ACTH	0.014	0.011	0.066	1.308	0.192
Cortisol	0.001	0.001	0.027	0.553	0.580
Gender	2.224	0.519	0.212	4.285	<0.001
Age	0.004	0.022	0.009	0.172	0.863
Age at onset	–0.033	0.032	–0.054	–1.047	0.296
BMI	–0.027	0.062	–0.020	–0.432	0.666
CPZ-equivalent dose	0.002	0.001	0.059	1.248	0.213
PANSS general					
Constant	35.158	2.543		13.824	<0.001
TSH	–0.132	0.170	–0.037	–0.776	0.438
FT3	0.051	0.331	0.008	0.154	0.878
FT4	–0.015	0.076	–0.010	–0.193	0.847
ACTH	–0.004	0.012	–0.017	–0.322	0.768
Cortisol	0.004	0.001	0.166	3.267	0.001
Gender	0.711	0.544	0.066	1.307	0.192
Age	0.037	0.023	0.090	1.607	0.109
Age at onset	–0.008	0.033	–0.013	–0.238	0.812
BMI	–0.025	0.065	–0.018	–0.383	0.702
CPZ-equivalent dose	0.001	0.001	–0.027	–0.563	0.574

[a]*Unstandardized Coefficients.* [b]*Standardardized Coefficients.*
TSH, thyroid-stimulating hormone; TT3, total triiodothyronine; FT3, free triiodothyronine; TT4, total thyroxine; FT4, free thyroxine; ACTH, adrenocorticotrophic hormone and COR, cortisol; PANSS, Positive and Negative Syndrome Scale.

The elevated FT4 level among schizophrenics compared to HC is not enough to overcome the overall reduction in TT4 levels. The increased FT4 may be overconsumed by humoral regulation for meeting the needs of pathological and physiological conditions. Thus, TT4 is drained continuously through the active transformation. The chronic toxicological change of the active component FT4 may be one of the causes of chronic psychopathology in schizophrenia (42). The present pattern of TT4 reduction was observed among patients with schizophrenia with a median illness duration of nine years. A longitudinal study had previously confirmed increased hypothyroidism as the disease progresses (43). It is also possible that hypoalbuminemia, hypotransthyretin (44) and a variety of drugs (androgens, glucocorticoids, growth hormones, and so on) can reduce the thyroglobulin content (45), producing a false-positive or false-negative outcome in HPTA among patients with schizophrenia. Transportation is the main factor to explain the difference between serum thyroid function and

actual thyroid hormone state of brain tissue. Transthyretin synthesized in the choroid plexus is involved in movement of thyroxine from the blood into the cerebrospinal fluid and the distribution of thyroid hormones in the brain. Besides the serum concentration of thyroid hormones, the dysregulation of transthyretin plays several roles in neurobiological function of schizophrenia, including neurodevelopment and endocrine disruption (46).

Classic negative feedback regulation cannot explain the synchronous increasing levels of ACTH and COR in the present study as they are negatively correlated. It may be that the synthesis, storage, reabsorption, decomposition, and release process of thyroid hormone depend on humoral regulation for a long period of time (19). Furthermore, illness duration can lead to long-term changes that reform endocrine negative feedback. For acute onset, therefore, negative feedback regulation shows little effect on the secretion of TSH at neuroregulation stage (47).

Under stress, both ACTH and COR levels increased in schizophrenia by means of depending on neural regulation (48), which is more rapid than humoral regulation. The peripheral hypersecretion of both FT4 and COR can maintain excitability of the nervous system. The thyroid–adrenergic interactions produce heat, maintain body temperature, and coordinate emotional regulation (49). The elevated COR level in the acute phase is in line with traditional biomedical models in the developmental course of schizophrenia (50). However, the adrenal fascicular cells present a pulse synthesis of COR, so the secretion is not only regulated by ACTH, but also regulated by vasopressin secreted by hypothalamus (51), stress (52), expression of peripheral clock genes (53), and so on. There is a diurnal variation, a COR awakening response. The secretion of COR appears to be aligned with circadian rhythm fluctuations and seasonal differences. After controlling the time for collecting samples, this result may explain the coelevation of ACTH and COR levels with both acute and chronic stress.

In terms of disease progression, FT3 and FT4 levels of acute stage patients were higher than those of chronic patients, consistent with previous findings of elevated levels of thyroxine during the acute stage of the disease (42). These findings suggest that the chronic course of the disease may have long-lasting effects on HPTA. In the present study, the chronic schizophrenic course presented a median illness duration of nine years. However, the free components of triiodothyronine and thyroxine reduced to normal levels and deactivate the biological changes from negative feedback and also have an effect on inhibiting metabolism, reducing the excitability of central and peripheral nervous systems. On the other hand, Vedal et al. (54) found that lower FT4 is associated with use of antipsychotics, which is contrary to our results in general. Out first-episode schizophrenic patients had never taken antipsychotics, and elevated FT4 is responsible for the hypermetabolism at the acute stage in particular. The effect of FT4 was then clarified after comparison among the first-episode schizophrenia, recurrent schizophrenia, and HC groups. In **Table 2**, the standardized mean effect sizes (*Cohen's d)* of TT3, TT4, FT4, ACTH, and COR range from 0.264 to 0.912 of different clinical relevance. This was also the case for gender subgroups when comparing between schizophrenia and HC. However, subgroup analysis in **Table 3** indicates that the effect size is miniscule. This suggests that while the differences are statistically significant, they are likely of weak clinical relevance in different periods of schizophrenia. Therefore, we assume that the gender factor may play a more important role.

The main analyses also explored differences between diagnostic groups with a sex-stratified approach. From another perspective of morbidity, thyroid-related diseases in women have an incidence 5–20 times more than in men (55). At certain physiological periods such as pregnancy, HPTA function becomes altered. The increase of estrogen during this period can result in increases in thyroxine-binding globulin (56) and risk of schizophrenia (57). Recent studies have established models in pregnant and nonpregnant women, suggesting that pregnancy factors could result in decreases in FT4 level (58). We excluded pregnant women for homogenous of samples. Then, we tested potential interactions with sex (age, BMI, and CPZ-equivalent dose) and found that sex difference is interacted with age and BMI in analyzing TT3 and FT3, but not with medication. Results from subgroup analysis also showed gender differences in HPAA and HPTA (24). We alleviated part of the drug effect by means of conversing CPZ-equivalent dose between gender subgroups. Then, compared to male patients, TSH level of female patients was higher, while TT3, FT3, and ACTH levels were lower. *Cohen's d* values of TSH (0.488), TT3 (0.503), FT3 (0.587), and ACTH (0.588) showed relatively clear differences when comparing between genders in the schizophrenia group in **Table 5**. Principles of the autoimmune system indicate immune changes for females after puberty and in the *postpartum* period by increasing 5-HT (59). The elevated ACTH and T3 (high functioning) may play important roles in the acute illness of male hospitalized schizophrenics with an intense stress response (1). The reference ranges of those axes should, therefore, be set out for schizophrenia separately for the genders. Some have suggested that there is an alteration in levels of HPAA hormones based on the menstrual cycle (60). Further, a study of chronic stress and the HPAA found that individuals who had suffered child maltreatment also presented with gender differences in HPAA function; namely, COR activation responses in females were significantly slower than in males (61). In the aspect of molecular structure, sex hormones and glucocorticoids both belong to neurosteroids. For example, mifepristone achieves its antiprogesterone effect by competing to bind to progesterone receptor while it also shows the ability of attaching to glucocorticoid receptors (62, 63). Affected by the concentration of these hormones, nonspecific receptors may produce different biological effects, which can better explain the higher morbidity of neuroendocrine diseases in women. Due to the special complexity with large physiological fluctuations of hormone levels in the menstrual cycle, the functional study of sex hormones was very limited in schizophrenia research.

We observed the AUC of the predictive model to distinguish between schizophrenia and HC to be 0.737 among total samples and between first-episode and recurrent schizophrenia to be 0.890 among subgroups. The pathophysiological parameters provide fair to good accuracy of these models, with great improvement in the subgroups. A study indicated an

association between use of antipsychotics and lower FT4 (54). Although we are unable to clarify the risk of psychotropic pharmacy, the changes of HPTA and HPAA have taken place. Age, gender, TSH, FT3, FT4, ACTH, and COR took effect on the discrimination models.

From the perspective of clinical medicine, the severity of schizophrenia symptoms may be correlated with the clinical features of hospitalized patients and their hormone levels in HPTA and HPAA (42). Looking at PANSS subscales, the weak correlations between positive symptom score and FT4 level and gender, between negative symptom score and FT3, FT4 levels and gender, and between general psychopathology score and COR level remained. It indicates that thyroid hormones may be associated with main symptoms of schizophrenia. Severity of symptom is associated with increased FT4 level in male schizophrenia (64), while thyroid dysfunction (TSH, FT3, and FT4) is associated with schizophrenia, especially in female patients (65).

The general psychopathology score reflects the emotional response and part of the cognitive functioning of patients, with higher scores previously found among those who attempt suicide (66). The present findings indicate that the chronic stress reaction of schizophrenia maintained at a high level for an extended period of time, and is directly related to the severity of psychotic symptoms. Elevated COR levels during the disease likely impair cognitive functions by producing chronic stress.

CONCLUSION

The present study compared hormone levels in HPTA and HPAA between patients with schizophrenia and HCs, between female and male patients, and between first-episode and recurrent patients. We also examined relationships between patient clinical features (age, illness duration, PANSS scores, and subscale scores) and HPTA and HPAA hormone levels. Decreased TT3 and TT4 levels and elevated ACTH and COR levels were found to be associated with the hospitalized patients. In addition, in this specified research on schizophrenia, both thyroid function and adrenal function varied with gender to some extent. Further studies are needed to fully explain the differences observed. The recurrent and chronic trait of schizophrenia may cause long-term effects on thyroid function, such as impacting TSH, FT3, and FT4 levels. Lastly, we found that symptom severity evaluated by the PANSS in hospitalized patients yielded a weak linear correlation with COR level. Looking at PANSS subscales, the weak correlation with COR level remained for the general psychopathology score.

Limitations

The use of antipsychotic drugs in patients with mental disorders affects their neuroendocrine function (54). Therefore, we recruited first-episode schizophrenic patients who had never taken antipsychotics or recurrent schizophrenic patients who had used medication in a lower dose for at least one year because they had experienced the acute phase and the consolidation phase. Samples chosen from the consolidation phase can avoid excessive interference of multiple antipsychotics on the axes. Hence, the endocrine effect of drugs was not studied in this study in order to avoid confounding caused by analysis of different drugs between groups. Transformed variables using CPZ-equivalent dose (39) may reduce the impact of confounder, but cannot eliminate it. We hope to include patients in different phases of the illness in future studies. If resources allow, we recommend a longitudinal study design involving patients who are in a stable phase initially and treated with only one antipsychotic medication.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/supplementary material.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Institutional Ethical Committee for Clinical Research of Shanghai Changning Mental Health Center, Shanghai, China. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

YZ and FW designed the study, collected and analyzed data, and wrote the first draft of the manuscript. HJ contributed to data collection and statistical analysis. LT, QC, WJ, and GL discussed and commented on the manuscript. YF reviewed and edited the manuscript. All authors read and approved the manuscript.

FUNDING

The work was supported by the National Key Research and Development Program of China (2016YFC1307100), the National Natural Science Foundation of China (81771465, 81930033), the National Key Technologies R&D Program of China (2012BAI01B04), the Sanming Project of Medicine in Shenzheng (SZSM201612006), the Innovative Research Team of High-level Local Universities in Shanghai, the Innovation Team Project of Shanghai Changning District, and the Project of Shanghai Yangpu Mental Health Center (YJY2018-3).

ACKNOWLEDGMENTS

The authors thank GL for his comments on an earlier version of the paper.

REFERENCES

1. Gajsak LR, Gelemanovic A, Kuzman MR, Puljak L. Impact of stress response in development of first-episode psychosis in schizophrenia: an overview of systematic reviews. *Psychiatr Danub* (2017) 29:14–23. doi: 10.24869/psyd.2017.14
2. Barbero JD, Gutiérrez-Zotes A, Montalvo I, Creus M, Cabezas Á., Solé M, et al. Free thyroxine levels are associated with cognitive abilities in subjects with early psychosis. *Schizophr Res* (2015) 166:37–42. doi: 10.1016/j.schres.2015.04.030
3. Carol EE, Mittal VA. Resting cortisol level, self-concept, and putative familial environment in adolescents at ultra high-risk for psychotic disorders. *Psychoneuroendocrinology* (2015) 57:26–36. doi: 10.1016/j.psyneuen.2015.03.018
4. Premkumar P, Bream D, Sapara A, Fannon D, Anilkumar AP, Kuipers E, et al. Pituitary volume reduction in schizophrenia following cognitive behavioural therapy. *Schizophr Res* (2018) 192:416–22. doi: 10.1016/j.schres.2017.04.035
5. Tamada D, Kitamura T, Onodera T, Hamasaki T, Otsuki M, Shimomura I. Clinical significance of fluctuations in thyroid hormones after surgery for Cushing's syndrome. *Endocr J* (2015) 62:805–10. doi: 10.1507/endocrj.EJ15-0001
6. Hamson DK, Roes MM, Galea LA. Sex hormones and cognition: neuroendocrine influences on memory and learning. *Compr Physiol* (2016) 6:1295–337. doi: 10.1002/cphy.c150031
7. Goldstein JM, Lancaster K, Longenecker JM, Abbs B, Holsen LM, Cherkerzian S, et al. Sex differences, hormones, and fMRI stress response circuitry deficits in psychoses. *Psychiatry Res* (2015) 232:226–36. doi: 10.1016/j.pscychresns.2015.03.006
8. Karanikas E, Garyfallos G. Role of cortisol in patients at risk for psychosis mental state and psychopathological correlates: a systematic review. *Psychiatry Clin Neurosci* (2015) 69:268–82. doi: 10.1111/pcn.12259
9. Sprah L, Dernovsek MZ, Wahlbeck K, Haaramo P. Psychiatric readmissions and their association with physical comorbidity: a systematic literature review. *BMC Psychiatry* (2017) 17:016–1172. doi: 10.1186/s12888-016-1172-3
10. Telo S, Bilgic S, Karabulut N. Thyroid hormone levels in chronic schizophrenic patients: association with psychopathology. *West Indian Med J* (2016) 65:312–5. doi: 10.7727/wimj.2015.186
11. Ustohal L, Hlavacova N, Mayerova M, Ceskova E, Jezova D. Aldosterone and aldosterone/cortisol ratio is higher in serum of long-term compared to first episode schizophrenia patients: a pilot study. *J Psychiatr Res* (2018) 104:46–9. doi: 10.1016/j.jpsychires.2018.06.012
12. Guest PC, Schwarz E, Krishnamurthy D, Harris LW, Leweke FM, Rothermundt M, et al. Altered levels of circulating insulin and other neuroendocrine hormones associated with the onset of schizophrenia. *Psychoneuroendocrinology* (2011) 36:1092–6. doi: 10.1016/j.psyneuen.2010.12.018
13. Radhakrishnan R, Calvin S, Singh JK, Thomas B, Srinivasan K. Thyroid dysfunction in major psychiatric disorders in a hospital based sample. *Indian J Med Res* (2013) 138:888–93.
14. Nordholm D, Rostrup E, Mondelli V, Randers L, Nielsen MO, Wulff S, et al. Multiple measures of HPA axis function in ultra high risk and first-episode schizophrenia patients. *Psychoneuroendocrinology* (2018) 92:72–80. doi: 10.1016/j.psyneuen.2018.03.015
15. Starr LR, Dienes K, Li YI, Shaw ZA. Chronic stress exposure, diurnal cortisol slope, and implications for mood and fatigue: moderation by multilocus HPA-Axis genetic variation. *Psychoneuroendocrinology* (2019) 100:156–63. doi: 10.1016/j.psyneuen.2018.10.003
16. Ichioka S, Terao T, Hoaki N, Matsushita T, Hoaki T. Triiodothyronine may be possibly associated with better cognitive function and less extrapyramidal symptoms in chronic schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* (2012) 39:170–4. doi: 10.1016/j.pnpbp.2012.06.008
17. Tas C, Brown EC, Eskikurt G, Irmak S, Aydin O, Esen-Danaci A, et al. Cortisol response to stress in schizophrenia: associations with oxytocin, social support and social functioning. *Psychiatry Res* (2018) 270:1047–52. doi: 10.1016/j.psychres.2018.05.011
18. Sapin R, Schlienger JL. Thyroxine (T4) and tri-iodothyronine (T3) determinations: techniques and value in the assessment of thyroid function. *Ann Biol Clin* (2003) 61:411–20.
19. Ortiga-Carvalho TM, Chiamolera MI, Pazos-Moura CC, Wondisford FE. Hypothalamus-pituitary-thyroid axis. *Compr Physiol* (2016) 6:1387–428. doi: 10.1002/cphy.c150027
20. Zhou JJ, Gao Y, Kosten TA, Zhao Z, Li DP. Acute stress diminishes M-current contributing to elevated activity of hypothalamic-pituitary-adrenal axis. *Neuropharmacology* (2017) 114:67–76. doi: 10.1016/j.neuropharm.2016.11.024
21. Ambroziak M, Pachucki J, Stachlewska-Nasfeter E, Nauman J, Nauman A. Disturbed expression of type 1 and type 2 iodothyronine deiodinase as well as titf1/nkx2-1 and pax-8 transcription factor genes in papillary thyroid cancer. *Thyroid* (2005) 15:1137–46. doi: 10.1089/thy.2005.15.1137
22. Brown SB, MacLatchy DL, Hara TJ, Eales JG. Effects of cortisol on aspects of 3,5,3'-triiodo-L-thyronine metabolism in rainbow trout (Oncorhynchus mykiss). *Gen Comp Endocrinol* (1991) 81:207–16. doi: 10.1016/0016-6480(91)90005-Q
23. Pruessner M, Boekestyn L, Bechard-Evans L, Abadi S, Vracotas N, Joober R, et al. Sex differences in the cortisol response to awakening in recent onset psychosis. *Psychoneuroendocrinology* (2008) 33:1151–4. doi: 10.1016/j.psyneuen.2008.04.006
24. Bicikova M, Hampl R, Hill M, Ripova D, Mohr P, Putz Z. Neuro- and immunomodulatory steroids and other biochemical markers in drug-naive schizophrenia patients and the effect of treatment with atypical antipsychotics. *Neuro Endocrinol Lett* (2011) 32:141–7.
25. Vuksan-Ćusa B, Šagud M, Mihaljević-Peleš A, Jakšić N, Jakovljević M. Metabolic syndrome and cortisol/DHEAS ratio in patients with bipolar disorder and schizophrenia. *Psychiatria Danubina* (2014) 26:187–9.
26. Halari R, Kumari V, Mehrotra R, Wheeler M, Hines M, Sharma T. The relationship of sex hormones and cortisol with cognitive functioning in Schizophrenia. *J Psychopharmacol* (2004) 18:366–74. doi: 10.1177/0269881104018003007
27. Labad J, Gutierrez-Zotes A, Creus M, Montalvo I, Cabezas A, Sole M, et al. Hypothalamic-pituitary-adrenal axis measures and cognitive abilities in early psychosis: are there sex differences? *Psychoneuroendocrinology* (2016) 72:54–62. doi: 10.1016/j.psyneuen.2016.06.006
28. Bale TL. Neuroendocrine and immune influences on the CNS: it's a matter of sex. *Neuron* (2009) 64:13–6. doi: 10.1016/j.neuron.2009.09.036
29. Zorn JV, Schur RR, Boks MP, Kahn RS, Joels M, Vinkers CH. Cortisol stress reactivity across psychiatric disorders: a systematic review and meta-analysis. *Psychoneuroendocrinology* (2017) 77:25–36. doi: 10.1016/j.psyneuen.2016.11.036
30. Szpunar MJ, Parry BL. A systematic review of cortisol, thyroid-stimulating hormone, and prolactin in peripartum women with major depression. *Arch Womens Ment Health* (2018) 21:149–61. doi: 10.1007/s00737-017-0787-9
31. Pasqualetti G, Pagano G, Rengo G, Ferrara N, Monzani F. subclinical hypothyroidism and cognitive impairment: systematic review and meta-analysis. *J Clin Endocrinol Metab* (2015) 100:4240–8. doi: 10.1210/jc.2015-2046
32. Yang ZQ, Zhao Q, Jiang P, Zheng SB, Xu B. Prevalence and control of hypertension among a community of elderly population in changning district of shanghai: a cross-sectional study. *BMC Geriatr* (2017) 17:017–0686. doi: 10.1186/s12877-017-0686-y
33. Buysse DJ, Reynolds CF3rd, Monk TH, Berman SR, Kupfer DJ. The pittsburgh sleep quality index: a new instrument for psychiatric practice and research. *Psychiatry Res* (1989) 28:193–213. doi: 10.1016/0165-1781(89)90047-4
34. Sheehan DV, Lecrubier Y, Sheehan KH, Amorim P, Janavs J, Weiller E, et al. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. *J Clin Psychiatry* (1998) 20:22–33. doi: 10.1037/t18597-000
35. Wu BJ, Lan TH, Hu TM, Lee SM, Liou JY. Validation of a five-factor model of a Chinese Mandarin version of the positive and negative syndrome scale (CMV-PANSS) in a sample of 813 schizophrenia patients. *Schizophr Res* (2015) 169:489–90:2015. doi: 10.1016/j.schres.2015.09.011
36. Montagna G, Balestra S, D'Aurizio F, Romanelli F, Benagli C, Tozzoli R, et al. Establishing normal values of total testosterone in adult healthy men by the use of four immunometric methods and liquid chromatography-mass spectrometry. *Clin Chem Lab Med* (2018) 56:1936–44. doi: 10.1515/cclm-2017-1201
37. Friis-Hansen L, Hilsted L. Reference intervals for thyreotropin and thyroid hormones for healthy adults based on the NOBIDA material and determined using a Modular E170. *Clin Chem Lab Med* (2008) 46:1305–12. doi: 10.1515/CCLM.2008.258

38. Christensen M, Madsen RF, Moller LR, Knudsen CS, Samson MH. Whole blood samples for adrenocorticotrophic hormone measurement can be stored at room temperature for 4 hours. *Scand J Clin Lab Invest* (2016) 76:653–6. doi: 10.1080/00365513.2016.1230887
39. Leucht S, Samara M, Heres S, Davis JM. Dose equivalents for antipsychotic drugs: the DDD method. *Schizophr Bull* (2016) 42:S90–4. doi: 10.1093/schbul/sbv167
40. Zhu Y, Wu Z, Sie O, Cai Y, Huang J, Liu H, et al. Causes of drug discontinuation in patients with major depressive disorder in China. *Prog Neuropsychopharmacol Biol Psychiatry* (2020) 96:24. doi: 10.1016/j.pnpbp.2019.109755
41. Pecori Giraldi F, Moro M, Cavagnini F. Gender-related differences in the presentation and course of Cushing's disease. *J Clin Endocrinol Metab* (2003) 88:1554–8. doi: 10.1210/jc.2002-021518
42. Baumgartner A, Pietzcker A, Gaebel W. The hypothalamic-pituitary-thyroid axis in patients with schizophrenia. *Schizophr Res* (2000) 44:233–43. doi: 10.1016/S0920-9964(99)00187-5
43. Hendrie HC, Tu W, Tabbey R, Purnell CE, Ambuehl RJ, Callahan CM. Health outcomes and cost of care among older adults with schizophrenia: a 10-year study using medical records across the continuum of care. *Am J Geriatric Psychiatry* (2014) 22:427–36. doi: 10.1016/j.jagp.2012.10.025
44. Huang JT, Leweke FM, Oxley D, Wang L, Harris N, Koethe D, et al. Disease biomarkers in cerebrospinal fluid of patients with first-onset psychosis. *PloS Med* (2006) 3:0030428. doi: 10.1371/journal.pmed.0030428
45. Manojlovic-Stojanoski MN, Filipovic BR, Nestorovic NM, Sosic-Jurjevic BT, Ristic NM, Trifunovic SL, et al. Morpho-functional characteristics of rat fetal thyroid gland are affected by prenatal dexamethasone exposure. *Steroids* (2014) 84:22–9. doi: 10.1016/j.steroids.2014.03.006
46. Alshehri B, D'Souza DG, Lee JY, Petratos S, Richardson SJ. The diversity of mechanisms influenced by transthyretin in neurobiology: development, disease and endocrine disruption. *J Neuroendocrinol* (2015) 27:303–23. doi: 10.1111/jne.12271
47. Akiibinu MO, Ogundahunsi OA, Ogunyemi EO. Inter-relationship of plasma markers of oxidative stress and thyroid hormones in schizophrenics. *BMC Res Notes* (2012) 5:1756–0500. doi: 10.1186/1756-0500-5-169
48. Jiang Y, Coleman FH, Kopenhaver Doheny K, Travagli RA. Stress adaptation upregulates oxytocin within hypothalamo-vagal neurocircuits. *Neuroscience* (2018) 390:198–205. doi: 10.1016/j.neuroscience.2018.08.021
49. Silva JE, Bianco SD. Thyroid-adrenergic interactions: physiological and clinical implications. *Thyroid* (2008) 18:157–65. doi: 10.1089/thy.2007.0252
50. Walker E, Mittal V, Tessner K. Stress and the hypothalamic pituitary adrenal axis in the developmental course of schizophrenia. *Annu Rev Clin Psychol* (2008) 4:189–216. doi: 10.1146/annurev.clinpsy.4.022007.141248
51. Fodor A, Pinter O, Domokos A, Langnaese K, Barna I, Engelmann M, et al. Blunted HPA axis response in lactating, vasopressin-deficient Brattleboro rats. *J Endocrinol* (2013) 219:89–100. doi: 10.1530/JOE-13-0224
52. Richardson CM, Rice KG, Devine DP. Perfectionism, emotion regulation, and the cortisol stress response. *J Couns Psychol* (2014) 61:110–8. doi: 10.1037/a0034446
53. Pierre K, Rao RT, Hartmanshenn C, Androulakis IP. Modeling the influence of seasonal differences in the HPA axis on synchronization of the circadian clock and cell cycle. *Endocrinology* (2018) 159:1808–26. doi: 10.1210/en.2017-03226
54. Vedal TSJ, Steen NE, Birkeland KI, Dieset I, Reponen EJ, Laskemoen JF, et al. Free thyroxine and thyroid-stimulating hormone in severe mental disorders: a naturalistic study with focus on antipsychotic medication. *J Psychiatr Res* (2018) 106:74–81. doi: 10.1016/j.jpsychires.2018.09.014
55. Gietka-Czernel M. The thyroid gland in postmenopausal women: physiology and diseases. *Prz Menopauzalny* (2017) 16:33–7. doi: 10.5114/pm.2017.68588
56. Gaberscek S, Zaletel K. Thyroid physiology and autoimmunity in pregnancy and after delivery. *Expert Rev Clin Immunol* (2011) 7:697–706. doi: 10.1586/eci.11.42
57. Brown JS Jr. Association of increased prenatal estrogen with risk factors for schizophrenia. *Schizophr Bull* (2011) 37:946–9. doi: 10.1093/schbul/sbp161
58. Cai J, Zhao X, Lei T, Meng Q, Zhou H, Zhang M. Urinary thyroid hormone parameters test for evaluating the thyroid function during pregnancy. *Syst Biol Reprod Med* (2014) 60:171–6. doi: 10.3109/19396368.2014.900138
59. Zhou Y, Wang X, Zhao Y, Liu A, Zhao T, Zhang Y, et al. Elevated thyroid peroxidase antibody increases risk of post-partum depression by decreasing prefrontal cortex BDNF and 5-HT levels in mice. *Front Cell Neurosci* (2017) 10:307. doi: 10.3389/fncel.2016.00307
60. Stephens MA, Mahon PB, McCaul ME, Wand GS. Hypothalamic-pituitary-adrenal axis response to acute psychosocial stress: effects of biological sex and circulating sex hormones. *Psychoneuroendocrinology* (2016) 66:47–55. doi: 10.1016/j.psyneuen.2015.12.021
61. Kaess M, Whittle S, O'Brien-Simpson L, Allen NB, Simmons JG. Childhood maltreatment, pituitary volume and adolescent hypothalamic-pituitary-adrenal axis - evidence for a maltreatment-related attenuation. *Psychoneuroendocrinology* (2018) 98:39–45. doi: 10.1016/j.psyneuen.2018.08.004
62. Sun Y, Fang M, Davies H, Hu Z. Mifepristone: a potential clinical agent based on its anti-progesterone and anti-glucocorticoid properties. *Gynecol Endocrinol* (2014) 30:169–73. doi: 10.3109/09513590.2013.856410
63. Soria V, Gonzalez-Rodriguez A, Huerta-Ramos E, Usall J, Cobo J, Bioque M, et al. Targeting hypothalamic-pituitary-adrenal axis hormones and sex steroids for improving cognition in major mood disorders and schizophrenia: a systematic review and narrative synthesis. *Psychoneuroendocrinology* (2018) 93:8–19. doi: 10.1016/j.psyneuen.2018.04.012
64. Jose J, Nandeesha H, Kattimani S, Meiyappan K, Sarkar S, Sivasankar D. Association between prolactin and thyroid hormones with severity of psychopathology and suicide risk in drug free male schizophrenia. *Clin Chim Acta* (2015) 444:78–80. doi: 10.1016/j.cca.2015.02.003
65. Zhang Y, Tang Z, Ruan Y, Huang C, Wu J, Lu Z, et al. Prolactin and Thyroid Stimulating Hormone (TSH) levels and sexual dysfunction in patients with schizophrenia treated with conventional antipsychotic medication: a cross-sectional study. *Med Sci Monit* (2018) 24:9136–43. doi: 10.12659/MSM.913759
66. Papadopoulou A, Douzenis A, Christodoulou C, Gournellis R, Papageorgiou C, Markianos M. Association of plasma cortisol levels with clinical characteristics of suicide attempters. *Neuropsychobiology* (2017) 76:161–5. doi: 10.1159/000489782

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 25 May 2020
doi: 10.3389/fpsyt.2020.00455

Glycated Haemoglobin Is Associated With Poorer Cognitive Performance in Patients With Recent-Onset Psychosis

*Itziar Montalvo[1], Alexandre González-Rodríguez[1], Ángel Cabezas[2], Alfonso Gutiérrez-Zotes[2], Montse Solé[2], Maria José Algora[2], Laura Ortega[3], Lourdes Martorell[2], Vanessa Sánchez-Gistau[2], Elisabet Vilella[2] and Javier Labad[1]**

[1] Department of Mental Health, Parc Taulí Hospital Universitari, Institut d'Investigació Sanitària Parc Taulí (I3PT), Universitat Autònoma de Barcelona, CIBERSAM, Sabadell, Spain, [2] Hospital Universitari Institut Pere Mata, Institut d'Investigació Sanitària Pere Virgili (IISPV), Universitat Rovira i Virgili, CIBERSAM, Reus, Spain, [3] Nursing Department, Universitat Rovira i Vigili, Tarragona, Spain

OPEN ACCESS

Edited by:
Grazia Rutigliano, University of Pisa, Italy

Reviewed by:
Domenico Tricò, University of Pisa, Italy
Zhiqiang Li, Qingdao University, China

***Correspondence:**
Javier Labad
jlabad@tauli.cat

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *25 February 2020*
Accepted: *05 May 2020*
Published: *25 May 2020*

Citation:
Montalvo I, González-Rodríguez A, Cabezas Á, Gutiérrez-Zotes A, Solé M, Algora MJ, Ortega L, Martorell L, Sánchez-Gistau V, Vilella E and Labad J (2020) Glycated Haemoglobin Is Associated With Poorer Cognitive Performance in Patients With Recent-Onset Psychosis. Front. Psychiatry 11:455. doi: 10.3389/fpsyt.2020.00455

Background: Glucose abnormalities and cognitive alterations are present before the onset of schizophrenia. We aimed to study whether glucose metabolism parameters are associated with cognitive functioning in recent-onset psychosis (ROP) patients while adjusting for hypothalamic-pituitary adrenal (HPA) axis measures.

Methods: Sixty ROP outpatients and 50 healthy subjects (HS) were studied. Cognitive function was assessed with the MATRICS Consensus Cognitive Battery. Glycated haemoglobin ($HbA1_c$), glucose, insulin, and C-peptide levels were determined in plasma. The HOMA-insulin resistance index was calculated. Salivary samples were obtained at home on another day to assess the cortisol awakening response and cortisol levels during the day. Univariate analyses were conducted to explore the association between glucose metabolism parameters and cognitive tasks. For those parameters that were more clearly associated with the cognitive outcome, multiple linear regression analyses were conducted to adjust for covariates. Each cognitive task was considered the dependent variable. Covariates were age, sex, education level, diagnosis, antipsychotic and benzodiazepine treatment, body mass index (BMI), smoking, and HPA axis measures. Potential interactions between diagnosis and glucose parameters were tested.

Results: There were no significant differences in HPA axis measures or glucose parameters, with the exception of C-peptide (that was higher in ROP patients), between groups. ROP patients had a lower performance than HS in all cognitive tasks ($p < 0.01$ for all tasks). Of all glucose metabolism parameters, HbA1c levels were more clearly associated with cognitive impairment in cognitive tasks dealing with executive functions and visual memory in both ROP patients and HS. Multivariate analyses found a significant negative association between HbA1c and cognitive functioning in five cognitive tasks dealing with executive functions, visual memory and attention/vigilance (a ROP

diagnosis by $HbA1_c$ negative interaction was found in this latter cognitive domain, suggesting that $HBA1_c$ levels are associated with impaired attention only in ROP patients).

Conclusions: Our study found that $HbA1_c$ was negatively associated with cognitive functioning in both ROP patients and HS in tasks dealing with executive functions and visual memory. In ROP patients, $HbA1_c$ was also associated with impaired attention. These results were independent of BMI and measures of HPA axis activity.

Keywords: glucose, glycated haemoglobin, cognition, early psychosis, cortisol

INTRODUCTION

Cognitive alterations are well-known predictors of social functioning in people with schizophrenia and related psychotic disorders (1). These cognitive alterations are present at early stages of the psychotic illness, even before the development of positive psychotic symptoms (delusions, hallucinations) (2). Biological mechanisms explaining these cognitive deficits are complex and include the potential role of hypothalamic-pituitary-adrenal (HPA) axis hormones (3, 4), thyroid hormones (5, 6), prolactin (7, 8), inflammatory markers (9, 10) and the genetic background (11).

In our current study, we aimed to explore whether glucose metabolism parameters might contribute to the cognitive impairment of people with recent-onset psychosis (ROP). Previous studies including drug-naïve first-episode psychosis and healthy controls have reported increased glucose and insulin resistance (12) and impaired glucose tolerance (13), suggesting that glucose-related parameters may be altered in patients with psychosis at the early stages of the illness. Previous studies have also reported an increased prevalence of type 2 diabetes in the parents of people with non-affective psychosis (14). It was initially thought that this association may be due to shared environmental or genetic risk factors, or both. However, a recent study (15) exploring the association between polygenic risk score of schizophrenia and glycated haemoglobin ($HbA1_c$) while adjusting for polygenic risk score of type 2 diabetes, and clinical and demographic covariables suggests that the mechanisms of hyperglycemia or diabetes are at least partly independent from genetic predisposition to schizophrenia.

It is also known that comorbidity with diabetes mellitus is associated with more severe cognitive deficits in schizophrenia (16). Type 2 diabetes is a risk factor for cognitive decline (17), mild cognitive impairment (18), and progression to dementia (19). Although the exact pathophysiology of mild cognitive impairment in type 2 diabetes is unclear, many studies suggest that several coexisting risk factors contribute to the cognitive impairment (20): chronic hyperglycaemia, diabetic complications (macrovascular and microvascular disease), inflammatory reaction and advanced glycation end products, and psychological status (e.g. depressive symptoms). In a systematic review (21) that included 86 studies exploring the role of glucose regulation (glycaemia, hypoglycaemic events, insulin concentration, insulin resistance, and glucose-lowering treatment) and cognitive function in people with type 2 diabetes without dementia, high $HbA1_c$ and glucose variability were negatively associated with cognitive function. $HbA1_c$ is a promising biomarker for cognitive impairment because it is also associated with poorer cognitive abilities in people without diabetes (17, 22). A recent study suggests that $HbA1_c$ is associated to both cognitive performance and white matter integrity in healthy young adults (22).

Few studies have explored the potential relationship between glucose metabolism indices and cognitive functioning in patients with psychotic disorders. In a study conducted in first-episode drug-naïve patients with schizophrenia, glucose intolerance (measured with a 75 g oral glucose tolerance test) was associated with more negative symptoms and poorer social cognition, but not with poorer neurocognitive performance (23). In another study that measured $HbA1_c$, this parameter was associated with poorer global cognition and attention in men (but not women) with schizophrenia (24). Insulin resistance has been associated with alterations in dopaminergic reward systems and homeostatic signals affecting food intake, glucose metabolism, body weight, and cognitive performance (25), being a potential moderator of the cognitive outcome in patients with psychotic disorders. Although several studies have reported an association between diabetes and cognition on schizophrenia, few investigations have explored the role of glucose parameters on cognition in patients with early psychosis.

We aimed to explore the previous hypothesis while adjusting for HPA axis measures, as some indices, such as a blunted cortisol awakening response (CAR) (3, 26, 27) and elevated cortisol diurnal levels during the day (26), have been reported to be associated with a poorer cognitive outcome in people with ROP. Moreover, elevated glucocorticoids contribute to the cognitive impairment of patients with type 2 diabetes (28).

Taking into account that $HbA1_c$ has been associated with poorer cognitive performance in both patients with schizophrenia (24) and healthy individuals (22), we hypothesized that $HbA1_c$ would be associated with poorer cognitive performance in both people with ROP and healthy individuals. We also aimed to conduct exploratory analyses regarding the contribution of other glucose-related parameters (fasting glucose, c-peptide, insulin resistance) on poorer cognitive functioning.

METHODS

Sample

Sixty ROP outpatients and 50 healthy subjects (HS) were studied. All patients (aged between 18 and 35 years) were attending the Early Intervention Service for Psychosis from Reus (Hospital Universitari Institut Pere Mata, Spain) had a DSM-IV diagnosis of a psychotic disorder [schizophreniform disorder (n=14); schizophrenia (n=10); schizoaffective disorder (n=8) or a psychotic disorder not otherwise specified (n=28)]. All patients had a duration of illness of <3 years (65% were patients with first-episode psychosis). A control population of 50 HS matched by sex and age was recruited from the community using advertisements. The sample of the study belongs to a project aiming to study the relationship between hormones and cognitive abilities in early psychosis. For this reason, participants of our study participated in a previous study that tested a different hypothesis focused on HPA axis hormones (3). The exclusion criteria were severe neurological disease or mental retardation; pregnancy; language difficulties; visual impairment; alcohol, heroin or cocaine dependence; or treatment with glucocorticoids.

The research protocol was approved by the Ethics Committee of Hospital Universitari Sant Joan, and all participants provided written informed consent after having received a full explanation of the study.

Clinical Assessment

All patients were interviewed by an experienced psychiatrist using the Schedules for Clinical Assessment in Neuropsychiatry (29). The OPCRIT checklist version 4.0 (available at http://sgdp.iop.kcl.ac.uk/opcrit/) was used to obtain DSM-IV diagnoses. The severity of positive, negative and general symptoms was assessed with the Positive and Negative Syndrome Scale (PANSS) (30, 31) to assess the severity of psychotic symptoms.

The Spanish version of the MATRICS Consensus Cognitive Battery (MCCB) was used to assess neurocognitive functioning (32), and it includes 10 cognitive tests assessing 7 cognitive domains: processing speed, attention and vigilance, working memory, verbal learning, visual learning, reasoning and problem solving, and social cognition.

Sociodemographic and clinical variables were obtained in a semi-structured interview. Substance use was recorded as the consumption of alcohol (standard units/day), tobacco (cigarettes/day), and cannabis (joints/day). Current psychopharmacological treatment was recorded during the neuropsychological assessment, as described previously (3). The dose of antipsychotics was converted to chlorpromazine equivalents following an international consensus of antipsychotic dosing (33).

Weight, height, waist circumference, and blood pressure were assessed by physical examination. Body mass index (BMI) was calculated with the formula weight (kg)/height $(m)^2$.

Biochemical Measures

A fasting morning blood analysis (between 8:30 a.m. and 9:30 a.m.) was obtained by antecubital venepuncture. $HbA1_c$, glucose, insulin, and C-peptide were determined in plasma. The Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index was calculated using the formula HOMA-IR = [insulin (μUI/mL) x glucose (mg/dL)]/405.

Salivary samples were obtained at home on another day with Salivette® tubes to assess the cortisol awakening response (calculated as the area under the curve with respect to the increase, considering three samples: awakening, 30' post-awakening, and 60' post-awakening) and cortisol levels during the day (calculated as the area under the curve with respect to the ground, considering 5 samples: awakening, 30' post-awakening, 60' post-awakening, 10:00 a.m., and 11:00 p.m.). Both formulas were computed using the trapezoid formula (34). A full explanation of the processing of the samples and cortisol determination with a high-sensitivity enzyme-linked immunosorbent assay (ELISA) kit has been described elsewhere (3).

Statistical Analyses

We used SPSS v 23.0 for conducting statistical analyses. Cortisol measures were transformed with a Box-Cox transformation (35), and the Trail Making Test (part A) was log transformed (ln) to reduce skewness. Chi-squared tests and T-tests were used to compare categorical and continuous data between both diagnostic groups. Non-parametric tests (Mann Whitney U test) were used to compare ordinal variables or continuous measures that were skewed (e.g. insulin, c-peptide). Pearson correlation analyses (and Spearman when needed) were used to explore associations between continuous variables. A p value <0.05 (two-tailed) was considered to be significant.

As $HbA1_c$ was considered the glucose-related parameter to be studied in our main hypothesis, we verified that this value followed a normal distribution and checked for potential outliers with the 1.5 quartile (Q) rule for outliers (36). With this definition, any observation is a suspected outlier if it falls more than 1.5 x interquartile range (IQR) above the third quartile or below the first quartile. In our sample, the distribution for $HbA1_c$ values was: minimum value= 4.6, Q1 = 4.9, Q2 (median)= 5.1, Q3 (5.3), maximum value 5.8, IQR= 0.4. Therefore, none of the values were considered outliers because they were within the limits 1.5xIQR (lower interval: 4.3, upper interval: 5.9).

Although our main hypothesis was conducted with multiple linear regression analyses, we first conducted an exploratory and univariate analysis to test the associations between different glucose-related parameters and cognitive outcomes. We also included exploratory correlational analyses between cognitive scores. These exploratory analyses were not adjusted for multiple comparisons following some recommendations that indicate that is it not strictly necessary to correct for multiple testing in analyses that are exploratory in nature (37). Multiple linear regression analyses were conducted for testing the association between $HbA1_c$ and cognitive variable while adjusting for covariates. Several multiple linear regression analyses were conducted, considering each cognitive task as the dependent variable. $HbA1_c$ was considered the main independent variable. We avoided the inclusion of different glucose metabolism parameters in the same equation because they were highly

correlated. The following covariates were included in each equation with the enter procedure: age, sex, education level, diagnosis, antipsychotic and benzodiazepine treatment, BMI, smoking, and HPA axis measures. Potential interactions between diagnosis and $HbA1_c$ were tested, and those significant interactions were included in the final model.

Sample Size and Power Analysis

G* Power 3.1.9.4. was used for sample size and power calculations. The original sample was calculated for detecting a moderate effect size ($f^2 = 0.2$) with multiple linear regression analyses, considering an alpha error of 0.05 and a beta error of 0.15 (statistical power of 85%), and 12 predictors. The needed sample size was 108. It is important to note that our sample is small for detecting small effects. Moreover, in the stratified analysis by diagnosis (e.g. correlation analyses), the statistical power can decrease: the statistical power for detecting moderate effect sizes (r= 0.3) was 59% for healthy individuals and 67% for ROP patients.

RESULTS

Clinical and hormonal variables from the sample are described in **Table 1**. Both groups were well matched in age and sex, although ROP patients had a lower education status and reported more smoking and alcohol consumption. In relation to glucose-related parameters, C-peptide concentrations were higher in ROP patients than in HS. There were no significant differences in HPA axis measures between groups.

ROP patients had a lower performance in all cognitive tasks than HS (**Table 2**). The correlation matrix between cognitive measures in all participants is described in **Table 3**.

Of all glucose metabolism parameters, $HbA1_c$ levels were more clearly associated with cognitive impairment in cognitive tasks dealing with executive functions and visual memory in both ROP patients and HS (**Table 4**).

A multivariate analysis conducted in all participants (ROP patients and HS) found a significant negative association between $HbA1_c$ and cognitive functioning in five cognitive

TABLE 1 | Clinical characteristics and hormonal measures from the sample.

	HS N= 50		ROP patients N=60		p value
Female sex, N (%)	22	44%	21	35%	0.335
Age (years)	23.8	4.8	24.5	5.4	0.465
Education level (years of study)	13.4	2.7	11.3	2.8	**<0.001**
Smoking, N (%)	10	20%	41	68.3%	**<0.001**
Smoking (cig/day), all participants	1.6	4.4	9.0	9.6	**<0.001**
Smoking (cig/day), only smokers	8.0	7.0	13.1	9.0	0.101
Cannabis use, N (%)					
No	38	76%	43	71.7%	0.215
Sporadic	10	20%	9	15%	
Continuous	2	4%	8	13.3%	
Alcohol consumption, N (%)					
No	5	10%	27	45%	**<0.001**
Sporadic	44	88%	28	46.7%	
Continuous	1	2%	5	8.3%	
BMI (kg/m^2)	22.5	3.2	24.1	4.1	0.053
Antipsychotic treatment:	0.0	0.0	371.1	334.0	**<0.001**
Chlorpromazine equivalents (mg/day)					
Benzodiazepine treatment:	0.0	0.0	2.6	7.7	**0.021**
Diazepam equivalents (mg/day)					
Glucose metabolism parameters					
Glucose (mg/dL)	78.6	11.2	78.7	11.1	0.956
Insulin (μIU/mL)	6.6	0 to 22.9	9.5	0 to 114	0.595
C-peptide (μg/L)	1.3	0.6 to 4.2	1.6	0.7 to 12.7	**0.003**
$HbA1_c$ (%)	5.1	0.3	5.1	0.3	0.250
HOMA-IR	1.4	0 to 4.9	1.3	0 to 26.18	0.802
HPA axis measures					
Cortisol at awakening (nmol/L)	14.7	9.0	13.0	8.6	0.381
Cortisol 30' post-awakening (nmol/L)	23.8	13.4	20.3	10.7	0.342
Cortisol 60' post-awakening (nmol/L)	21.0	12.8	16.3	7.5	0.234
10:00 a.m.	12.5	7.4	12.0	6.4	0.971
11:00 p.m.	2.7	2.1	3.0	3.0	0.971
CAR (AUC_i)	39.7	63.6	40.5	53.7	0.942
Cortisol during the day (AUC_g)	2049.6	837.3	2082.3	733.4	0.834

Data are mean (SD), median (range) or N (%).

Cortisol raw data are shown. However, p values were obtained using transformed cortisol values (Box-Cox transformation).

BMI, Body mass index; $HbA1_c$, Glycated haemoglobin; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HPA, Hypothalamic-pituitary-adrenal; CAR, Cortisol awakening response; AUC_i, Area under the curve (calculated with respect to the increase); AUC_g, Area under the curve (calculated with respect to the ground).

Significant associations ($p < 0.05$) are represented in bold.

TABLE 2 | Cognitive functioning by diagnostic group.

	HS N=50		ROP patients N=60		p value
	Mean	**SD**	**Mean**	**SD**	
Verbal learning and memory					
HVLT-R	27.5	3.8	22.8	4.9	**<0.001**
Visual learning and memory					
BVMT-R	27.0	5.9	19.6	7.2	**<0.001**
Working memory					
WMS-III-SS (nonverbal)	16.2	2.9	14.2	3.7	**0.002**
LNS (verbal)	14.2	2.9	12.0	2.3	**<0.001**
Processing speed					
TMT-A† (seconds)	24.7	8.5	38.2	13.4	**<0.001**
BACS SC	61.9	9.6	45.9	12.3	**<0.001**
Category Fluency	24.1	5.1	18.3	5.2	**<0.001**
Reasoning and problem solving					
NAB Mazes	27.0	5.9	19.6	7.2	**<0.001**
Attention/vigilance					
CPT-IP	2.7	0.6	2.0	0.7	**<0.001**
Social cognition					
MSCEIT-ME	94.4	9.2	86.8	9.8	**<0.001**

†As this cognitive test is measured in seconds, higher scores reflect poorer cognitive performance.
HVLT-R, Hopkins Verbal Learning Test-Revised; BVMT-R, Brief Visuospatial Memory Test-Revised; WMS-III-SS, Corsi Block-Tapping Test; Weschler Memory Scale (3rd edition) spatial span; LNS, Letter Number Span; TMT-A, Trail Making Test part A; BACS-SC, Brief Assessment of Cognition in Schizophrenia-Symbol Coding; NAB, Neuropsychological Assessment Battery; CPT-IP, Continuous Performance Test – Identical Pairs; MSCEIT-ME, Mayer-Salovey-Caruso Emotional Intelligence Test – Managing emotions.
Significant associations ($p < 0.05$) are represented in bold.

tasks dealing with executive functions, visual memory and attention/vigilance (**Table 5**). A diagnosis by $HbA1_c$ interaction was found in this latter cognitive domain, which means that the pattern in the relationship between $HbA1_c$ and attention/vigilance differs between ROP patients and HS: in ROP patients, higher $HbA1_c$ levels are associated with impaired attention and vigilance, but this pattern does not apply to HS. This interaction is also described in **Figure 1**. As it is observed in this figure, the interaction is driven by higher $HbA1_c$ values. Although there were no outliers in our sample, we repeated a sensitivity analysis excluding the two higher values ($HbA1_c$= 5.8%) to explore whether the interaction was influenced for these

TABLE 3 | Pearson's correlations studying the relationship between cognitive tasks in 110 participants.

		HVLT-R	BVMT-R	WMS-III SS	LNS	TMT-A†	BACS SC	Fluency	NAB Mazes	CPT-IP	MSCEIT-ME
HVLT-R	r	1	0.596	0.469	0.539	-0.601	0.675	0.594	0.256	0.504	0.401
	p value		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	.007	< 0.001	< 0.001
BVMT-R	r	0.596	1	0.599	0.554	-0.622	0.690	0.624	0.549	0.491	0.168
	p value	< 0.001		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.086
WMS-III SS	r	0.469	0.599	1	0.387	-0.546	0.504	0.417	0.553	0.376	0.300
	p value	< 0.001	< 0.001		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.002
LNS	r	0.539	0.554	0.387	1	-0.536	0.605	0.473	0.367	0.610	0.286
	p value	< 0.001	< 0.001	< 0.001		< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	0.004
TMT-A†	r	-0.601	-0.622	-0.546	-0.536	1	-0.707	-0.559	-0.545	-0.573	-0.308
	p value	< 0.001	< 0.001	< 0.001	< 0.001		< 0.001	< 0.001	< 0.001	< 0.001	.001
BACS SC	r	0.675	0.690	0.504	0.605	-0.707	1	0.653	0.494	0.635	0.312
	p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		< 0.001	< 0.001	< 0.001	0.001
Fluency	r	0.594	0.624	0.417	0.473	-0.559	0.653	1	0.495	0.519	0.220
	p value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		< 0.001	< 0.001	0.024
NAB Mazes	r	0.256	0.549	0.553	0.367	-0.545	0.494	0.495	1	0.447	0.173
	p value	0.007	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		< 0.001	0.078
CPT-IP	r	0.504	0.491	0.376	0.610	-0.573	0.635	0.519	0.447	1	0.294
	value	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001		0.002
MSCEIT-ME	r	0.401	0.168	0.300	0.286	-0.308	0.312	0.220	0.173	0.294	1
	p value	< 0.001	0.086	0.002	0.004	0.001	0.001	0.024	0.078	0.002	

†As TMT-A is measured in seconds, greater scores in this variable reflect poorer cognitive performance. For this reason, this variable showed negative correlations with other cognitive tasks. The log transformed variable (ln TMT-A) was used.
HVLT-R, Hopkins Verbal Learning Test-Revised; BVMT-R, Brief Visuospatial Memory Test-Revised; WMS-III-SS, Corsi Block-Tapping Test; Weschler Memory Scale (3rd edition) spatial span; LNS, Letter Number Span; TMT-A, Trail Making Test part A; BACS-SC, Brief Assessment of Cognition in Schizophrenia-Symbol Coding; NAB, Neuropsychological Assessment Battery; CPT-IP, Continuous Performance Test – Identical Pairs; MSCEIT-ME, Mayer-Salovey-Caruso Emotional Intelligence Test – Managing emotions.

TABLE 4 | Correlations between glucose-related parameters and cognitive tasks: Stratified analyses by diagnosis.

		HS					ROP patients				
		HbA1c	**Glucose**	**C-peptide**	**Insulin**	**HOMA-IR**	**HbA1c**	**Glucose**	**C-peptide**	**Insulin**	**HOMA-IR**
HVLT-R	r	0.012	-0.080	-0.014	-0.152	-0.160	-0.076	0.120	0.023	0.076	0.063
	p value	0.935	0.582	0.921	0.292	0.271	0.567	0.366	0.863	0.567	0.634
BVMT-R	r	-0.350	-0.238	0.000	-0.169	-0.184	-0.262	-0.002	-0.031	0.033	0.028
	p value	0.013	0.096	0.999	0.242	0.205	0.047	0.985	0.817	0.806	0.835
WMS-III SS	r	-0.295	-0.285	-0.155	-0.395	-0.370	-0.218	0.052	-0.343	-0.102	-0.072
	p value	0.037	0.045	0.283	0.005	0.009	0.097	0.695	0.008	0.442	0.590
LNS	r	-0.157	-0.092	0.055	-0.231	-0.258	-0.348	-0.134	-0.128	-0.104	-0.102
	p value	0.276	0.524	0.707	0.106	0.073	0.010	0.332	0.357	0.454	0.461
TMT-A	r	0.135	0.047	-0.050	0.091	0.081	0.330	-0.046	0.199	0.016	-0.008
	p value	0.349	0.747	0.732	0.528	0.579	0.011	0.729	0.131	0.901	0.955
BACS SC	r	-0.329	-0.250	-0.152	-0.351	-0.375	-0.176	0.048	-0.002	0.055	0.050
	p value	0.020	0.080	0.292	0.013	0.008	0.182	0.721	0.986	0.677	0.706
Fluency	r	-0.002	-0.070	0.076	-0.215	-0.202	-0.106	0.214	-0.044	0.061	0.073
	p value	0.987	0.629	0.598	0.134	0.165	0.426	0.107	0.745	0.648	0.585
NAB Mazes	r	-0.243	-0.093	0.151	0.020	0.010	-0.425	-0.067	-0.174	0.021	0.050
	p value	0.089	0.520	0.295	0.890	0.944	0.001	0.620	0.191	0.877	0.712
CPT-IP	r	-0.037	-0.042	-0.123	-0.248	-0.265	-0.262	0.049	-0.015	0.138	0.145
	p value	0.800	0.775	0.397	0.082	0.066	0.047	0.717	0.912	0.303	0.277
MSCEIT-ME	r	-0.252	-0.268	-0.038	-0.295	-0.323	-0.137	0.036	-0.155	0.024	0.018
	p value	0.078	0.060	0.793	0.037	0.024	0.318	0.797	0.259	0.863	0.897

HVLT-R, Hopkins Verbal Learning Test-Revised; BVMT-R, Brief Visuospatial Memory Test-Revised; WMS-III-SS, Corsi Block-Tapping Test; Weschler Memory Scale (3rd edition) spatial span; LNS, Letter Number Span; TMT-A, Trail Making Test part A; BACS-SC, Brief Assessment of Cognition in Schizophrenia-Symbol Coding; NAB, Neuropsychological Assessment Battery; CPT-IP, Continuous Performance Test – Identical Pairs; MSCEIT-ME, Mayer-Salovey-Caruso Emotional Intelligence Test – Managing emotions.
Pearson's correlation analyses were used for exploring associations between HbA1c and glucose measures and cognitive tasks. Spearman's correlation analyses were used for exploring associations between c-peptide, insulin and HOMA-IR, and cognitive tasks.

values, and this was the case because the interaction term lost its significance when these two higher values were excluded from the analyses.

DISCUSSION

In our study that explored whether glucose metabolism parameters may contribute to the explanation, at least in part, of the cognitive deficits of individuals diagnosed with a ROP, we found that $HbA1_c$ contributed to a poorer cognitive performance in domains related to processing speed, executive functions and visual memory in both the ROP patients and the HS, whereas it was associated with poorer attention and vigilance only in the ROP group.

With the main aim of a better understanding of the molecular, cellular, and other system disturbances in patients with schizophrenia, biomarkers of diagnosis, prognosis, or treatment response have been recommended (38). The study of glucose metabolism parameters in this population is of special interest since patients with schizophrenia have a three-fold risk of

TABLE 5 | Multiple linear regression analyses exploring the relationship between $HbA1_c$ and cognitive abilities.

	Unadjusted model			Final model (adjusted for covariates† and interactions between $HbA1_c$ and diagnosis)			
	R^2	β	p value	R^2	β	p value	Significant interactions
HVLT-R	0.0002	0.016	0.871	0.465	-0.014	0.862	None
BVMT-R	0.038	-0.194	**0.043**	0.486	-0.189	**0.017**	None
WMS-III SS	0.041	-0.202	**0.034**	0.371	-0.143	0.100	None
LNS	0.029	-0.169	0.077	0.292	-0.170	0.065	None
TMT-A	0.02	0.143	0.137	0.474	0.160	**0.044**	None
BACS SC	0.015	-0.124	0.197	0.617	-0.136	**0.044**	None
Category fluency	0.0001	0.010	0.914	0.417	-0.014	0.864	None
NAB Mazes	0.082	-0.287	**0.002**	0.407	-0.290	**0.001**	None
CPT-IP	0.008	-0.089	0.355	0.489	0.021	0.816	ROP x $HbA1_c$: β= -1.888; p=**0.016**
MSCEIT-ME	0.015	-0.121	0.208	0.337	-0.099	0.266	None

β, Standardized beta regression coefficient (for $HbA1_c$); HVLT-R, Hopkins Verbal Learning Test-Revised; BVMT-R, Brief Visuospatial Memory Test-Revised; WMS-III-SS, Corsi Block-Tapping Test; Weschler Memory Scale (3rd edition) spatial span; LNS, Letter Number Span; TMT-A, Trail Making Test part A; BACS-SC, Brief Assessment of Cognition in Schizophrenia-Symbol Coding; NAB, Neuropsychological Assessment Battery; CPT-IP, Continuous Performance Test – Identical Pairs; MSCEIT-ME, Mayer-Salovey-Caruso Emotional Intelligence Test – Managing emotions.
†Covariates: age, sex, education level, diagnosis, antipsychotic and benzodiazepine treatment, BMI, smoking, cortisol at awakening, cortisol awakening response, cortisol levels during the day (AUC_g).
Significant associations ($p < 0.05$) are represented in bold.

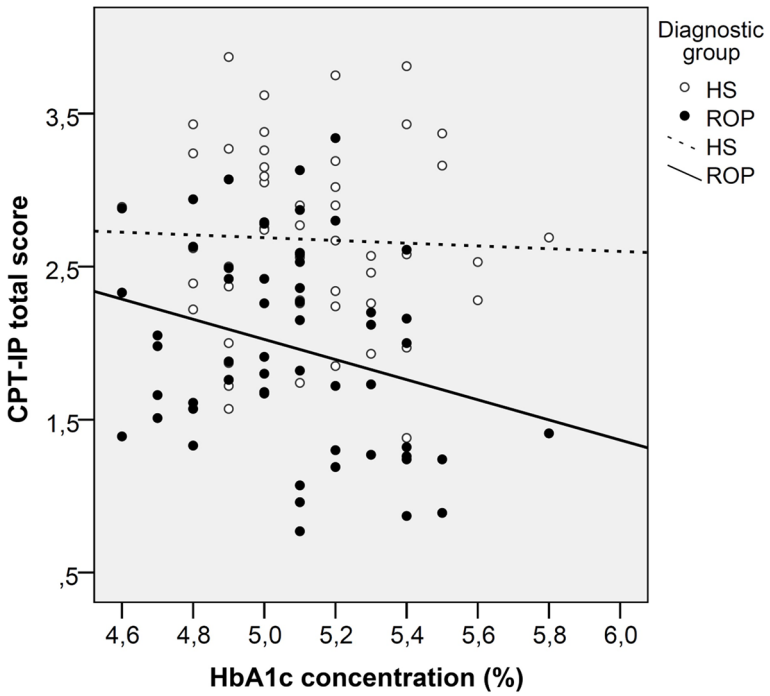

FIGURE 1 | Scatterplot graph of the association between glycated haemoglobin and attention in patients with a recent-onset psychosis and healthy subjects. ROP, Recent-onset psychosis; HS, Healthy subjects.

diabetes compared to the general population (39), and it gives us the opportunity to investigate pathogenic processes underlying both disturbances, with the aim of discovering new treatment strategies.

Biomarkers of schizophrenia have been widely used in recent years. They are frequently divided into two groups: peripheral and central biomarkers. The central nervous system and the periphery are strongly connected, a fact that has revealed the relevance of blood-based parameters as biomarkers in schizophrenia. Once again, several classifications for biomarkers have examined molecules modulating brain functions. Biomarkers have then been divided into inflammatory biomarkers, neuroendocrine biomarkers, neurotransmitters (well-documented and deeply understood), and metabolic biomarkers (38). The last may include indicators of metabolic syndrome or insulin resistance (13) that have been proven to discriminate between patients with or without metabolic syndrome. Furthermore, for many years, increased glucose concentrations, insulin resistance, and impaired glucose tolerance (13, 40) have been reported to be present in drug-naïve first-episode psychosis patients compared to HS. However, in our study, we did not find significant differences in most glucose-related parameters, except for C-peptide between ROP patients and HS.

In our study, in terms of all glucose parameters, $HbA1_c$ levels were found to be associated with poorer cognitive performance, particularly in those cognitive tasks assessing executive function and visual memory in both groups, ROP patients and HS. These findings agree with a recent study carried out by Zhang et al. (24), who found this parameter to be correlated with poorer global cognition and attention in men suffering from schizophrenia. Other studies (23) have reported an association between glucose intolerance and more severe negative symptoms and poorer social cognition, although no associations were found for neurocognitive performance. There is also a meta-analysis suggesting that type 2 diabetes is associated with more severe cognitive deficits in schizophrenia (16). Interestingly, in a meta-analysis (21) exploring different glucose-related biomarkers and cognitive impairment in people with type 2 diabetes, high $HbA1_c$ was negatively associated with cognitive function. Our study is in accordance with this last study, as we found a greater association for $HbA1_c$ than for other fasting-related glucose parameters. These results are seemingly in agreement with a recent study investigating glucose metabolic parameters associated with cognition and white matter microstructure in healthy young populations (22). $HbA1_c$ levels (even under the diagnostic values for diabetes mellitus) were inversely correlated with measures of cognitive performance. Moreover, this low-grade $HbA1_c$ variation negatively affected white matter integrity, that also correlated with cognitive function. This study supports our findings related to the transdiagnostic relationship between $HbA1_c$ and cognitive function, as associations between these measures were observed in both ROP patients and healthy controls.

In contrast with other cognitive domains, attention was negatively associated with $HbA1_c$ in ROP patients but not in HS. It is not clear why a distinct pattern could exist between ROP patients and HS in this particular domain. Previous studies that have measured allostatic load, an index that reflects systemic biological dysregulations including glucose homeostasis

parameters (glucose and insulin), have reported associations with poorer attention in ROP patients but not in HS (41). In another double-blind, placebo-controlled experimental study that assessed the effects of multiple-dose oral glucose administration on cognition in younger and older patients with schizophrenia and HS, a decrease in attentional performance at the 75 g glucose dose, when compared to placebo, was found in younger patients with schizophrenia (42). These findings suggest that glucose metabolism parameters might differentially affect ROP patients and HS in the attention and vigilance domains. However, it is also important to mention that this interaction was driven by higher $HbA1_c$ values, as the interaction lost its significance when analyses were restricted to people with $HbA1_c$ below 5.8%. The $HbA1_c$ range of our sample was also low, which limits the possibility of finding associations between cognitive outcomes and this glucose-related biomarker. Further studies might improve this issue by including a sample with a greater proportion of patients with glucose intolerance. This can be achieved by recruiting patients with a longer duration of the illness, because pre-diabetes and diabetes might increase over time. As only 16% of patients with first episode psychosis show abnormal glucose tolerance (13), the recruitment of patients with psychotic disorders at early stages of the illness might explain the narrow range of $HbA1_c$ levels in our sample.

The negative correlation between $HbA1_c$ and cognitive performance found in our study may be partially explained by the fact that cognitive tasks associated with $HbA1_c$ are mainly those implicating hippocampal functions and the prefrontal cortex, a hypothesis that is supported by a recent review (43). Continuous exposure to glucose and prediabetes have been significantly associated with structural brain abnormalities such as decreased brain volume and grey matter and white matter volume (44). Further, the risk of brain infarcts and decreased hippocampal volume may be associated with continuous exposure to glucose, which is reflected by higher levels of $HbA1_c$. This indicator of long-term glycaemic control is also thought to impact negatively on white matter structure even in healthy individuals (22). This last study suggests that biological processes other than microvascular and macrovascular disease could be playing a role in this associations. As pointed out by Repple et al. (22), it is plausible that inflammatory processes might constitute one of several biological mechanisms potentially mediating the relationship between glycose dysregulation and brain structural damage. This is a particularly important issue to be studied because the low-grade inflammation is also found in people with psychotic disorders (45–47), even before the onset of the disease (48, 49), and is associated with poorer cognitive function (9, 50). The duration of postpandrial glucose increase, a major contributor to chronic hyperglycaemia (and higher $HbA1_c$), is also thought to contribute to excessive protein glycation, generation of oxidative stress and inflammation (51). Future longitudinal studies are needed to explore whether the association between $HbA1_c$ levels and impaired cognitive function could be explained by changes in inflammatory markers.

Recently, some authors have hypothesized that central insulin resistance could have an important role in the relationship between metabolic and cognitive disorders (52). There is consistent evidence pointing out that the dopamine system has an important role in glucose homeostasis control because the dopaminergic and insulin signalling pathways influence each other (53–55) and that both central nervous system insulin and striatal dopamine can regulate peripheral glucose homeostasis (56). Given the central importance of dopaminergic dysregulation, cognitive deficits, and metabolic dysfunction in schizophrenia (57–59), the potential role of central nervous system insulin signalling in the pathophysiology of schizophrenia is an interesting field to be explored. This knowledge could help in the exploration and development of future therapeutic strategies.

For most cognitive domains, with the exception of attention and vigilance, the association between $HbA1_c$, and cognition was not specific for patients with ROP. Moreover, ROP patients and HS had similar $HbA1_c$ levels, which were in the normal range. These findings suggest that subtle differences in $HbA1_c$ could have a significant impact on cognitive processing independent of diagnosis. As our study has a cross-sectional design and includes psychotic patients who are at early stages of the disease, our study does not allow us to infer causality. Future longitudinal studies are needed to explore whether longitudinal changes in glucose metabolic parameters (mainly $HbA1_c$) could contribute to cognitive impairment in patients with schizophrenia. This is an important hypothesis to be tested, as chronic antipsychotic treatment is associated with weight gain and metabolic abnormalities, including the risk of type 2 diabetes (60). If our results are replicated in longitudinal studies, therapeutic agents that improve insulin sensitivity and promote neurogenesis, such as metformin (61), could be considered cognitive enhancement options for people with ROP and higher $HbA1_c$ values.

The results regarding $HbA1_c$ and cognitive performance were independent of HPA axis activity, as all multivariate analyses were adjusted for cortisol levels during the day and the CAR. These two HPA axis measures have been reported to be associated with impaired cognitive functioning in ROP patients (4): a blunted CAR has been associated with poorer verbal memory in ROP patients (27), higher afternoon plasma cortisol levels are associated with poorer verbal memory (62), and a more flattened diurnal cortisol slope has been associated with poorer working memory in women with ROP (3). It is also important to control for HPA axis activity when exploring the role of glucose-related parameters on cognition because a central dysregulation of the HPA axis has been reported in type 2 diabetes and because higher morning cortisol levels are associated with poorer cognitive abilities in people with type 2 diabetes (28). In patients with type 2 diabetes, there are elevated basal plasma cortisol and ACTH levels (63–65), higher late-night cortisol (66), and increased cortisol levels following overnight dexamethasone suppression (67, 68).

Several strengths of our study should be noted. The patients in our study seemed to be representative of our catchment area, and our first-episode psychosis unit is a referral center in our area. On the other hand, to the best of our knowledge, few studies have tried to specifically investigate metabolic biomarkers for cognition in schizophrenia. Although some studies have

explored the association between diabetes mellitus and poorer cognitive functioning in patients with schizophrenia (16, 24), our study is the first to specifically highlight the relationship between $HbA1_c$ and cognitive function in the early stages of the psychotic illness. The search for biomarkers in cognition has been the focus of interest in research on patients with schizophrenia (50). However, in recent decades, the vast majority of studies have investigated whether neurotrophic factors or inflammatory markers may be correlated with cognitive deficits or cognitive recovery in schizophrenia (50), and metabolic parameters have been a second focus of interest.

The main limitation of our study is the cross-sectional design. Therefore, no causal relationships between glucose metabolism parameters and cognitive functioning can be inferred. The relatively small sample size limits the possibility of the conduction of specific subanalyses regarding sex differences or psychotic phenotypes. Larger studies are needed to test whether the role of $HbA1_c$ on cognitive outcomes differs by subtypes of psychotic disorders. The CAR was obtained only on one day, as consensus guidelines (69) recommend obtaining repeated measures for this parameter on separate days. As in the original study (3), we administered dexamethasone to all participants, and we decided to measure the CAR only on one day. Finally, a replication dataset was not included.

In summary, the present study suggests that long-term exposure to higher glucose levels, although within the normal range, is negatively associated with cognitive performance in both ROP patients and HS in tasks dealing with executive functions and visual memory, two cognitive domains that involve the hippocampus and prefrontal cortex. Our study suggests that this association is independent of BMI and HPA axis functioning. These findings highlight the importance of the consideration of the inclusion of $HbA1_c$ in future longitudinal studies exploring cognitive changes in patients with a psychotic disorder at early stages of the disease to disentangle a potential negative effect on cognitive outcomes.

DATA AVAILABILITY STATEMENT

The datasets generated for this study will not be made publicly available. The datasets are not publicly available due to privacy or ethical restrictions. However, additional analyses might be available from authors by request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Hospital Sant Joan Ethics Committee. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

JL, AG-Z, and EV designed the study. IM, ÁC, MS, VS-G, MA, and LO participated in the recruitment of participants. LM and EV participated in the biochemical determinations. JL and IM analyzed the data. IM and AG-R reviewed the scientific literature and wrote the first draft of the manuscript. All authors participated in the discussion of the results and approved the final manuscript.

FUNDING

This work was funded in part by the Instituto de Salud Carlos III (PI10/01607; PI15/01386) and by La Fundació de la Marató de TV3 (92230). JL and IM have received an Intensification of the Research Activity Grant by the Health Department from the Generalitat de Catalunya (SLT006/17/00012 and SLT008/18/00074). JL has also received an Intensification of the Research Activity Grant by the Instituto de Salud Carlos III in 2020 (INT19/00071).

REFERENCES

1. Fett AKJ, Viechtbauer W, Dominguez M de G, Penn DL, van Os J, Krabbendam L. The relationship between neurocognition and social cognition with functional outcomes in schizophrenia: A meta-analysis. *Neurosci Biobehav Rev* (2011) 35:573–88. doi: 10.1016/j.neubiorev.2010.07.001
2. Fusar-Poli P, Deste G, Smieskova R, Barlati S, Yung AR, Howes O, et al. Cognitive functioning in prodromal psychosis: a meta-analysis. *Arch Gen Psychiatry* (2012) 69:562–71. doi: 10.1001/archgenpsychiatry.2011.1592
3. Labad J, Gutiérrez-Zotes A, Creus M, Montalvo I, Cabezas Á, Solé M, et al. Hypothalamic-pituitary-adrenal axis measures and cognitive abilities in early psychosis: Are there sex differences? *Psychoneuroendocrinology* (2016) 72:54–62. doi: 10.1016/j.psyneuen.2016.06.006
4. Labad J. The role of cortisol and prolactin in the pathogenesis and clinical expression of psychotic disorders. *Psychoneuroendocrinology* (2019) 102:24–36. doi: 10.1016/j.psyneuen.2018.11.028
5. Barbero JD, Gutiérrez-Zotes A, Montalvo I, Creus M, Cabezas Á, Solé M, et al. Free thyroxine levels are associated with cognitive abilities in subjects with early psychosis. *Schizophr Res* (2015) 166:37–42. doi: 10.1016/j.schres.2015.04.030
6. Labad J, Barbero JD, Gutiérrez-Zotes A, Montalvo I, Creus M, Cabezas Á, et al. Free thyroxine levels are associated with cognitive changes in individuals with a first episode of psychosis: A prospective 1-year follow-up study. *Schizophr Res* (2016) 171:182–6. doi: 10.1016/j.schres.2016.01.036
7. Montalvo I, Nadal R, Armario A, Gutiérrez-Zotes A, Creus M, Cabezas Á, et al. Sex differences in the relationship between prolactin levels and impaired processing speed in early psychosis. *Aust N Z J Psychiatry* (2018) 52:585–95. doi: 10.1177/0004867417744254
8. Montalvo I, Gutiérrez-Zotes A, Creus M, Monseny R, Ortega L, Franch J, et al. Increased prolactin levels are associated with impaired processing speed in subjects with early psychosis. *PloS One* (2014) 9:e89428. doi: 10.1371/journal.pone.0089428
9. Cabrera B, Bioque M, Penadés R, González-Pinto A, Parellada M, Bobes J, et al. Cognition and psychopathology in first-episode psychosis: are they related to inflammation? *Psychol Med* (2016) 46:2133–44. doi: 10.1017/S0033291716000659

10. Ribeiro-Santos A, Lucio Teixeira A, Salgado JV. Evidence for an immune role on cognition in schizophrenia: a systematic review. *Curr Neuropharmacol* (2014) 12:273–80. doi: 10.2174/1570159X1203140511160832
11. Fanous AH, Kendler KS. Genetics of clinical features and subtypes of schizophrenia: A review of the recent literature. *Curr Psychiatry Rep* (2008) 10:164–70. doi: 10.1007/s11920-008-0028-z
12. Ryan MCM, Collins P, Thakore JH. Impaired fasting glucose tolerance in first-episode, drug-naive patients with schizophrenia. *Am J Psychiatry* (2003) 160:284–9. doi: 10.1176/appi.ajp.160.2.284
13. Fernandez-Egea E, Bernardo M, Donner T, Conget I, Parellada E, Justicia A, et al. Metabolic profile of antipsychotic-naive individuals with non-affective psychosis. *Br J Psychiatry* (2009) 194:434–8. doi: 10.1192/bjp.bp.108.052605
14. Fernandez-Egea E, Miller B, Bernardo M, Donner T, Kirkpatrick B. Parental history of Type 2 diabetes in patients with nonaffective psychosis. *Schizophr Res* (2008) 98:302–6. doi: 10.1016/j.schres.2007.10.002
15. Habtewold TD, Islam MA, Liemburg EJ, Bartels-Velthuis AAA, van Beveren NJ, Cahn W, et al. Polygenic risk score for schizophrenia was not associated with glycemic level (HbA1c) in patients with non-affective psychosis: Genetic Risk and Outcome of Psychosis (GROUP) cohort study. *J Psychosom Res* (2020) 132:109968. doi: 10.1016/j.jpsychores.2020.109968
16. Bora E, Akdede BB, Alptekin K. The relationship between cognitive impairment in schizophrenia and metabolic syndrome: A systematic review and meta-analysis. *Psychol Med* (2017) 47:1030–40. doi: 10.1017/S0033291716003366
17. Marden JR, Mayeda ER, Tchetgen Tchetgen EJ, Kawachi I, Glymour MM. High Hemoglobin A1c and Diabetes Predict Memory Decline in the Health and Retirement Study. *Alzheimer Dis Assoc Disord* (2017) 31:48–54. doi: 10.1097/WAD.0000000000000182
18. Gao Y, Xiao Y, Miao R, Zhao J, Cui M, Huang G, et al. The prevalence of mild cognitive impairment with type 2 diabetes mellitus among elderly people in China: A cross-sectional study. *Arch Gerontol Geriatr* (2016) 62:138–42. doi: 10.1016/j.archger.2015.09.003
19. Cooper C, Sommerlad A, Lyketsos CG, Livingston G. Modifiable predictors of dementia in mild cognitive impairment: A systematic review and meta-analysis. *Am J Psychiatry* (2015) 172:323–34. doi: 10.1176/appi.ajp.2014.14070878
20. Yuan XY, Wang XG. Mild cognitive impairment in type 2 diabetes mellitus and related risk factors: A review. *Rev Neurosci* (2017) 28:715–23. doi: 10.1515/revneuro-2017-0016
21. Geijselaers SLC, Sep SJS, Stehouwer CDA, Biessels GJ. Glucose regulation, cognition, and brain MRI in type 2 diabetes: A systematic review. *Lancet Diabetes Endocrinol* (2015) 3:75–89. doi: 10.1016/S2213-8587(14)70148-2
22. Repple J, Karliczek G, Meinert S, Förster K, Grotegerd D, Goltermann J, et al. Variation of HbA1c affects cognition and white matter microstructure in healthy, young adults. *Mol Psychiatry* (2019). doi: 10.1038/s41380-019-0504-3
23. Chen DC, Du XD, Yin GZ, Yang KB, Nie Y, Wang N, et al. Impaired glucose tolerance in first-episode drug-naïve patients with schizophrenia: Relationships with clinical phenotypes and cognitive deficits. *Psychol Med* (2016) 46:3219–30. doi: 10.1017/S0033291716001902
24. Zhang BH, Han M, Zhang XY, Hui L, Jiang SR, De YF, et al. Gender differences in cognitive deficits in schizophrenia with and without diabetes. *Compr Psychiatry* (2015) 63:1–9. doi: 10.1016/j.comppsych.2015.07.003
25. Agarwal SM, Kowalchuk C, Castellani L, Costa-Dookhan KA, Caravaggio F, Asgariroozbehani R, et al. Brain insulin action: Implications for the treatment of schizophrenia. *Neuropharmacology* (2019) 168:107655. doi: 10.1016/j.neuropharm.2019.05.032
26. Cullen AE, Zunszain PA, Dickson H, Roberts RE, Fisher HL, Pariante CM, et al. Cortisol awakening response and diurnal cortisol among children at elevated risk for schizophrenia: Relationship to psychosocial stress and cognition. *Psychoneuroendocrinology* (2014) 46:1–13. doi: 10.1016/j.psyneuen.2014.03.010
27. Aas M, Dazzan P, Mondelli V, Toulopoulou T, Reichenberg A, Di Forti M, et al. Abnormal cortisol awakening response predicts worse cognitive function in patients with first-episode psychosis. *Psychol Med* (2011) 41:463–76. doi: 10.1017/S0033291710001170
28. Reynolds RM, Strachan MWJ, Labad J, Lee AJ, Frier BM, Fowkes FG, et al. Morning cortisol levels and cognitive abilities in people with type 2 diabetes: The Edinburgh type 2 diabetes study. *Diabetes Care* (2010) 33:714–20. doi: 10.2337/dc09-1796
29. Wing JK, Babor T, Brugha T, Burke J, Cooper JE, Giel R, et al. SCAN. Schedules for Clinical Assessment in Neuropsychiatry. *Arch Gen Psychiatry* (1990) 47:589–93. doi: 10.1001/archpsyc.1990.01810180089012
30. Kay SR, Fiszbein A, Vital-Herne M, Fuentes LS. The Positive and Negative Syndrome Scale–Spanish adaptation. *J Nerv Ment Dis* (1990) 178:510–7. doi: 10.1097/00005053-199017880-00007
31. Peralta V, Cuesta MJ. Validacion de la escala de los síndromes positivo y negativo (PANSS) en una muestra de esquizofrénicos españoles. *Actas Luso Esp Neurol Psiquiatr Cienc Afines* (1994) 22:171–7.
32. Nuechterlein KH, Green MF, Kern RS, Baade LE, Barch DM, Cohen JD, et al. The MATRICS Consensus Cognitive Battery, part 1: test selection, reliability, and validity. *Am J Psychiatry* (2008) 165:203–13. doi: 10.1176/appi.ajp.2007.07010042
33. Gardner DM, Murphy AL, O'Donnell H, Centorrino F, Baldessarini RJ. International consensus study of antipsychotic dosing. *Am J Psychiatry* (2010) 167:686–93. doi: 10.1176/appi.ajp.2009.09060802
34. Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology* (2003) 28:916–31. doi: 10.1016/S0306-4530(02)00108-7
35. Miller R, Plessow F. Transformation techniques for cross-sectional and longitudinal endocrine data: Application to salivary cortisol concentrations. *Psychoneuroendocrinology* (2013) 38:941–6. doi: 10.1016/j.psyneuen.2012.09.013
36. Olsen W. Outliers. In: *Data Collection: Key Debates and Methods in Social Research*. London: SAGE Publications Ltd. (2012). doi: 10.4135/9781473914230.n32
37. Bender R, Lange S. Adjusting for multiple testing - When and how? *J Clin Epidemiol* (2001) 54:343–9. doi: 10.1016/S0895-4356(00)00314-0
38. Perkovic MN, Erjavec GN, Strac DS, Uzun S, Kozumplik O, Pivac N. Theranostic biomarkers for schizophrenia. *Int J Mol Sci* (2017) 18:pii: E733. doi: 10.3390/ijms18040733
39. Rajkumar AP, Horsdal HT, Wimberley T, Cohen D, Mors O, Børglum AD, et al. Endogenous and antipsychotic-related risks for diabetes mellitus in young people with schizophrenia: A danish population-based cohort study. *Am J Psychiatry* (2017) 174:686–94. doi: 10.1176/appi.ajp.2016.16040442
40. Petrikis P, Tigas S, Tzallas AT, Papadopoulos I, Skapinakis P, Mavreas V. Parameters of glucose and lipid metabolism at the fasted state in drug-naïve first-episode patients with psychosis: Evidence for insulin resistance. *Psychiatry Res* (2015) 229:901–4. doi: 10.1016/j.psychres.2015.07.041
41. Piotrowski P, Kotowicz K, Rymaszewska J, Beszłej JA, Plichta P, Samochowiec J, et al. Allostatic load index and its clinical correlates at various stages of psychosis. *Schizophr Res* (2019) 210:73–80. doi: 10.1016/j.schres.2019.06.009
42. Fucetola R, Newcomer JW, Craft S, Melson AK. Age- and dose-dependent glucose-induced increases in memory and attention in schizophrenia. *Psychiatry Res* (1999) 88:1–13. doi: 10.1016/S0165-1781(99)00063-3
43. Zhou J, Zhang Z, Zhou H, Qian G. Diabetic Cognitive Dysfunction: From Bench to Clinic. *Curr Med Chem* (2019). doi: 10.2174/1871530319666190206225635
44. van Agtmaal MJM, Houben AJHM, de Wit V, Henry RMA, Schaper NC, Dagnelie PC, et al. Prediabetes is associated with structural brain abnormalities: The Maastricht study. *Diabetes Care* (2018) 41:2535–43. doi: 10.2337/dc18-1132
45. Kirkpatrick B, Miller BJ. Inflammation and schizophrenia. *Schizophr Bull* (2013) 39:1174–9. doi: 10.1093/schbul/sbt141
46. Soria V, Uribe J, Salvat-Pujol N, Palao D, Menchón JM, Labad J. Psychoneuroimmunology of mental disorders. *Rev Psiquiatr Salud Ment* (2018) 11:115–24. doi: 10.1016/j.rpsm.2017.07.006
47. Miller BJ, Culpepper N. Rapaport MH. C-reactive protein levels in schizophrenia: a review and meta-analysis. *Clin Schizophr Relat Psychoses* (2014) 7:223–30. doi: 10.3371/CSRP.MICU.020813
48. Stojanovic A, Martorell L, Montalvo I, Ortega L, Monseny R, Vilella E, et al. Increased serum interleukin-6 levels in early stages of psychosis: Associations with at-risk mental states and the severity of psychotic symptoms. *Psychoneuroendocrinology* (2014) 41:23–32. doi: 10.1016/j.psyneuen.2013.12.005
49. Khandaker GM, Pearson RM, Zammit S, Lewis G, Jones PB. Association of serum interleukin 6 and C-reactive protein in childhood with depression and

psychosis in young adult life. *JAMA Psychiatry* (2014) 71:1121–8. doi: 10.1001/jamapsychiatry.2014.1332
50. Penadés R, García-Rizo C, Bioque M, González-Rodríguez A, Cabrera B, Mezquida G, et al. The search for new biomarkers for cognition in schizophrenia. *Schizophr Res Cognit* (2015) 2:172–8. doi: 10.1016/j.scog.2015.10.004
51. Blaak EE, Antoine JM, Benton D, Björck I, Bozzetto L, Brouns F, et al. Impact of postprandial glycaemia on health and prevention of disease. *Obes Rev* (2012) 13:923–84. doi: 10.1111/j.1467-789X.2012.01011.x
52. Kullmann S, Heni M, Hallschmid M, Fritsche A, Preissl H, Häring HU. Brain insulin resistance at the crossroads of metabolic and cognitive disorders in humans. *Physiol Rev* (2016) 96:1169–209. doi: 10.1152/physrev.00032.2015
53. Lopez Vicchi F, Luque GM, Brie B, Nogueira JP, Garcia Tornadu I, Becu-Villalobos D. Dopaminergic drugs in type 2 diabetes and glucose homeostasis. *Pharmacol Res* (2016) 109:74–80. doi: 10.1016/j.phrs.2015.12.029
54. Nash AI. Crosstalk between insulin and dopamine signaling: A basis for the metabolic effects of antipsychotic drugs. *J Chem Neuroanat* (2017) 83-84:59–68. doi: 10.1016/j.jchemneu.2016.07.010
55. Daws LC, Avison MJ, Robertson SD, Niswender KD, Galli A, Saunders C. Insulin signaling and addiction. *Neuropharmacology* (2011) 61:1123–8. doi: 10.1016/j.neuropharm.2011.02.028
56. ter Horst DM, Schene AH, Figueroa CA, Assies J, Lok A, Bockting CLH, et al. Cortisol, dehydroepiandrosterone sulfate, fatty acids, and their relation in recurrent depression. *Psychoneuroendocrinology* (2019) 100:203–12. doi: 10.1016/j.psyneuen.2018.10.012
57. Tripathi A, Kar SK, Shukla R. Cognitive deficits in schizophrenia: Understanding the biological correlates and remediation strategies. *Clin Psychopharmacol Neurosci* (2018) 16:7–17. doi: 10.9758/cpn.2018.16.1.7
58. Pillinger T, Beck K, Gobjila C, Donocik JG, Jauhar S, Howes OD. Impaired glucose homeostasis in first-episode schizophrenia: A systematic review and meta-analysis. *JAMA Psychiatry* (2017) 74:261–9. doi: 10.1001/jamapsychiatry.2016.3803
59. Caravaggio F, Hahn M, Nakajima S, Gerretsen P, Remington G, Graff-Guerrero A. Reduced insulin-receptor mediated modulation of striatal dopamine release by basal insulin as a possible contributing factor to hyperdopaminergia in schizophrenia. *Med Hypotheses* (2015) 85:391–6. doi: 10.1016/j.mehy.2015.06.011
60. Galling B, Roldán A, Nielsen RE, Nielsen J, Gerhard T, Carbon M, et al. Type 2 diabetes mellitus in youth exposed to antipsychotics: A systematic review and meta-analysis. *JAMA Psychiatry* (2016) 73:247–59. doi: 10.1001/jamapsychiatry.2015.2923
61. Ahmed S, Mahmood Z, Javed A, Hashmi SN, Zerr I, Zafar S, et al. Effect of Metformin on Adult Hippocampal Neurogenesis: Comparison with Donepezil and Links to Cognition. *J Mol Neurosci* (2017) 62:88–98. doi: 10.1007/s12031-017-0915-z
62. Havelka D, Prikrylova-Kucerova H, Prikryl R, Ceskova E. Cognitive impairment and cortisol levels in first-episode schizophrenia patients. *Stress* (2016) 19:383–9. doi: 10.1080/10253890.2016.1193146
63. Cameron OG, Thomas B, Tiongco D, Hariharan M, Greden JF. Hypercortisolism in diabetes mellitus. *Diabetes Care* (1987) 10:662–4. doi: 10.2337/diacare.10.5.662
64. Lee ZSK, Chan JCN, Yeung VTF, Chow CC, Lau MSW, Ko GTC, et al. Plasma insulin, growth hormone, cortisol, and central obesity among young Chinese type 2 diabetic patients. *Diabetes Care* (1999) 22:1450–357. doi: 10.2337/diacare.22.9.1450
65. Reynolds RM, Walker BR, Syddall HE, Andrew R, Wood PJ, Whorwood CB, et al. Altered control of cortisol secretion in adult men with low birth weight and cardiovascular risk factors. *J Clin Endocrinol Metab* (2001) 86:245–50. doi: 10.1210/jcem.86.1.7145
66. Liu H, Bravata DM, Cabaccan J, Raff H, Ryzen E. Elevated late-night salivary cortisol levels in elderly male type 2 diabetic veterans. *Clin Endocrinol (Oxf)* (2005) 63:642–9. doi: 10.1111/j.1365-2265.2005.02395.x
67. Cameron OG, Kronfol Z, Greden JF, Carroll BJ. Hypothalamic-Pituitary-Adrenocortical Activity in Patients With Diabetes Mellitus. *Arch Gen Psychiatry* (1984) 41:1090–5. doi: 10.1001/archpsyc.1983.01790220080013
68. Hudson JI, Hudson MS, Rothschild AJ, Vignati L, Schatzberg AF, Melby JC. Abnormal Results of Dexamethasone Suppression Tests in Nondepressed Patients With Diabetes Mellitus. *Arch Gen Psychiatry* (1984) 41:1086–9. doi: 10.1001/archpsyc.1983.01790220076012
69. Stalder T, Kirschbaum C, Kudielka BM, Adam EK, Pruessner JC, Wüst S, et al. Assessment of the cortisol awakening response: Expert consensus guidelines. *Psychoneuroendocrinology* (2016) 63:414–32. doi: 10.1016/j.psyneuen.2015.10.010

Conflict of Interest: JL and VS-G have received honoraria for lectures or advisory boards from Jannsen, Otsuka and Lundbeck.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

SYSTEMATIC REVIEW
published: 26 May 2020
doi: 10.3389/fpsyt.2020.00479

Women Undergoing Hormonal Treatments for Infertility: A Systematic Review on Psychopathology and Newly Diagnosed Mood and Psychotic Disorders

Alexandre González-Rodríguez[1], Jesús Cobo[1], Virginia Soria[2], Judith Usall[3], Clemente Garcia-Rizo[4], Miquel Bioque[4], José Antonio Monreal[1], on behalf of PNECAT Group and Javier Labad[1]*

[1] Department of Mental Health, Parc Tauli Hospital Universitari, Institut d'Investigació i Innovació Parc Tauli (I3PT), Autonomous University of Barcelona (UAB), Centro de Investigación Biomédica en Red en Salud Mental (CIBERSAM), Sabadell, Spain, [2] Department of Psychiatry, Bellvitge University Hospital, Bellvitge Biomedical Research Institute (IDIBELL), Department of Clinical Sciences, University of Barcelona (UB), Centro de Investigación Biomédica en Red en Salud Mental (CIBERSAM), Hospitalet de Llobregat, Barcelona, Spain, [3] Mental Health Services, Parc Sanitari Sant Joan de Déu, Sant Boi de Llobregat, Spain, [4] Barcelona Clinic Schizophrenia Unit, Neuroscience Institute, Hospital Clinic de Barcelona, Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS), Centro de Investigación Biomédica en Red en Salud Mental (CIBERSAM), Department of Medicine, University of Barcelona, Barcelona, Spain

OPEN ACCESS

Edited by:
Grazia Rutigliano, University of Pisa, Italy

Reviewed by:
Alexis E. Cullen, King's College London, United Kingdom
Ivana Zivoder, University North, Croatia
Maarten Van Den Buuse, La Trobe University, Australia

***Correspondence:**
Alexandre González-Rodríguez
agonzalezro@tauli.cat

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *11 March 2020*
Accepted: *11 May 2020*
Published: *26 May 2020*

Citation:
González-Rodríguez A, Cobo J, Soria V, Usall J, Garcia-Rizo C, Bioque M, Monreal JA and Labad J (2020) Women Undergoing Hormonal Treatments for Infertility: A Systematic Review on Psychopathology and Newly Diagnosed Mood and Psychotic Disorders. Front. Psychiatry 11:479. doi: 10.3389/fpsyt.2020.00479

Background: The association between infertility treatments and mental disorders has been poorly addressed. This work aims to review current evidence on the psychopathological effects of hormonal treatments used for infertility on women and the occurrence of newly diagnosed mood and psychotic disorders.

Methods: A systematic review was performed by searching PubMed and clinicaltrials.gov databases from inception until September 2019. Clinical trials on hormone treatments for infertility in patients with mood or psychotic disorders, as well as those evaluating the onset of symptoms, were included. Selected studies were published in English, Spanish, and Dutch language peer-reviewed journals. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines were followed. Observational studies and case reports were excluded. Effect sizes for changes in depressive symptoms were calculated with Hedges'g and Cohen's d confidence intervals. A meta-analysis was not performed due to the heterogeneity of hormonal compounds in protocols.

Results: From 1,281 retrieved records, nine trials were included; all of them were conducted in non-clinical populations. Four trials compared Gonadotropin-releasing hormone (GnRH) agonists and GnRH antagonists, showing a better mood profile for hormonal protocols including antagonists in one trial. Two trials compared protocols using GnRH agonists/antagonists versus natural cycle protocols (without gonadotropin stimulation), with a better mood profile (less depressive symptoms) in those protocols

without gonadotropin stimulation. Other studies compared long and short protocols of GnRH agonists (no differences); two GnRH agonists, buserelin, and goserelin (no differences); and two patterns of clomiphene vs placebo administration (no differences). None of the selected studies investigated the risk of relapse in women with a previous diagnosis of depressive or psychotic disorders. When exploring pre-post changes in depressive symptoms, effect sizes suggested mild mood worsenings for most protocols (effect sizes ≤ -0.4), with the following pattern (worse to better): GnRH agonist > GnRH antagonist > no gonadotropin stimulation.

Conclusions: This is the first systematic review exploring the psychopathological effects of hormonal infertility treatments. Our study suggests that protocols without gonadotropin stimulation show a better mood profile when compared to those using GnRH antagonists or GnRH agonists. Future studies need to include patients with major mood and psychotic disorders.

Keywords: infertility, sex hormones, fertility treatments, psychosis, affective

INTRODUCTION

Women with fertility problems have been extensively found to suffer from psychological burden and may experience this clinical situation as very stressful (1). The relationship between assisted reproductive technologies (ARTs) and mental distress is complex, and it calls for a careful examination of the direction of the effects between both variables (2–4).

ART treatments may increase mental distress in women undergoing these therapeutic options (1). Mental health consequences may be partially explained by psychological factors, such as neuroticism, as well as by biological factors derived from the biochemical nature of treatment compounds (3).

When focusing on reproductive medicine, it seems to be crucial to determine whether a woman is infertile (5). Medical history (e.g., health history, the use of other medications); physical examination; blood tests including assessments of hormone levels; and ultrasonography exploring the ovaries, uterus and Fallopian tubes appear to be mandatory (5, 6). In some cases, infertility in women might be secondary to an ovulation problem or an obstruction of the Fallopian tubes (7). However, in 5%–15% of cases, all tests and physical examinations are normal, and specific causes cannot be determined (8).

Once the cause of infertility is found, all potential options for treatment should be considered (9). Briefly, four main types of infertility treatment are available: gamete intrafallopian transfer (GIFT) and zygote intrafallopian transfer (ZIFT), intrauterine insemination (IUI), and *in vitro* fertilization (IVF) techniques (10). IUI is a fertility treatment based on placing sperm directly into the uterus while the woman is ovulating (11). IVF is an ART process based on controlled ovarian stimulation, egg retrieval from women's ovaries and the fertilization of these eggs with sperm in the laboratory (11). Finally, the embryo is placed in the woman's uterus. GIFT and ZIFT refer to the collection and placement of gametes or zygotes, respectively, into the Fallopian tube (12).

Regarding IVF techniques, we will focus on the use of biological compounds, mainly hormones, aiming to achieve controlled ovarian stimulation. These molecules are factors that can be associated with psychopathological changes in healthy women and women with previous mental disorders (9).

Many biological compounds have been developed for ovulation induction in IVF, with the main aim of obtaining more oocytes (13), including clomiphene citrate, aromatase inhibitors, gonadotrophins, and gonadotropin-releasing hormone (GnRH) analogues (9). Clomiphene citrate blocks oestrogen receptors and increases follicle-stimulating hormone (FSH) levels. Aromatase inhibitors block the conversion of androgens to oestrogens. Gonadotrophins include recombinant FSH and luteinizing hormone (LH), and GnRH analogues include agonists and antagonists.

Conventional controlled ovarian stimulation protocols include the administration of gonadotropin-releasing hormone (GnRH) agonists with the main aim of the desensitization of the pituitary gland through the suppression of the release of both pituitary follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (13). This technique has been successfully associated with IVF; however, several adverse effects have also been described, such as mood disturbances (3). More recently, GnRH antagonist protocols have been found to be an alternative for ovarian stimulation. They seem to show lower rates of ovarian hyperstimulation syndrome and may reduce the length of ovulatory stimuli compared with other ovulation induction protocols (14). GnRH antagonist protocols are based on the daily administration of recombinant follicle stimulating hormone (r-FSH) analogue to start ovarian stimulation (2–3 cycle days). Some studies have found GnRH protocols to have higher rates of pregnancies than treatment with clomiphene or aromatase inhibitors (13). More recently, a short GnRH antagonist protocol has been recommended for younger women undergoing their first ART cycle (14).

Nevertheless, sex hormones have been found to regulate mood and may play an important role on the pathophysiology of affective disorders as well as schizophrenia and other psychotic disorders (15). Rubinow and Schmidt proposed several models to

explain how sex may influence brain function by means of hormonal effects and how the regulation of affect may be sex dependent (16). These investigations have also been conducted in patients with psychotic disorders. If the response to stress may be dysregulated in psychosis, Goldstein and co-workers (17) reported that the interplay between steroid hormones and neural activity may be sex dependent in psychosis, particularly for brain functions implicating the prefrontal cortex (17, 18).

In the particular case of GnRH agonists, it should be noted that they combine hormonal control of the cycle by means of a hypoestrogenism state. This short induced-hormonal state may determine subsequent mental health consequences that may be partially explained through the hypothalamic-pituitary-gonadal axis (19). Hormonal changes and withdrawal of oestrogens may determine an increased vulnerability to depression in women as well as a poorer prognosis (worsening of psychotic symptoms, greater risk of relapse and higher needs of antipsychotic dosages) in female patients with schizophrenia (20, 21). Therefore, those fertility treatments that induce hypoestrogenism may have a negative effect on depressive or psychotic symptoms.

Although some studies have addressed the effects of sex steroids in brain functions in individuals with and without mental illnesses (15–18), no previous systematic reviews have explored the effects of infertility hormonal treatments on psychopathology in women. The vast majority of the studies have investigated pregnancy outcomes or other somatic concerns, and psychopathological symptoms have been widely neglected.

Several observational studies have investigated the prevalence rates for psychiatric disorders in women undergoing fertility treatments (22). The authors found a prevalence of mood disorders around 26% in women, highlighting that major depression was the most common diagnosis. In contrast with these findings, Salih Joelsson et al. (23) compared depressive symptoms in pregnant and non-pregnant women, and pregnant women receiving fertility treatment (23). Pregnant women did not differ in depressive symptoms compared to the other two groups, suggesting a lack of negative effect of fertility treatments on psychopathological symptoms. More recently, Freeman et al. (24) carried out a prospective observational study in women with a previous history of unipolar or bipolar depression undergoing fertility treatment (24). They concluded that maintenance of psychotropic medication was not sufficient for avoiding affective recurrences. With regard to schizophrenia and other psychoses, a national register study compared the success rates of fertility in women with and without psychotic disorders (25), although exacerbation of psychotic symptoms was not investigated.

In brief, findings from cohort and observational studies seem to be contradictory and inconclusive. Therefore, we aimed to systematically review current evidence on the psychopathological effects of hormonal treatments for infertility on women and the influence of these treatments on the occurrence of newly diagnosed mental disorders. We focused mainly on investigations concerning women with mood disorders and psychotic disorders.

METHODS

Search Strategy

A systematic computerized search was performed by focusing on trials evaluating the effect of hormone treatments for infertility in major depression, bipolar disorder, schizophrenia, and other psychotic disorders. PubMed database and ClinicalTrials.gov were searched from inception until September 2019 in keeping with the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines (26). Electronic search was also completed by revising all references of including studies for potential papers to be included.

The following search terms were used: (clomiphene OR GnRH OR letrozole OR vorozole OR anastrozole OR aromatase inhibitors OR FSH OR LH OR r-FSH OR r-LH OR *in vitro* fertilization OR IVF OR ICSI OR intracytoplasmic sperm injection OR ovarian stimulation OR controlled ovarian hyperstimulation) AND (psychiatric OR psychopathology OR psychopathological OR relapse OR recurrence OR depression OR bipolar OR mania OR psychosis OR schizophrenia) AND trial.

Inclusion Criteria

Studies that met the following inclusion criteria were considered: a) trials focused on evaluating the effect of hormonal treatment for infertility on women; b) studies that evaluated psychopathological effects of infertility treatments on women without a diagnosis of mental disorders (newly diagnosed mental disorder) or in women with a previous mental disorder (major depression, bipolar disorder, schizophrenia and other psychotic disorders); c) published trials in peer-reviewed journals or registered in clinicaltrials.gov; d) articles written in English, Spanish or Dutch language; and e) studies that assessed the effectiveness of hormone compounds for the treatment of infertility.

The exclusion criteria were as follows: a) naturalistic studies and case reports, b) studies assessing the effect of hormone compounds administered for uses other than the treatment of infertility, and c) studies exploring the effectiveness of hormone drugs to treat mental disorders.

Data Collection and Extraction

Titles and abstracts of studies identified in the initial searches were screened independently by two review authors (AG-R and JC). The same authors extracted data independently. Any disagreements or discrepancies between them were resolved by consensus and were explored with a third additional reviewer. Full-text documents were also reviewed. From the initially selected articles, those that did not meet our inclusion criteria or met any exclusion criterion were excluded. The last search was conducted on 29th October 2019. The PICO method for the systematic review is detailed in **Table S1**.

Risk of Bias

The risk of bias was evaluated using the Cochrane risk of bias tool. Studies were classified into three categories attending to their quality: good, fair, and poor. A meta-analysis was not performed due to the heterogeneity of the hormonal treatment protocols.

Effect Sizes for Changes in Depressive Symptoms

For those clinical trials that included information on depressive symptoms at baseline and after the hormonal treatment for infertility, effect sizes were calculated. Hedges' g was used as a measure of the effect size for changes in depressive symptoms after the hormonal protocol using an on-line calculator that allows the calculation of the effect size for paired samples tests (https://effect-size-calculator.herokuapp.com/#paired-samples-t-test). Another effect size measure considered in the systematic review was confidence intervals (lower and upper limits) using Cohen's d. These measures were calculated using pre-post scores (depressive symptoms) and standard deviation measures of all studies with available data. If data were available as the median (interquartile range), the mean and SD were estimated as described previously (27). In a few cases in which data were only available in figures, we extracted this information from figures using the same procedure explained in a recent systematic review (28). Previous studies suggest that data extraction from figures shows high precision and seems to be a useful option to perform a meta-analysis when randomized clinical trials publish figures as the only source of outcome data (29).

Because the correlation between pre- and post-scores is required to impute the standard deviation within groups from the standard deviation of the difference, this correlation needs to be known for calculating the confidence intervals in effect sizes (30). As these correlations were not reported in studies, we calculated different confidence intervals using different estimated correlations (r = 0, r = 0.25, r = 0.5, r = 0.75). The confidence intervals calculated with a correlation of 0.5 will be included in a table in the article and the confidence intervals calculated with different correlations (sensitivity analyses) will be included in the supplementary material (**Table S3**).

Hedges'g and Cohen's d are effect size measures that indicate the standardized difference between two means. It is assumed that 0.2 can be considered a "small" effect size, 0.5 represents a "medium" effect size and 0.8 a "large" effect size (31). In our systematic review, as effect sizes represent changes in depressive symptoms, positive effect sizes would imply mood improvement (reduction in depressive scores after hormonal treatment) whereas negative effect sizes would indicate mood worsening. Effect sizes closer to zero correspond to hormonal treatments that were relatively "neutral" in terms of changing the mood status.

RESULTS

A total of 1,281 abstracts were identified, 1,257 in PubMed and 24 through other sources (www.clinicaltrials.gov). Further details of the screening and selection process are described in **Figure 1**.

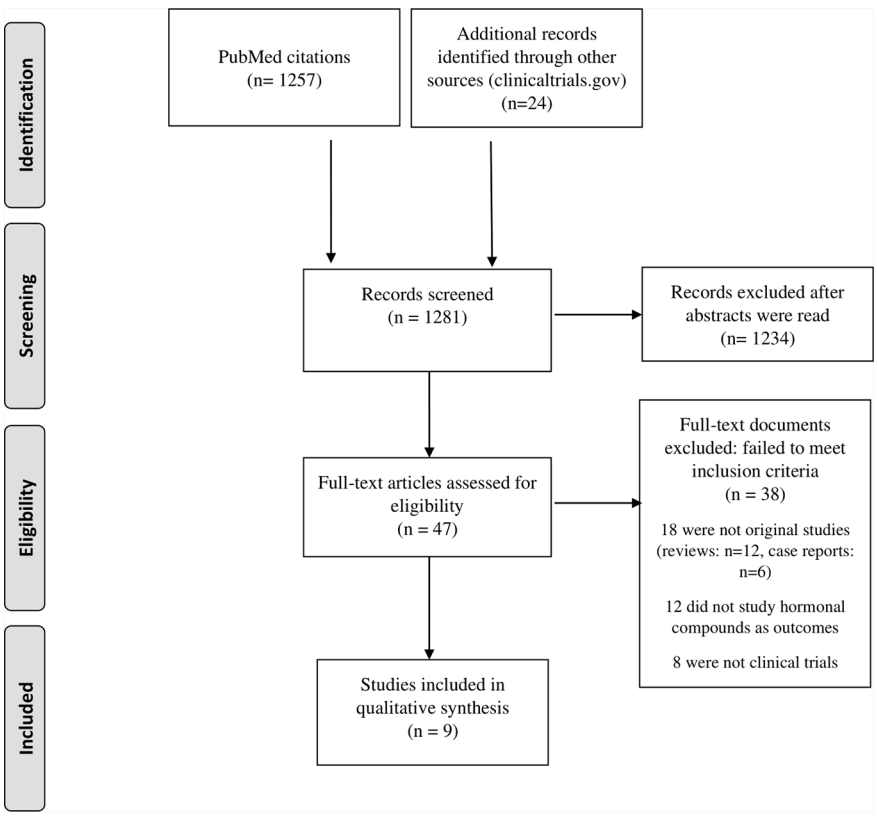

FIGURE 1 | Flowchart for studies inclusion.

Published Articles Reporting Psychopathological Effects of Hormonal Treatments Used for Infertility

Eight trials investigated the effects of hormones used in women undergoing fertility treatments (2, 3, 32–37). All of them evaluated the effect of GnRH antagonists or GnRH agonists on the occurrence of newly diagnosed depressive symptoms in women (**Tables 1**, **2** and **3**). None of them explored the effects of ART on women with a previous history of affective or psychotic disorders or the occurrence of psychotic or manic symptoms in women without a previous diagnosis of mental disorder.

Haemmerli-Keller et al. (32) carried out a non-randomized trial comparing women undergoing IVF with (cIVF) and without gonadotropin stimulation (NC-IVF) (32). In the cIVF arm (gonadotropin stimulation), human menopausal gonadotropin (HMG) was initiated between days 3 and 5 of the menstrual cycle, and GnRH antagonists were first administered subcutaneously between days 6 and 7 of the menstrual cycle and continued until ovulation induction with urinary human chorionic gonadotropin (hCG). NC-IVF patients received no stimulation at all or very low dosages of clomiphene citrate from day 6 or 7 of the menstrual cycle until the day of ovulation induction with hCG. Depressive symptoms were evaluated in all women by means of the Center for Epidemiologic Studies Depression Scale. The authors found that patients who were given gonadotropin stimulation showed higher rates of depression than women who were not given gonadotropin stimulation.

Greco et al. (33) included 236 women who underwent infertility treatment in a prospective controlled randomized trial (33). Two methods of endometrial preparation for blastocyst transfer were applied: modified natural cycle and artificial cycle. Frozen-thawed single euploid blastocyst transfer by means of a modified natural cycle was applied to 118 women. The hormonal protocol for this treatment arm did not include gonadotropin stimulation and final oocyte maturation was induced with hCG. One hundred 18 women were included in the artificial cycle arm. The hormonal protocol for this treatment arm consisted of the administration of a GnRH agonist

TABLE 1 | Main characteristics of published clinical trials reporting psychopathological complications with hormonal treatments for infertility (n=8).

Author and year of publication	Sample size (n)	Country	Mean age (SD) or Median [IQR]	Inclusion criteria	Treatment (main arm and control group)
Haemmerli Keller et al. (32)	119 women	Switzerland	NC-IVF: 34.0 (7.2) c-IVF: 34.9 (4.5)	Women between 18 and 42 years with indication of IVF c-IVF or NC-IVF depending on medical indication and women's wishes	Non-randomized trial: a) c-IVF (IVF with HMG and GnRH antagonists) (n=62) b) NC-IVF (IVF without gonadotropin stimulation) (n=57)
Greco et al. (33)	236 women	Italy	Modified-NC: 35.2 (3.6) AC: 35.5 (3.8)	Women with indication of IVF at their first IVF cycle	Randomized controlled trial, method of preparation, blastocyst transfer: a) modified natural cycle (n=118) b) artificial protocol (n=118), GnRH agonist combined with oestradiol valerate
Mamata et al. (34)	692 women	India	GnRH antagonists: 30.6 (3.83) GnRH agonists: 30.7 (4.21)	Women from 12 sites in India, aged 18- 45 years undergoing for first cycle of IVF/ICSI	Non-randomized trial: a) Group A - GnRH antagonist (n=232) b) Group B - GnRH agonist (n=460)
Stenbæk et al. (3)	83 women,	Denmark	GnRH antagonist: 31.2 [35.5-28.4] GnRH agonist: 36.4 [37.6-32.7]	Women undergoing first infertility treatment cycle of IVF or ICSI	Randomized trial: a) GnRH antagonist (n=42): daily injections with r-FSH b) GnRH agonist (n=41)
Bloch et al. (2)	108 women	Israel	Total sample: 31.8 (5.4)	Women admitted, period 2006-2007, first or second IVF cycle Age < 42 years No endometriosis No psychopharmacological treatment	Prospective randomized trial: a) Short protocol (GnRH agonist triptorelin plus daily r-FSH (n=60) b) Long protocol (GnRH agonist triptorelin for 14 days, followed by r-FSH (n=48)
de Klerk et al. (36)	391 women	The Netherlands	Mild strategy: 33.0 (3.0) Standard strategy: 32.8 (3.3)	Women who planned IVF, randomization into one protocol No previous history of unsuccessful IVF	Randomized controlled two-center trial: a) Mild ovarian stimulation (GnRH antagonist co-treatment) and single embryo transfer (n=197) b) Standard GnRH agonist long-protocol ovarian stimulation with double embryo transfer (n=194)
Heijnen et al. (35)	404 women	The Netherlands	Mild strategy: 32.9 (3.1) Standard strategy: 32.8 (3.2)	Women who planned IVF or intracytoplasmatic sperm injection with no previous IVF history	Randomized non-inferiority trial: a) GnRH antagonist combined with single embryo transfer (n=205) b) GnRH agonist long protocol and transfer of two embryos (n=199)
Tapanainen et al. (37)	100 women	Finland	Goserelin: 33.6 (4.0) Buserelin: 33.6 (3.8)	Women who planned IVF	Randomized trial: a) Long-acting s.c. goserelin (LHRH agonist) (n=49) b) Buserelin acetate i.n. (LHRH agonist) (n=51) plus 150 IU of HMG/day after 11 days of GnRH-a treatment

AC, artificial cycle; GnRH-a, gonadotrophin-releasing hormone agonist; HADS, Hospital Anxiety and Depression Scale; HMG, human menopausal gonadotrophin; i.n., intranasal; c-IVF, conventional in vitro fertilization; n-IVF, non-conventional in vitro fertilization; IQR, interquartile range; NC, natural cycle; LHRH, luteinizing hormone-releasing hormone; r-FSH, recombinant follicle-stimulating hormone; s.c., subcutaneous; SD, standard deviation.

TABLE 2 | Methods and results of published clinical trials reporting psychopathological complications with hormonal treatments for infertility (n=8).

Author and year of publication	Trial objectives	Psychopathological assessment (scales, interviews)	Outcomes	Main results
Haemmerli Keller et al. (32)	To compare the psychological burden of conventional IVF with gonadotropin stimulation and IVF without gonadotropin stimulation	CES-D Brief Symptom Inventory	Mean (pre-post) differences in CES-D between both arms†	NC-IVF patients had significantly lower level of depression than cIVF patients.
Greco et al. (33)	To evaluate clinical pregnancy rates of two methods of endometrial preparation for frozen-thawed single euploid blastocyst transfer: modified natural and artificial cycle with GnRH agonist suppression To compare psychological distress between both strategies	HADS	Mean (pre-post) differences in HADS scores between both arms†	No significant differences were found between both groups in anxiety and depression scores before starting treatment, on the days of progesterone administration, at the blastocyst transfer, and at the pregnancy test
Mamata et al. (34)	To understand physical and psychological burden in women under fertility treatment To compared GnRH antagonist protocol with GnRH agonist in terms of physical and psychological burden	HADS HSCL Somatization subscale	Mean (pre-post) differences in HADS scores between both arms†	No statistically significant differences were found in physical or psychological burden between the protocols (GnRH antagonist and GnRH agonist)
Stenbæk et al. (3)	To investigate whether women exposed to GnRH agonist protocols exhibit higher levels of mental distress compared to women under GnRH antagonist protocols	Profile of Mood States Perceived Stress Scale Major Depression Inventory	Mean (pre-post) differences in MDI scores between both arms†	Although the GnRH antagonist protocol was associated with mood fluctuations during the stimulation phase, mood disturbances were not induced by either of the protocols
Bloch et al. (2)	To determine whether affective symptoms appear with the use of GnRH agonists inducing hypogonadic states during IVF cycles To compare long- (inducing hypogonadism) and short-protocols (without hypogonadism)	Brief Symptom Inventory State-Trait Anxiety Inventory CES-D	Mean (pre-post) differences in CES-D scores between long- and short-protocols†	GnRH agonist-induced hypogonadal states were not associated with increased mood symptoms. Both protocols (short and long) were comparable in their effects on the induction of affective symptoms
de Klerk et al. (36)	To compare the impact of unsuccessful IVF on women's psychological symptoms between mild and standard protocols To compare self-reported symptoms of depression between both protocols	HADS	Mean (SD) on HADS (depression scores) after treatment % of significant depression	At the first IVF treatment cycle, mild ovarian stimulation (including GnRH antagonists) was associated with fewer short-term depressive symptoms than conventional protocols
Heijnen et al. (35)	To investigate whether mild and standard protocols differ in the proportion of term livebirths and women's well- being, and cost per couple	Assessment (baseline, 1 week after outcomes): HADS HSCL Somatization subscale Subjective Sleep Quality Scale	Differences in HADS scores after each IVF cycle (represented in Figure) between both arms	No statistically significant differences in depressive or anxious symptoms between the protocols
Tapanainen et al. (37)	To investigate successful outcomes of IVF treatment with goserelin depot versus buserelin acetate	Ad hoc questionnaire with a subjective estimation scale of different side effects (e.g., tiredness, depression and irritability) (scores from 1 = absent to 5 = severe)	Mean (SD) scores after treatment†	Buserelin group: higher incidence of tiredness, depression, headache and abdominal pain than the goserelin group. No differences between the groups in mental irritability, nausea, or swelling.

†*Effect sizes for the change (pre-post) in depressive symptoms for each treatment arm could be calculated (see* ***Table 3****).*

CES-D, Center for Epidemiologic Studies Depression Scale; HSCL, Hopkins Symptom Checklist; HADS, Hospital Anxiety and Depression Scale; HMG, human menopausal gonadotrophin (HMG); IVF, in vitro fertilization; NC-IVF, non-conventional in vitro fertilization; SD, standard deviation.

(buserelin acetate) combined with oestradiol valerate (33). The authors did not find statistically significant differences between both groups in terms of anxiety and depressive symptoms, neither before the beginning of treatment, nor on the following days after progesterone administration, blastocyst transfer or at the pregnancy test (33), suggesting that the use of GnRH agonists show similar psychological effects compared to blastocyst transfer in a modified natural cycle. However, when calculating the effect sizes for longitudinal changes in depressive symptoms (**Table 3**), both treatment arms were associated with worsening of depressive symptoms, although a greater negative effect was seen in the protocol using GnRH agonists (g = -1.22) when compared to the natural cycle protocol (g = -0.53), that did not include gonadotropin stimulation. It is important to underscore that mean (SD) HADS scores for both groups were below 7, which suggests that depressive symptoms were mild, because a cut-off score ≥ 8 is thought to be appropriate for detecting major depression in the general practice (38).

TABLE 3 | Effect sizes of changes in depressive symptoms in studies exploring the effect of hormonal treatments for infertility.

Study	Assessment scale	Arm	N	Before treatment		After treatment		Effect size measures	
				Mean	SD	Mean	SD	g	d 95% CI limits
Haemmerli Keller et al. (32)	CES-D	NC-IVF (no gonadotropin stimulation or very low doses of clomiphene)	Pre (N = 57) Post (N = 44)	12.7	7.3	13.4	10.9	-0.07	-0.38 to 0.23
	CES-D	cIVF (HMG + GnRH antagonist)	Pre (N = 62) Post (N = 45)	12.2	8.6	15.7	7.9	-0.42	-0.73 to -0.12
Greco et al. (33)	HADS-D	Modified-NC (no gonadotropin stimulation; oocyte maturation with hCG)	109	5.3	1.8	6.1	1.1	-0.53	-0.75 to -0.32
	HADS-D	Artificial cycle (GnRH agonist)	113	4.9	1.5	6.8	1.6	-1.22	-1.47 to -0.98
Mamata et al. (34)[§]	HADS-D	GnRH antagonist	232			-0.1	3.6	-0.03	NA[#]
	HADS-D	GnRH agonist	460			0.1	3.7	0.03	NA[#]
Stenbæk et al. (3)[†]	MDI	GnRH antagonist	42	8.7	9.6	8.2	7.3	0.06	-0.25 to 0.37
	MDI	GnRH agonist	41	6.3	3.8	7	5.4	-0.15	-0.47 to 0.17
Bloch et al. (2)	CES-D	Long-protocol (GnRH agonist for 14 days+ r-FSH)	48	32.5	7.6	36.2	9.4	-0.43	-0.073 to -0.13
	CES-D	Short- protocol (GnRH agonist + r-FSH from first day of the cycle)	60	30.6	8.5	34.3	9.7	-0.40	-0.67 to -0.14
Tapanainen et al. (37)[‡]	Subjective depressive symptoms	Goserelin (GnRH agonist)	49	1.11	0.50	1.27	0.55	-0.30	-0.59 to -0.02
	Subjective depressive symptoms	Buserelin (GnRH agonist)	51	1.35	0.87	1.59	1.04	-0.25	-0.53 to 0.03

g, Hedges' g; d, Cohen's d; SD, standard deviation; CI, confidence intervals; CES-D, Center for Epidemiologic Studies Depression Scale; cIVF, in vitro fertilization with gonadotropin stimulation; AC, artificial cycle; NC, natural cycle; NC-IVF, in vitro fertilization without gonadotropic stimulation; GnRH, gonadotropin- releasing hormone; HMG, human menopausal gonadotropin; r-FSH, recombinant follicle-stimulating hormone; HADS-D, Hospital Anxiety and Depression Scale—depression subscore; MDI, Major Depression Inventory; NA, not assessed.

[§]*In the Mamata et al. (34) study, mean differences in HADS-D were reported. Hedges g' was calculated taking into account this information.*

[†]*As median and interquartile range were reported in the study by Stenbæk et al. (3), mean (SD) was computed as suggested by Wan et al.*

[‡]*In the Tapanainen et al. (37) study, mean (SD) changes in subjective symptoms of depression were obtained from figures with t he procedure described by Labad et al. (28).*

[#]*d Cohen confidence interval limits were not calculated because means and standard deviations for baseline and final visits were not available.*

Stenbaek et al. (3) carried out a randomized trial in a total of 83 women undergoing treatment for infertility (3). Patients were randomized to the GnRH antagonist protocol, which included daily injections with r-FSH treatment, and GnRH agonists, which were administered intranasally. Mood symptoms and neuroticism traits were evaluated by self-reported assessment scales. The authors found that neuroticism was associated with higher scores on psychological distress, independent of the protocol received. Mental distress associated with ART treatments may not be attributed to hypogonadism or any protocol (3). In terms of depressive symptoms, although GnRH agonists seemed to show a slightly poorer profile than GnRH antagonists, effect sizes were very small (**Table 3**), without significant pre-post changes.

Similarly, Mamata et al. (34) carried out a trial across 12 IVF centres in India (34). The authors divided participants into two groups according to the type of treatment they received: a) GnRH antagonists and b) GnRH agonists. Psychopathological symptoms and somatic distress symptoms were assessed by means of the Hospital Anxiety and Depression Scale (HADS) and the Hopkins Symptom Check List (HSCL) during two treatment visits. Although no statistically significant differences in depressive or anxiety symptoms were found between both protocol groups, the percentage of individuals who reported depressive symptoms was higher in those receiving GnRH agonists compared to those under GnRH antagonists, suggesting that women undergoing IVF/ICSI may suffer from higher depressive symptoms than women not undergoing these treatments, irrespective of the protocol assigned.

With the main hypothesis that GnRH agonists may be responsible for the induction of depressive symptoms in women undergoing IVF, Bloch and co-workers (2) carried out a prospective randomized trial with two main treatment groups: a short protocol and a long protocol (2). The long protocol consisted of the subcutaneous administration of the GnRH agonist triptorelin for 14 days. 225 IU of recombinant FSH (r-FSH) were administered in a second step. The short protocol was defined by the administration of the GnRH agonist from the first day of the cycle, followed by concomitant 225 IU r-FSH. The authors hypothesized that a long protocol may induce prolonged hypogonadism compared to a short protocol, a fact that may increase levels of psychological distress and may be associated with higher depressive symptoms (2). The authors found a significant increase in depressive and anxiety symptoms during IVF-ET cycles, which were higher between the hypogonadal phase and the peak in gonadotropin stimulation, at later points in treatment. The hypogonadal state which was GnRH agonist-induced was not found to be associated with increased mood

symptoms, suggesting that neither long nor short protocols were correlated with an increase in depression. Effect sizes for the change in depressive symptoms suggest that both protocols are associated mild mood worsening (**Table 3**).

De Klerk et al. (36) investigated the psychopathological effect of IVF treatment on women undergoing mild ovarian stimulation (including GnRH antagonist and single embryo transfer) compared to an standard strategy (long-protocol with GnRH agonist and double embryo transfer) (36). After IVF treatment, women with negative outcome were more likely to present depressive symptoms 1 week after the end of treatment compared to women who received mild IVF treatment. The prevalence of possible depressive disorder (defined as a HADS score>7) was 38.8% for the women in the standard IVF group who underwent multiple IVF cycles against 19.4% of the women in the mild IVF group ($p = 0.04$). The authors associated these results with the prolonged ovarian suppression by GnRH agonists in those women who underwent the standard IVF, suggesting that these treatments may lead to more symptoms of depression.

Heijnen et al. (35) carried out a randomized, open-label, non-inferiority trial in 404 women with an indication for IVF or ICSI in the Netherlands, of whom 205 received mild ovarian stimulation (single embryo transfer with the administration of GnRH antagonists) and 199 were given standard ovarian stimulation with the transfer of two embryos and with the administration of GnRH agonists (35). The authors assessed depressive symptoms and anxiety by means of the HADS and found no statistically significant differences in psychopathological symptoms between both groups (35).

One of the most relevant studies was the randomized trial carried out by Tapanainen et al. (37). In this IVF study, patients received two types of GnRH agonists. Individuals were randomized to treatment with long-acting subcutaneous goserelin or with intranasally administered buserelin acetate. They both stimulate the production of testosterone and oestrogen in a non-pulsatile manner, which results in the downregulation of both sex hormone systems (37). After 11 days of the administration of either goserelin or buserelin, the administration of 150 IU of HMG/day was started. The authors found that patients receiving buserelin reported more depressive mood, tiredness, and headache 1 week after starting the GnRH agonist, when compared to those treated with goserelin (37). However, when considering the effect sizes for the change in depressive symptoms during the study, both protocols had similar effects (mild worsening) on mood (**Table 3**).

Non-Published Studies Designed to Assess Psychopathology in Women Receiving Hormonal Treatments for Infertility (Completed or Ongoing)

One trial was registered at clinicaltrials.gov and reported to be completed.

In 2010, Pittman et al. started a double-blind, placebo-controlled, crossover clinical trial in 20 menstruating women with regular menstrual cycles who suffered from unexplained infertility (39). The main goal of this trial was to explore psychopathological symptoms and physical concerns in women receiving clomiphene citrate for superovulation with intrauterine insemination. Women were randomized to treatment with clomiphene citrate or placebo and received this intervention on days 3–7 of their menstrual cycle. Ten patients received clomiphene citrate 50 mg daily and then placebo daily, and 10 women received placebo and then clomiphene citrate daily. Physical, behavioral, and mental symptoms were explored by using the following assessment scales: Follicular Cycle Total Physical Score for the Calendar of Premenstrual Experiences (COPE) self-assessment, the Follicular Cycle Total Behavioral Score for the Calendar of Premenstrual Experiences (COPE) self-assessment, the Luteal Cycle Total Behavioral Score for the Calendar of Premenstrual Experiences (COPE) self-assessment and the Luteal Cycle Total Physical Score for the Calendar of Premenstrual Experiences (COPE) self-assessment. Although not statistically relevant, patients receiving placebo showed higher scores than those receiving clomiphene citrate on the Follicular Cycle Behavioral and Physical Scores (COPE). No other differences in median scores were found in behavioral, mental, and physical symptoms as measured by the luteal scales.

Quality Assessment and Risk of Bias

The assessment of the risk of bias has been presented in detail in **Table S2**. Of all nine clinical trials included in our systematic review, four were considered to be good (2, 3, 36, 39), four fair (33–35, 37), and only one (32) had poor quality.

We did not identify any trial publication or non-publication of results regarding the occurrence of psychotic symptoms in women undergoing fertility treatments.

DISCUSSION

In this paper, we carried out a systematic review on available work on the psychopathological effects of hormonal treatments for infertility on women and the effects of these therapies on the occurrence of newly diagnosed mood and psychotic disorders. Finally, nine trials were included, all of them in non-clinical populations. Four trials compared GnRH agonists and GnRH antagonists (3, 34–36), showing a better mood profile (less depressive symptoms) for those hormonal protocols including antagonists in one trial. Two trials compared protocols using either GnRH agonists or GnRH antagonists versus natural cycle protocols that did not use gonadotropin stimulation, reporting a better mood profile in those protocols without gonadotropin stimulation (32, 33). Other two studies comparing long and short protocols of GnRH agonists (2) and two GnRH agonists (buserelin and goserelin) (37) did not find significant differences in mood changes between treatment arms. An unpublished study compared two patterns of clomiphene vs placebo administration with no differences between groups (39).

Most previous published trials evaluated the effect of either GnRH antagonists or GnRH agonists on the occurrence of newly diagnosed depressive disorder or depressive symptoms for the first time in women. None of them investigated the risk or prevalence of relapses in women with a previous diagnosis of depressive disorders or psychotic disorders. When focusing on hormonal compounds other than GnRH agonists or antagonists, one recent trial has been developed to study the effect of clomiphene citrate on women under the IVF protocol (39). Moreover, in the study by Haemmerli Keller et al. (32), patients under the protocol without gonadotropin stimulation could receive low doses of clomiphene (32). This latter study suggests that the lack of gonadotropin stimulation is associated with fewer changes in mood, as the effect size was very small (g = -0.07).

Celano et al. (40) and Wilkins and collaborators (41) pointed out that GnRH agonists (e.g., leuprolide and goserelin) induce hypogonadism (40, 41), which could be related to different psychiatric side effects. In the information about these hormonal drugs (42, 43), depressive symptoms also seem to be reported. These findings have been replicated in subsequent case series (44, 45) and retrospective studies, including the report of a patient treated with leuprolide (46). Furthermore, prophylactic treatment with SSRIs has been reported to prevent the development of depressive symptoms in these patients (45).

In our systematic review, we found that patients receiving ovarian stimulation with GnRH antagonists had increased depressive symptoms compared to patients who did not receive ovarian stimulation (32). One study suggested that treatment with one GnRH agonist, buserelin, had a poorer mood profile than other GnRH agonist (goserelin) 1 week later but showed similar mood changes at the end of the trial (37). However, GnRH agonists did not seem to differ from GnRH antagonists in their associated occurrence of depressive symptoms, suggesting that neither protocol has a psychopathological effect on women undergoing fertility treatments (3, 34–36).

In contrast, Ben Dor et al. (47) studied the effect of GnRH agonists on a sample of 72 healthy women (medication-free; not pregnant; no significant past or current medical illness; reported regular menstrual cycles; and normal physical, gynaecological examinations, and laboratory results) (47). The absence of current or past Axis I psychiatric illness was confirmed by Structured Clinical Interview for DSM-IV. The objective of the study was to better understand whether the acute induction of hypogonadism may determine significant depressive symptoms in healthy premenopausal women, which individual symptoms may be associated with hypogonadism and increase susceptibility to depression and whether changes in plasma levels of ovarian hormones correlate with changes in mood symptoms (47). After a 2-month screening phase, every woman received the first dose of depot leuprolide acetate. Relative to baseline, induced hypogonadism with GnRH agonist was associated with significantly decreased sexual interest, disturbed sleep, and hot flashes but no significant change in any mood-related symptom scores (47). The authors proposed that depressive symptoms associated with GnRH agonist ovarian suppression in menopause could reflect mainly the effects of ovarian suppression on women who are more vulnerable to the development of depression or who are currently depressed (47). However, once again, no clinical trials have explored the effect of leuprolide on women undergoing fertility treatments.

Bloch and collaborators (2) investigated the relationship between affective symptoms and gonadal steroids during *in vitro* fertilization (2). They found that the drop from high oestradiol levels at the oestradiol phase to lower levels at the progesterone phase was correlated with increasing depressive symptoms, suggesting that the abrupt decline in oestrogen levels can precipitate negative mood states. This fact may be responsible for the variability of the presence of depressive symptoms in women undergoing IVF (2). This may be partially in line with some observational studies exploring the prevalence of depressive symptoms in women undergoing fertility treatments. Particularly, Volgsten and co-workers (22) carried out an observational study including couples undergoing IVF or ICSI and applied the Primary Care Evaluation of Mental Disorders (PRIME-MD) system, which is a tool to evaluate the prevalence of psychiatric disorders according to 2% in men, being major depression the most commonly found, irrespective of the hormonal compound they received. More recently, other studies have reported that pregnant women after fertility treatment did not differ in terms of depressive symptoms compared to those naturally pregnant, suggesting that the treatment did not have a negative impact on mood (23). However, the hormonal compounds were not included in the discussion. Evans-Hoeker et al. (48) carried out a cohort study including participants in two previous randomized trials, namely PPCOS II and AMIGOS (48). The first trial compared patients treated with clomiphene citrate versus letrozole, and the second compared gonadotropins, clomiphene citrate and letrozole. Both studies assessed whether maternal depression would have an influence on pregnancy outcomes after non-IVF fertility therapies. They did not find any negative effect of current active depression on non-IVF outcomes. The effect of hormonal compounds used in fertility treatment on the recurrence of major depression have been poorly investigated. Sejbaek et al. (49) carried out a register-based national cohort study including women undergoing IVF, ICSI, and other embryo transfer treatments (49). Women with a previous diagnosis of depression had lower rates of live births. The authors did not find statistically significant differences in rates of depression, but they recommended that women with previous depression may require specific psychiatric attention before starting a new treatment. In the same line, other authors highlight that a history of major depression would be a significant predictor for major depression during fertility treatment (50). Psychosocial support and interventions addressed to women with a previous history of depression have been recommended when initiating infertility treatment.

We did not find any clinical trial specifically investigating the effects of hormonal treatments on depressive symptoms in

unipolar or bipolar disorder patients. In a recent prospective observational study that included women receiving infertility treatments with a history of major depressive disorder or bipolar disorder (24), a high risk of depressive relapse was found, even in those women maintaining psychotropic medication. These results suggest that maintenance of medication is not sufficient to avoid affective relapses. In our systematic review, we did not find clinical trials assessing recurrences in patients with affective disorders.

Women with polycystic ovary syndrome (PCOS) show an increased prevalence of higher depression and anxiety scores and increased odds of moderate and severe depressive and anxiety symptoms compared with controls (51). On the other hand, a few studies that have evaluated the impact of PCOS-related treatments (lifestyle interventions and pharmacotherapy) on mood have reported no detrimental effect or even some improvement in depressive and anxiety symptoms and quality of life (51). In addition, clomiphene citrate, a selective oestrogen receptor modulator used to induce ovulation, even in patients with PCOS, has been associated with mood lability and depressed mood in two cross-sectional studies (52, 53). In the cross-sectional, self-report survey of Choi et al. (53), 41% of clomiphene-treated women experienced depressed mood, and 75 of 162 (45%) experienced mood swings during treatment (53). Mood changes (54), suicidal behavior (55), and visual hallucinations (56) have also been associated with clomiphene treatment in case reports.

None of the selected published clinical trials in our systematic review specifically investigated the psychopathological effects of the use of clomiphene citrate in the treatment of infertility on women. In an unpublished randomized double-blind crossover trial in 20 women (53), clomiphene treatment was not associated with mood or behavioral changes, a result that contrasts those of other studies suggesting that clomiphene induces psychological side effects quite frequently (53).

In relation to patients with psychotic disorders, to the best of our knowledge, no clinical trials have evaluated the psychopathological effects of hormonal treatments for infertility on women diagnosed with bipolar disorder, schizophrenia, or other psychotic disorders. A case report linked the use of leuprolide acetate for *in vitro* fertilization (IVF) treatment to a psychotic exacerbation in a 37-year-old woman who suffered from a previous schizoaffective psychosis (57). In this line, a case series of Purvin (58) showed different visual disturbances (some of them persistent) secondary to clomiphene citrate treatment in three women treated for infertility with clomiphene for 4 to 15 months (58). In a review by Seeman (59), there were five case reports of a self-limited psychotic disorder induced by clomiphene (59). All cases were reported to start psychotic symptoms during treatment, to have a paranoid component and to stop when the drug was withdrawn. We did not find any trial reporting the potential effects of hormones used in fertility treatments on women with psychosis or the occurrence of psychotic symptoms in non-diagnosed women. However, some case reports have been reported on the use of clomiphene. A recent national register study compared success rates of fertility treatments in women with and without psychotic disorders prior to the treatment (25), as a part of a cohort of 42,915 Danish women undergoing fertility treatments. Women with previous diagnosis of psychotic disorder had lower rates of success compared to non-psychotic women. The authors did not evaluate depressive symptoms as the main outcomes.

Several limitations in this systematic review should be considered. The most important limitation of this systematic review is the absence of a sufficient number of published articles on the psychopathological effects of hormone compounds used in infertility treatments in women. Furthermore, most clinical trials included in our systematic review excluded patients with a psychiatric history or psychopathological treatment. Therefore, these exclusion criteria might bias the evidence because most studies excluded women with mental illnesses. Although the quality of selected clinical trials was fair to good in most cases (8/9 = 89%), as none of them included patients with serious mental illnesses, the research field needs to conduct more inclusive clinical trials to overcome this limitation.

The prediction of acute exacerbations or the occurrence of newly diagnosed mental disorders is limited by the scarce literature in the field. Furthermore, most studies were focused on depressive or anxiety symptoms, and they did not assess, in general, psychopathological symptoms according to the most commonly used assessment scales for depression. Several authors have reported that psychotic symptoms may occur during fertility treatments, during or after the use of hormone compounds; however, to date, few studies have focused on patients suffering from schizophrenia and other psychotic disorders. Most of them are case reports or case series, and trials are still lacking on this topic. To the best of our knowledge, this is the first systematic review on the psychopathological effects of hormonal treatments for infertility. Although the number of selected clinical trials was low, our systematic review opens new avenues on the investigation of hormone effects in women undergoing infertility treatments. Future studies need to include patients with major mood and psychotic disorders.

AUTHOR CONTRIBUTIONS

AG-R and JC conducted the screening and selection processes. AG-R, JC, VS, CG-R, MB, JM and JL contributed to the conceptualization and writing of this manuscript. All authors have approved the final version of the manuscript.

FUNDING

This study was supported in part by a grant from the Catalan Agency for the Management of University and Research Grants (AGAUR 2017 SGR 632). The funder had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.

ACKNOWLEDGMENTS

JL received an Intensification of the Research Activity Grant (SLT006/17/00012) from the Health Department of the Generalitat de Catalunya.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.00479/full#supplementary-material

REFERENCES

1. Rooney KL, Domar AD. The relationship between stress and infertility. *Dialogues Clin Neurosci* (2018) 20:41–7.
2. Bloch M, Azem F, Aharonov I, Ben Avi I, Yagil Y, Schreiber S, et al. GnRH-agonist induced depressive and anxiety symptoms during in vitro fertilization-embryo transfer cycles. *Fertil Steril* (2011) 95:307–9. doi: 10.1016/j.fertnstert.2010.07.1073
3. Stenbæk DS, Toftager M, Hjordt LV, Jensen PS, Holst KK, Bryndorf T, et al. Mental distress and personality in women undergoing GnRH agonist versus GnRH antagonist protocols for assisted reproductive technology. *Hum Reprod* (2015) 30:103–10. doi: 10.1093/humrep/deu294
4. Stanhiser J, Steiner AZ. Psychosocial Aspects of Fertility and Assisted Reproductive Technology. *Obstet Gynecol Clin North Am* (2018) 45:563–74. doi: 10.1016/j.ogc.2018.04.006
5. Hanson B, Johnstone E, Dorais J, Silver B, Peterson CM, Hotaling J. Female infertility, infertility-associated diagnoses, and comorbidities: a review. *J Assist Reprod Genet* (2017) 34:167–77. doi: 10.1007/s10815-016-0836-8
6. Santos C, Sobral MP, Martins MV. Effects of life events on infertility diagnosis: comparison with presumably fertile men and women. *J Reprod Infant Psychol* (2017) 35:1–13. doi: 10.1080/02646838.2016.1249834
7. Briceag I, Costache A, Purcarea VL, Cergan R, Dumitru M, Briceag I, et al. Fallopian tubes–literature review of anatomy and etiology in female infertility. *J Med Life* (2015) 8:129–31.
8. Chambers GM, Harrison C, Raymer J, Petersen Raymer AK, Britt H, Chapman M, et al. Ledger W5, Norman RJ6. Infertility management in women and men attending primary care-patient characteristics, management actions and referrals. *Hum Reprod* (2019) 34:2173–83. doi: 10.1093/humrep/dez172
9. Lunenfeld B, Bilger W, Longobardi S, Alam V, D'Hooghe T, Sunkara SK. The Development of Gonadotropins for Clinical Use in the Treatment of Infertility. *Front Endocrinol (Lausanne)* (2019) 10:429. doi: 10.3389/fendo.2019.00429
10. Niederberger C, Pellicer A. Introduction: IVF's 40th world birthday. *Fertil Steril* (2018) 110:4. doi: 10.1016/j.fertnstert.2018.05.017
11. Bai F, Wang DY, Fan YJ, Qiu J, Wang L, Dai Y, et al. Assisted reproductive technology service availability, efficacy and safety in mainland China: 2016. *Hum Reprod* (2020) 35:446–52 doi: 10.1093/humrep/dez245
12. Ray A, Shah A, Gudi A, Homburg R. Unexplained infertility: an update and review of practice. *Reprod Biomed Online* (2012) 24:591–602. doi: 10.1016/j.rbmo.2012.02.021
13. Lai Q, Zhang H, Zhu G, Li Y, Jin L, He L, et al. Comparison of the GnRH agonist and antagonist protocol on the same patients in assisted reproduction during controlled ovarian stimulation cycles. *Int J Clin Exp Pathol* (2013) 6:1903–10.
14. Toftager M, Bogstad J, Bryndorf T, Løssl K, Roskær J, Holland T, et al. Risk of severe ovarian hyperstimulation syndrome in GnRH antagonist versus GnRH agonist protocol: RCT including 1050 first IVF/ICSI cycles. *Hum Reprod* (2016) 31:1253–64. doi: 10.1093/humrep/dew051
15. Soria V, González-Rodríguez A, Huerta-Ramos E, Usall J, Cobo J, Bioque M, et al. Targeting hypothalamic-pituitary-adrenal axis hormones and sex steroids for improving cognition in major mood disorders and schizophrenia: a systematic review and narrative synthesis. *Psychoneuroendocrinology* (2018) 93:8–19. doi: 10.1016/j.psyneuen.2018.04.012
16. Rubinow DR, Schmidt PJ. Sex differences and the neurobiology of affective disorders. *Neuropsychopharmacology* (2019) 44:111–28. doi: 10.1038/s41386-018-0148-z
17. Goldstein JM, Lancaster K, Longenecker JM, Abbs B, Holsen LM, Cherkerzian S, et al. Sex differences, hormones, and fMRI stress response circuitry deficits in psychoses. *Psychiatry Res* (2015) 232:226–36. doi: 10.1016/j.pscychresns.2015.03.006
18. Holka-Pokorska J, Jarema M, Wichniak A. Clinical determinants of mental disorders occurring during the infertility treatment. *Psychiatr Pol* (2015) 49:965–82. doi: 10.12740/PP/35958
19. Soares CN, Zitek B. Reproductive hormone sensitivity and risk for depression across the female life cycle: a continuum of vulnerability? *J Psychiatry Neurosci* (2008) 33:331–43.
20. González-Rodríguez A, Catalán R, Penadés R, Bernardo M. The oestrogen dysfunction hypothesis in schizophrenia: The need for an integrative approach to treat postmenopausal women. *Aust N Z J Psychiatry* (2016) 50:1207–8. doi: 10.1177/0004867416649033
21. Riecher-Rössler A. Oestrogens, prolactin, hypothalamic-pituitary-gonadal axis, and schizophrenic psychoses. *Lancet Psychiatry* (2017) 4:63–72. doi: 10.1016/S2215-0366(16)30379-0
22. Volgsten H, Skoog Svanberg A, Ekselius L, Lundkvist O, Sundström Poromaa I. Prevalence of psychiatric disorders in infertile women and men undergoing in vitro fertilization treatment. *Hum Reprod* (2008) 23:2056–63. doi: 10.1093/humrep/den154
23. Salih Joelsson L, Tydén T, Wanggren K, Georgakis MK, Stern J, Berglund A, et al. Anxiety and depression symptoms among sub-fertile women, women pregnant after infertility treatment, and naturally pregnant women. *Eur Psychiatry* (2017) 45:212–9. doi: 10.1016/j.eurpsy.2017.07.004
24. Freeman MP, Lee H, Savella GM, Sosinsky AZ, Marfurt SP, Murphy SK, et al. Predictors of Depressive Relapse in Women Undergoing Infertility Treatment. *J Womens Health (Larchmt)* (2018) 27:1408–14. doi: 10.1089/jwh.2017.6878
25. Ebdrup NH, Assens M, Hougaard CO, Pinborg A, Hageman I, Schmidt L. Assisted reproductive technology (ART) treatment in women with schizophrenia or related psychotic disorder: a national cohort study. *Eur J Obstet Gynecol Reprod Biol* (2014) 177:115–20. doi: 10.1016/j.ejogrb.2014.03.013
26. Moher D, Shamseer L, Clarke M, Ghersi D, Liberati A, Petticrew M, et al. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015 statement. *Syst Rev* (2015) 4:1. doi: 10.1186/2046-4053-4-1
27. Wan X, Wang W, Liu J, Tong T. Estimating the sample mean and standard deviation from the sample size, median, range and/or interquartile range. *BMC Med Res Methodol* (2014) 14:135. doi: 10.1186/1471-2288-14-135
28. Labad J, Montalvo I, González-Rodríguez A, García-Rizo C, Crespo-Facorro B, Monreal JA, et al. Pharmacological treatment strategies for lowering prolactin in people with a psychotic disorder and hyperprolactinaemia: a systematic review and meta-analysis. *Schiz Res* (2020). In press.
29. Silva V, Carvalho A, Grande A, Martimbianco A, Riera R, Atallah A. *Can data extraction from figures perform a meta-analysis? Abstract retrieved from Abstracts of the 20th Cochrane Colloquium*. Auckland, New Zealand: Cochrane (2012).
30. Borenstein M, Hedges LV, Higgins J, Rothstein HR. *Introduction to Meta-Analysis*. John Wiley and Sons (2009). pp. 1–421. doi: 10.1002/9780470743386
31. Cohen J. *Statistical Power Analysis for the Behavioral Sciences*. New York, NY: Routledge Academic (1988).
32. Haemmerli Keller K, Alder G, Loewer L, Faeh M, Rohner S, von Wolff M. Treatment-related psychological stress in different in vitro fertilization therapies with and without gonadotropin stimulation. *Acta Obstet Gynecol Scand* (2018) 97:269–76. doi: 10.1111/aogs.13281
33. Greco E, Litwicka K, Arrivi C, Varricchio MT, Caragia A, Greco A, et al. The endometrial preparation for frozen-thawed euploid blastocyst transfer: a prospective randomized trial comparing clinical results from natural modified cycle and exogenous hormone stimulation with GnRH agonist. *J Assist Reprod Genet* (2016) 33:873–84. doi: 10.1007/s10815-016-0736-y
34. Mamata D, Ray SK, Pratap K, Firuza P, Birla AR, Manish B. Impact of different controlled ovarian stimulation protocols on the physical and psychological burdens in women undergoing in vitro fertilization/intra

cytoplasmic sperm injection. *J Hum Reprod Sci* (2015) 8:86–92. doi: 10.4103/0974-1208.158615
35. Heijnen EM, Eijkemans MJ, De Klerk C, Polinder S, Beckers NG, Klinkert ER, et al. A mild treatment strategy for in-vitro fertilisation: a randomised non-inferiority trial. *Lancet* (2007) 369:743–9. doi: 10.1016/S0140-6736(07)60360-2
36. de Klerk C, Macklon NS, Heijnen EM, Eijkemans MJ, Fauser BC, Passchier J, et al. The psychological impact of IVF failure after two or more cycles of IVF with a mild versus standard treatment strategy. *Hum Reprod* (2007) 22:2554–8. doi: 10.1093/humrep/dem171
37. Tapanainen J, Hovatta O, Juntunen K, Martikainen H, Ratsula K, Tulppala M, et al. Subcutaneous goserelin versus intranasal buserelin for pituitary down-regulation in patients undergoing IVF: a randomized comparative study. *Hum Reprod* (1993) 8:2052–5. doi: 10.1093/oxfordjournals.humrep.a137980
38. Olssøn I, Mykletun A, Dahl AA. The Hospital Anxiety and Depression Rating Scale: a cross-sectional study of psychometrics and case finding abilities in general practice. *BMC Psychiatry* (2005) 5:46. doi: 10.1186/1471-244X-5-46
39. https://clinicaltrials.gov/ct2/show/NCT01291056.
40. Celano CM, Freudenreich O, Fernandez-Robles C, Stern TA, Caro MA, Huffman JC. Depressogenic effects of medications: a review. *Dialogues Clin Neurosci* (2011) 13:109–25.
41. Wilkins KM, Warnock JK, Serrano E. Depressive symptoms related to infertility and infertility treatments. *Psychiatr Clin N Am* (2010) 33:309–21. doi: 10.1016/j.psc.2010.01.009
42. Goserelin depot. *Product Monograph Zoladex® LA Goserelin Depot (10.8 mg Goserelin/depot as goserelin acetate), Luteinizing Hormone - Releasing Hormone Analog (LHRH Analog)*. (2019). Retrieved from: https://documents.tersera.com/zoladex-ca/10.8mg_ProductMonograph.pdf.
43. Lupron Depot. *Center for Drug Evaluation and Research Approval Package for Lupron Depot*. (2011). Retrieved from: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/020517orig1s025s030s032Review.pdf.
44. Warnock JK, Bundren JC. Anxiety and mood disorders associated with gonadotropin-releasing hormone agonist therapy. *Psychopharmacol Bull* (1997) 33:311–6.
45. Warnock JK, Bundren JC, Morris DW. Sertraline in the treatment of depression associated with gonadotropin-releasing hormone agonist therapy. *Biol Psychiatry* (1998) 43:464–5. doi: 10.1016/S0006-3223(97)00396-X
46. Warnock JK, Bundren JC, Morris DW. Depressive symptoms associated with gonadotropin-releasing hormone agonists. *Depress Anxiety* (1998) 7:171–7. doi: 10.1002/(SICI)1520-6394(1998)7:4<171::AID-DA5>3.0.CO;2-D
47. Ben Dor R, Harsh VL, Fortinsky P, Koziol DE, Rubinow DR, Schmidt PJ. Effects of pharmacologically induced hypogonadism on mood and behavior in healthy young women. *Am J Psychiatry* (2013) 170:426–33. doi: 10.1176/appi.ajp.2012.12010117
48. Evans-Hoeker EA, Eisenberg E, Diamond MP, Legro RS, Alvero R, Coutifaris C, et al. Major depression, antidepressant use, and male and female fertility. *Fertil Steril* (2018) 109:879–87. doi: 10.1016/j.fertnstert.2018.01.029
49. Sejbaek CS, Hageman I, Pinborg A, Hougaard CO, Schmidt L. Incidence of depression and influence of depression on the number of treatment cycles and births in a national cohort of 42,880 women treated with ART. *Hum Reprod* (2013) 28:1100–9. doi: 10.1093/humrep/des442
50. Holley SR, Pasch LA, Bleil ME, Gregorich S, Katz PK, Adler NE. Prevalence and predictors of major depressive disorder for fertility treatment patients and their partners. *Fertil Steril* (2015) 103:1332–9. doi: 10.1016/j.fertnstert.2015.02.018
51. Dokras A, Stener-Victorin E, Yildiz BO, Li R, Ottey S, Shah D, et al. Androgen Excess- Polycystic Ovary Syndrome Society: position statement on depression, anxiety, quality of life, and eating disorders in polycystic ovary syndrome. *Fertil Steril* (2018) 109:888–99. doi: 10.1016/j.fertnstert.2018.01.038
52. Blenner JL. Clomiphene-induced mood swings. *J Obstet Gynecol Neonatal Nurs* (1991) 20:321–7. doi: 10.1111/j.1552-6909.1991.tb01695.x
53. Choi SH, Shapiro H, Robinson GE, Irvine J, Neuman J, Rosen B, et al. Psychological side-effects of clomiphene citrate and human menopausal gonadotrophin. *J Psychosom Obstet Gynaecol* (2005) 26:93–100. doi: 10.1080/01443610400022983
54. Aussedat M, Jean-Louis J, Djahangirian O, Brochet MS. Clomiphene for the Treatment of Male Infertility: A Case Report of Mood Change and a Literature Overview. *Curr Drug Saf* (2017) 12:208–15. doi: 10.2174/1574886312666170616092036
55. Knight JC, Pandit AS, Rich AM, Trevisani GT, Rabinowitz T. Clomiphene-Associated Suicide Behavior in a Man Treated for Hypogonadism: Case Report and Review of The Literature. *Psychosomatics* (2015) 56:598–602. doi: 10.1016/j.psym.2015.06.003
56. Venkatesh R, Gujral GS, Gurav P, Tibrewal S, Mathur U. Clomiphene citrate-induced visual hallucinations: a case report. *J Med Case Rep* (2017) 11:60. doi: 10.1186/s13256-017-1228-0
57. Abu-Tair F, Strowitzki T, Bergemann N. Exacerbation of a schizoaffective psychosis after in vitro fertilization with leuproreline acetate. *Nervenarzt* (2007) 78:691–2. doi: 10.1007/s00115-007-2276-2
58. Purvin VA. Visual disturbance secondary to clomiphene citrate. *Arch Ophthalmol* (1995) 113:482–4. doi: 10.1001/archopht.1995.01100040102034
59. Seeman M. Transient psychosis in women on clomiphene, bromocriptine, domperidone and related endocrine drugs. *Gynecol Endocrinol* (2015) 31:751–4. doi: 10.3109/09513590.2015.1060957

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 29 May 2020
doi: 10.3389/fpsyt.2020.00480

Assessment of Appetite-Regulating Hormones Provides Further Evidence of Altered Adipoinsular Axis in Early Psychosis

Michał Lis[1], *Bartłomiej Stańczykiewicz*[2], *Lilla Pawlik-Sobecka*[2], *Agnieszka Samochowiec*[3], *Artur Reginia*[4] *and Błażej Misiak*[5]*

[1] Clinical Department of Internal Diseases, Endocrinology and Diabetology, The Central Clinical Hospital of the Ministry of the Interior in Warsaw, Warsaw, Poland, [2] Department of Nervous System Diseases, Wroclaw Medical University, Wrocław, Poland, [3] Department of Clinical Psychology, Institute of Psychology, University of Szczecin, Szczecin, Poland, [4] Department of Psychiatry, Pomeranian Medical University, Szczecin, Poland, [5] Department of Genetics, Wroclaw Medical University, Wrocław, Poland

OPEN ACCESS

Edited by:
Grazia Rutigliano, University of Pisa, Italy

Reviewed by:
Milica Milovan Borovcanin, University of Kragujevac, Serbia
Maria Giuseppina Petruzzelli, University of Bari Aldo Moro, Italy

***Correspondence:**
Błażej Misiak
mblazej@interia.eu

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *26 February 2020*
Accepted: *11 May 2020*
Published: *29 May 2020*

Citation:
Lis M, Stańczykiewicz B, Pawlik-Sobecka L, Samochowiec A, Reginia A and Misiak B (2020) Assessment of Appetite-Regulating Hormones Provides Further Evidence of Altered Adipoinsular Axis in Early Psychosis. Front. Psychiatry 11:480. doi: 10.3389/fpsyt.2020.00480

It has been found that antipsychotic-naïve patients with first-episode psychosis (FEP) present with impaired hormonal regulation of appetite in terms of low leptin and high insulin levels (the adipoinsular axis). These findings imply that certain intrinsic mechanisms might play a role in the development of metabolic dysregulation in early psychosis. However, clinical correlates of this phenomenon remain unknown. Moreover, these alterations have not been tested in individuals at familial high risk of psychosis (FHR-P). In this study we aimed to assess the levels of adiponectin, insulin, leptin, glucose, total cholesterol, lipoproteins and triglycerides in FEP patients, unaffected offspring of schizophrenia patients (FHR-P individuals) and healthy controls (HCs) with respect to cognitive performance and psychopathological manifestation. Participants were 35 FEP patients, 33 FHR-P individuals, and 32 HCs. Cognitive performance was assessed using the Repeatable Battery for Assessment of Neuropsychological Status (RBANS). The levels of leptin and high-density lipoproteins (HDL) were significantly lower (leptin: 10.7 ± 15.7 vs. 12.6 ± 10.1, $p = 0.046$, and HDL: 48.0 ± 16.9 vs. 59.8 ± 17.5 mg/dl, $p = 0.007$), while the levels of triglycerides and insulin were significantly higher (triglycerides: 137.4 ± 58.8 vs. 77.5 ± 33.2 mg/dl, $p < 0.001$, and insulin: 15.2 ± 13.1 vs. 9.6 ± 5.0 μIU/ml, $p = 0.023$) in FEP patients compared to HCs. These differences were significant after controlling for the effects of potential confounding factors. No significant differences in the levels of serum markers between FHR-P individuals and HCs were found. There was a significant negative correlation between the level of leptin and the RBANS language score after covarying for potential confounding factors in FEP patients ($B = –0.226$, $p = 0.006$) but not in other subgroups of participants. Our findings confirm impairment of adipoinsular axis in early psychosis. However, results of our study do not support the hypothesis that familial liability to psychosis might be associated with metabolic dysregulation. Leptin levels might be associated with cognitive deficits in FEP patients. Longitudinal studies of individuals at

risk of psychosis are needed to provide insights into causal mechanisms underlying our results.

Keywords: adipose tissue, lipid, hormone, schizophrenia, obesity

INTRODUCTION

Cardiovascular comorbidities largely contribute to reduced life expectancy in patients with schizophrenia-spectrum disorders (1). Although environmental factors that underlie this phenomenon have been widely recognized, accumulating evidence indicates that certain intrinsic mechanisms might also be relevant. Indeed, antipsychotic-naïve or minimally-medicated patients with first-episode psychosis (FEP) present with a number of cardio-metabolic, immune and the hypothalamic-pituitary-adrenal (HPA) axis dysregulations (2). One of hypotheses beyond these observations states that psychotic disorders and cardiovascular risk factors share overlapping genetic backgrounds (3). However, the role of environmental factors (e.g., unhealthy life style or early-life stress) that act in the premorbid phase of illness cannot be excluded.

Our group performed a systematic review and meta-analysis of studies investigating the levels of appetite-regulating hormones in (FEP) patients (4). We found that patients with FEP present with increased levels of insulin and reduced levels of leptin. Subgroup analysis of antipsychotic-naïve patients confirmed these findings. No significant differences in the levels of other hormones (adiponectin, ghrelin, orexin, resistin, and visfatin) between FEP and healthy controls (HCs) were observed in this meta-analysis. Notably, studies of multiple-episode schizophrenia patients revealed increased levels of leptin and insulin (5). Although our meta-analysis did not demonstrate altered levels of adiponectin in FEP, low adiponectin levels were reported in multiple-episode schizophrenia patients (6). The authors of a recent meta-analysis of studies in this field found that low adiponectin levels in this group of patients might occur as a consequence of antipsychotic treatment (6). Notably, adiponectin is another hormone that regulates secretion of insulin. It has been found that adiponectin not only increases insulin sensitivity but it can also exert antiangiogenic, antiatherogenic and neuroprotective effects (7). Although it has been observed that adiponectin levels might be associated with cognitive performance in the general population, these observations have not been confirmed in patients with schizophrenia (8).

Leptin is a hormone released by white adipose tissue and is able to pass through the blood-brain barrier. It reduces appetite *via* interactions with receptors located in the arcuate nucleus of the hypothalamus. Moreover, leptin plays an important role in the brain development and may be responsible for learning and memory processes (9). Apart from the hypothalamus, leptin receptors are expressed by neurons of the cerebral cortex, hippocampus, basal ganglia and cerebellum (10, 11). Leptin-deficient mice not only develop extreme obesity and other components of the metabolic syndrome but also show decreased brain weight and cortical volumes (9, 12, 13). There is evidence that leptin suppresses secretion of insulin by central actions and direct effects on pancreatic cells. This peripheral regulation of insulin secretion has been named as the adipoinsular axis (14).

Insulin receptors are expressed by various cells of the central nervous system. The highest concentration of insulin receptors has been found in the olfactory bulb, cerebral cortex, hippocampus, cerebellum, and hypothalamus (15). Notably, insulin resistance has been associated with cognitive impairment in FEP patients (16). There are various mechanisms underlying the effects of central insulin signaling on cognitive performance. Indeed, central insulin plays an important role in maintaining neuronal plasticity (17). More specifically, it has been demonstrated that central insulin is involved in spatial learning and memory processes through its interactions with receptors located in the hippocampus (18). In addition, patients with type 2 diabetes are at risk of cognitive impairment across various domains, including, i.e., attention, learning, memory and executive function (19).

It should be noted that insulin resistance is not the only cardiovascular risk factor associated with cognitive impairment in schizophrenia. A recent meta-analysis revealed that a diagnosis of metabolic syndrome and its single components (hypertension, abdominal obesity, insulin resistance and dyslipidemia) are also related to cognitive deficits (20). However, this meta-analysis included only two studies that investigated the effects of dyslipidemia on cognition in schizophrenia (21, 22). These studies revealed that dyslipidemia is related to impairments of executive function, verbal memory and attention. Dyslipidemia may impact cognitive performance through various mechanisms associated with the injury of the blood-brain barrier and blood vessels as well as increased amyloid deposition (23).

Although there is evidence that subclinical indices of metabolic dysregulation, such as lipid profile disturbances, decreased leptin levels and decreased insulin sensitivity, occur in patients with FEP, it remains unknown whether these alterations are present in unaffected individuals at familial high risk of psychosis (FHR-P). Moreover, clinical correlates of metabolic dysregulation in early psychosis are yet to be established. Therefore, we aimed to compare the levels of glucose, insulin, adiponectin and leptin as well as lipid profile in FEP patients, FHR-P individuals and HCs. In addition, we investigated whether these metabolic parameters are related to psychopathological manifestation and cognitive performance.

MATERIAL AND METHODS

Participants

We enrolled 35 FEP inpatients, 33 FHR-P individuals, and 32 HCs. The group of FHR-P individuals represented unaffected offspring of patients with schizophrenia, who were diagnosed according to the ICD-10 criteria. A diagnosis of FEP was established based on the DSM-IV criteria using the Operational Criteria for Psychotic Illness (OPCRIT) checklist (24). All patients were recruited during their first inpatient treatment and they had a negative history of antipsychotic treatment before admission to the inpatient unit. The following diagnoses were established in FEP patients: schizophrenia, schizoaffective disorder, schizophreniform disorder, brief psychotic disorder, and delusional disorder. In turn, HCs were recruited through advertisements and had a negative family history of psychotic and mood disorders in first- and second-degree relatives. Participants were matched for age, sex, and parental education level (the proxy measure of socioeconomic status). The exclusion criteria were as follows: (1) comorbid neurological disorders; (2) intellectual disability; (3) physical health impairment that might affect biochemical markers measured in the study (diabetes, hypertension, coronary artery disease, autoimmune disorders, inflammatory diseases, endocrine disorders); (4) drug and/or alcohol dependence (except for nicotine) and (5) duration of antipsychotic treatment longer than 30 days. Participants were recruited in two big Polish cities (Wroclaw and Szczecin) in the time period between October, 2016 and December, 2019. The study protocol was approved by the Ethics Committee of Wroclaw Medical University (Poland) and all participants gave a written informed consent.

We used the following measures of psychopathology: the Positive and Negative Syndrome Scale (PANSS) (25), the Hamilton Depression Rating Scale (HDRS) (26), the Young Mania Rating Scale (YMRS) (27), the Global Assessment of Functioning (GAF) (28) and the Social and Occupational Assessment of Functioning (SOFAS) (28). Cognitive performance was recorded using the Repeatable Battery for Assessment of Neuropsychological Status (RBANS) (29). The RBANS includes several tests that are grouped into the following domains: immediate memory (list learning and story memory), visuospatial/constructional functions (figure copy and line orientation), language (picture naming and semantic fluency), attention (digit span and coding), and delayed memory (list recall, list recognition, story memory, and figure recall).

Anthropometric Measures and Biochemical Parameters

All participants underwent physical examination to record the waist-to-hip ratio (WHR) and the body mass index (BMI). Blood samples were collected between 7 a.m. and 9 a.m. after overnight fasting. Subsequently, they were centrifuged to obtain serum samples. Colorimetric methods were used to determine the levels of glucose, total cholesterol, triglycerides and high-density lipoproteins (HDL) in the Konelab 60 analyzer (Argenta). The level of low-density lipoproteins (LDL) was calculated using the Friedewald formula. Electrochemiluminescence analysis was applied to measure the levels of insulin (the Cobas e411 analyzer, Roche). The levels of adiponectin and leptin were determined using the Enzyme-Linked Immunosorbent Assay (ELISA) kits.

Statistics

Bivariate comparisons were performed using the χ^2 test (categorical variables) and the Mann-Whitney U test or t-tests (continuous variables). Normality of data distribution was assessed using the Kolmogorov-Smirnov test. The Spearman rank correlation coefficients were used to test bivariate correlations. The analysis of covariance (ANCOVA) was performed to test for between-group differences in metabolic parameters after adjustment for age, sex, BMI, and cigarette smoking status. Similarly, linear regression analysis was performed in case of significant bivariate correlations between the measures of psychopathology or cognitive performance and metabolic parameters. Age, sex, BMI, cigarette smoking status, and chlorpromazine equivalent dosage (CPZeq) were included as covariates. Results were considered statistically significant if the p-value was less than 0.05. All analyses were conducted using the Statistical Package for Social Sciences, version 20 (SPSS Inc., Chicago, Illinois, USA).

RESULTS

General characteristics of the participants were shown in **Table 1**. There were no significant between-group differences in age, sex, parental education level, BMI, and WHR. As expected, cigarette smoking rates were significantly higher, while the number of education years was significantly lower, in FEP patients. Similarly, patients with FEP scored significantly lower on all domains of cognitive performance compared to HCs or FHR-P individuals (except for visuospatial/constructional abilities). In turn, FHR-P individuals had significantly lower scores of visuospatial/constructional abilities, attention and delayed memory in comparison with HCs.

Metabolic parameters in distinct groups of participants were presented in **Table 2**. Patients with FEP had significantly lower levels of leptin and HDL than HCs. The difference in HDL levels between FEP patients and FHR-P individuals was also significant. In turn, the levels of triglycerides and insulin were significantly higher in FEP patients compared to HCs. Moreover, patients with FEP had significantly higher levels of triglycerides than HCs. These between-group differences remained significant after covarying for age, sex, BMI, and cigarette smoking status (**Table 2**).

There were several significant bivariate correlations between metabolic parameters, psychopathological manifestation and cognitive performance, especially in FEP patients (**Table 3**). However, only a negative correlation between leptin levels and the RBANS language score remained significant ($B = -0.226$, $p = 0.006$) in FEP patients after controlling for the effects of age

TABLE 1 | General characteristics of participants.

	FEP, n = 35	FHR-P, n = 33	HCs, n = 32	p (FEP vs. HCs)	p (FEP vs. FHR-P)	p (FHR-P vs. HCs)
Age, years	34.2 ± 12.5	37.3 ± 11.2	32.3 ± 8.4	0.477	0.283	0.054
Sex, M(%)	18 (51.4)	12	11	0.218	0.232	1.000
Years of education	13.7 ± 3.0	15.4 ± 3.6	15.7 ± 2.5	**0.007**	**0.044**	0.743
Paternal education, higher/other than higher (%)	7 (20.0)	3 (9.1)	7 (21.9)	1.000	0.314	0.301
Maternal education, higher/other than higher (%)	7 (20.0)	6 (18.2)	9 (28.1)	0.566	1.000	0.554
BMI, kg/m^2	23.9 ± 4.1	24.7 ± 4.2	23.7 ± 3.1	0.876	0.400	0.281
WHR	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.072	0.788	0.151
Cigarette smoking (%)	12 (34.3)	5 (15.2)	4 (12.5)	**0.047**	0.158	0.732
CPZeq, mg/day	347.2 ± 174.2	–	–	–	–	–
HDRS	8.8 ± 7.8	–	–	–	–	–
YMRS	2.3 ± 5.3	–	–	–	–	–
PANSS-P	15.0 ± 5.3	–	–	–	–	–
PANSS-N	19.3 ± 8.0	–	–	–	–	–
SOFAS	48.5 ± 13.1	92.4 ± 9.8	93.7 ± 6.1	**<0.001**	**<0.001**	0.519
GAF	48.0 ± 15.2	–	–	–	–	–
RBANS – immediate memory	41.7 ± 9.4	49.4 ± 6.9	49.7 ± 6.3	**<0.001**	**0.002**	0.851
RBANS – visuospatial/constructional	35.0 ± 4.5	36.1 ± 4.1	38.1 ± 2.2	**0.001**	0.291	**0.022**
RBANS – language	27.5 ± 5.8	32.1 ± 6.1	32.4 ± 6.0	**0.001**	**0.004**	0.942
RBANS – attention	50.1 ± 13.8	59.8 ± 11.7	66.4 ± 8.5	**<0.001**	**0.003**	**0.016**
RBANS – delayed memory	46.1 ± 9.4	51.1 ± 5.3	54.4 ± 4.9	**<0.001**	**0.041**	**0.009**

Significant bivariate differences (p < 0.05) were marked with bold characters.
BMI, body mass index; CPZeq, chlorpromazine equivalent dosage; FEP, first-episode psychosis; FHR-P, individuals at familial high risk of psychosis; GAF, the Global Assessment of Functioning; HCs, healthy controls; HDRS, the Hamilton Depression Rating Scale; PANSS-N, the Positive and Negative Syndrome Scale (negative symptoms subscale); PANSS-P, the Positive and Negative Syndrome Scale (positive symptoms subscale); RBANS, the Repeatable Battery for Assessment of Neuropsychological Status; SOFAS, the Social and Occupational Assessment of Functioning; WHR, waist-to-hip ratio; YMRS, the Young Mania Rating Scale.

TABLE 2 | Metabolic parameters in FEP patients, FHR-P individuals and HCs.

	FEP, n = 35	FHR-P, n = 33	HCs, n = 32	p (FEP vs. HCs)	p (FEP vs. FHR-P)	p (FHR-P vs. HCs)
Total cholesterol, mg/dl	179.6 ± 37.6	191.6 ± 39.3	183.4 ± 36.8	0.682	0.207	0.395
LDL, mg/dl	104.1 ± 35.0	112.6 ± 36.5	108.2 ± 34.8	0.633	0.335	0.627
HDL, mg/dl	48.0 ± 16.9	60.6 ± 14.1	59.8 ± 17.5	**0.007**[a]	**0.002**[b]	0.836
Triglycerides, mg/dl	137.4 ± 58.8	91.8 ± 46.3	77.5 ± 33.2	**<0.001**[c]	**0.001**[d]	0.168
Leptin, ng/ml	10.7 ± 15.7	12.6 ± 10.1	17.6 ± 19.0	**0.046**[e]	0.086	0.475
Adiponectin, µg/ml	8.2 ± 4.0	7.6 ± 5.2	7.5 ± 4.0	0.533	0.587	0.995
Glucose, mg/dl	87.5 ± 19.4	87.0 ± 17.5	85.8 ± 11.3	0.995	0.999	0.998
Insulin, µlU/ml	15.2 ± 13.1	11.2 ± 6.4	9.6 ± 5.0	**0.023**[f]	0.152	0.205

Significant bivariate differences (p < 0.05) were marked with bold characters.
[a]*ANCOVA: group (FEP vs. HCs): F = 4.84, p = 0.032; BMI: F = 6.16, p = 0.016; cigarette smoking: F = 3.94, p = 0.052; age: F = 0.544, p = 0.464; sex: F = 2.71, p = 0.105.*
[b]*ANCOVA: group (FEP vs. FHR-P): F = 6.84, p = 0.003, BMI: F = 8.53, p = 0.005, cigarette smoking: F = 3.01, p = 0.088, age: F = 0.28, p = 0.602, sex: F = 2.32, p = 0.133.*
[c] *ANCOVA: group (FEP vs. HCs): F = 25.00, p < 0.001; BMI: F = 8.92, p = 0.004; cigarette smoking: F = 0.001, p = 0.990; age: F = 0.202, p = 0.655; sex: F = 6.74, p = 0.012.*
[d]*ANCOVA: group (FEP vs. FHR-P): F = 15.06, p < 0.001, BMI: F = 5.56, p = 0.022, cigarette smoking: F = 0.34, p = 0.563, age: F = 0.49, p = 0.486, sex: F = 5.35, p = 0.024.*
[e]*ANCOVA: group (FEP vs. HCs): F = 5.04, p = 0.028; BMI: F = 13.70, p < 0.001, cigarette smoking: F = 0.559, p = 0.457; age: F < 0.001, p = 0.990; sex: F = 1.41, p = 0.239.*
[f]*ANCOVA: group (FEP vs. FHR-P): F = 6.45, p = 0.014; BMI: F = 1.68, p = 0.199, cigarette smoking: F = 0.197, p = 0.659; age: F = 1.05, p = 0.310, sex: F = 1.80, p = 0.185.*
FEP, first-episode psychosis; FHR-P, individuals at familial high risk of psychosis; HCs, healthy controls; HDL, high-density lipoproteins; LDL, low-density lipoproteins.

(B = 0.023, p = 0.795), sex (B = 2.221, p = 0.314), BMI (B = −0.071, p = 0.817), cigarette smoking status (B = −3.771, p = 0.047) and CPZeq (B = −0.004, p = 0.358) in linear regression analysis. Other bivariate correlations between metabolic parameters, psychopathological manifestation and cognitive performance were not significant in linear regression analysis (data not shown).

DISCUSSION

Our results provide further evidence that impaired adipoinsular axis is an early sign of metabolic dysregulation in psychosis. These findings are consistent with results of our meta-analysis showing increased levels of insulin and decreased levels of leptin in FEP patients compared to HCs (4). Leptin is an anorexigenic hormone released by adipose tissue and suppresses the release of insulin by direct interactions with its receptors expressed by β-cells (14). Therefore, leptin deficiency can contribute to excessive release of insulin that is an adipogenic hormone. Increased storage of adipose tissue leads to overproduction of leptin and subsequent leptin resistance. Indeed, there is evidence that multiple-episode schizophrenia patients present with increased leptin levels (5). Impaired leptin signaling might also be related to the pathophysiology of psychosis. It has been found that leptin reduces dopamine neuronal firing in the mesolimbic system (30).

The observation that higher leptin levels are associated with lower RBANS scores of language performance also support the

TABLE 3 | Correlations between metabolic parameters, psychopathological manifestation, and cognitive performance.

	TC	LDL	HDL	Triglycerides	Leptin	Adiponectin	Glucose	Insulin
FEP:								
HDRS	r = 0.101	r = 0.213	r = –0.301	r = 0.391[a]	**r = 0.357[b]**	r = –0.255	r = 0.194	r = 0.321
YMRS	r = –0.266	r = –0.234	r = 0.094	r = –0.399[a]	r = –0.179	r = –0.037	r = 0.091	r = –0.157
PANSS-P	r = –0.307	r = –0.216	r = –0.064	r = –0.175	r = –0.203	r = 0.048	r = –0.061	r = –0.220
PANSS-N	r = 0.047	r = 0.086	r = –0.179	r = 0.357[a]	r = 0.069	r = –0.209	r = –0.240	r = –0.112
SOFAS	r = 0.287	r = 0.181	**r = 0.340[a]**	r = –0.216	r = –0.125	r = –0.021	r = –0.092	r = –0.107
GAF	r = 0.238	r = 0.164	r = 0.329	r = –0.246	r = –0.125	r = 0.004	r = –0.041	r = –0.066
Immediate memory	r = 0.205	r = –0.023	**r = 0.437[a]**	r = –0.201	r = –0.082	r = 0.294	r = 0.243	r = 0.140
Visuospatial/constructional	r = 0.111	r = 0.067	r = 0.148	r = –0.123	r = –0.112	r = –0.216	r = –0.140	r = 0.007
Language	r = –0.205	**r = –0.343[a]**	**r = 0.440[b]**	**r = –0.484[b]**	**r = 0.552[b]**	r = 0.175	r = –0.129	**r = –0.375[a]**
Attention	r = 0.096	r = 0.063	r = 0.144	r = –0.225	r = 0.003	r = 0.038	r = –0.180	r = 0.005
Delayed memory	r = 0.285	r = 0.139	r = 0.219	r = 0.014	r = –0.037	r = 0.765	r = 0.003	r = 0.025
FHR–P:								
SOFAS	r = 0.313	r = 0.247	r = 0.083	r = 0.169	r = –0.167	r = 0.331	r = 0.127	r = –0.252
Immediate memory	r = 0.224	r = 0.222	r = 0.225	r = –0.067	r = 0.267	r = 0.302	r = 0.029	r = 0.045
Visuospatial/constructional	r = 0.071	r = 0.096	r = 0.108	r = –0.182	r = –0.027	r = 0.119	r = –0.057	r = –0.148
Language	r = 0.268	r = 0.274	r = 0.055	r = 0.154	r = 0.053	r = 0.087	r = 0.173	r = 0.091
Attention	r = 0.031	r = 0.017	r = 0.081	r = –0.085	r = 0.080	r = 0.106	r = –0.105	r = 0.001
Delayed memory	r = –0.189	r = –0.139	r = –0.043	r = –0.243	r = 0.141	r = 0.142	r = –0.270	r = 0.012
HCs:								
SOFAS	r = –0.112	r = –0.066	r = –0.050	r = –0.014	r = –0.021	r = –0.310	r = –0.008	r = –0.034
Immediate memory	r = –0.027	r = –0.059	r = 0.158	**r = –0.527[b]**	r = 0.162	**r = 0.415[a]**	r = –0.368	r = –0.146
Visuospatial/constructional	r = 0.157	r = 0.189	r = 0.055	r = –0.115	r = 0.141	r = 0.061	r = 0.081	r = –0.012
Language	r = –0.067	r = –0.177	r = 0.286	r = –0.289	r = 0.267	r = 0.234	r = –0.104	r = –0.146
Attention	r = 0.245	**r = –0.379[a]**	r = –0.108	r = –0.324	r = 0.316	r = –0.012	r = –0.040	r = –0.085
Delayed memory	r = –0.063	r = –0.167	r = 0.288	r = –0.314	r = 0.265	r = 0.279	r = –0.193	r = –0.154

Significant correlations ($p < 0.05$) were marked with bold characters.
[a]$p < 0.05$, [b]$p < 0.01$.
BMI, body mass index; FEP, first-episode psychosis; FHR-P, individuals at familial high risk of psychosis; GAF, the Global Assessment of Functioning; HCs, healthy controls; HDL, high-density lipoproteins; HDRS, the Hamilton Depression Rating Scale; LDL, low-density lipoproteins; PANSS-N, the Positive and Negative Syndrome Scale (negative symptoms subscale); PANSS-P, the Positive and Negative Syndrome Scale (positive symptoms subscale); SOFAS, the Social and Occupational Assessment of Functioning; TC, total cholesterol; WHR, waist-to-hip ratio; YMRS, the Young Mania Rating Scale.

involvement of leptin in the pathophysiology of psychosis. Notably, the RBANS language score is composed of two cognitive tasks—picture naming and semantic fluency. Patients with schizophrenia show robust deficits of verbal fluency, with semantic fluency being more impaired than phonemic fluency (31). These impairments can be attributed to attenuated frontal activation (32). There is evidence that neonatal leptin deficiency reduces the frontal cortex volumes (33). Desensitization of leptin receptors in the prefronal cortex has been associated with upregulation of dopaminergic genes in this brain region (34). Another potential explanation is related to the effects of leptin on immune-inflammatory processes. Elevated levels of leptin in obesity might contribute to the release of pro-inflammatory cytokines (35). In turn, elevated levels of proinflammatory cytokines have been associated with cognitive impairment in patients with schizophrenia (36). Surprisingly, our study demonstrated a negative correlation between leptin levels and performance of the language domain. However, a cross-sectional study design does not allow to conclude regarding direction of causality. One of potential scenarios is that higher secretion of leptin is a response to neurostructural alterations of the frontal cortex and related cognitive impairment in FEP. A lack of significant correlations between the RBANS scores and leptin levels in FHR-P individuals as well as HCs further support this interpretation.

Furthermore, we demonstrated significantly lower levels of HDL as well as significantly higher levels of triglycerides in FEP patients than in HCs. These findings are also in line with those provided by recent meta-analyses of lipid profile alterations in FEP patients (37, 38). Notably, we did not confirm the hypothesis that familial liability to psychosis is related to metabolic alterations and impaired appetite regulation. However, it should be noted that our operationalization of familial liability might be insufficient to detect a significant association as we did not assess prodromal symptoms. Moreover, due to low sample size, we were not able to test our hypotheses in a subgroup of individuals meeting the criteria of at-risk mental state (genetic risk and deterioration syndrome) (39). Indeed, there is evidence that individuals at clinical high risk of psychosis show a high percentage of metabolic syndrome components prior to exposure to antipsychotic treatment (40). Moreover, it has been shown that measuring the levels of fatty acids in subjects at ultra-high risk of psychosis may improve prediction of transition to overt psychotic episode (41). Another study demonstrated that FEP patients have significantly higher levels of prolactin, fasting glucose, glycosylated hemoglobin and insulin resistance compared to individuals at clinical high risk of psychosis (42). It cannot also be excluded that results of our study simply reflect the effects of environmental factors or unhealthy lifestyle characteristics that are highly prevalent in early psychosis and include nutritional deficiencies as well as low exercise activity (43–45).

There are some important limitations of this study that need to be acknowledged. Firstly, our sample size was not large. Therefore, it cannot be excluded that our sample had insufficient power to detect the association between familial liability to psychosis and metabolic alterations. Moreover, we did not perform a detailed clinical assessment of FHR-P individuals, especially with respect to prodromal symptoms of psychosis. In light of these two limitations, we were unable to test the hypothesis whether metabolic dysregulation assessed in this study appears in individuals at clinical high risk of psychosis. Another limitation is that we cannot exclude medication effects. However, exposure to antipsychotic treatment was low in our study and linear regression analyses did not confirm a significant effect of CPZeq. It is also important to note that we did not record initial sample of individuals approached for participation and reasons for nonparticipation. Therefore, it is difficult to evaluate representativeness of our sample. Finally, a lack of longitudinal study design does not allow to establish conclusions regarding causality and temporal patterns of changes in metabolic parameters.

In summary, this study provides additional evidence of impaired adipoinsular axis, in terms of low leptin and high insulin levels, in early psychosis. Leptin levels might be related to cognitive impairment in FEP patients; however, causal mechanisms of this association need to be confirmed. Our findings provide novel insights into potential mechanisms of early metabolic disturbances and cognitive impairment in psychotic disorders. Moreover, we confirmed that FEP is associated with specific lipid profile disturbances. Longitudinal studies investigating our findings in subjects at clinical high risk of psychosis, especially in those with genetic risk and deterioration syndrome, are needed to confirm direction of causality and address limitations of our study.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by The Ethics Committee at Wroclaw Medical University, Poland. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

ML and BM designed the study. ML, BM, BS, AS and AR were involved in recruitment of participants. LP-S measured the levels of leptin and adiponectin. BM performed data analysis. ML and BM wrote the first draft of the manuscript. All authors contributed to reviewing and editing the first draft of the manuscript.

FUNDING

This study was funded from science budget resources granted for the years 2016–2019 (the Iuventus Plus grant awarded by the Ministry of Science and Higher Education, grant number: IP2015 052474).

REFERENCES

1. Piotrowski P, Gondek TM, Królicka-Deręgowska A, Misiak B, Adamowski T, Kiejna A. Causes of mortality in schizophrenia: An updated review of European studies. *Psychiatr Danub* (2017) 29:108–20. doi: 10.24869/psyd.2017.108
2. Pillinger T, D'Ambrosio E, McCutcheon R, D Howes O. Is psychosis a multisystem disorder? A meta-review of central nervous system, immune, cardiometabolic, and endocrine alterations in first-episode psychosis and perspective on potential models. *Mol Psychiatry* (2018) 24:776–94. doi: 10.1038/s41380-018-0058-9
3. Andreassen OA, Djurovic S, Thompson WK, Schork AJ, Kendler KS, O'Donovan MC, et al. Improved detection of common variants associated with schizophrenia by leveraging pleiotropy with cardiovascular-disease risk factors. *Am J Hum Genet* (2013) 92:197–209. doi: 10.1016/j.ajhg.2013.01.001
4. Misiak B, Bartoli F, Stramecki F, Samochowiec J, Lis M, Kasznia J, et al. Appetite regulating hormones in first-episode psychosis: A systematic review and meta-analysis. *Neurosci Biobehav Rev* (2019) 102:362–70. doi: 10.1016/j.neubiorev.2019.05.018
5. Stubbs B, Wang AK, Vancampfort D, Miller BJ. Are leptin levels increased among people with schizophrenia versus controls? A systematic review and comparative meta-analysis. *Psychoneuroendocrinology* (2016) 63:144–54. doi: 10.1016/j.psyneuen.2015.09.026
6. Bartoli F, Lax A, Crocamo C, Clerici M, Carrà G. Plasma adiponectin levels in schizophrenia and role of second-generation antipsychotics: A meta-analysis. *Psychoneuroendocrinology* (2015) 56:179–89. doi: 10.1016/j.psyneuen.2015.03.012
7. Lihn AS, Pedersen SB, Richelsen B. Adiponectin: Action, regulation and association to insulin sensitivity. *Obes Rev* (2005) 6:13–21. doi: 10.1111/j.1467-789X.2005.00159.x
8. Lee EE, Sears DD, Liu J, Jin H, Tu XM, Eyler LT, et al. A novel biomarker of cardiometabolic pathology in schizophrenia? *J Psychiatr Res* (2019) 117:31–7. doi: 10.1016/j.jpsychires.2019.06.011
9. Farr OM, Tsoukas MA, Mantzoros CS. Leptin and the brain: Influences on brain development, cognitive functioning and psychiatric disorders. *Metabolism* (2015) 64:114–30. doi: 10.1016/j.metabol.2014.07.004
10. Tang BL. Leptin as a neuroprotective agent. *Biochem Biophys Res Commun* (2008) 368:181–5. doi: 10.1016/j.bbrc.2008.01.063
11. Venkatasubramanian G, Chittiprol S, Neelakantachar N, Shetty TK, Gangadhar BN. A longitudinal study on the impact of antipsychotic treatment on serum leptin in schizophrenia. *Clin Neuropharmacol* (2010) 33:288–92. doi: 10.1097/WNF.0b013e3181fa2a6f
12. Bereiter DA, Jeanrenaud B. Altered neuroanatomical organization in the central nervous system of the genetically obese (ob/ob) mouse. *Brain Res* (1979) 165:249–60. doi: 10.1016/0006-8993(79)90557-2
13. Vannucci SJ, Gibbs EM, Simpson IA. Glucose utilization and glucose transporter proteins GLUT-1 and GLUT-3 in brains of diabetic (db/db) mice. *Am J Physiol - Endocrinol Metab* (1997) 272:E267–74. doi: 10.1152/ajpendo.1997.272.2.e267
14. Kieffer TJ, Habener JF. The adipoinsular axis: Effects of leptin on pancreatic β-cells. *Am J Physiol - Endocrinol Metab* (2000) 278:E1–E14. doi: 10.1152/ajpendo.2000.278.1.e1
15. Park CR. Cognitive effects of insulin in the central nervous system. *Neurosci Biobehav Rev* (2001) 25:311–23. doi: 10.1016/S0149-7634(01)00016-1

16. Zhang X, Yang M, Du X, Liao W, Chen D, Fan F, et al. Glucose disturbances, cognitive deficits and white matter abnormalities in first-episode drug-naive schizophrenia. *Mol Psychiatry* (2019). doi: 10.1038/s41380-019-0478-1
17. Ferrario CR, Reagan LP. Insulin-mediated synaptic plasticity in the CNS: Anatomical, functional and temporal contexts. *Neuropharmacology* (2018) 136:182–91. doi: 10.1016/j.neuropharm.2017.12.001
18. Agarwal SM, Caravaggio F, Costa-Dookhan KA, Castellani L, Kowalchuk C, Asgariroozbehani R, et al. Brain insulin action in schizophrenia: Something borrowed and something new. *Neuropharmacology* (2020) 163:107633. doi: 10.1016/j.neuropharm.2019.05.010
19. Zhang X, Jiang X, Han S, Liu Q, Zhou J. Type 2 Diabetes Mellitus Is Associated With the Risk of Cognitive Impairment: A Meta-Analysis. *J Mol Neurosci* (2019) 68:251–60. doi: 10.1007/s12031-019-01290-3
20. Bora E, Akdede BB, Alptekin K. The relationship between cognitive impairment in schizophrenia and metabolic syndrome: A systematic review and meta-analysis. *Psychol Med* (2017) 47:1030–40. doi: 10.1017/S0033291716003366
21. Wysokiński A, Dzienniak M, Kłoszewska I. Effect of metabolic abnormalities on cognitive performance and clinical symptoms in schizophrenia. *Arch Psychiatry Psychother* (2013) 4:13–25. doi: 10.12740/APP/19967
22. Botis AC, Miclutia I, Vlasin N. Cognitive function in female patients with schizophrenia and metabolic syndrome. *Eur Psychiatry* (2016) 33:S97. doi: 10.1016/j.eurpsy.2016.01.070
23. Li R, Wang TJ, Lyu PY, Liu Y, Chen WH, Fan MY, et al. Effects of Plasma Lipids and Statins on Cognitive Function. *Chin Med J (Engl)* (2018) 131:471–6. doi: 10.4103/0366-6999.225062
24. McGuffin P. A Polydiagnostic Application of Operational Criteria in Studies of Psychotic Illness. *Arch Gen Psychiatry* (1991) 48:764. doi: 10.1001/archpsyc.1991.01810320088015
25. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull* (1987) 13:261–76. doi: 10.1093/SCHBUL/13.2.261
26. Hamilton M. A rating scale for depression. *J Neurol Neurosurg Psychiatry* (1960) 23:56–62. doi: 10.1136/jnnp.23.1.56
27. Young RC, Biggs JT, Ziegler VE, Meyer DA. A rating scale for mania: reliability, validity and sensitivity. *Br J Psychiatry* (1978) 133:429–35. doi: 10.1192/bjp.133.5.429
28. Goldman HH, Skodol AE, Lave TR. Revising axis V for DSM-IV: A review of measures of social functioning. *Am J Psychiatry* (1992) 149:1148–56. doi: 10.1176/ajp.149.9.1148
29. Randolph C, Tierney MC, Mohr E, Chase TN. The Repeatable Battery for the Assessment of Neuropsychological Status (RBANS): Preliminary Clinical Validity. *J Clin Exp Neuropsychol (Neuropsychology Dev Cognit Sect A)* (1998) 20:310–9. doi: 10.1076/jcen.20.3.310.823
30. Hommel JD, Trinko R, Sears RM, Georgescu D, Liu ZW, Gao XB, et al. Leptin Receptor Signaling in Midbrain Dopamine Neurons Regulates Feeding. *Neuron* (2006) 51:801–10. doi: 10.1016/j.neuron.2006.08.023
31. Henry JD, Crawford JR. A meta-analytic review of verbal fluency deficits in schizophrenia relative to other neurocognitive deficits. *Cognit Neuropsychiatry* (2005) 10:1–33. doi: 10.1080/13546800344000309
32. Curtis VA, Bullmore ET, Brammer MJ, Wright IC, Williams SCR, Morris RG, et al. Attenuated frontal activation during a verbal fluency task in patients with schizophrenia. *Am J Psychiatry* (1998) 155:1056–63. doi: 10.1176/ajp.155.8.1056
33. Dexter BC, Rahmouni K, Cushman T, Hermann GM, Ni C, Nopoulos PC, et al. Neonatal leptin deficiency reduces frontal cortex volumes and programs adult hyperactivity in mice. *Behav Brain Res* (2014) 263:115–21. doi: 10.1016/j.bbr.2014.01.021
34. Del Rio D, Del Olmo N, Ruiz-Gayo M. Desensitization of leptin receptors is coincident with the upregulation of dopamine-related genes in the prefrontal cortex of adolescent mice. *Neuroreport* (2016) 27:516–21. doi: 10.1097/WNR.0000000000000574
35. Iikuni N, Kwan Lam Q, Lu L, Matarese G, Cava A. Leptin and Inflammation. *Curr Immunol Rev* (2008) 4:70–9. doi: 10.2174/157339508784325046
36. Misiak B, Beszłej JA, Kotowicz K, Szewczuk-Bogusławska M, Samochowiec J, Kucharska-Mazur J, et al. Cytokine alterations and cognitive impairment in major depressive disorder: From putative mechanisms to novel treatment targets. *Prog Neuropsychopharmacol Biol Psychiatry* (2017) 80:177–88. doi: 10.1016/j.pnpbp.2017.04.021
37. Misiak B, Stańczykiewicz B, Łaczmański Ł, Frydecka D. Lipid profile disturbances in antipsychotic-naive patients with first-episode non-affective psychosis: A systematic review and meta-analysis. *Schizophr Res* (2017) 190:18–27. doi: 10.1016/j.schres.2017.03.031
38. Pillinger T, Beck K, Stubbs B, Howes OD. Cholesterol and triglyceride levels in first-episode psychosis: systematic review and meta-analysis. *Br J Psychiatry* (2017) 211:339–49. doi: 10.1192/bjp.bp.117.200907
39. Fusar-Poli P, Cappucciati M, Borgwardt S, Woods SW, Addington J, Nelson B, et al. Heterogeneity of psychosis risk within individuals at clinical high risk: A meta-analytical stratification. *JAMA Psychiatry* (2016) 73:113–20. doi: 10.1001/jamapsychiatry.2015.2324
40. Cadenhead KS, Minichino A, Kelsven S, Addington J, Bearden C, Cannon TD, et al. Metabolic abnormalities and low dietary Omega 3 are associated with symptom severity and worse functioning prior to the onset of psychosis: Findings from the North American Prodrome Longitudinal Studies Consortium. *Schizophr Res* (2019) 204:96–103. doi: 10.1016/j.schres.2018.09.022
41. Clark SR, Baune BT, Schubert KO, Lavoie S, Smesny S, Rice SM, et al. Prediction of transition from ultra-high risk to first-episode psychosis using a probabilistic model combining history, clinical assessment and fatty-acid biomarkers. *Transl Psychiatry* (2016) 6:e897. doi: 10.1038/tp.2016.170
42. Petruzzelli MG, Margari M, Peschechera A, de Giambattista C, De Giacomo A, Matera E, et al. Hyperprolactinemia and insulin resistance in drug naive patients with early onset first episode psychosis. *BMC Psychiatry* (2018) 18:246. doi: 10.1186/s12888-018-1827-3
43. Borgan F, O'Daly O, Hoang K, Veronese M, Withers D, Batterham R, et al. Neural Responsivity to Food Cues in Patients With Unmedicated First-Episode Psychosis. *JAMA Netw Open* (2019) 2:e186893. doi: 10.1001/jamanetworkopen.2018.6893
44. Firth J, Carney R, Stubbs B, Teasdale SB, Vancampfort D, Ward PB, et al. Nutritional Deficiencies and Clinical Correlates in First-Episode Psychosis: A Systematic Review and Meta-analysis. *Schizophr Bull* (2017) 44:1275–92. doi: 10.1093/schbul/sbx162
45. Stubbs B, Firth J, Berry A, Schuch FB, Rosenbaum S, Gaughran F, et al. How much physical activity do people with schizophrenia engage in? A systematic review, comparative meta-analysis and meta-regression. *Schizophr Res* (2016) 176:431–40. doi: 10.1016/j.schres.2016.05.017

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

MINI REVIEW
published: 10 June 2020
doi: 10.3389/fpsyt.2020.00543

Polycystic Ovary Syndrome and Psychotic Disorder

Larissa Doretto[1], *Flora Chaves Mari*[2] *and Ana Cristina Chaves*[1]*

[1] First Episode Psychosis Program, Department of Psychiatry, Universidade Federal de São Paulo (UNIFESP), Sao Paulo, Brazil, [2] Santa Casa Medical School of Sao Paulo, Sao Paulo, Brazil

Polycystic ovary syndrome (PCOS), a disease that usually emerges during adolescence, is characterized by hormonal imbalance and ovarian dysfunction. The prevalence can vary between 5.6 to 21.3% in women and 6% in adolescent girls. This discrepancy is related to the population studied and the diagnostic criteria used. The underlying pathophysiology of PCOS is not fully understood, but it can lead to a number of co-morbidities, including hypertension, diabetes, dyslipidemia, and also, mental health disorders. Clinical and preclinical data indicate neuroendocrine involvement with dysfunction in gamma-Aminobutyric acid (GABA) signaling and neuronal androgen receptors that might reduce hypothalamic sensitivity and lead to an impairment of estradiol and progesterone feedback. Based on these assumptions, the aims of this paper are to review the association of PCOS and psychotic disorders in order to address the burden of women comorbid for both conditions.

Keywords: polycystic ovary syndrome, psychotic disorder, women, antipsychotic drugs, schizophrenia

OPEN ACCESS

Edited by:
Mary V. Seeman,
University of Toronto, Canada

Reviewed by:
Gary Remington,
Centre for Addiction and Mental Health (CAMH), Canada
Anna Comparelli,
Sapienza University of Rome, Italy

***Correspondence:**
Ana Cristina Chaves
anachaves@terra.com.br

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *26 March 2020*
Accepted: *27 May 2020*
Published: *10 June 2020*

Citation:
Doretto L, Mari FC and Chaves AC (2020) Polycystic Ovary Syndrome and Psychotic Disorder. Front. Psychiatry 11:543. doi: 10.3389/fpsyt.2020.00543

INTRODUCTION

Polycystic ovary syndrome (PCOS) is among the most common endocrine disorders, affecting 5.6–21.3% of women of reproductive age worldwide (1, 2). It is a heterogenous clinical condition, with a range of different phenotypes, a clinical reality that results in divergent opinions regarding diagnosis and treatment. International guidelines have been developed with the aim of integrating the best available evidence on diagnosis, assessment, and treatment and improving clinical care (2). These guidelines highlight the necessity to avoid over diagnosis, especially in adolescents. They emphasize the uncertainty of the natural history of this syndrome and its clinical implications. They point to hyperandrogenism, ovulatory dysfunction, and polycystic ovaries (as seen on ultrasound) as the key diagnostic features. Other potential features mentioned are: menstrual irregularity, subfertility, obesity, hirsutism, acne, and abnormal biochemistry, namely elevations of serum testosterone, androstenedione, luteinizing hormone, and insulin. The guidelines warn that affected women are at increased risk for hypertension, dyslipidemia, insulin resistance, glucose intolerance, type 2 diabetes, coagulation disorders, as well as cardiovascular morbidity and mortality.

Psychiatric symptoms such as anxiety and depression are additional common features of the syndrome (3, 4), but they may be unrecognized by the treating physician. On the other hand, physicians who see patients for psychological problems may not ask about features of PCOS, or if they see clinical signs such as obesity, acne, and hirsutism, may automatically attribute them to the effects of psychiatric medications. Publications on comorbidity between PCOS and severe psychiatric disorders are more frequently seen in the gynecology and endocrinology literature than in papers on psychiatry. Lack of recognition of the co-existence of PCOS and psychiatric

syndromes impacts negatively on affected women by delaying appropriate treatment. A large population-based study from Sweden found that 22.4% of the 22,385 women participants with PCOS had received at least one lifetime psychiatric diagnosis (5). When compared to a matched comparison sample, these women showed a higher prevalence not only of depression and anxiety, but also of less common disorders, such as bipolar disorder, schizophrenia spectrum disorder, eating disorder, and personality disorder.

There are several competing theories regarding the etiology of PCOS, but there is a consensus that the syndrome results from multiple causes. Significant interactions are likely among genetic and epigenetic factors, primary ovarian abnormalities, and neuroendocrine alterations (6). Recent evidence shows that neuroendocrine brain circuits, particularly the hypothalamic GnRH neuronal, are involved in the etiology of at least some forms of PCOS (7).

Similar neuroendocrine dysfunctions of the hypothalamic-pituitary axis are found in psychotic disorder (5). Few studies, however, have assessed the impact on clinical, course and prognosis of comorbid PCOS and psychosis. The aim of this paper, therefore, is to review this literature starting with the variety of theories explaining the pathophysiology of PCOS, followed by the consequences of the simultaneous occurrence of PCOS and psychosis, the role of antipsychotic medications, recommendations, and conclusion.

METHODS

This review is based on papers retrieved from PubMed searches using the following terms: "psychosis", "schizophrenia", "affective psychosis", "antipsychotics", "polycystic ovary syndrome", "menstrual dysfunction", "hyperandrogenism". First, abstracts in English from papers published from 2005 to 2019 were assessed to evaluate their relevance to the combined clinical effect of the two conditions, psychosis and PCOS. The final inclusion/exclusion of articles was not based on a protocol developed before as in a systematic review.

Theories About the Pathophysiology of PCOS

Based on a recent extensive review (8), we summarize in this section recent theories about the pathophysiology of PCOS. PCOS begins during the pubertal years, but the diagnosis is usually made later in life once the disorder has become relatively more severe. Ovarian dysfunction is thought to be caused by an impairment in the feedback loop of the steroid-hormone gonadotropin-releasing hormone (GnRH) produced in the hypothalamus. While there are other biologic systems and interconnected signaling networks also involved in the pathophysiology of PCOS, these latter networks may not be impaired in all affected women (6, 8)

In healthy women, the frequency of GnRH pulses in the hypothalamus regulate the pulsatile release of luteinizing hormone (LH) and follicle stimulating hormone (FSH) from the anterior pituitary gland: faster frequencies favor LH secretion and slower frequencies favor FSH release. In turn, LH and FSH secretion regulates the production of follicles and gonadal steroids in the ovary. The level of sex steroid hormones produced by the ovaries—estrogens, progestogens, and androgens—provides critical feedback to the hypothalamus and pituitary gland, thus regulating the degree of GnRH, LH, and FSH secretion. In PCOS, this physiological feedback loop is compromised, resulting in hyperactivity of the hypothalamus-pituitary-gonadal axis and an abnormally high LH : FSH ratio. This then impairs follicle generation in the ovary and interferes with the production of steroid hormones (6). Ovarian follicles remain in their immature state, a pre-ovulatory stage characterized by the cystic appearance of the ovary on ultrasound. The increased secretion of LH, acting on ovarian theca cells, stimulates the production of androgens. While androgens would normally be transformed into estradiol *via* an FSH-induced aromatase synthesized in ovarian granulosa cells (see **Figure 1**), in women with PCOS, this transformation is impaired due to the altered LH/FSH ratio. This leads to a state of hyperandrogenism (9). It is unclear whether the problem starts with a dysfunction in GnRH neurons in the hypothalamus or whether this occurs secondarily due to pathology of upstream neuronal systems (6).

Hyperandrogenism and KNDy Neurons

Some pre-clinical models tested in animals are trying to understand how neuronal systems are causing hyperandrogenism in PCOS. Studies that promote hyperandrogenic environments during pre-natal life and/or post-natal life in female mice found alterations in gamma-Aminobutyric acid (GABA) neurons in the medial basal hypothalamus and kisspeptin/neurokinin B/dynorphin neurons (KNDy neurons) in the arcuate nucleus (ARN) (9). These hypothalamic neurons have receptors for progesterone (PR), estradiol receptor α (ERα), and androgens (AR) and are responsible for the control of GnRH neurons and their feedback. As GnRH neurons have only ERβ receptor and not ERα, which is the most important receptor in estrogen activity, the investigators concluded that the presence of these receptors in kisspeptin neurons and in GABAergic neurons could be essential for modulating the negative feedback to GnRH neurons (see **Figure 1**). However, the findings concerning KNDy neurons are still controversial: in some models, KNDy neuronal population is high, in others, low or even not different at all. However, inhibition of KNDy neurons has shown to ameliorate the signs and symptoms of women with PCOS, by decreasing LH pulse frequency and LH and testosterone serum levels (10).

Hyperandrogenism and GABA

Based on clinical findings showing elevated concentrations of GABA in the cerebrospinal fluid of women with PCOS, animal studies have also addressed the involvement of GABA (9). A hyperandrogenic environment in female mice in prenatal life can cause an increase in the frequency and degree of GABAergic postsynaptic firing onto GnRH neurons. Unlike its function in other brain circuits, GABA seems to exert an excitatory effect on GnRH neurons attributable to their high chloride content. This

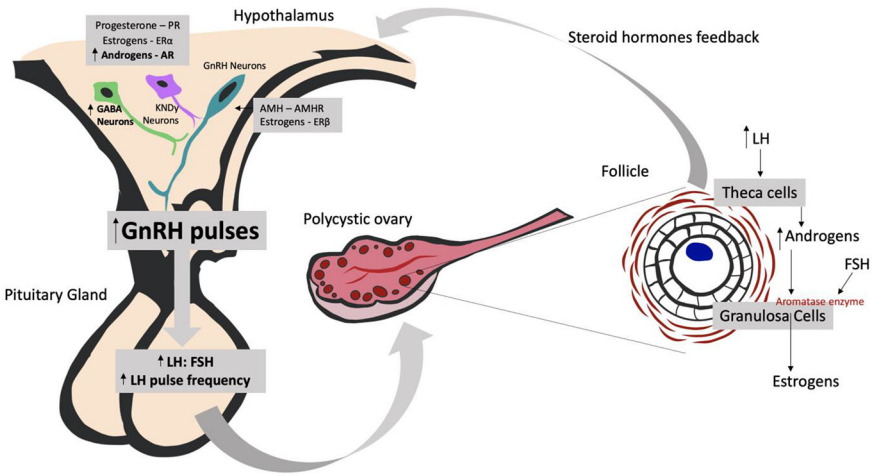

FIGURE 1 | Understanding the neuroendocrine effects of the hyperandrogenic environment on PCOS development. AMH, Anti-Müllerian Hormone; AMHR, Anti-Müllerian Hormone Receptor; AR, Androgen Receptor; ERα, Estrogen Receptor α; ERβ, Estrogen Receptor β; FSH, Follicle-stimulating Hormone; GnRH, Gonadotropin-releasing Hormone; GABA neurons, gamma-Aminobutyric acid neurons; KNDy neurons, kisspeptin/dynorphin/neurokinin B neurons; LH, Luteinizing Hormone.

leads to greater secretion of LH by the pituitary gland, as occurs in PCOS. GnRH antagonists prevent this effect (9, 11).

Hyperandrogenism and Anti-Mullerian Hormone

Normally, a dynamic equilibrium exists between growing and dormant follicles in the ovaries, regulated by a balance between androgens, anti-Müllerian hormone (AMH), and FSH. In PCOS, this balance is disrupted, leading to follicular arrest. It is known that women with PCOS have higher than normal serum levels of AMH, and that these levels remain high even during pregnancy (11). Tata et al. (11) conducted a study in which they classified 63 women with PCOS and 66 control women into lean or obese groups according to their body mass index. They found that lean pregnant women with PCOS had higher AMH levels than lean pregnant controls. However, there was no difference in AMH levels between PCOS pregnant obese women and controls. These findings led to the creation of a mouse model prenatally treated with AMH, which induced brain masculinization of female offspring that then showed neuroendocrine and reproductive PCOS-like features. It was therefore concluded that high AMH levels during pregnancy may be the cause of a hyperandrogenic environment that ultimately leads to PCOS pathology (11).

Hyperandrogenism and Androgen Receptors (AR)

When animal experiments compared female knock out mice with no brain AR with mice with no AR in the granulosa cells of the ovary, the mice with no brain AR showed some of the reproductive and metabolic features of PCOS, whilst the group with no ovarian AR showed all the features. Despite this difference, the conclusion reached was that hyperandrogenic changes in the brain came first and were, therefore, primarily responsible for what followed (12).

Hyperandrogenism and Microbiota

It has been recently discovered that gut microbiota plays a role in host sex differences (8). The composition of commensal microbes of males and females appears to diverge at puberty, thus implicating sex hormone levels. Torres et al. (13) have suggested that hyperandrogenism may play a direct role in altering the gut microbiome of women with PCOS. (14), studying rats, demonstrated a shift in the distribution of microbiota that was associated with sex hormone levels. In addition, they showed that fecal transplants were able to decrease androgen blood levels and increase estrogen levels, thereby improving estrus cycles in rat models of PCOS. Gut dysbiosis, therefore, may turn out to be an important contributing factor in the genesis of PCOS.

Classification of PCOS

The many disparate findings of animal studies mirror the multiple hypotheses that have been formulated to address the potential etiology of PCOS. In order to minimize controversy, guidelines to improve diagnosis and treatment have been developed (2). The following classification system has been introduced: phenotype A (hyperandrogenism + ovulatory disfunction + polycystic ovarian morphology); phenotype B (hyperandrogenism + ovarian dysfunction); phenotype C (hyperandrogenism + polycystic ovulatory morphology); phenotype D (ovarian dysfunction + polycystic ovulatory morphology). The guidelines recognize that PCOS is also a metabolic disorder, but do not incorporate insulin resistance into the diagnostic criteria because tests for insulin resistance show poor accuracy (2). The prevalence of psychological features is also acknowledged, and recommendations are made for the investigation and treatment of psychiatric illness associated with PCOS.

Women With PCOS and a Psychotic Disorder

Estrogen appears to exert an antipsychotic effect. When levels decline, the emergence of psychotic symptoms is facilitated (15). For instance, several literature reviews suggest that, in women with chronic psychotic illness, symptoms are aggravated during the premenstrual period (16), after delivery (17) and at menopause (15).

PCOS women, might, therefore, be vulnerable to psychosis because they are exposed to long durations of high levels of unopposed estrogen as a result of infrequent ovulation (18). When they do ovulate, they experience a precipitous reduction in estrogen, mimicking a postpartum state. This could explain the vulnerability of women with PCOS to psychotic symptoms (19).

Women with psychosis often show menstrual irregularity or amenorrhea, attributable to the hyperprolactinemia induced by the use of antipsychotic drugs (15). Hyperprolactinemia can also interfere with fertility (20), a problem for up to 72% of PCOS women (21). Antipsychotic medications impact negatively on personal appearance because of associated weight gain, hirsutism, acne, dental problems, halitosis, alopecia, rash, tremor, stiff gait, unsightly mouth movements, voice changes, or incontinence (22, 23). Similar symptoms are associated with PCOS, leading to a negative body image (24), low self-esteem, perceived stigma (25, 26), and a high prevalence of anxiety and depression (27).

The high degree of symptom overlap between the two conditions may be what prevents the recognition of primary PCOS in psychiatric patients. Additionally, for women suffering from both conditions, these symptoms are all aggravated.

The Role of Antipsychotic Medication

Besides leading to PCOS-like symptoms, the chronic administration of antipsychotics has been shown to exert a negative impact on gut microbiota (28–32), increasing the dysbiosis caused by PCOS. Indeed, Davey et al. (30) demonstrated that the administration of olanzapine for 3 weeks in rats induced identifiable alterations in the microbiome. Antipsychotics have antibacterial properties. Olanzapine, for instance, is able to completely inhibit the growth of E. coli (32). For women with both conditions, hyperandrogenism could, in this way, be aggravated by the use of antipsychotics.

Furthermore, the weight gain induced by antipsychotics affects more than appearance. Elevated body mass index and intra-abdominal adiposity predict insulin resistance and type 2 diabetes (T2DM) in patients treated for long periods of time (33, 34). Particular drugs (especially clozapine and olanzapine) are more likely than others to cause weight gain (35, 36). Compared to untreated psychiatric controls, Galling and colleagues (37) reported an incidence of T2DM three times higher in youth treated with antipsychotics for over three months. Antipsychotic-induced diabetes has been confirmed in animal models; both olanzapine and clozapine have been shown to decrease the plasma level of insulin and to cause hyperglycemia and insulin resistance in rats (16).

Women with PCOS and without psychosis also show symptoms of obesity and insulin resistance, both conditions closely related to T2DM (38, 39). The prevalence of overweight and obesity in PCOS is significantly greater than that of the general female population. Impaired glucose tolerance in women with PCOS was found to be 3-fold higher than in women of similar age by the National Health and Nutrition Survey (NHANES) II. When age and weight were controlled, the comparative prevalence was two-fold (39). Even lean women with PCOS show an increased risk for T2DM (40). The risk for women suffering from both PCOS and psychosis that requires antipsychotic treatment is, therefore, very high.

Antipsychotic drugs predispose to metabolic syndrome (37), to which women with PCOS are already susceptible (41). The available evidence shows that dyslipidemia is very common in PCOS (42), and that elevated values of triglycerides and total and low-density lipoprotein-cholesterol are frequently present. It is well known that dyslipidemia, obesity, and diabetes are all potent cardiovascular risk factors, but it is not currently certain whether the increased cardiovascular risk seen in PCOS is mediated through obesity or through other metabolic factors. Despite uncertainty about the pathway, the risk of cardiovascular illness is significantly elevated in PCOS (43), as it is in patients with psychosis.

Cardiovascular disease is the major cause of mortality in patients with psychosis. The rate is approximately two times higher than it is in the general population (44) and antipsychotic drugs are, at least in part, responsible (45, 46). The similarities between both metabolic and cardiovascular side effects of patients in treatment for psychosis and patients with PCOS have profound health implications for women who suffer from both conditions.

Valproate can be used together or alone to treat bipolar disorder and it is known to induce PCOS-like symptoms. Therefore, patients maintained chronically on valproic acid should be monitored to avoid the development of menstrual irregularities and signs of PCOS, since the reproductive endocrine effects of valproate are reversible after the treatment is discontinued (47).

CONCLUSIONS AND RECOMMENDATIONS

Although PCOS is the most frequent of all endocrine disorders among women of reproductive age, many women do not receive adequate treatment because of a too late diagnosis. To facilitate accurate diagnosis and timely treatment, clinicians who see female patients need to be familiar with the diversity of PCOS phenotypes. Patients with severe mental illness, on the other hand, have limited access to physical healthcare services (48). For this reason, it is important that psychiatrists be aware of the possibility of PCOS in their patients. When they learn about menstrual cycle irregularity in their women patients or find signs of hyperandrogenism, such as acne, hirsutism, and acanthosis

nigricans (49), *they must not automatically attribute them to antipsychotic drug effects*. Routine referral for a gynecology/endocrinology consult is indicated.

Moreover, for women with PCOS and psychosis, treatment with antipsychotic drugs can worsen PCOS symptomatology and lead to negative consequences for a woman's reproductive potential and her quality of life. Antipsychotic-induced weight gain is an important concern in the management of these patients. Prevention of weight gain (choosing the right drug, keeping the dose as low as possible, instruction about diet, exercise, and substance use) is more effective than after-the-fact attempts at intervention. Adequate monitoring of body mass index, fasting glucose, and prolactin levels in patients on antipsychotics is vital for patients suffering from both conditions. Given the seriousness of psychotic conditions in women with PCOS, further study of epidemiology, clinical features, neurobiology, disability, quality of life, and treatment in different settings is needed to more fully understand this association.

AUTHOR CONTRIBUTIONS

LD, FM, and AC reviewed literature and wrote this paper.

REFERENCES

1. Lizneva D, Suturina L, Walker W, Brakta S, Gavrilova-Jordan L, Azziz R. Criteria, prevalence, and phenotypes of polycystic ovary syndrome. *Fertil Steril* (2016) 106:6–15 doi: 10.1016/j.fertnstert.2016.05.003
2. Teede H, Misso M, Costello M, Dokras A, Laven J, Moran L, et al. International evidence-based guideline for the assessment and management of polycystic ovary syndrome 2018. National Health and Medical Research Council (NHMRC). (Melbourne Australia: Monash University) (2018).
3. Cesta CE, Månsson M, Palm C, Lichtenstein P, Iliadou AN, Landén M. Polycystic ovary syndrome and psychiatric disorders: Co-morbidity and heritability in a nationwide Swedish cohort. *Psychoneuroendocrinology*. (2016) 73:196–203. doi: 10.1016/j.psyneuen.2016.08.005
4. Blay SL, Aguiar JVA, Passos IC. Polycystic ovary syndrome and mental disorders: A systematic review and exploratory meta-analysis. *Neuropsychiatr Dis Treat* (2016) 12:2895–903. doi: 10.2147/NDT.S91700
5. Ibáñez L, Oberfield SE, Witchel S, Auchus RJ, Chang RJ, Codner E, et al. An International Consortium Update: Pathophysiology, Diagnosis, and Treatment of Polycystic Ovarian Syndrome in Adolescence. *Horm Res Paediatr* (2017) 88:371–95. doi: 10.1159/000479371
6. Coyle C, Campbell RE. Pathological pulses in PCOS. *Mol Cell Endocrinol* (2019) 498:110561. doi: 10.1016/j.mce.2019.110561
7. Nordholm D, Rostrup E, Mondelli V, Randers L, Nielsen M, Wulff S, et al. Multiple measures of HPA axis function in ultra high risk and first-episode schizophrenia patients. *Psychoneuroendocrinology* (2018) 92:72–80. doi: 10.1016/j.psyneuen.2018.03.015
8. Witchel SF, Oberfield SE, Peña AS. Polycystic Ovary Syndrome: Pathophysiology, Presentation, and Treatment With Emphasis on Adolescent Girls. *J Endocr Soc* (2019) 3(8):1545–73. doi: 10.1210/js.2019-00078
9. Ruddenklau A, Campbell RE. *Neuroendocrine Impairments of Polycystic Ovary Syndrome*. Endocrinology: Oxford University Press; (2019) Vol. 160. p. 2230–42.
10. Deng Y, Zhang Y, Li S, Zhou W, Ye L, Wang L, et al. Steroid hormone profiling in obese and nonobese women with polycystic ovary syndrome. *Sci Rep* (2017) 7(1). doi: 10.1038/s41598-017-14534-2
11. Tata B, Mimouni NEH, Barbotin AL, Malone SA, Loyens A, Pigny P, et al. Elevated prenatal anti-Müllerian hormone reprograms the fetus and induces polycystic ovary syndrome in adulthood. *Nat Med* (2018) 24(6):834–46. doi: 10.1038/s41591-018-0035-5
12. Caldwell ASL, Edwards MC, Desai R, Jimenez M, Gilchrist RB, Handelsman DJ, et al. Neuroendocrine androgen action is a key extraovarian mediator in the development of polycystic ovary syndrome. *Proc Natl Acad Sci U. S. A.* (2017) 114(16):E3334–43. doi: 10.1073/pnas.1616467114
13. Torres PJ, Siakowska M, Banaszewska B, Pawelczyk L, Duleba AJ, Kelley ST, et al. Gut Microbial Diversity in Women with Polycystic Ovary Syndrome Correlates with Hyperandrogenism. *J Clin Endocrinol Metab* (2018) 103 (4):1502–11. doi: 10.1210/jc.2017-02153
14. Guo Y, Qi Y, Yang X, Zhao L, Wen S, Liu Y, et al. Association between polycystic ovary syndrome and gut microbiota. *PloS One* (2016) 11(4):1–15. doi: 10.1371/journal.pone.0153196
15. Riecher-Rössler A. Oestrogens, prolactin, hypothalamic-pituitary-gonadal axis, and schizophrenic psychoses. *Lancet Psychiatry Elsevier Ltd* (2017) 4:63–72. doi: 10.1016/S2215-0366(16)30379-0
16. Seeman MV. Menstrual exacerbation of schizophrenia symptoms. *Acta Psychiatr Scand* (2012) 125(5):363–71. doi: 10.1111/j.1600-0447.2011.01822.x
17. González-Rodríguez A, Seeman MV. The association between hormones and antipsychotic use: a focus on postpartum and menopausal women. *Ther Adv Psychopharmacol* (2019) 9:204512531985997. doi: 10.1177/2045125319859973
18. Franks S, Stark J, Hardy K. Follicle dynamics and anovulation in polycystic ovary syndrome. *Hum Reprod Update*. (2008) 14(4):367–78. doi: 10.1093/humupd/dmn015
19. Deuchar N, Brockington I. Puerperal and menstrual psychoses: The proposal of a unitary etiological hypothesis. *J Psychosom Obstet Gynaecol*. (1998) 19 (2):104–10. doi: 10.3109/01674829809048503
20. Zhang-Wong JH, Seeman MV. Antipsychotic drugs, menstrual regularity and osteoporosis risk. *Arch Womens Ment Health* (2002) 5(3):93–8. doi: 10.1007/s00737-002-0002-4
21. Joham AE, Teede HJ, Ranasinha S, Zoungas S, Boyle J. Prevalence of infertility and use of fertility treatment in women with polycystic ovary syndrome: Data from a large community-based cohort study. *J Women's Heal* (2015) 24 (4):299–307. doi: 10.1089/jwh.2014.5000
22. Haddad PM, Sharma SG. Adverse Effects of Atypical Antipsychotics Differential Risk and Clinical Implications. *CNS Drugs* (2007) 21: (11):911–36. doi: 10.2165/00023210-200721110-00004
23. Seeman MV. Antipsychotics and physical attractiveness. *Clin Schizophr Relat Psychoses* (2011) 5(3):142–6. doi: 10.3371/CSRP.5.3.4
24. Moran L, Gibson-Helm M, Teede H, Deeks A. Polycystic ovary syndrome: A biopsychosocial understanding in young women to improve knowledge and treatment options. *J Psychosom Obstet Gynecol*. (2010) 31(1):24–31. doi: 10.3109/01674820903477593
25. Conaglen HM, Conaglen JV. Sexual desire in women presenting for antiandrogen therapy. *J Sex Marital Ther* (2003) 29(4):255–67. doi: 10.1080/00926230390195498
26. Chachamovich JR, Chachamovich E, Ezer H, Fleck MP, Knauth D, Passos EP. Investigating quality of life and health-related quality of life in infertility: A systematic review. *J Psychosom Obstet Gynecol*. (2010) 31(2):101–10. doi: 10.3109/0167482X.2010.481337
27. Veltman-verhulst SM, Boivin J, Eijkemans MJC, Fauser BJCM. Emotional distress is a common risk in women with polycystic ovary syndrome: A systematic review and meta-analysis of 28 studies. *Hum Reprod Update*. (2012) 18(6):638–51. doi: 10.1093/humupd/dms029
28. Bahr SM, Tyler BC, Wooldridge N, Butcher BD, Burns TL, Teesch LM, et al. Use of the second-generation antipsychotic, risperidone, and secondary weight gain are associated with an altered gut microbiota in children. *Transl Psychiatry* (2015) 5(9):e652–6. doi: 10.1038/tp.2015.135
29. Davey KJ, Cotter PD, O'Sullivan O, Crispie F, Dinan TG, Cryan JF, et al. Antipsychotics and the gut microbiome: Olanzapine-induced metabolic dysfunction is attenuated by antibiotic administration in the rat. *Transl Psychiatry* (2013) 3:1–7. doi: 10.1038/tp.2013.83

30. Davey KJ, O'Mahony SM, Schellekens H, O'Sullivan O, Bienenstock J, Cotter PD, et al. Gender-dependent consequences of chronic olanzapine in the rat: Effects on body weight, inflammatory, metabolic and microbiota parameters. *Psychopharmacol (Berl)* (2012) 221(1):155–69. doi: 10.1007/s00213-011-2555-2
31. Kao ACC, Spitzer S, Anthony DC, Lennox B, Burnet PWJ. Prebiotic attenuation of olanzapine-induced weight gain in rats: Analysis of central and peripheral biomarkers and gut microbiota. *Transl Psychiatry* (2018) 8(1). doi: 10.1038/s41398-018-0116-8
32. Morgan AP, Crowley JJ, Nonneman RJ, Quackenbush CR, Miller CN, Ryan AK, et al. The antipsychotic olanzapine interacts with the gut microbiome to cause weight gain in mouse. *PloS One* (2014) 9(12):1–20. doi: 10.1371/journal.pone.0115225
33. Bou Khalil R. Atypical antipsychotic drugs, schizophrenia, and metabolic syndrome in non-euro-american societies. *Clin Neuropharmacol.* (2012) 35 (3):141–7. doi: 10.1097/WNF.0b013e31824d5288
34. Manu P, Correll CU, van Winkel R, Wampers M. Hert M De. Prediabetes in Patients Treated With Antipsychotic Drugs. *J Clin Psychiatry* (2012) 73 (04):460–6. doi: 10.4088/JCP.10m06822
35. Leucht S, Cipriani A, Spineli L, Mavridis D, Örey D, Richter F, et al. Comparative efficacy and tolerability of 15 antipsychotic drugs in schizophrenia: A multiple-treatments meta-analysis. *Lancet* (2013) 382 (9896):951–62. doi: 10.1016/S0140-6736(13)60733-3
36. Musil R, Obermeier M, Russ P, Hamerle M. Weight gain and antipsychotics: A drug safety review. *Expert Opin Drug Saf.* (2015) 14(1):73–96. doi: 10.1517/14740338.2015.974549
37. Galling B, Roldán A, Nielsen RE, Nielsen J, Gerhard T, Carbon M, et al. Type 2 diabetes mellitus in youth exposed to antipsychotics: A systematic review and meta-analysis. *JAMA Psychiatry* (2016) 73(3):247–59. doi: 10.1001/jamapsychiatry.2015.2923
38. Kahn SE, Cooper ME, Del Prato S. Pathophysiology and treatment of type 2 diabetes: Perspectives on the past, present, and future. *Lancet.* (2014) 383 (9922):1068–83. doi: 10.1016/S0140-6736(13)62154-6
39. Verma S, Hussain ME. Obesity and diabetes: An update. *Diabetes Metab Syndr Clin Res Rev* (2017) 11(1):73–9. doi: 10.1016/j.dsx.2016.06.017
40. Zhang N, Shi YH, Hao CF, Gu HF, Li Y, Zhao YR, et al. Association of +45G15G(T/G) and +276(G/T) polymorphisms in the ADIPOQ gene with polycystic ovary syndrome among Han Chinese women. *Eur J Endocrinol* (2008) 158(2):255–60. doi: 10.1530/EJE-07-0576
41. Bhattacharya SM. Metabolic syndrome in females with polycystic ovary syndrome and International Diabetes Federation criteria. *J Obstet Gynaecol. Res* (2008) 34(1):62–6. doi: 10.1111/j.1447-0756.2007.00685.x
42. Dunaif A, Legro RS. Prevalence and Predictors of Risk for Type 2 Diabetes Mellitus and Impaired Glucose Tolerance in Polycystic Ovary Syndrome-Authors' Response. *J Clin Endocrinol Metab* (1999) 84(8):297–2976. doi: 10.1210/jc.84.8.2975
43. Orio F, Palomba S, Colao A. Cardiovascular risk in women with polycystic ovary syndrome. *Fertil. Steril.* (2006) 86:S20–1. doi: 10.1016/j.fertnstert.2006.03.003
44. Page H, Morgan C, Lappin J, Dazzan P, Murray R, Fearon P. @ a Systematic Review of Coping in Schizophrenia. *Schizophr Res* (2008) 102(1–3):218. doi: 10.1016/S0920-9964(08)70659-5
45. Johannesen L, Garnett C, Luo M, Targum S, Sørensen JS, Mehrotra N. Quantitative Understanding of QTc Prolongation and Gender as Risk Factors for Torsade de Pointes. *Clin Pharmacol Ther* (2018) 103(2):304–9. doi: 10.1002/cpt.783
46. Axelsson S, Hägg S, Eriksson AC, Lindahl TL, Whiss PA. In vitro effects of antipsychotics on human platelet adhesion and aggregation and plasma coagulation. *Clin Exp Pharmacol Physiol* (2007) 34(8):775–80. doi: 10.1111/j.1440-1681.2007.04650.x
47. Joffe H, Hayes FJ. Menstrual Cycle Dysfunction Associated with Neurologic and Psychiatric Disorders. *Ann N Y Acad Sci* (2008) 1135(1):219–29. doi: 10.1196/annals.1429.030
48. De Hert M, Cohen D, Bobes J, Cetrovik-Barmas M, Leucht S, Ndetei DM, et al. Physical illness in patients with severe mental disorders. II. Barriers to care, monitoring and treatment guidelines, plus recommendations at the system and individual level. *World Psychiatry* (2011) 10(2):138–51. doi: 10.1002/j.2051-5545.2011.tb00036.x
49. Azziz R, Carmina E, Chen Z, Dunaif A, Laven JSE, Legro RS, et al. Polycystic ovary syndrome. *Nat Rev Dis Prim* (2016) 2. doi: 10.1038/nrdp.2016.57

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

SYSTEMATIC REVIEW
published: 11 June 2020
doi: 10.3389/fpsyt.2020.00513

Cortisol Responses to Naturally Occurring Psychosocial Stressors Across the Psychosis Spectrum: A Systematic Review and Meta-Analysis

Alexis E. Cullen[1*], *Sushma Rai*[1], *Meghna S. Vaghani*[1], *Valeria Mondelli*[2,3] *and Philip McGuire*[1,3]

[1] Department of Psychosis Studies, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, United Kingdom, [2] Department of Psychological Medicine, Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, United Kingdom, [3] NIHR Biomedical Research Centre, South London and Maudsley NHS Foundation Trust, London, United Kingdom

OPEN ACCESS

Edited by:
Grazia Rutigliano, University of Pisa, Italy

Reviewed by:
Fabian Streit, University of Heidelberg, Germany
Thomas Ruben Vaessen, KU Leuven, Belgium

***Correspondence:**
Alexis E. Cullen
alexis.cullen@kcl.ac.uk

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *26 March 2020*
Accepted: *19 May 2020*
Published: *11 June 2020*

Citation:
Cullen AE, Rai S, Vaghani MS, Mondelli V and McGuire P (2020) Cortisol Responses to Naturally Occurring Psychosocial Stressors Across the Psychosis Spectrum: A Systematic Review and Meta-Analysis. Front. Psychiatry 11:513. doi: 10.3389/fpsyt.2020.00513

Background: Individuals with established psychosis and those at high-risk for the disorder have been found to show abnormalities within the hypothalamic-pituitary-adrenal (HPA) axis, including elevations in basal and diurnal cortisol, but a blunted cortisol awakening response. However, the extent to which these features are associated with psychosocial stressors encountered in the natural environment (which are known to be more commonly experienced by these groups, and more distressing) is currently unclear. We therefore conducted a systematic review and meta-analysis to investigate the concordance between naturally-occurring psychosocial stressors and cortisol levels in these populations.

Methods: PubMed, PsycINFO, and EMBASE were searched up to November 2019 to identify studies examining the concordance between psychosocial stressors and cortisol in healthy controls and individuals on the psychosis spectrum (patients with established psychosis and/or high-risk individuals). An overall meta-analysis (including data for all stressor-cortisol pairings) was performed to determine the degree of concordance irrespective of group status, with meta-regression employed to test whether the degree of concordance differed in established psychosis and high-risk groups compared to controls. Planned stratified analyses were then performed to examine group differences (where established psychosis and high-risk groups were combined) within individual stressor-cortisol pairings.

Results: Eighteen studies (16 datasets) were eligible for inclusion. The overall model, comprising 134 effect sizes, showed that stressors and cortisol measures were only weakly correlated [r=0.05 (95% CI: -0.00 to 0.10), p=0.059] and that neither established psychosis status (r=0.01, p=0.838) nor high-risk status (r=0.02, p=0.477) had a significant effect of the strength of correlation. In stratified analyses, significant differences between

healthy controls and psychosis spectrum groups were observed for only one of the six stressor-cortisol pairings examined, where life event exposure and diurnal cortisol were positively correlated in controls [*r*=0.25 (95% CI: 0.01 to 0.46)], but negatively correlated in the psychosis spectrum group [*r*=-0.28 (95% CI: -0.49 to -0.04)].

Conclusions: Overall, we observed poor concordance between naturally-occurring psychosocial stressors and cortisol irrespective of stressor type, cortisol measure, or group status. We consider a range of methodological factors that may have obscured the ability to detect "true" associations and provide recommendations for future studies in this field.

Keywords: schizophrenia, psychosis, hypothalamic-pituitary-adrenal axis, stress responsivity, cortisol, concordance, trauma, adversity

INTRODUCTION

Research conducted over the past four decades has provided evidence to suggest that psychosocial stress contributes to the onset and exacerbation of psychosis. Meta-analyses indicate that major life events and childhood trauma (typically encompassing experiences of neglect and abuse) are associated with increased risk of developing psychotic disorders (1, 2). Furthermore, in patients with established psychosis, minor daily stressors have been associated with psychotic symptom intensity (3–6) and illness relapse (7, 8). More recently, focus has shifted to individuals identified as being at increased risk for psychosis by virtue of a family history (FHx) of illness and/or clinical features, the latter including individuals who fulfil ultra-high risk (UHR) criteria, present with schizotypal personality traits, or report psychotic experiences (PEs). Studying these populations overcomes some of the potential confounds that often arise in studies of patients with established psychosis (e.g., retrospective recall, antipsychotic medication, and stress associated with illness onset). Such studies demonstrate that high-risk individuals are also more frequently exposed to childhood trauma, major life events, and minor daily stressors, experience greater distress in relation to these events, and report higher levels of perceived stress compared to their healthy peers (4, 9–17). Although these studies lend support to the notion that stress may play a causal role in the development of psychosis, the biological mechanisms underlying this relationship remain unclear.

One leading hypothesis, the neural diathesis-stress model (18–20), proposes that the hypothalamic-pituitary-adrenal (HPA) axis plays a major role in mediating the effects of stress on psychosis development. Specifically, it is hypothesized that individuals with increased vulnerability for psychosis are more sensitive to the effects of psychosocial stress due to abnormalities within the HPA axis (e.g., HPA hyperactivity/dysregulation or increased glucocorticoid sensitivity) and that these HPA axis abnormalities in turn trigger the onset of psychosis by acting on dopaminergic and glutamatergic pathways (20). The model is supported by individual studies and meta-analyses reporting elevations in basal and diurnal cortisol (21–28), a blunted cortisol awakening response [CAR (23, 29–31)], and enlarged pituitary volume (23, 32, 33) among high-risk individuals and psychosis patients. It is important to note that these measures represent different attributes of HPA axis function: While the increases in basal/diurnal cortisol levels and pituitary volume likely reflect chronic hyper-activation of the HPA axis, it is thought that the CAR is a distinct HPA axis component driven by endogenous processes, possibly related to anticipation of the demands of the upcoming day (34, 35). Together, these findings imply that HPA axis dysfunction characterizes individuals on the psychosis spectrum; however, evidence linking these HPA axis changes to psychosocial stressors is lacking.

Several systematic reviews/meta-analyses have been published concerning the "stress response" in psychosis [for an overview see (36)]; however, the majority have considered HPA axis abnormalities and psychosocial stressors in isolation rather than the concordance (i.e., degree of association) between these measures. Of those that specifically examined HPA axis responsivity to stress (37–39) all three looked exclusively at responses to acute psychosocial stressor tasks, concurring that individuals with schizophrenia and psychosis show a blunted cortisol response relative to healthy controls. While recent work indicates that laboratory-based psychosocial stressor tasks can be considered ecologically valid [i.e., associations observed between cortisol responses to these tasks and responses to real-world examination stress (40)] these "performance-related" stressors likely differ in both nature and frequency to the stressors shown to be etiologically relevant to psychosis (e.g., major life events and childhood trauma). Understanding the concordance between psychosocial stressors encountered in the natural environment and HPA axis function is important for several reasons: If psychosocial stressors are found to correlate with HPA axis markers, then this provides a plausible biological mechanism for how stress might contribute to the onset and maintenance of psychosis, further strengthening the case for this being a causal factor. Similarly, if high concordance between these measures is found, then this supports the notion that the HPA axis abnormalities observed among individuals on the psychosis spectrum are driven by psychosocial stressors, as opposed to being simply epiphenomena (perhaps indicative of global metabolic abnormalities). Furthermore, comparing the degree of concordance in healthy individuals and those on the

psychosis spectrum will help to clarify the extent to which abnormal stress responsivity (either hyper- or hypo-responsivity) is a feature of psychosis. Such work may ultimately enable targeted interventions to be delivered to those who are more sensitive, at least biologically, to the effects of psychosocial stress.

To this end, we conducted a systematic review and meta-analysis of studies examining the concordance between naturally-occurring psychosocial stressors and HPA axis function among individuals on the psychosis spectrum. Given that cortisol is the most widely used measure of HPA axis function (18, 35), we restricted our review to studies examining stressor-cortisol concordance only. Our primary aim was to test whether the degree of concordance differed among healthy individuals and those on the psychosis spectrum (patients with established psychosis and high-risk individuals). Meta-analytic evidence indicates that the degree and direction of concordance varies across different cortisol measures and stressor types; for example, chronic stress has been found to correlate positively with overall diurnal output, afternoon/evening levels, and the CARi (increase in cortisol following awakening) but negatively with basal morning levels (41, 42). We were therefore concerned that combining all effect sizes in a single analysis could lead to a neutral effect overall. To mitigate against this, in addition to performing an overall meta-analysis (which included all effect sizes), we also conducted analyses within individual stressor-cortisol pairings.

As this was the first review to address these specific questions, we tested four possible (and competing) hypotheses regarding the pattern of findings across controls and psychosis spectrum groups (see **Figure 1**). The "normal/adaptive" hypothesis (blue) proposes that the degree of concordance between naturally-occurring psychosocial stressors and cortisol is moderate-to-strong in both healthy and psychosis spectrum individuals, but that there is no difference in the degree of concordance across groups. If supported, this would imply that the HPA axis abnormalities observed among psychosis spectrum groups reflects a normal/adaptive response to the high levels of psychosocial stress experienced by this population, thus, the HPA axis itself is responding to stress appropriately. The "hyper-responsive" hypothesis (yellow) proposes that psychosocial stressors will be associated with cortisol in both groups, but that this relationship will be stronger among those on the psychosis spectrum. Support for this hypothesis would suggest that psychosocial stressors measured in concordance studies are, at least partially, responsible for the HPA axis abnormalities in psychosis spectrum individuals, but that the HPA axis responds excessively to these stressors in this population. The reverse situation is represented by the "hypo-responsivity" hypothesis (green), whereby the degree of concordance is moderate-to-high in controls but is blunted (perhaps due to glucocorticoid sensitization) in psychosis spectrum groups. If supported, this would suggest any HPA axis abnormalities observed in the psychosis spectrum group occur despite the fact that this group experiences greater psychosocial stress exposure/distress. Alternatively, the pattern observed may be that presented in the "unrelated" hypothesis (red), whereby concordance in both groups is similar but weak. Such findings would indicate that the psychosocial stressors commonly measured in concordance studies are unrelated to HPA axis function, implying that any cortisol abnormalities observed in psychosis spectrum groups must be driven by other factors (e.g., unmeasured stressors, genetic variations, or a manifestation of a globally dysregulated physiological system). However, poor concordance could also reflect measurement error (of psychosocial stressors, cortisol levels, or both). These

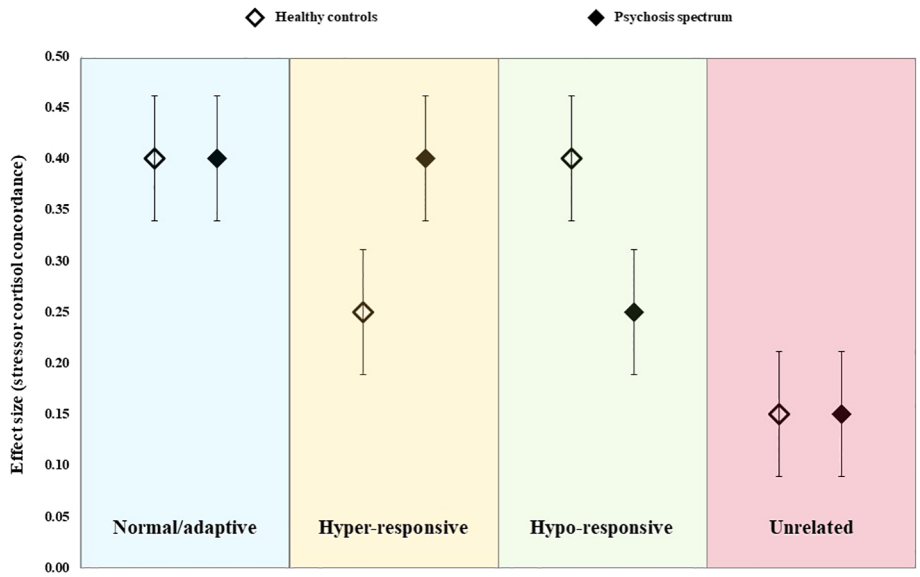

FIGURE 1 | Schematic representation of the four alternative hypotheses tested in the current meta-analysis.

competing hypotheses were tested statistically by comparing pooled effect sizes in psychosis spectrum and healthy control groups.

METHODS

The protocol for this systematic review and meta-analysis was registered prospectively on PROSPERO (CRD42019159290), the search strategy and reporting was conducted in compliance with the Meta-Analysis of Observational Studies in Epidemiology (MOOSE) guidelines (43).

Search Strategy

PubMed and Ovid (PsycINFO, EMBASE) databases were searched for articles published up to November 2019 using the following terms: (genetic high risk) OR family history) OR prodrom*) OR at risk mental state) OR clinical high risk) OR ultra high risk) OR sibling*) OR offspring*) OR relative*) AND (psychotic) OR psychosis) OR schizophren*) OR schizotyp*) OR psychotic experiences) OR subclinical psycho*) OR psychotic) OR psychosis) OR schizophren*) OR schizotyp*) OR psychotic experiences) OR subclinical psycho*) AND (trauma) OR advers*) OR neglect) OR stress*) OR hassles) OR life events) OR maltreatment) OR abuse) AND (HPA axis) OR stress response) OR cortisol) OR glucocorticoid). The searches were performed independently by two researchers (SR, MV). No restrictions were applied for year of publication. Reference lists of eligible studies and relevant reviews were manually searched to identify additional studies.

Study Selection

We included observational studies (cross-sectional, case-control, and cohort studies) that examined the relationship between naturally-occurring psychosocial stressors and cortisol in individuals on the psychosis spectrum (patients with established psychosis or those at elevated risk for psychosis) and healthy controls. Patients with diagnoses of first-episode psychosis, multi-episode psychosis, schizophrenia, and schizoaffective disorder were eligible for the established psychosis group. Consistent with a previous review by our group (33), we defined high-risk individuals as those who met criteria for one of the following groups: i) "ultra-high risk" for psychosis [also known as "clinical high-risk" or an "at-risk mental state" (44, 45)] as determined using a well-defined assessment tool, ii) family history (FHx), as conferred by a first- or second-degree relative with psychosis, iii) schizotypal personality disorder (SPD) or high scores on a schizotypal personality checklist, or iv) presence of psychotic-experiences (PEs: also known as psychotic-like experiences or subclinical psychotic symptoms).

All articles identified in the search were independently rated for eligibility by two authors (SR, MV). Disagreements were resolved following a discussion with a third author (AC). Article titles and abstracts were first screened to remove those that were clearly not relevant to the review; a full text review was then performed for all potentially eligible articles (both phases performed in duplicate). Original studies meeting the following criteria were eligible: i) inclusion of a psychosis spectrum group (established or high-risk, as defined above) and healthy control group, ii) assessment of naturally-occurring psychosocial stressors (e.g., exposure to daily stressors, life events, trauma, adversity, distress associated with these events, or perceived stress), iii) measurement of cortisol (basal, diurnal, or CAR as measured in saliva, blood, or hair), iv) concordance between psychosocial stressor and cortisol reported, and v) published in English in a peer-reviewed journal. Articles that did not include a control group or report the association between psychosocial stressors and cortisol were excluded. Conference abstracts were not included (as none included sufficient data), but where relevant abstracts were identified, additional searches (by author name) were conducted to determine whether a full text article had been subsequently published and/or corresponding authors were contacted for further details. Where studies with potentially overlapping samples were identified we contacted study authors to clarify.

Data Extraction

Two researchers (SR, MV) extracted study characteristic data from eligible articles, this included: author(s), year of publication, psychosis spectrum group(s), psychosis spectrum group recruitment/identification method, sample size, mean age and sex of psychosis spectrum and control groups, proportion treated with antipsychotic medication, stressor measurement method, cortisol measure (tissue and type), and lapse-of-time between stress measurement and cortisol collection. Researchers were not blind to the names of authors, journals, or institutions. A third author (AC) then checked all study details for accuracy and extracted data necessary for effect size computation. The latter varied across studies and included any statistical value representing a within-group measure of the association between psychosocial stressors and cortisol (e.g., correlation coefficient, beta coefficient, or mean and standard deviation of cortisol for participants exposed and not exposed to stressor). Where these data were not provided for each group separately, we contacted study authors for additional details (46–56) which were provided in all instances.

Assessment of Study Quality

A modified version of the Newcastle-Ottawa scale [NOS (57)], a quality appraisal tool for case-control, cohort, and cross-sectional studies, was created for the purposes of the review to capture pertinent features. The modified tool included 11 items covering three domains (selection, comparability, exposure/outcome) and was designed to be applicable to any of the above study designs (see **Supplementary Table 1** for a detailed description of the items). The maximum score available across the 11 items was 16. All studies were rated independently against these criteria by two authors (AC, SR) with disagreements resolved by discussion.

Statistical Analyses

All computations and statistical analyses were conducted using Stata version 16 (58). In order to facilitate pooling of effect sizes (representing the association between psychosocial stressors and cortisol, in each group separately) it was necessary to first derive a common effect size for all studies. As correlational coefficients (r) were the most commonly-reported effect sizes, and are easily-interpretable [values of 0.1, 0.3, and 0.5 reflecting small, moderate, and large magnitudes of effect, respectively (Cohen, 1988)] we requested r values from study authors where these were not provided, or derived these from alternative statistics where possible. Specifically, for studies reporting means and standard deviations (SD), we first computed standardized mean differences (d), representing the difference in cortisol levels between those with and without stressor exposure, which were then converted to correlation coefficients (59). As beta coefficients (B) derived from regression models examining the effect of stressors (measured as continuous variables) on cortisol could not be converted to r values without prior standardization of variables, effect sizes from studies reporting these values (53, 60) could not be included in meta-analyses; these results were, however, retained in the systematic review. In order to perform meta-analyses, all correlation coefficients were transformed to a Fisher's z score (59); for presentation purposes, pooled z scores and associated confidence intervals were reverse-transformed to the original units for ease of interpretation.

As nearly all studies included in the review provided multiple effect sizes derived from the same study sample, thereby violating the independence assumption, it was necessary to account for dependence of effects. For the overall meta-analysis (which included data for all stressor-cortisol pairings) we therefore used robust variance estimation (RVE) which accounts for correlated effects (61). We first derived the unconditional overall effect size (degree of concordance), irrespective of group status, by estimating the constant term only (62). This analysis was performed to determine whether the pooled correlation across all groups and studies was statistically different from zero. Next we tested the effect of group status by including two dummy variables, "established psychosis" and "high risk", to determine whether the effect sizes (degree of concordance) in these groups differed from controls. To derive pooled effect sizes for all groups (including the control group) we then performed stratified analyses to derive the mean effect size in each group separately. Finally, in a univariate meta-regression model, we tested the effect of NOS scores on effect sizes. For all RVE models we applied a random effects weighting scheme which assumes that effect sizes from the same study are correlated with each other. The assumed value of rho was set at 0.5 after sensitivity analyses performed on the entire sample showed that there were no differences when rho values of 0.1, 0.3, 0.5, and 0.8 were applied. Heterogeneity was assessed by means of the Tau statistic (62), which provides an estimate of the standard deviation of the true effect (59). Small sample bias (i.e., publication bias) was assessed visually by means of a funnel plot but was not tested statistically due to dependence of effects.

As we anticipated that the degree and direction of concordance would vary across different cortisol measures and stressor types (41, 42), we next performed planned stratified analyses to examine group differences within individual stressor-cortisol pairings. However, as this greatly reduced the number of studies contributing to each analysis, and RVE performs poorly when the degrees of freedom are small (63), it was necessary to employ a different approach to deal with dependent effects. Thus, for studies that included more than one psychosis spectrum group (for example, an established psychosis group and a high-risk group), we first computed within-study pooled effect sizes for each stressor-cortisol pairing, which combined data from all psychosis spectrum groups. As such, each study contributed only two effect sizes to each stressor-cortisol pairing: one for the control group, and the other a pooled effect size derived from all psychosis spectrum groups. Stratified analyses were performed when three or more studies were available using the default settings within Stata 16 (random effects model with restricted maximum likelihood weighting applied). We used the subgroup command which enables the derivation of subgroup specific pooled effect sizes (and heterogeneity estimates) and a between-group comparison of effect sizes. Statistical significance for all analyses was set at $p < 0.05$ (two-tailed). Heterogeneity was assessed *via* the Cochran Q and the I^2 statistics, where classification of the latter as likely unimportant (0%–40%), moderate (30%–60%), substantial (50%–90%), or considerable (75%–100%) is dependent on the magnitude and/or direction of effects and statistical significance (Cochran Q) of heterogeneity (64).

RESULTS

Search Results

After removing duplicates, 3,354 studies were identified in the initial search (**Figure 2**). Of these, 3,141 were excluded following a preliminary review of the title and abstract, with a full-text review performed for 213 articles. After screening studies for eligibility, 18 met criteria for inclusion in the review and meta-analysis (31, 46–56, 60, 65–69), all of which were published in the last decade. Details of the 18 studies are provided in **Table 1**.

Partially overlapping samples were identified for five studies. Three studies authored by Labad and colleagues (48–50) included overlapping study groups and stressor/cortisol measures. The corresponding author provided a combined dataset that included data for the largest available UHR, first-episode psychosis (FEP), and healthy control subgroups which was used for all meta-analyses (studies retained as separate when describing characteristics). The combined dataset is herein referred to as (70). Two studies included participants from the UK Genetic and Psychosis (GAP) study (46, 65); as both examined the association between cortisol and childhood trauma, we used the largest sample for this analysis (46); however, the earlier study (smaller sample) was retained as it included additional stress measures not examined in the later study.

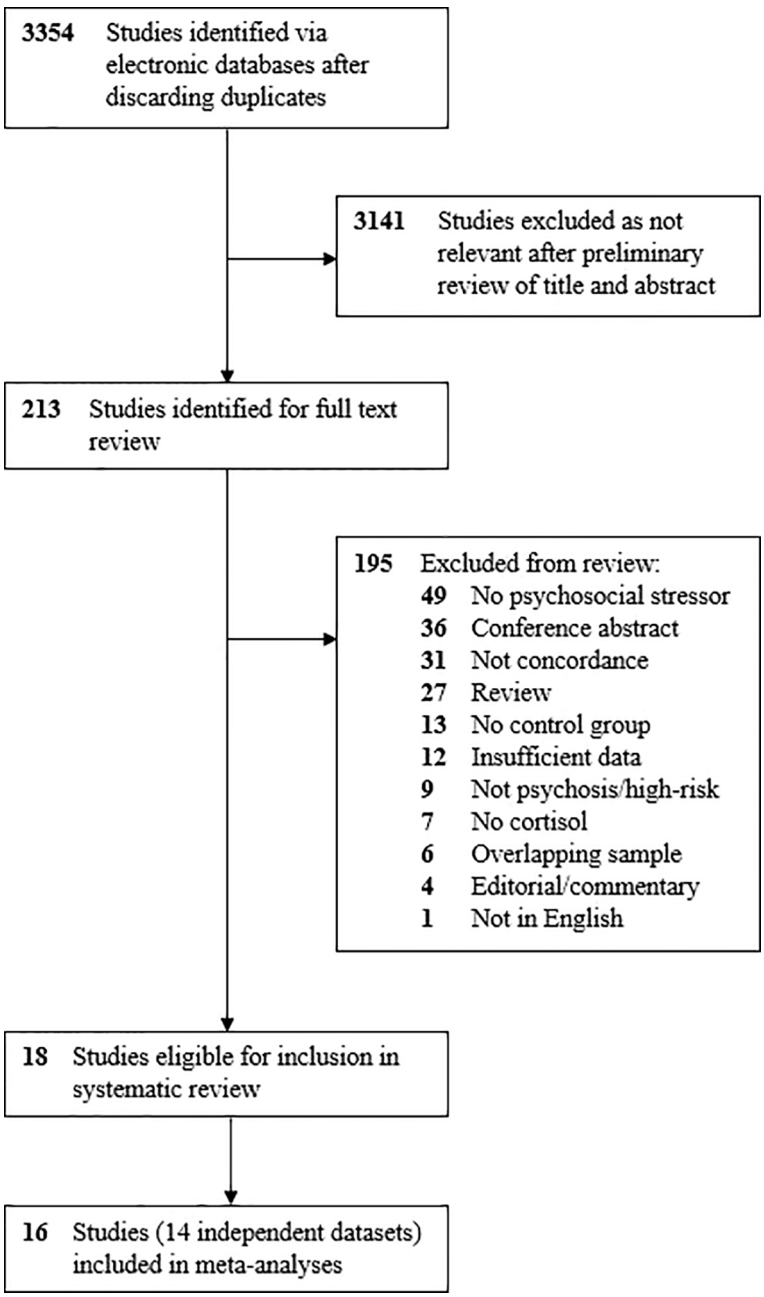

FIGURE 2 | Search process.

Study Characteristics

Group Status and Psychosis Spectrum Definitions

As dictated by our inclusion criteria, all studies included a healthy control group and at least one psychosis spectrum group. Four studies included both high-risk and established psychosis subgroups (48, 51, 53, 55), eight included established psychosis groups only (46, 49, 52, 54, 65, 67–69), and six examined high-risk groups only (31, 47, 50, 56, 60, 66). The most commonly-examined established psychosis subgroup was FEP (n=5), a further study examined recent-onset psychosis (49) which included FEP patients, two studies included patients with psychotic disorder where the stage of illness was not indicated (53, 67), three studies included patients with schizophrenia (52, 54, 69), while a further study distinguished between patients with early and chronic schizophrenia (55). With regards to high-risk groups, these were most commonly youth meeting UHR criteria (n=5) and individuals with a FHx of psychosis/schizophrenia (n=4). One of the five studies examining young people at UHR

TABLE 1 | Characteristics of studies included in systematic review and meta-analyses.

Author	Group	*N*	Age[1]	% Male	Stress type (measure)	Cortisol measures
Aas et al. (69)	SCZ	28	33.6	54%	Childhood trauma (CTQ)	Hair (3 cm)
	HC	94	35.3	51%		
Ciufolini et al. (46)	FEP	169	28.1	65%	Childhood trauma (CECA)	Saliva (CAR and diurnal)
	HC	133	26.9	36%		
Collip et al. (60)[2]	FHx	60	28.8	37%	Daily event stress (ESM)	Saliva (ESM)
	HC	63	33.3	29%		
Cullen (31)	PE	33	12.8	70%	Negative life events; Daily hassles	Saliva (CAR and diurnal)
	FHx	22	13.3	50%		
	HC	40	13.1	43%		
Faravelli et al. (67)	PD	54	43.7	56%	Childhood trauma (CECA)	Saliva (basal morning and evening)
	HC	102	43.5	52%		
Garner et al. (68)	FEP	39	20.6	67%	Perceived stress (PSS)	Serum (basal morning)
	HC	25	22.5	84%		
Heinze et al. (2015)	UHR^{+1a}	30	21.0	13%	Perceived stress (PSS), Childhood trauma (CTQ)	Hair (3 cm)
	HC	28	20.0	7%		
Hirt et al. (55)	UHR	29	22.5	79%	Childhood trauma (MACE)	Hair (3 cm)
	ESCZ	34	24.0	65%		
	CSCZ	24	35.5	79%		
	HC	38	24.0	63%		
Labad et al. (50)[3]	UHR	39	22.3	69%	Perceived stress (PSS); Stressful life events (HRSS)	Saliva (basal morning and CAR); Serum (basal morning)
	HC	44	23.2	65%		
Labad et al. (48)[3]	UHR	21	22.1	71%	Perceived stress (PSS); Stressful life events (HRSS)	Saliva (CAR and diurnal slope)
	FEP	34	23.9	71%		
	HC	34	24.3	71%		
Labad et al. (49)[3]	ROP	56	24.8	63%	Childhood trauma (CTQ); Stressful life events (HRSS)	Saliva (CAR and diurnal slope)
	HC	47	23.8	53%		
Mondelli et al. (65)	FEP	50	29.2	64%	Life events (BLEQ); Perceived stress (PSS); Childhood trauma (CECA)	Saliva (CAR and diurnal)
	HC	36	27.3	72%		
Moskow (2016)	UHR	348	15.6	56%	Daily stress (DSI)	Saliva (basal morning)
	HC	93	15.2	65%		
Nordholm et al. (51)	UHR	41	23.9	43%	Perceived stress (PSS); Life events (BLEQ)	Saliva (CAR and diurnal)
	FEP	40	24.1	55%		
	HC	46	24.7	58%		
Seidenfaden et al. (52)	SCZ	37	32.3	46%	Childhood trauma (CATS); Perceived stress (PSS)	Plasma (basal morning); Saliva (diurnal)
	HC	39	31.7	51%		
Soder et al. (56)	PE	43	26.2	33%	SES; Migration; Minority status; Perceived discrimination; Social undermining; Ostracism experience; Child abuse; Bullying victimization; Trauma	Hair (3 cm)
	FHx	32	33.3	31%		
	HC	35	27.3	37%		
Streit et al. (54)	SCZ	159	40.3	36%	Perceived stress (SSCS)	Hair (3 cm)
	HC	82	32.9	40%		
Vaessen et al. (53)[2]	FHx	47	42.9	36%	Daily event stress (ESM)	Saliva (ESM)
	PD	73	43.8	55%		
	HC	67	39.9	52%		

[1]Mean age in years; [2] Data from these studies are not included in meta-analyses as a common effect size could not be derived from these studies; [3] Due to partially-overlapping samples and measures, the corresponding author provided a single dataset comprising the largest study groups which is used in all subsequent analyses in this review (70). FEP, first-episode psychosis; HC, healthy control; PE, psychotic experiences; FHx, family history of psychosis; SCZ, schizophrenia; UHR, ultra-high risk; UHR^{+1a}, group includes help-seeking youth meeting UHR (stage 1b) and stage 1a criteria; ESCZ, early-stage schizophrenia; CSZC, chronic schizophrenia; ROP, recent-onset psychosis; PD, psychotic disorders; CECA, Childhood Experience of Care and Abuse Questionnaire; ESM, experience sampling method; PSS, perceived stress scale; MACE, Maltreatment and Abuse Chronology of Exposure; HRSS, Holmes-Rahe Social Readjustment Scale; CTQ, Childhood Trauma Questionnaire; BLEQ, Brief Life Events questionnaire; DSI, Daily Stress Inventory; CATS, Child Abuse and Trauma Scale; SES, socioeconomic status; CAR, cortisol awakening response.

also included those who presented with clinical stage 1a symptoms (47). Soder and colleagues included a high-risk group comprising individuals who scored above threshold on a measure of psychotic experiences (PEs), whilst a further study (31) included children who at age 9–12 years presented with PEs in combination with other antecedents of schizophrenia.

Sample Sizes and Demographic Characteristics

The total number of healthy controls, established psychosis patients, and high-risk individuals was N=1046, N=797, and N=745, respectively. Control groups varied in size, ranging from 25 (68) to 133 (46), the latter study also comprised the largest established psychosis group (n=169). With regards to high-risk group sizes, the smallest comprised of 21 UHR individuals (48) with the largest including 348 UHR youth from the NAPLS-2 study (66). Participants in the high-risk groups were the youngest on average (mean age = 23.7 years; range: 12.8 to 42.9 years), followed by healthy controls (mean age = 27.2 years; range 13.1 to 43.5 years), with the oldest being those with established psychosis (mean age = 31.1 years; range 20.6 to 43.8 years). When averaged across studies, the percentage male was broadly similar across healthy control, established psychosis,

and high-risk groups (52%, 60%, and 49%, respectively); however, this varied substantially across studies, from as low as 7% in the control group of one study (47) to 84% in the control group of another (68).

Psychosocial Stress Measures

Perceived stress and childhood trauma were the most common types of psychosocial stressor examined across studies (n=8 for both), followed by life event exposure (n=6). There was consistency across studies in the measures of perceived stress employed, with most studies using the Perceived Stress Scale (71). For childhood trauma, there was less consistency, with the most commonly-used measures being the Childhood Experience of Care and Abuse (CECA) questionnaire (72) and the Childhood Trauma Questionnaire (73). Daily stressors were examined in two studies (albeit using different measures), one reported both exposure and distress scores separately (31), whilst the other reported a single score that accounted for both exposure and associated distress (66); the former study also reported distress scores (both current distress and distress at the time of the event) for negative life events. The experience sampling method (ESM), a structured diary technique in which participants are prompted at multiple time-points throughout the day to report the extent to which their current activity is stressful, was used in two studies (53, 60). One study examined nine individual psychosocial stressors (56), including, socioeconomic status, migration, minority status, perceived discrimination, social undermining, ostracism experience, bullying victimization, childhood abuse, and trauma experiences.

Cortisol Measures

Across the 18 studies, cortisol was most frequently measured in saliva (n=12), two of these studies also examined cortisol in blood samples [serum (50); plasma (52)] with a further study examining serum only (68). Hair sampling was the second most common method used to determine cortisol levels (n=5), with all studies obtaining at least one 3 cm segment for analysis (47, 54–56, 69). With regards to the timing of cortisol collection, basal samples (saliva, plasma, and serum) were the most commonly-examined, with four studies obtaining a single measure (50, 52, 67, 68), typically in the morning, and a further study deriving a mean cortisol value from three samples obtained at 1-h intervals (66). The cortisol awakening response (CAR) was measured in saliva in seven studies (31, 46, 48–51, 65): All of these studies computed the area-under-the-curve with respect to increase (AUCi) which captures the increase in cortisol from awakening levels; one of these studies (46) also calculated the AUC with respect to ground (AUCg) representing the total amount of cortisol secreted in the hour following awakening. Diurnal cortisol was examined in saliva in six studies, five of which calculated the total cortisol output over the entire day using the AUCg (31, 46, 51, 52, 65), with the remaining study calculating the diurnal slope between samples collected at awakening and late evening (49). Two studies used the ESM method to obtain multiple salivary cortisol samples throughout the day (53, 60) with repeated observations handled using multilevel (hierarchical) models.

Quality Assessment

Study quality scores are presented in **Table 2**. Total scores ranged from 6 to 12 (max=16) with an average score of 8 across the 18 studies. Sample size was a concern for most studies; only three were awarded a single point for this item and none were awarded two points. Of the three studies obtaining a single point, two (46, 66) included at least 85 participants in each group and so were sufficiently large to detect a moderate correlation with 80% power at the 0.05 level. Only one study conducted an *a priori* power calculation (56); however, this was used to determine the total sample size (comprising controls, FHx, and PE groups) and so each individual group did not meet the criteria outlined above (n≥85). With regards to participants, all studies used an adequate/validated measure to confirm diagnosis (established psychosis) or high-risk status; however, only 11 studies applied the same measures to the healthy control group to confirm that these participants were free from psychotic disorder and/or did not meet high-risk criteria. A major area of weakness across the studies was the extent to which psychosis spectrum and control groups were representative/unbiased. In general, very few details were available to be able to assess the extent to which patients with established psychosis and at-risk groups were representative of the target populations, and none reported that patients were randomly selected from a registry. However, two studies reported that they attempted to recruit all patients who were newly admitted to psychiatric services operating within a large catchment area (65, 67) and so were awarded a point for this item. Similarly, details of methods used to identify and recruit controls were minimal in most studies, with only one study (60) reporting that controls were selected through random mailings to addresses in the residential areas of patients and siblings. Only three studies (31, 47, 52) reported the response rate (proportion of individuals approached who agreed to participate) for any group. One strength was that all studies either deliberately matched psychosis spectrum and control groups on age and/or sex (two points) or compared groups on these characteristics (one point).

With regards to measures, all studies employed a widely used measure of psychosocial stress (one point), with seven reporting the reliability/validity of these measures (two points). Descriptions of the cortisol collection procedure varied from brief to very detailed, with half of the studies providing a reference for the procedure and/or assessing compliance. Only three studies reported details of the timing of cortisol collection with regards to psychosocial stress measurement; two of these studies used the ESM method, where cortisol samples were collected within 10 min of the event stress rating (53, 60), the other study reported the mean lapse-of-time between completion of stress measures and collection of cortisol samples (31). Assessment of potential confounders varied across studies, ranging from very few confounders examined (age, sex, and

TABLE 2 | Study quality ratings with regards to assessment of stressor-cortisol concordance.

Study	Sample size adequate/determined *a priori* (max 2)	Psychosis spectrum definition valid (max 1)	Psychosis spectrum cases unbiased (max 1)	Control group unbiased (max 1)	Control status confirmed (max 1)	Response rate reported/same in both groups (max 1)	Psychosis spectrum and control groups matched (max 2)	Stress measure reliable/valid (max 2)	Cortisol measure reliable/valid (max 2)	Lapse of time between measures reported (max 1)	Potential confounds examined (max 2)	Total score (max 16)
Aas et al. (69)	0	1	0	0	1	0	1	2	1	0	1	7
Ciufolini et al. (46)	1	1	0	0	1	0	2	1	2	0	1	9
Collip et al. (60)	0	1	0	1	1	0	2	2	2	1	2	12
Cullen et al. (31)	0	1	0	0	1	1	1	2	2	1	2	11
Faravelli et al. (67)	0	1	1	0	0	0	2	1	1	0	2	8
Garner et al. (68)	0	1	0	0	1	0	2	1	1	0	0	6
Heinze et al. (47)	0	1	0	0	0	1	2	1	2	0	2	9
Hirt et al. (55)	0	1	0	0	0	0	1	1	2	0	2	7
Labad et al. (50)	0	1	0	0	0	0	2	1	1	0	2	7
Labad et al. (48)	0	1	0	0	0	0	2	1	1	0	2	7
Labad et al. (49)	0	1	0	0	0	0	2	2	1	0	2	8
Mondelli et al. (65)	0	1	1	0	1	0	1	1	2	0	2	9
Moskow (66)	1	1	0	0	1	0	1	2	1	0	0	7
Nordholm et al. (51)	0	1	0	0	1	0	2	2	1	0	1	8
Seidenfaden et al. (52)	0	1	0	0	1	1	1	1	1	0	0	6
Soder et al. (56)	1	1	0	0	1	0	1	1	1	0	2	8
Streit et al. (54)	0	1	0	0	0	0	1	2	2	0	0	6
Vaessen et al. (53)	0	1	0	0	1	0	1	1	2	1	2	9

one other measure: n=4) to a wide range of variables that were compared across groups and/or examined in relation to cortisol/stress measures (n=11).

Description of Stressor-Cortisol Concordance Findings Across Studies

From the 16 datasets, 139 separate effect sizes were available (124 correlation coefficients, 10 standardized mean differences converted to correlation coefficients, and five beta coefficients). Of these, 123 (88%) were not statistically significant (indicating no association between stressor and cortisol), 11 (8%) were statistically significant positive associations, and five (4%) were significant negative associations. At the study level, nine (56%) of the datasets included at least one statistically significant association (31, 46, 53, 55, 56, 60, 65, 67, 70). With regards to magnitude of effect irrespective of sign (positive or negative), after excluding the five beta coefficients (which were not standardized and therefore not comparable), 53 (40%) were negligible, 62 (47%) were small, 14 (11%) were moderate, and three (2%) were large effect sizes.

Basal Cortisol

Basal cortisol was examined in six studies, yielding 20 separate effect sizes (morning=18; evening=2), only three of which were statistically significant. The pattern of findings varied across studies and stressor types. In one large study of patients with psychotic disorder (67), morning salivary cortisol showed a significant positive association with childhood trauma in patients [r=0.29 (95% CI: 0.02 to 0.52)] that was not observed in healthy controls [r=-0.06 (95% CI: -0.25 to 1.33)], yet evening cortisol was significantly associated with childhood trauma in controls [r=0.22 (95% CI: 0.03 to 0.40)] but not patients [r=0.14 (95% CI: -0.13 to 0.39)]. In contrast, Labad and colleagues (70), who assessed morning basal cortisol in plasma, observed no relationship with childhood trauma, instead finding a significant negative relationship with stressful life events in controls [r=-0.30 (95% CI: -0.54 to -0.01)] that was not present in either UHR individuals [r=-0.01 (95% CI: -0.34 to 0.33)] or FEP patients [r=0.14 (95% CI: -0.20 to 0.45)]. Plasma morning cortisol was not, however, associated with childhood trauma in either controls or individuals with schizophrenia in a further study (52). One consistent finding was that basal morning cortisol was not significantly associated with perceived stress in any group (52, 68, 70); moreover, no relationship was found between basal cortisol and daily stressors in UHR youth and healthy controls (66).

Cortisol Awakening Response (CAR)

In total, 37 individual effect sizes were available for the CAR; nearly all (n=35) calculated the increase in cortisol following awakening (CARi) with only two pertaining to the total output of cortisol in the hour following awakening (CARg). Five effect sizes achieved statistical significance: A study of children at elevated risk of schizophrenia observed that the CARi was strongly associated with both current [r=0.52 (95% CI: 0.13 to 0.77)] and previous distress [r=0.51 (95% CI: 0.11 to 0.77)] in relation to negative life events in children with a FHx of schizophrenia, but found no significant associations in children presenting antecedents of illness (including PEs) or controls (31). Moreover, this study found that negative life event exposure, daily stressor exposure, and daily stressor distress were not associated with the CARi in any group. Similarly, no significant associations were found in any group between the CARi and life event exposure in three further studies that between them included controls, UHR individuals, and FEP patients (51, 65, 70). With regards to childhood trauma, significant associations were observed with the CARi in healthy controls that were not observed in FEP patients in two studies; however, in one study (46), the association in controls was negative [r=-0.43 (95% CI: -0.56 to -0.28)] whilst in the other study (70) the relationship was positive [r=0.39 (95% CI: 0.11 to 0.62)]. Interestingly, Ciufolini and colleagues also calculated the CARg and found a significant positive association in the control group [r=0.21 (95% CI: 0.04 to 0.37)] that was not present in the FEP group. In contrast, there were no significant associations in controls, UHR individuals, or FEP patients in any of three studies examining the relationship between perceived stress and the CARi (51, 65, 70).

Diurnal Cortisol

Thirty-nine effect sizes were available for diurnal cortisol, the majority of which (n=31) were AUCg values (i.e., the total cortisol output throughout the day), with the remaining (n=8) representing the diurnal slope (i.e., the decrease in cortisol from awakening to evening). Significant associations were found in a single study (65), in which life event exposure was negatively associated with diurnal AUCg cortisol in FEP patients [r=-0.36 (95% CI -0.58 to -0.09)] but positively associated in healthy controls [r=0.42 (95% CI: 0.11 to 0.66)]. In contrast, two further studies that assessed diurnal cortisol using the same sampling procedure as this study found no significant relationships with life event exposure or life event distress in any group (31, 51). Moreover, neither of the diurnal cortisol measures (AUCg or slope) were associated with childhood trauma, perceived stress, or daily stressor exposure/distress in any group (31, 46, 51, 52, 65, 70).

Hair Cortisol

We identified 38 individual effect sizes for hair cortisol, of which four were statistically significant. A single study (56) reported positive associations of hair cortisol with socioeconomic status [r=0.42 (95% CI: 0.08 to 0.67)] and lifetime trauma [r=0.48 (95% CI: 0.15 to 0.71)] among individuals with a FHx of illness, and similarly a positive association with lifetime trauma in individuals reporting PEs [r=0.31 (95% CI 0.01 to 0.56)]; none of these associations were significant in controls. However, this study observed no significant associations between hair cortisol and any other stressor (migration, minority status, perceived discrimination, social undermining, ostracism experience, bullying victimization, childhood abuse) in any group. The only other study to report a significant effect (55), found a negative relationship between childhood trauma and hair cortisol among patients with chronic schizophrenia [r=-0.66

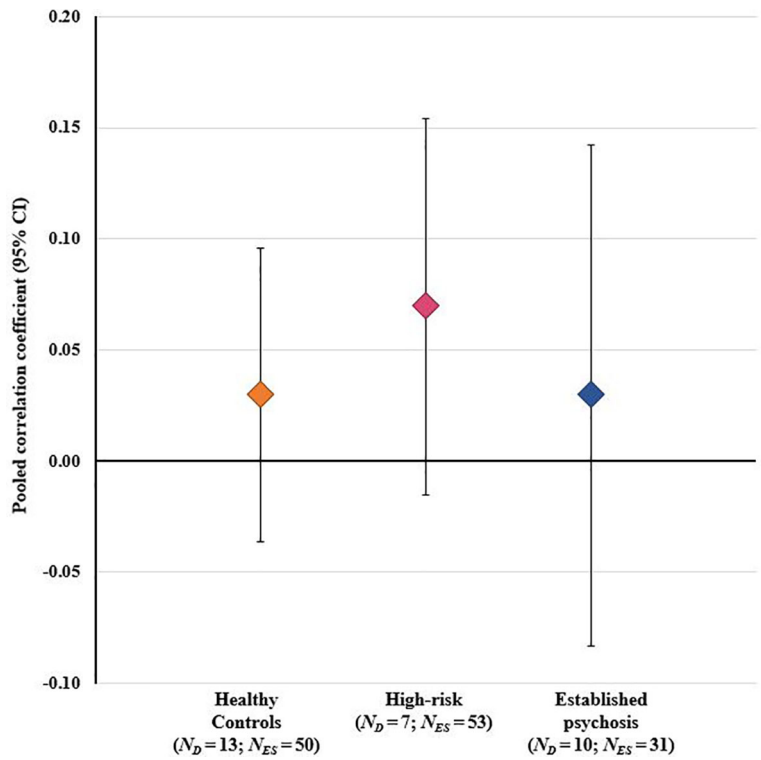

FIGURE 3 | Results of overall meta-analysis comparing healthy controls, high-risk individuals, and patients with established psychosis on the degree of concordance between psychosocial stressors and cortisol across all stressor-cortisol pairings. CI, confidence interval; N_D, number of study datasets contributing effect sizes; N_{ES}, number of effect sizes included in pooled effect size.

(95% CI: -0.89 to 0.17)] that was not observed among patients with early schizophrenia, individuals at UHR, or healthy controls. In contrast, a further study of patients with established schizophrenia found no association between hair cortisol and childhood trauma (69); however, as childhood trauma data was not collected in controls, no comparison is available. Neither of the studies examining perceived stress reported significant associations in controls or psychosis spectrum groups (47, 54).

Experience Sampling Method (ESM) Cortisol

Two studies, yielding five effect sizes, assessed stressor-cortisol concordance using the ESM method. The first of these (60), reported that event stress was positively associated with cortisol in individuals with a FHx of psychosis [*B*=0.04 (95% CI: 0.00 to 0.08)], a relationship that was not present among healthy controls [*B*=0.00 (95% CI: -0.01 to 0.02)]. A later study by the same group (53) tested both linear and quadratic effects of event stress on cortisol, finding the latter to be a better fit. When using linear predictor terms, a significant positive association was observed among patients with psychotic disorder [*B*=0.28 (95% CI: 0.01 to 0.05)] that was not present among FHx individuals [*B*=-0.00 (95% CI: -0.02 to 0.02)] or controls [*B*=0.02 (95% CI: -0.04 to 0.01)]. Similarly, in the quadratic model a significant negative relationship (inverted U-shape) was detected in patients with psychotic disorder [*B*=-0.02 (95% CI: -0.03 to -0.00)] whereas positive (U-shaped), non-significant associations were found in the FHx [*B*=0.00 (95% CI: -0.01 to -0.02)] and control [*B*=0.12 (95% CI: -0.03 to 0.03)] groups.

Meta-Analysis of Stressor-Cortisol Concordance

Overall Meta-Analysis of Stressor-Cortisol Concordance

The overall RVE model, which included data from all stressor-cortisol pairings, was performed on 134 effect sizes (beta coefficients were excluded as they could not be converted to a common metric). This model indicated a weak, positive association between stressors and cortisol that did not achieve statistical significance [*r*=0.05 (95% CI: -0.00 to 0.10), *p*=0.059]. A second model testing for group differences also showed no significant effect of either established psychosis status [*r*=0.01 (95% CI: -0.01 to 0.16), *p*=0.838] or high-risk status [*r*=0.02 (95% CI: -0.05 to 0.10), *p*=0.477] on effect sizes, indicating that the degree of concordance in these groups did not differ from healthy controls (see **Figure 3**). A further univariate regression model

TABLE 3 | Subgroup meta-analyses comparing stressor-cortisol concordance in psychosis spectrum and healthy control groups.

Stressor-cortisol pairing	Datasets contributing to analysis	Healthy Controls					Psychosis Spectrum					HC vs. PS
		N_{ES}	*r*	(95% CI)	*P* for Q	I^2	N_{ES}	*r*	(95% CI)	*P* for Q	I^2	*P*
Childhood trauma & basal (morning)	Faravelli et al. (67); Labad et al. (70); Seidenfaden et al. (52)	3	-0.08	(-0.22–0.06)	0.93	0%	3	0.13	(-0.22–0.44)	0.03	71%	0.285
Perceived stress & basal (morning)	Garner et al. (68); Labad et al. (70); Seidenfaden et al. (52)	3	-0.05	(-0.26–0.16)	0.73	0%	4	0.07	(-0.34–0.47)	0.10	56%	0.611
Life events & CAR (AUCi)	Cullen et al. (2014); Labad et al. (70); Mondelli et al. (65); Nordholm et al. (51)	4	0.09	(-0.08–0.25)	0.85	0%	7	0.11	(-0.13–0.33)	0.96	0%	0.872
Perceived stress & CAR (AUCi)	Labad et al. (70); Mondelli et al. (65); Nordholm et al. (51)	3	-0.14	(-0.34–0.07)	0.36	0%	5	0.12	(-0.12–0.35)	0.62	0%	0.105
Life events & diurnal (AUCg)	Cullen et al. (2014); Mondelli et al. (65); Nordholm et al. (51)	**3**	**0.25**	**(0.01–0.46)**	**0.23**	**29%**	**5**	**-0.28**	**(-0.49–-0.04)**	**0.47**	**0%**	**0.002**
Perceived stress & diurnal (AUCg)	Mondelli et al. (65); Nordholm et al. (51); Seidenfaden et al. (52)	3	-0.03	(-0.23–0.18)	0.57	0%	4	-0.09	(-0.35–0.18)	0.25	34%	0.698

CAR, cortisol awakening response; AUCi, area-under-the-curve with respect to increase; AUCg, area-under-the-curve with respect to ground; N_{ES}, total number of effect sizes included before within-study pooling; CI, confidence interval; P for Q, P value associated with Cochran's Q; HC, healthy control; PS, psychosis spectrum.
Bold text indicates that the effect size comparison between HC and PS groups is statistically significant at P<0.05.

indicated no effect of study quality (NOS scores) on effect sizes [r = -0.01 (95% CI: -0.05 to 0.03), p=0.525]; moreover, as the funnel plot was not asymmetric (**Supplementary Figure 1**) there was no evidence of small sample bias. Heterogeneity estimates derived from the RVE model (τ^2 = 0.016) indicated that 95% of the "true effects" were estimated to lie between r values of -0.20 and 0.30.

Stratified Analyses Examining Concordance Within Individual Stressor-Cortisol Pairings

Sufficient data were available to examine six individual stressor-cortisol pairings (i.e., these pairings were examined in three or more studies): i) childhood trauma and basal morning cortisol; ii) perceived stress and basal morning cortisol; iii) life event exposure and the CARi; iv) perceived stress and the CARi; v) life event exposure and diurnal cortisol (AUCg); and vi) perceived stress and diurnal cortisol (AUCg). Results of these stratified analyses are presented in **Table 3**. As illustrated in **Figure 4**, pooled effect sizes in both healthy control and psychosis spectrum groups were in the small-to-moderate range with both positive and negative associations observed. Statistically significant group differences were found for the association between life event exposure and diurnal cortisol (p=0.002); in controls a significant positive correlation was observed [r=0.25

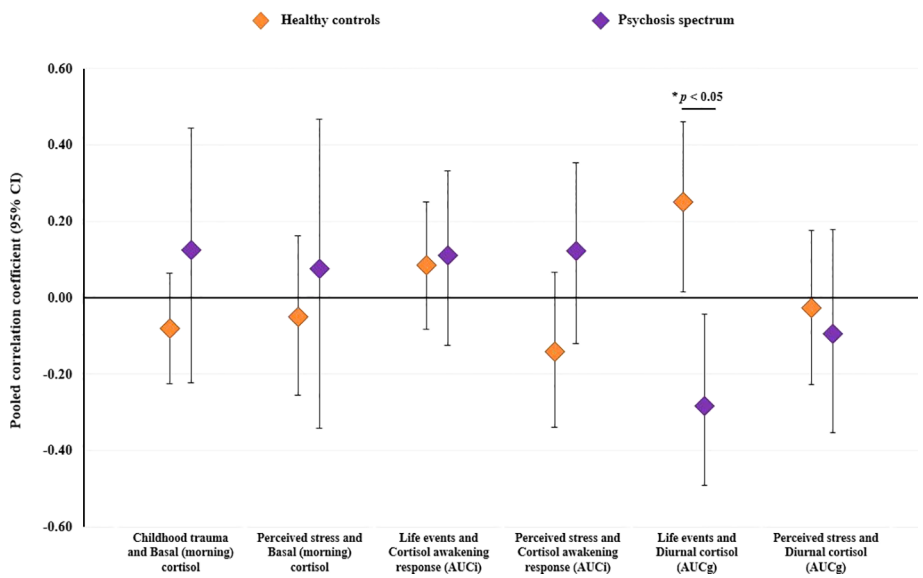

FIGURE 4 | Results of stratified meta-analyses comparing healthy controls and individuals on the psychosis spectrum (established psychosis and high-risk groups combined) on the degree of concordance between psychosocial stressors and cortisol within individual stressor-cortisol pairings. CI, confidence interval.

(95% CI: 0.01 to 0.46)], whereas a significant negative correlation was observed in the psychosis spectrum group [*r*=-0.28 (95% CI: -0.49 to -0.04)]. No other group differences or individual effects achieved statistical significance. Overall, heterogeneity estimates ranged from low (particularly in the control group) to moderate, except for the association between childhood trauma and basal (morning) cortisol in the psychosis spectrum group, where substantial and significant heterogeneity was observed (I^2 = 71%, *P* for Cochran's Q=0.03).

DISCUSSION

In the first meta-analysis to compare associations between naturally-occurring psychosocial stressors and cortisol in individuals on the psychosis spectrum and healthy controls, we observed poor concordance irrespective of stressor type, cortisol measure, or group status. The overall model, comprising 134 effect sizes, showed that stressors and cortisol measures were only weakly (and not significantly) correlated. Moreover, meta-regression analyses indicated that effect sizes among individuals with established psychosis and those at high-risk for psychosis did not differ from controls. In stratified analyses, performed to test for group differences within individual stressor-cortisol pairings, significant differences between healthy controls and psychosis spectrum groups were observed for only one of the six stressor-cortisol pairings examined. Thus, we found little evidence to suggest that cortisol responses to naturally-occurring stressors are any different in individuals on the psychosis spectrum compared to healthy controls.

Of the four *a priori* alternative hypotheses presented (**Figure 1**), our findings are most consistent with the "unrelated" hypothesis: Regardless of whether analyses were conducted using all effect sizes (excluding those derived from ESM studies which could not be pooled), or within individual stressor-cortisol pairings, the degree of concordance was weak, and, in most instances, did not differ across groups. The only significant group difference that we observed was for the relationship between life events and diurnal cortisol where significant associations were found in both controls and psychosis spectrum groups, but the direction of these effects differed (a positive correlation was observed in controls and negative correlation in the psychosis spectrum group). However, this finding appeared to be driven by a single study (65) which reported significant, opposing relationships between life events and diurnal cortisol in FEP patients and controls. Indeed, two subsequent studies which used the same protocol for obtaining cortisol samples in the home environment as Mondelli and colleagues found no significant associations between life events and diurnal cortisol in any group (31, 51), which is particularly surprising given that that the latter employed the same life event measure and included FEP patients (51). Our findings contrast with those observed in studies examining cortisol responses to acute psychosocial stressor tasks, in which healthy controls show a robust cortisol response whilst patients with psychosis and schizophrenia (37–39) and those at high-risk for psychosis (74, 75) demonstrate a blunted cortisol response. Together, these findings suggest that the cortisol abnormalities previously observed among psychosis spectrum groups (e.g., elevated basal and diurnal cortisol and a blunted CAR) are unlikely to be driven by greater exposure and/or sensitivity to these psychosocial stressors (which do not appear to elicit a robust cortisol response). Instead, these HPA axis abnormalities might be epiphenomenal, perhaps secondary to medication effects, substance use, or a manifestation of global physiological dysregulation. Moreover, these findings suggest that psychosocial stressors may contribute to the onset and exacerbation of psychotic illness *via* other mechanisms (e.g., cognitive processes, or the immuno-inflammatory system) as opposed to cortisol fluctuations.

There are several reasons why we should be cautious about drawing these conclusions; these fall into the broad domains of statistical power, analytical approaches, heterogeneity (relating to study measures and populations), and timing of cortisol collection in relation to stressor onset/measurement, which will now be discussed in turn. First, low statistical power at both the study level and meta-analysis level may explain the poor concordance we observed between stressors and cortisol. Our systematic review indicated that only a small number of studies (2/18) included sufficient participants in each group to be able to detect a moderate correlation. Given that the majority of studies (87%) reported negligible-to-small correlations, this might explain why so few statistically significant correlations were observed at the study level. At the meta-analysis level, we were only able to include data from 16 separate datasets, far less than the minimum number (N=40) recommended (63). As such, our meta-analyses were almost certainly underpowered.

The analytic approaches adopted across studies may have also contributed to poor concordance. With regards to the analysis of cortisol data, a study of healthy females reported that while perceived stress scores showed no relationship with absolute cortisol levels (mean of multiple samples obtained throughout a single day), they were significantly associated with change in cortisol levels (76), implying that cortisol measures indexing deviation from normal HPA axis activity may be more sensitive to psychosocial stress. Indeed, this might explain why studies employing acute psychosocial stressor tasks observe a robust "stressor effect" (i.e., an increase in cortisol from baseline as a result of task anticipation and commencement). However, it should be noted that we did not observe this pattern in our review: In fact, more than half of the individual effect sizes that achieved statistical significance (10/16) pertained to absolute measures of cortisol (i.e., basal levels, diurnal AUCg, hair cortisol). Another analytical issue pertains to adjustment for potentially confounding factors, which substantially varied across studies. Cortisol levels have been associated with a range of participant factors, including age, sex, ethnicity, socioeconomic status, and psychotropic medication, factors which often distinguish psychosis spectrum and healthy

control groups (20). As such, failure to account for these factors may mask important group differences.

Heterogeneity across studies with regards to study measures may have impacted on our ability to detect a significant overall association between psychosocial stressors and cortisol. In this review, we examined a broad range of psychosocial stressors, including: exposure to specific, pre-defined events (daily stressors, recent and major life events, childhood trauma); distress related to these specific events; subjectively-rated stressfulness of current activities (ESM activity stress); and appraisals of the degree to which life is stressful, unpredictable, and uncontrollable (perceived stress). While all of these measures are relevant to the concept of "stress" (either because they index events that most individuals would consider to be stressful, or because they capture subjective experiences of stress/distress) there is likely substantial variability in the extent to which they are associated with a biological stress response. Moreover, perceived stress has been found to correlate with both personality traits and depressive symptoms (77), suggesting that it can be considered a trait-like feature rather than a measure of stress exposure per se. Indeed, this might explain why perceived stress was not associated with cortisol in any of the studies included in our review. Coupled with the fact that, as noted above, cortisol measures also varied substantially across studies, it is perhaps unsurprising that we observed substantial heterogeneity in effect sizes across studies. In addition to this, heterogeneity in the study populations examined may have contributed to our inability to detect significant differences between individuals on the psychosis spectrum and healthy controls. In our overall analysis, we were able to differentiate between individuals with established psychosis and those at high-risk for the disorder; however, even within these subcategories there was substantial variability. The established psychosis group included patients with diagnoses of first-episode psychosis, early stage schizophrenia, and chronic schizophrenia who likely differed with regards to exposure to antipsychotic medication and other confounding factors known to influence cortisol levels (20). However, there was perhaps even greater variability within the high-risk groups, which included help-seeking individuals meeting UHR criteria (who present features consistent with the prodromal phase of psychosis); adolescents and adults with a family history of illness; and individuals reporting PEs. Within these groups, the proportion of individuals who will go on to develop full psychosis varies considerably (45, 78); indeed, it is likely that FHx individuals who reach adulthood without developing psychosis do so due to protective factors. In our review, we chose to include populations that are frequently defined in the literature as being at "high-risk" for psychosis on the basis that the neural diathesis-stress model describes a mechanism that may operate in those with increased vulnerability for psychosis, irrespective of cause (18, 20). Nevertheless, it is important to note that this may have contributed to substantial heterogeneity in effect sizes across studies.

A further possible explanation for the poor concordance we observed is that cortisol samples are unlikely to have been collected at the time of stressor exposure. A previous meta-analysis (41) found that the degree of concordance between chronic stress and cortisol is strongly influenced by the lapse-of-time between stressor exposure and cortisol measurement (i.e., as time since stressor onset increases, the degree of concordance diminishes). However, this pattern did not emerge in the present review; rather, significant associations with cortisol were observed for both distal (e.g., childhood trauma) and proximal events (e.g., ESM event stress). As a related issue, it is possible that the time-lapse between stress *measurement* and cortisol collection might be a contributing factor. A recent study using data from a large sample of individuals at UHR for psychosis and healthy controls indicated that the degree of concordance between psychosocial stressors and basal cortisol was moderated by the lapse-of-time between collection of these measures (79): Specifically, daily stressors, life events, and childhood trauma, were only associated with basal cortisol measures when these stress measures were completed on the same day as cortisol collection. The fact that this pattern was observed for daily stressors occurring within the last 24-h and life events/childhood trauma (which did not occur on the day of testing) suggests that distress associated with recalling these events might elicit a cortisol response that enables a significant association to be observed. Importantly, after accounting for the lapse-of-time between assessments, analyses indicated that the degree of concordance was stronger among CHR individuals who later converted to psychosis when compared to those who did not (79); thus, accounting for the time-lapse between assessments may improve precision and reveal important group differences. In the present review, we found that only three studies reported the lapse-of-time between stress measurement and cortisol collection, and only two confirmed that measures were completed on the same day. Both of these studies used the ESM approach to obtain cortisol samples within 10 min of stressor ratings (53, 60); however, even with this short lapse-of-time, significant associations between event stress and salivary cortisol were not observed in healthy controls, only those on the psychosis spectrum (i.e., relatives and patients with psychotic disorders). Interestingly, a recent ESM study examining individuals with 22q11.2 deletion syndrome (a syndrome associated with learning difficulties, a range of physical health problems, and psychiatric comorbidity—including psychosis), reported that cortisol levels in the healthy control group, but not the 22q11.2 deletion syndrome group, increased in parallel with activity related stress, but that this association in controls was only significant at the trend level (80). Together, these findings suggest that the activity-/event-related stress captured using existing ESM approaches may not be sufficiently "stressful" to elicit robust changes in cortisol levels in healthy controls.

In summary, there are a number of important methodological issues that contribute to complexity when examining the relationship between psychosocial stressors encountered in the natural environment and cortisol. While none of these potential explanations can fully account for the poor concordance that we observed across a range of stressor and cortisol measures, it is certainly possible that methodological issues obscured the ability to detect "true" associations between these measures.

Limitations

As noted above, given that we were only able to include data from 18 studies (representing 16 independent datasets) our

meta-analyses were likely underpowered. This would have affected our ability to detect statistically significant correlations between stressors and cortisol (which were largely within the small-to-moderate range), and to test for group differences in the degree of concordance. However, it is important to note that previous meta-analyses have observed group differences in cortisol responses to psychosocial stressor tasks with far fewer studies (37, 39). The small number of studies identified also meant that in our stratified analyses (testing group differences within individual stressor-cortisol pairings) it was necessary to combine effect sizes derived from patients with established psychosis and individuals at high-risk for psychosis in a single "psychosis spectrum" group. As such, the psychosis spectrum group was highly heterogeneous. It is possible that the inclusion of individuals at different stages of illness (from adolescents reporting isolated psychotic experiences to adult patients with chronic schizophrenia) with varying degrees of psychopathology may have diluted any group differences; indeed, recent theories propose that different stages of illness may be associated with different patterns of HPA axis dysregulation (35). However, in our overall analysis (which included all effect sizes) we were able to distinguish between established psychosis patients and high-risk participants and found no substantial difference in effect sizes in these groups. As noted above, there was also substantial heterogeneity across studies with regards to both psychosocial stressors (ranging from minor daily stressors to major life events and childhood trauma) and cortisol measures (which included both dynamic measures such as the CAR, and chronic cortisol levels as measured in hair samples). This was reflected in the heterogeneity estimates derived from the overall model where the interval within which 95% of the "true effects" were estimated to lie was wide and crossed zero (-0.20 to 0.30). While this questions the extent to which these effect sizes could be pooled using meta-analytic techniques, we performed stratified analyses to reduce this heterogeneity. Moreover, pooling these results enabled us to quantify the level of heterogeneity and address the key question of whether the strength of association, irrespective of stressor-cortisol type, differed in healthy controls and those on the psychosis spectrum. A further limitation, noted above, pertains to the fact that our search was restricted to studies examining cortisol, as such, we did not consider other potential markers of HPA axis function (e.g., adrenocorticotropic hormone, hippocampal/pituitary volume, or glucocorticoid receptor density, distribution and/or affinity). However, cortisol is one of the most widely used indicators of HPA axis function and expanding our search parameters would have likely yielded an unmanageable number of studies to assess for eligibility. These limitations are balanced by several strengths. First, we employed robust statistical approaches to account for dependence of effect sizes, thereby allowing us to include multiple effect sizes from the same study. Second, to avoid potential cancelling effects (i.e., deriving a neutral effect by combining positive and negative associations) we additionally conducted stratified analyses where effect sizes were pooled within individual stressor-cortisol pairings (although the number of studies contributing to each analysis was substantially reduced). Finally, we included a wide range of psychosocial stressors and cortisol measures, increasing the number of studies in the review.

Implications

As noted above, there are several methodological issues that might explain the poor concordance that we observed between psychosocial stressors and cortisol. As such, we recommend that future studies in this field i) conduct *a priori* power calculations to determine the minimum number of participants required for each group and ensure that recruitment is matched to the target number; ii) investigate within-subject deviation from normative cortisol levels, whether this be daily fluctuations (i.e., increase from awakening or other time-point) or variations across days (i.e., changes from mean level), as these variations may be more strongly associated with psychosocial stressors; iii) move beyond simple cross-sectional analyses and instead attempt to obtain longitudinal measures of both stressors and cortisol in order to disentangle the temporal relationship between these measures; iv) report the lapse-of-time between stressor assessment and cortisol collection and test whether this variable moderates the strength of association (and, if so, account for interaction effects accordingly); and (v) investigate potential confounders and adjust analyses as appropriate. It is important to note that we found no association between study quality/bias scores (which considered some of these factors) and effect sizes. As such, it is possible that these recommendations will not necessarily increase the likelihood that a study is able to detect concordance between naturally-occurring psychosocial stressors and cortisol; however, this is an important first step to elucidating these relationships.

Our findings should be considered with reference to existing theories of psychosis aetiology. The neural diathesis-stress model of schizophrenia hypothesized that HPA axis dysregulation among those on the psychosis spectrum could be stress-induced, a manifestation of hippocampal dysfunction or glucocorticoid receptor abnormalities, or genetically determined (18–20). While the current review provides no evidence to suggest that cortisol abnormalities among individuals on the psychosis spectrum are stress-induced, we again emphasize the need to consider the range of methodological issues that might have contributed to this null finding. Aside from the aforementioned methodological issues, it is also possible that repeated exposure to psychosocial stressors among individuals on the psychosis spectrum leads to an initial increase in HPA axis function, that, when exhausted, leads to a dysregulated system that no longer responds to stress appropriately—as proposed in the tonic/phasic model of HPA axis dysregulation (35). However, this would not explain why healthy controls (who we know experience lower levels of psychosocial stressor exposure and distress) also showed poor concordance, and we would have also expected to see variability in the degree of concordance across illness phases had this been the case. Our review provides important findings regarding the relationship (or lack of) between psychosocial stressors and

cortisol that should be incorporated in future revisions to these theories.

Conclusions

This comprehensive systematic review and meta-analysis found no evidence to suggest that individuals on the psychosis spectrum are characterized by either hyper- or hypo-responsivity of the HPA axis to naturally-occurring psychosocial stressors. These findings are in contrast to the blunted cortisol response observed during psychosocial stressor tasks among patients with established illness and individuals at high-risk for psychosis. While our findings suggest that psychosocial stressors cannot explain the cortisol abnormalities that have been previously reported in psychosis spectrum groups, this might also reflect methodological issues that are common to studies of naturally-occurring psychosocial stressors (e.g., failure to acquire cortisol samples proximal to stress exposure/assessment) but are tightly controlled in experimental studies employing psychosocial stressor tasks. Moreover, without adequate assessment of potential confounders and moderating factors, no conclusions can be drawn regarding the true relationship between psychosocial stressors encountered in the natural environment and cortisol levels. Thus, we strongly advocate that future studies attempting to investigate stressor-cortisol concordance consider these factors during the study planning phase and when conducting analyses. Nevertheless, the current evidence suggests that cortisol responses to naturally-occurring stressors are not a robust marker of either risk for psychosis or established illness.

DATA AVAILABILITY STATEMENT

The dataset generated and analyzed in this study is available from AC on request.

AUTHOR CONTRIBUTIONS

AC conceived the study, oversaw all systematic searches, conducted all statistical analyses, and wrote the first draft of the manuscript. SR and MV contributed equally to the study and were responsible for conducting the systematic search, reviewing studies for eligibility, extracting study characteristic data, and rating study quality/bias. VM and PM contributed intellectually to the critical interpretation of results. All authors reviewed and contributed to the final manuscript.

FUNDING

AC is supported by a Sir Henry Welcome Postdoctoral Fellowship from the Wellcome Trust (107395/Z/15/Z), and a NARSAD Young Investigator Grant awarded by the Brain & Behavior Research Foundation (28336) and funded by the Evelyn Toll Family Foundation. This paper represents independent research part-funded by the National Institute for Health Research (NIHR) Biomedical Research Centre at South London and Maudsley NHS Foundation Trust and King's College London (IS-BRC-1215-20018 awarded to AC). The views expressed are those of the authors and not necessarily those of the NHS, the NIHR or the Department of Health and Social Care. The funders had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit the manuscript for publication.

ACKNOWLEDGMENTS

The authors are extremely grateful to Drs Aas, Ciufolini, Heinze, Hirt, Labad, Nordholm, Schalinski, Seidenfaden, Soder, Streit, and Vaessen for responding to queries and providing additional data for inclusion in this meta-analysis.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.00513/full#supplementary-material

REFERENCES

1. Varese F, Smeets F, Drukker M, Lieverse R, Lataster T, Viechtbauer W, et al. Childhood adversities increase the risk of psychosis: a meta-analysis of patient-control, prospective- and cross-sectional cohort studies. *Schizophr Bull* (2012) 38(4):661–71. doi: 10.1093/schbul/sbs050
2. Beards S, Gayer-Anderson C, Borges S, Dewey ME, Fisher HL, Morgan C. Life events and psychosis: a review and meta-analysis. *Schizophr Bull* (2013) 39 (4):740–7. doi: 10.1093/schbul/sbt065
3. Myin-Germeys I, Delespaul P, van Os J. Behavioural sensitization to daily life stress in psychosis. *psychol Med* (2005) 35(5):733–41. doi: 10.1017/S0033291704004179
4. Myin-Germeys I, van Os J, Schwartz JE, Stone AA, Delespaul PA. Emotional reactivity to daily life stress in psychosis. *Arch Gen Psychiatry* (2001) 58 (12):1137–44. doi: 10.1001/archpsyc.58.12.1137
5. Reininghaus U, Kempton MJ, Valmaggia L, Craig TK, Garety P, Onyejiaka A, et al. Stress Sensitivity, Aberrant Salience, and Threat Anticipation in Early Psychosis: An Experience Sampling Study. *Schizophr Bull* (2016) 42(3):712–22. doi: 10.1093/schbul/sbv190
6. Vaessen T, Viechtbauer W, van der Steen Y, Gayer-Anderson C, Kempton MJ, Valmaggia L, et al. Recovery from daily-life stressors in early and chronic psychosis. *Schizophr Res* (2019) 213:32–9. doi: 10.1016/j.schres.2019.03.011
7. Malla AK, Cortese L, Shaw TS, Ginsberg B. Life events and relapse in schizophrenia. A one year prospective study. *Soc Psychiatry Psychiatr Epidemiol* (1990) 25(4):221–4. doi: 10.1007/BF00782965
8. Norman RM, Malla AK. A prospective study of daily stressors and symptomatology in schizophrenic patients. *Soc Psychiatry Psychiatr Epidemiol* (1994) 29(6):244–9. doi: 10.1007/BF00802047
9. Cullen AE, Fisher HL, Roberts RE, Pariante CM, Laurens KR. Daily stressors and negative life events in children at elevated risk of developing schizophrenia. *Br J Psychiatry* (2014) 204:354–60. doi: 10.1192/bjp.bp.113.127001
10. Trotman HD, Holtzman CW, Walker EF, Addington JM, Bearden CE, Cadenhead KS, et al. Stress exposure and sensitivity in the clinical high-risk syndrome: initial findings from the North American Prodrome Longitudinal

Study (NAPLS). *Schizophr Res* (2014) 160(1-3):104–9. doi: 10.1016/j.schres.2014.09.017
11. Tessner KD, Mittal V, Walker EF. Longitudinal study of stressful life events and daily stressors among adolescents at high risk for psychotic disorders. *Schizophr Bull* (2011) 37(2):432–41. doi: 10.1093/schbul/sbp087
12. Palmier-Claus JE, Dunn G, Lewis SW. Emotional and symptomatic reactivity to stress in individuals at ultra-high risk of developing psychosis. *psychol Med* (2012) 42(5):1003–12. doi: 10.1017/S0033291711001929
13. Kelleher I, Harley M, Lynch F, Arseneault L, Fitzpatrick C, Cannon M. Associations between childhood trauma, bullying and psychotic symptoms among a school-based adolescent sample. *Br J Psychiatry* (2008) 193(5):378–82. doi: 10.1192/bjp.bp.108.049536
14. Mayo D, Corey S, Kelly LH, Yohannes S, Youngquist AL, Stuart BK, et al. The Role of Trauma and Stressful Life Events among Individuals at Clinical High Risk for Psychosis: A Review. *Front Psychiatry* (2017) 8:55. doi: 10.3389/fpsyt.2017.00055
15. Peh OH, Rapisarda A, Lee J. Childhood adversities in people at ultra-high risk (UHR) for psychosis: a systematic review and meta-analysis. *Psychol Med* (2019) 49(7):1–13. doi: 10.1017/S003329171800394X
16. Fusar-Poli P, Tantardini M, De Simone S, Ramella-Cravaro V, Oliver D, Kingdon J, et al. Deconstructing vulnerability for psychosis: Meta-analysis of environmental risk factors for psychosis in subjects at ultra high-risk. *Eur Psychiatry* (2017) 40:65–75. doi: 10.1016/j.eurpsy.2016.09.003
17. DeVylder JE, Koyanagi A, Unick J, Oh H, Nam B, Stickley A. Stress Sensitivity and Psychotic Experiences in 39 Low- and Middle-Income Countries. *Schizophr Bull* (2016) 42(6):1353–62. doi: 10.1093/schbul/sbw044
18. Walker E, Mittal V, Tessner K. Stress and the hypothalamic pituitary adrenal axis in the developmental course of schizophrenia. *Annu Rev Clin Psychol* (2008) 4:189–216. doi: 10.1146/annurev.clinpsy.4.022007.141248
19. Walker EF, Diforio D. Schizophrenia: a neural diathesis-stress model. *psychol Rev* (1997) 104(4):667–85. doi: 10.1037/0033-295X.104.4.667
20. Pruessner M, Cullen AE, Aas M, Walker EF. The neural diathesis-stress model of schizophrenia revisited: An update on recent findings considering illness stage and neurobiological and methodological complexities. *Neurosci Biobehav Rev* (2017) 73:191–218. doi: 10.1016/j.neubiorev.2016.12.013
21. Girshkin L, Matheson SL, Shepherd AM, Green MJ. Morning cortisol levels in schizophrenia and bipolar disorder: a meta-analysis. *Psychoneuroendocrinology* (2014) 49:187–206. doi: 10.1016/j.psyneuen.2014.07.013
22. Hubbard DB, Miller BJ. Meta-analysis of blood cortisol levels in individuals with first-episode psychosis. *Psychoneuroendocrinology* (2019) 104:269–75. doi: 10.1016/j.psyneuen.2019.03.014
23. Borges S, Gayer-Anderson C, Mondelli V. A systematic review of the activity of the hypothalamic-pituitary-adrenal axis in first episode psychosis. *Psychoneuroendocrinology* (2013) 38(5):603–11. doi: 10.1016/j.psyneuen.2012.12.025
24. Chaumette B, Kebir O, Mam-Lam-Fook C, Morvan Y, Bourgin J, Godsil BP, et al. Salivary cortisol in early psychosis: New findings and meta-analysis. *Psychoneuroendocrinology* (2016) 63:262–70. doi: 10.1016/j.psyneuen.2015.10.007
25. Mittal VA, Dhruv S, Tessner KD, Walder DJ, Walker EF. The relations among putative biorisk markers in schizotypal adolescents: minor physical anomalies, movement abnormalities, and salivary cortisol. *Biol Psychiatry* (2007) 61(10):1179–86. doi: 10.1016/j.biopsych.2006.08.043
26. Mittal VA, Orr JM, Pelletier A, Dean DJ, Smith A, Lunsford-Avery J. Hypothalamic-pituitary-adrenal axis dysfunction in non-clinical psychosis. *Psychiatry Res* (2013) 206(2-3):315–7. doi: 10.1016/j.psychres.2012.12.021
27. Yildirim O, Dogan O, Semiz M, Kilicli F. Serum cortisol and dehydroepiandrosterone-sulfate levels in schizophrenic patients and their first-degree relatives. *Psychiatry Clin Neurosci* (2011) 65(6):584–91. doi: 10.1111/j.1440-1819.2011.02252.x
28. Walker EF, Walder DJ, Reynolds F. Developmental changes in cortisol secretion in normal and at-risk youth. *Dev Psychopathol* (2001) 13(3):721–32. doi: 10.1017/S0954579401003169
29. Berger M, Kraeuter AK, Romanik D, Malouf P, Amminger GP, Sarnyai Z. Cortisol awakening response in patients with psychosis: Systematic review and meta-analysis. *Neurosci Biobehav Rev* (2016) 68:157–66. doi: 10.1016/j.neubiorev.2016.05.027
30. Day FL, Valmaggia LR, Mondelli V, Papadopoulos A, Papadopoulos I, Pariante CM, et al. Blunted cortisol awakening response in people at ultra high risk of developing psychosis. *Schizophr Res* (2014) 158(1-3):25–31. doi: 10.1016/j.schres.2014.06.041
31. Cullen AE, Zunszain PA, Dickson H, Roberts RE, Fisher HL, Pariante CM, et al. Cortisol awakening response and diurnal cortisol among children at elevated risk for schizophrenia: relationship to psychosocial stress and cognition. *Psychoneuroendocrinology* (2014) 46:1–13. doi: 10.1016/j.psyneuen.2014.03.010
32. Nordholm D, Krogh J, Mondelli V, Dazzan P, Pariante C, Nordentoft M. Pituitary gland volume in patients with schizophrenia, subjects at ultra high-risk of developing psychosis and healthy controls: a systematic review and meta-analysis. *Psychoneuroendocrinology* (2013) 38(11):2394–404. doi: 10.1016/j.psyneuen.2013.06.030
33. Saunders TS, Mondelli V, Cullen AE. Pituitary volume in individuals at elevated risk for psychosis: A systematic review and meta-analysis. *Schizophr Res* (2019) 213:23–31. doi: 10.1016/j.schres.2018.12.026
34. Fries E, Dettenborn L, Kirschbaum C. The cortisol awakening response (CAR): facts and future directions. *Int J Psychophysiol* (2009) 72(1):67–73. doi: 10.1016/j.ijpsycho.2008.03.014
35. Shah JL, Malla AK. Much ado about much: stress, dynamic biomarkers and HPA axis dysregulation along the trajectory to psychosis. *Schizophr Res* (2015) 162(1-3):253–60. doi: 10.1016/j.schres.2015.01.010
36. Gajsak LR, Gelemanovic A, Kuzman MR, Puljak L. Impact of stress response in development of first-episode psychosis in schizophrenia: An overview of systematic reviews. *Psychiatr Danub* (2017) 29(1):14–23. doi: 10.24869/psyd.2017.14
37. Zorn JV, Schur RR, Boks MP, Kahn RS, Joels M, Vinkers CH. Cortisol stress reactivity across psychiatric disorders: A systematic review and meta-analysis. *Psychoneuroendocrinology* (2017) 77:25–36. doi: 10.1016/j.psyneuen.2016.11.036
38. Dauvermann MR, Donohoe G. Cortisol stress response in psychosis from the high-risk to the chronic stage: a systematic review. *Ir J Psychol Med* (2019) 36(4):305–15. doi: 10.1017/ipm.2019.27
39. Ciufolini S, Dazzan P, Kempton MJ, Pariante C, Mondelli V. HPA axis response to social stress is attenuated in schizophrenia but normal in depression: evidence from a meta-analysis of existing studies. *Neurosci Biobehav Rev* (2014) 47:359–68. doi: 10.1016/j.neubiorev.2014.09.004
40. Henze GI, Zankert S, Urschler DF, Hiltl TJ, Kudielka BM, Pruessner JC, et al. Testing the ecological validity of the Trier Social Stress Test: Association with real-life exam stress. *Psychoneuroendocrinology* (2017) 75:52–5. doi: 10.1016/j.psyneuen.2016.10.002
41. Miller GE, Chen E, Zhou ES. If it goes up, must it come down? Chronic stress and the hypothalamic-pituitary-adrenocortical axis in humans. *Psychol Bull* (2007) 133(1):25–45. doi: 10.1037/0033-2909.133.1.25
42. Chida Y, Steptoe A. Cortisol awakening response and psychosocial factors: a systematic review and meta-analysis. *Biol Psychol* (2009) 80(3):265–78. doi: 10.1016/j.biopsycho.2008.10.004
43. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* (2000) 283(15):2008–12. doi: 10.1001/jama.283.15.2008
44. Yung AR, Yuen HP, McGorry PD, Phillips LJ, Kelly D, Dell'Olio M, et al. Mapping the onset of psychosis: the Comprehensive Assessment of At-Risk Mental States. *Aust New Z J Psychiatry* (2005) 39(11-12):964–71. doi: 10.1080/j.1440-1614.2005.01714.x
45. Fusar-Poli P, Borgwardt S, Bechdolf A, Addington J, Riecher-Rossler A, Schultze-Lutter F, et al. The psychosis high-risk state: a comprehensive state-of-the-art review. *JAMA Psychiatry* (2013) 70(1):107–20. doi: 10.1001/jamapsychiatry.2013.269
46. Ciufolini S, Gayer-Anderson C, Fisher HL, Marques TR, Taylor H, Di Forti M, et al. Cortisol awakening response is decreased in patients with first-episode psychosis and increased in healthy controls with a history of severe childhood abuse. *Schizophr Res* (2019) 205:38–44. doi: 10.1016/j.schres.2018.05.002
47. Heinze K, Lin A, Reniers R, Wood SJ. Longer-term increased cortisol levels in young people with mental health problems. *Psychiatry Res* (2016) 236:98–104. doi: 10.1016/j.psychres.2015.12.025
48. Labad J, Armario A, Nadal R, Sole M, Gutierrez-Zotes A, Montalvo I, et al. Clinical correlates of hypothalamic-pituitary-adrenal axis measures in individuals at risk for psychosis and with first-episode psychosis. *Psychiatry Res* (2018) 265:284–91. doi: 10.1016/j.psychres.2018.05.018
49. Labad J, Ortega L, Cabezas A, Montalvo I, Arranz S, Algora MJ, et al. Hypothalamic-pituitary-adrenal axis function and exposure to stress factors

and cannabis use in recent-onset psychosis. *World J Biol Psychiatry* (2019), 1–8. doi: 10.1080/15622975.2019.1628301
50. Labad J, Ortega L, Cabezas Á, et al. Hypothalamic-pituitary-adrenal axis function and exposure to stress factors and cannabis use in recent-onset psychosis [published online ahead of print, 2019 Jun 27]. *World J Biol Psychiatry* (2019) 1–8. doi: 10.1080/15622975.2019.1628301
51. Nordholm D, Rostrup E, Mondelli V, Randers L, Nielsen MO, Wulff S, et al. Multiple measures of HPA axis function in ultra high risk and first-episode schizophrenia patients. *Psychoneuroendocrinology* (2018) 92:72–80. doi: 10.1016/j.psyneuen.2018.03.015
52. Seidenfaden D, Knorr U, Soendergaard MG, Poulsen HE, Fink-Jensen A, Jorgensen MB, et al. The relationship between self-reported childhood adversities, adulthood psychopathology and psychological stress markers in patients with schizophrenia. *Compr Psychiatry* (2017) 72:48–55. doi: 10.1016/j.comppsych.2016.09.009
53. Vaessen T, Kasanova Z, Hernaus D, Lataster J, Collip D, van Nierop M, et al. Overall cortisol, diurnal slope, and stress reactivity in psychosis: An experience sampling approach. *Psychoneuroendocrinology* (2018) 96:61–8. doi: 10.1016/j.psyneuen.2018.06.007
54. Streit F, Memic A, Hasandedic L, Rietschel L, Frank J, Lang M, et al. Perceived stress and hair cortisol: Differences in bipolar disorder and schizophrenia. *Psychoneuroendocrinology* (2016) 69:26–34. doi: 10.1016/j.psyneuen.2016.03.010
55. Hirt V, Schalinski I, Rockstroh B. Decoding the impact of adverse childhood experiences on the progression of schizophrenia. *Ment Health Prev* (2019) 13:82–91. doi: 10.1016/j.mhp.2019.01.002
56. Soder E, Clamor A, Lincoln TM. Hair cortisol concentrations as an indicator of potential HPA axis hyperactivation in risk for psychosis. *Schizophr Res* (2019) 212:54–61. doi: 10.1016/j.schres.2019.08.012
57. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. (2011). Available at: http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. (Last Accessed 01/06/2020)
58. StataCorp. *Stata Statistical Software: Release 16*. College Station, TX: StataCorp LLC (2019).
59. Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. *Introduction to meta-analysis*. Chichester, U.K: John Wiley & Sons (2009). xxviii, 421 p. p.
60. Collip D, Nicolson NA, Lardinois M, Lataster T, van Os J, Myin-Germeys I. Daily cortisol, stress reactivity and psychotic experiences in individuals at above average genetic risk for psychosis. *psychol Med* (2011) 41(11):2305–15. doi: 10.1017/S0033291711000602
61. Hedges LV, Tipton E, Johnson MC. Robust variance estimation in meta-regression with dependent effect size estimates. *Res Synth Methods* (2010) 1 (1):39–65. doi: 10.1002/jrsm.5
62. Tanner-Smith EE, Tipton E. Robust variance estimation with dependent effect sizes: practical considerations including a software tutorial in Stata and spss. *Res Synth Methods* (2014) 5(1):13–30. doi: 10.1002/jrsm.1091
63. Tipton E. Small sample adjustments for robust variance estimation with meta-regression. *Psychol Methods* (2015) 20(3):375–93. doi: 10.1037/met0000011
64. Higgins JPT, Green S, Cochrane Collaboration. *Cochrane handbook for systematic reviews of interventions*. Chichester, England ; Hoboken, NJ: Wiley-Blackwell (2008). xxi, 649 p. p.
65. Mondelli V, Dazzan P, Hepgul N, Di Forti M, Aas M, D'Albenzio A, et al. Abnormal cortisol levels during the day and cortisol awakening response in first-episode psychosis: the role of stress and of antipsychotic treatment. *Schizophr Res* (2010) 116(2-3):234–42. doi: 10.1016/j.schres.2009.08.013
66. Moskow DM, Addington J, Bearden CE, Cadenhead KS, Cornblatt BA, Heinssen R, et al. The relations of age and pubertal development with cortisol and daily stress in youth at clinical risk for psychosis. *Schizophr Res* (2016) 172(1-3):29–34. doi: 10.1016/j.schres.2016.02.002
67. Faravelli C, Mansueto G, Palmieri S, Lo Sauro C, Rotella F, Pietrini F, et al. Childhood Adversity, Cortisol Levels, and Psychosis: A Retrospective Investigation. *J Nerv Ment Dis* (2017) 205(7):574–9. doi: 10.1097/NMD.0000000000000699
68. Garner B, Phassouliotis C, Phillips LJ, Markulev C, Butselaar F, Bendall S, et al. Cortisol and dehydroepiandrosterone-sulphate levels correlate with symptom severity in first-episode psychosis. *J Psychiatr Res* (2011) 45 (2):249–55. doi: 10.1016/j.jpsychires.2010.06.008
69. Aas M, Pizzagalli DA, Laskemoen JF, Reponen EJ, Ueland T, Melle I, et al. Elevated hair cortisol is associated with childhood maltreatment and cognitive impairment in schizophrenia and in bipolar disorders. *Schizophr Res* (2019) 213:65–71. doi: 10.1016/j.schres.2019.01.011
70. Labad J. (2020). Combined dataset provided by author.
71. Cohen S, Kamarck T, Mermelstein R. A global measure of perceived stress. *J Health Soc Behav* (1983) 24(4):385–96. doi: 10.2307/2136404
72. Bifulco A, Bernazzani O, Moran PM, Jacobs C. The Childhood Experience of Care and Abuse Questionnaire (CECA. Q): Validation in a community series. *Br J Clin Psychol* (2005) 44:563–81. doi: 10.1348/014466505X35344
73. Bernstein DP, Fink L. *Childhood Trauma Questionnaire: A Retrospective Self-report Manual*. New York: The Psychological Corporation (1998).
74. Pruessner M, Bechard-Evans L, Boekestyn L, Iyer SN, Pruessner JC, Malla AK. Attenuated cortisol response to acute psychosocial stress in individuals at ultra-high risk for psychosis. *Schizophr Res* (2013) 146(1-3):79–86. doi: 10.1016/j.schres.2013.02.019
75. Walter EE, Fernandez F, Snelling M, Barkus E. Stress induced cortisol release and schizotypy. *Psychoneuroendocrinology* (2018) 89:209–15. doi: 10.1016/j.psyneuen.2018.01.012
76. Vedhara K, Miles J, Bennett P, Plummer S, Tallon D, Brooks E, et al. An investigation into the relationship between salivary cortisol, stress, anxiety and depression. *Biol Psychol* (2003) 62(2):89–96. doi: 10.1016/S0301-0511(02)00128-X
77. Kim SE, Kim HN, Cho J, Kwon MJ, Chang Y, Ryu S, et al. Direct and Indirect Effects of Five Factor Personality and Gender on Depressive Symptoms Mediated by Perceived Stress. *PloS One* (2016) 11(4):e0154140. doi: 10.1371/journal.pone.0154140
78. Kaymaz N, Drukker M, Lieb R, Wittchen HU, Werbeloff N, Weiser M, et al. Do subthreshold psychotic experiences predict clinical outcomes in unselected non-help-seeking population-based samples? A systematic review and meta-analysis, enriched with new results. *Psychol Med* (2012) 42(11):1–15. doi: 10.1017/S0033291711002911
79. Cullen AE, Addington J, Bearden CE, Stone WS, Seidman LJ, Cadenhead KS, et al. Stressor-Cortisol Concordance Among Individuals at Clinical High-Risk for Psychosis: Novel Findings from the NAPLS Cohort. *Psychoneuroendocrinology* (2020) 115:104649. doi: 10.1016/j.psyneuen.2020.104649
80. van Duin EDA, Vaessen T, Kasanova Z, Viechtbauer W, Reininghaus U, Saalbrink P, et al. Lower cortisol levels and attenuated cortisol reactivity to daily-life stressors in adults with 22q11.2 deletion syndrome. *Psychoneuroendocrinology* (2019) 106:85–94. doi: 10.1016/j.psyneuen.2019.03.023

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The handling editor declared a past co-authorship with one of the authors, PM.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 30 June 2020
doi: 10.3389/fpsyt.2020.00628

Peripheral Endogenous Cannabinoid Levels Are Increased in Schizophrenia Patients Evaluated in a Psychiatric Emergency Setting

Stéphane Potvin[1,2]*, *Louiza Mahrouche*[3], *Roxane Assaf*[1,2], *Marjolaine Chicoine*[4], *Charles-Édouard Giguère*[1], *Alexandra Furtos*[3] and *Roger Godbout*[2,4]* *on behalf of the Signature Consortium*

[1] Department of Psychiatry, Centre de recherche de l'Institut Universitaire en Santé Mentale de Montréal, Montreal, QC, Canada, [2] Department of Psychiatry, University of Montreal, Montreal, QC, Canada, [3] Department of Chemistry, University of Montreal, Montreal, QC, Canada, [4] Sleep Laboratory and Clinic, CIUSSS du Nord-de-l'Île-de-Montréal, Hôpital en santé mentale Rivière-des-Prairies, Montréal, QC, Canada

OPEN ACCESS

Edited by:
Błażej Misiak,
Wroclaw Medical University, Poland

Reviewed by:
Cathrin Rohleder,
University of Sydney, Australia
Maria Scherma,
Università di Cagliari, Italy

***Correspondence:**
Stéphane Potvin
stephane.potvin@umontreal.ca
Roger Godbout
roger.godbout@umontreal.ca

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: 28 March 2020
Accepted: 16 June 2020
Published: 30 June 2020

Citation:
Potvin S, Mahrouche L, Assaf R, Chicoine M, Giguère C-É, Furtos A and Godbout R (2020) Peripheral Endogenous Cannabinoid Levels Are Increased in Schizophrenia Patients Evaluated in a Psychiatric Emergency Setting. Front. Psychiatry 11:628. doi: 10.3389/fpsyt.2020.00628

Background: The endogenous cannabinoid system mediates the psychoactive effects of cannabis in the brain. It has been argued that this system may play a key role in the pathophysiology of schizophrenia. While some studies have consistently shown that the levels of anandamide, an endogenous cannabinoid ligand, are increased in the cerebrospinal fluid of schizophrenia patients, inconsistent results have been observed in studies measuring anandamide levels in the periphery. Here, we sought to determine if the assessment of peripheral anandamide levels in patients evaluated in a psychiatric emergency setting would show robust increases.

Methods: One hundred seven patients with a schizophrenia-spectrum disorder from the psychiatric emergency settings of the *Institut Universitaire en Santé Mentale de Montréal* and 36 healthy volunteers were included in the study. A subsample of thirty patients were assessed at two time points: at the emergency and at their discharge from the hospital. Anxious and depressive symptoms, sleep and substance use were assessed using self-report questionnaires. In addition to anandamide, the levels of oleoylethanolamide (OEA), an anorexigenic fatty-acid ethanolamide, were also measured, since the prevalence of the metabolic syndrome is increased in schizophrenia. Plasma levels of anandamide and OEA were measured using liquid chromatography and mass spectrometry.

Results: Plasma anandamide and OEA levels were significantly increased in schizophrenia patients, relative to controls (Cohen's d=1.0 and 0.5, respectively). Between-group differences remained significant after controlling for metabolic measures. No differences were observed between schizophrenia patients with and without a comorbid substance use disorder at baseline. Importantly, the levels of both endocannabinoids significantly decreased after discharge from the emergency setting.

Conclusion: The current results add to the growing body of evidence of endocannabinoid alterations in schizophrenia. The strong elevation of plasma anandamide levels in schizophrenia patients assessed in the psychiatric emergency setting suggests that anandamide and OEA area potential biomarkers of the psychological turmoil associated with this context.

Keywords: anandamide, oleoylethanolamide, schizophrenia, emergency setting, cannabinoids

INTRODUCTION

Schizophrenia is a complex psychiatric disorder, and its pathophysiology is not fully understood. During the last decades, several longitudinal studies have shown that cannabis smoking is a risk factor for psychosis outcomes (1). In adult populations with schizophrenia, several studies have also shown that persistent cannabis smoking is associated with worse outcomes (2). Moreover, several experimental studies have shown that the administration of delta-9-tetrahydrocannabinol to healthy volunteers produces transient effects that are similar to the psychiatric symptoms and cognitive deficits seen in schizophrenia (3, 4). These findings have fueled interest in examining the potential role of the endogenous cannabinoid system, which mediates the psychoactive effects of cannabis in the brain, in the pathophysiology of schizophrenia (5, 6).

The endogenous cannabinoid (ECB) system is complex and is composed of two primary natural ligands, namely anandamide and 2-arachidonoylglycerol (2-AG), and two primary receptors, CB_1 and CB_2 (7). Virodhamine, N-arachidonoyl-dopamine and noladin ether are increasingly considered as ECB ligands (8), and vanilloid receptor 1 and GPR55 as potential ECB receptors (9, 10); however, their precise roles remain to be determined. Anandamide is synthesized from N-acetylphosphatidyl-ethanolamine (NAPE) by NAPE-hydrolysing phospholipidase D and degraded by fatty acid amid hydrolase (FAAH) (11) into ethanolamine and arachidonic acid (12). 2-AG is synthesized from diacylglycerol (DAG) by DAG lipase and degraded by monoacylglycerol lipase into glycerol and arachidonic acid (13). Interestingly, anandamide is involved in key functions that are known to be altered in schizophrenia, including reward processing, stress regulation and memory (14, 15). Moreover, CB_1 receptors are distributed in high densities in brain regions known to be impaired in schizophrenia, such as the prefrontal cortex, the hippocampus and the basal ganglia (7, 16).

Preliminary evidence suggests that the ECB system is involved in the pathophysiology of schizophrenia. Indeed, *postmortem* human brain studies using auto radiography have consistently shown that CB_1 receptor binding is elevated in the dorso-lateral prefrontal cortex in schizophrenia (6). The *postmortem* studies on CB_1 receptor mRNA levels in the dorso-lateral prefrontal cortex have produced mixed results however (17, 18). As for *in vivo* studies, a recent positron emission tomography (PET) study has shown an increase in CB_1 receptor binding in 67 schizophrenia patients in several brain regions, including the ventral striatum, the insula, the inferior frontal cortex and the medial temporal cortex (19). Likewise, Wong et al. (20) had also observed an increase in CB_1 receptor binding in the pons in a small sample of 9 schizophrenia patients. However, a more recent PET study actually showed a decrease in CB_1 receptor binding in several sub-cortical and limbic regions (21). Regarding the ECB ligands, an elevation of anandamide levels in the cerebrospinal fluid (CSF) of schizophrenia patients was initially reported by Leweke et al. (22) in 10 schizophrenia patients (22). Subsequently, CSF anandamide levels were found to be eight-fold higher in 47 schizophrenia patients than in 84 healthy controls and individuals with other psychiatric disorders (23). Importantly, the finding of elevated CSF anandamide levels in schizophrenia has been replicated since then (24). CSF anandamide levels were also found to be elevated during the initial prodromal stages of psychosis (25).

Due to the ease of measurement, a growing number of laboratories have examined peripheral levels of endogenous cannabinoids in schizophrenia, with results being inconsistent across studies thus far. In a study of 20 schizophrenia patients, the blood levels of anandamide were shown to be higher in patients with acute schizophrenia compared to healthy controls (26). Similarly, Koethe et al. (25) found that plasma levels of anandamide are elevated in twins discordant for schizophrenia compared to healthy twins (25). Furthermore, the expression of CNR1, the gene coding for the CB_1 receptor, was found to be up-regulated in the peripheral blood of schizophrenia patients (27). Despite these promising results, other studies looking at blood levels of anandamide did not detect any differences between schizophrenia and healthy controls (24, 28).

The heterogeneity of findings on peripheral levels of anandamide in schizophrenia could be explained by different factors. One important factor is the phase of illness. In the acute phase of illness or during emergency visits, when patients are experiencing significant stress, anandamide levels may be more increased. In fact, it has been shown in experimental studies performed in healthy volunteers that acute stress provokes increases in peripheral anandamide levels (29). Thus far, two studies have been performed in schizophrenia patients during the acute phase of illness—at least to our knowledge. A first study showed significantly higher levels of anandamide in patients compared to healthy controls (26), although it only included a small sample of 12 patients. However, a study from Giuffrida et al. (23) showed no significant alterations of serum anandamide levels in acutely paranoid schizophrenia patients.

As argued by Desfossés et al. (30), important comorbid factors such as substance use and metabolic problems may also influence results. Substance use disorders are highly prevalent in

schizophrenia and are associated with poorer clinical outcomes in this population (31). In a dually diagnosed population, our research team found that plasma anandamide levels were increased, relative to controls, and that there was a positive correlation between anandamide levels at baseline and substance use at 3-month follow-up (32). As for the metabolic syndrome, its prevalence is two to three times higher in patients with schizophrenia (30-40% prevalence) than in the general population (10-20%) (33). Given that anandamide is involved in food control intake *via* central and peripheral mechanisms (34), we performed a pilot functional neuroimaging study and showed that plasma levels of anandamide were positively correlated with amygdala hyper-activations in schizophrenia patients in response to appetizing food stimuli (35). In addition, an association has been observed between the CNR1 gene and the metabolic syndrome in 407 patients with schizophrenia (36). Although structurally related to anandamide, oleoylethanolamide (OEA) is a non-cannabinoid natural bioactive fatty-acid ethanolamide, which binds peroxisome-proliferator-activated receptors, and is degraded by FAAH into oleic acid and ethanolamide (37). OEA has well-demonstrated anorexic properties (37). In schizophrenia, most studies on CSF and blood levels of OEA have shown no significant alterations (22, 23, 25). However, it is crucial to point out that these studies have not accounted for comorbid metabolic problems.

The primary objective of the current study is to show that peripheral levels of anandamide are increased in schizophrenia patients evaluated in a psychiatric emergency setting. The secondary objective is to examine the clinical correlates of anandamide and OEA levels in schizophrenia.

METHODS

Participants

One hundred seven patients with schizophrenia or schizo-affective disorder, male or female, and aged between 18 and 50 years old, were recruited at the psychiatric urgency setting of the *Institut Universitaire en Santé Mentale de Montréal*. The sample was taken from the Signature Bank of the institute (http://www.iusmm.ca/recherche/signature.html). Patients enrolled in the Signature Bank were referred to the research team by the clinical emergency team (nurses, social worker, and emergency psychiatrist), who met each morning to discuss every new case. Psychiatric diagnoses were established by psychiatrists on the ward, and were coded according to the *World Health Organisation International Classification of Disease*, ICD-10 (38). Diagnoses were confirmed after psychiatric hospitalization. The mean number of psychiatric hospitalizations in the last two years was 2.5 (± 3.4), and 34.7% of patients experienced their first episode of psychosis. In this naturalistic study, substance use disorder and metabolic syndrome were not considered as exclusion criteria in the schizophrenia group. Out of 107 schizophrenia patients, 14 had a current comorbid substance use disorder (alcohol, cannabis and/or stimulants). Patients with substance-induced psychosis were however excluded if a schizophrenia of schizo-affective disorder was not confirmed by the psychiatrist. **Figure 1** shows the flowchart for the selection of the 108 patients. Schizophrenia patients were treated with antipsychotics (mean olanzapine equivalents: 13.0 ± 11.6 mg); among them, 22 were treated with two antipsychotics or more, and 10 were treated with clozapine. Thirty-eight healthy volunteers, with no history of severe mental illness or substance use disorder, were also recruited. None of the healthy controls were treated with medication affecting the central nervous system. Both groups did not differ in terms of age (schizophrenia: 31.5 ± 8.2 years; controls: 30.0 ± 7.3 years; t=1.0; p=0.32) and sex ratio (schizophrenia: 40 females; controls: 17 females; χ^2 = 0.36; p=0.55). None of the participants in either group had a history of neurologic disorder, an IQ lower than 70, or chronic and unstable medical diseases at the moment of participation in the study. A subsample of thirty patients had measures at two time points: at the emergency (T1; emergency phase) and at their discharge from the hospital (T2; stabilization phase). Patients were discharged on clinical decision resulting in a variable duration of admission. At baseline, the subsample of 30 patients did not differ from the rest of patients in terms of socio-demographic variables, psychiatric symptoms and metabolic markers (see **Supplementary Table**).

All participants signed a detailed consent form, and the study was approved by the ethics committee of the *Centre de Recherche de l'Institut Universitaire en Santé Mentale de Montréal*.

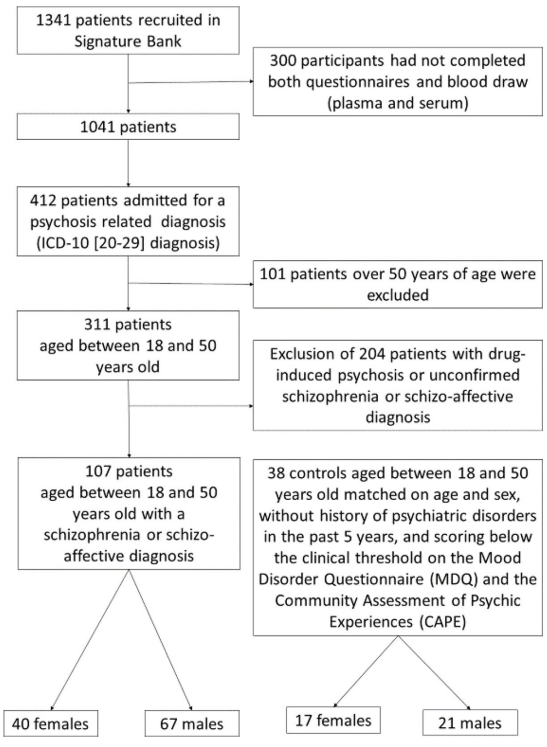

FIGURE 1 | Flowchart of the participants sample selection.

Clinical Assessments

Substance use disorder severity was assessed with the *Alcohol Use Disorders Identification Test* (AUDIT) (39) and the *Drug Abuse Screening Test* (DAST-10) (40). Psychotic symptoms, depressive symptoms and anxiety were measured, respectively, with the *Psychosis Screening Questionnaire* (PSQ) (41), the *Patient Health Questionnaire* (PHQ-9) (42), and the *State Trait Anxiety Inventory* (STAI) (43); sleep problems were assessed with a validated questionnaire from our team (44). The potential influence of antipsychotics on results was examined by calculating olanzapine equivalents (45).

Metabolic Syndrome

A 12-h fasting blood collection (38 ml) was obtained in the morning for the emergency patients and healthy controls. Within 2 h, the local hospital laboratory assayed serum total cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides, and fasting glucose, using standard hospital techniques. Resting seated systolic and diastolic blood pressure and heart rate was measured. Anthropometric measures (e.g. body mass index and waist-to-hip ratio) were also collected.

Metabolic syndrome was defined as the presence of 3 or more of the risk factors identified by the *International Diabetes Federation*: (i) *waist circumference*: males≥ 102 cm; females≥ 88 cm; (ii) *triglycerides*≥ 150 mg/dl; (iii) *high-density lipoprotein* (HDL): males≤ 40 mg/dl; females≤ 50 mg/dl; (iv) *arterial pressure*: systolic pressure≥ 130 mmHg; diastolic pressure≥ 85 mmHg; and (v) *fasting glucose*≥ 100 mg/dl) (46).

Analysis of Plasma Anandamide (AEA) and OEA Levels

We collected blood samples (10 ml) of participants in the morning after 12 h of fasting. Within 2 h, blood samples were centrifuged (2600 rpm for 15 min), and plasma (1 ml) was stored at −80°C in glass vials. Calibration curve standards were prepared in a pooled human plasma (Innovative research, Novi, MI) using standards of AEA-d4 and OEA-d4 (Cayman Chemical, Ann Arbour, MI) ranging from 0.02 ng/ml to 6 ng/ml and were kept frozen. Freshly thawed plasma aliquots and calibration curve standards (450 µL) were diluted with 900 µL of cold acetonitrile containing 10 ng/ml of the internal standard, AEA-d8 (Cayman Chemical, Ann Arbour, MI). Samples were then loaded to an Impact protein precipitation plate from (Phenomenex, Terrance, CA). The flow through was diluted with 500 µL HPLC grade water and submitted to solid phase extraction on Hydrophilic-Lipophilic Balance Oasis HLB 30 mg cartridges from (Waters). Eluted compounds were dried down under a nitrogen stream and reconstituted in 75 µL of the starting mobile phase. Aliquots of 15 µL were injected into the liquid chromatography-mass spectrometry (LC-MS) system. Chromatography was performed on an 1100 series from Agilent Technologies (Santa Clara, CA) using a Charged Surface Hybrid C18, 2.1x100 mm, 3.5 µm column from Waters (Milford, MA). The eluents consisted of 40% acetonitrile and 60% water (solvent A) and 90% isopropanol and 10% acetonitrile (solvent B), both containing 0.4% formic acid. The initial mobile phase contained 35% B and was increased to 45% B over 10 min. Endocannabinoids were monitored on a triple quadrupole mass spectrometer 6410 from Agilent Technologies (Santa Clara, CA) operated in positive Electrospray Ionization using the Multiple Reaction Monitoring mode. The LC-MS method was linear for both AEA and OEA from 0.02 to 6 ng/ml. Samples were run on four batches, over four days. Coefficients of Variation as measured for the Quality Controls across the four days were within 9.6 % for AEA and 7.4 % for OEA.

Statistical Analyses

Potential group differences for dichotomic variables and continuous variables were examined, respectively, with chi-square tests and two-sample *t* tests. The potential relationships between endocannabinoid levels and clinical variables (e.g. psychiatric symptoms, sleep and metabolic variables) were examined using linear regression analyses. For the regression analyses, the potential association between endocannabinoid levels and metabolic variables was examined by calculating the number of metabolic syndrome criteria met by each participant. Finally, a subsample of patients (n=30) were assessed at admission (T1) and at their discharge (T2). A paired *t* test was assessed to check for changes between T1 and T2. Pearson's correlation tests were performed to assess if antipsychotic dosage (in olanzapine equivalents) was associated with each of the endocannabinoid levels. Statistical analyses were performed with R version 3.6.3. The threshold for statistical significance was set at $p < 0.05$. For each series of variables, a false-discovery rate (FDR) was applied to the p-value to account for type-II error. Both corrected and uncorrected p-values are presented.

RESULTS

Between-Group Differences

Relative to controls, schizophrenia patients had increased psychiatric symptoms (e.g. anxiety, depression, psychosis and poor sleep efficiency), as well as higher scores on substance use scales (e.g. AUDIT and DAST) (**Table 1**). A higher proportion of patients presented the metabolic syndrome, compared to controls (schizophrenia: 34.0%; controls: 8.1%; $\chi^2 = 7.99$; p=0.005). Furthermore, metabolic markers (e.g. Waist circumference, Triglyceride, HDL) were found to be impaired in schizophrenia. More importantly, anandamide levels were strongly increased in schizophrenia patients, relative to controls, with a large effect size (Cohen's d=0.9; $p < 0.001$) (**Table 1** and **Figure 2**). OEA levels differed between patients and controls with a smaller effect-size (Cohen's d=0.5; p=0.013) (**Table 1** and **Figure 2**). Between-group differences in anandamide and OEA levels remained significant after controlling for the potential influence of metabolic markers. Likewise, there were no differences in ECB levels between patients with and without a substance use disorder (anandamide: p=0.56; OEA: p=0.43).

TABLE 1 | Differences between schizophrenia patients and healthy controls.

Type of variable	Variable	Schizophrenia (n=107)	Controls (n=38)	Statistics
Endogenous cannabinoids	Anandamide	653.7 (222.8)	469.8 (110.1)	t=6.5; p < 0.001
	OEA	2856.8 (969.7)	2416.2 (716.7)	t=2.9; p=0.005
Psychiatric symptoms	AUDIT	5.2 (6.9)	4.1 (2.8)	t=1.3; p=0.19
	DAST	3.1 (3.2)	0.5 (0.6)	t=8.0; p < 0.001
	Psychosis	1.5 (1.4)	0.0 (0.0)	t=11.1; p < 0.001
	Anxiety	47.8 (14.8)	34.3 (10.2)	t=6.2; p < 0.001
	Depression	10.6 (7.0)	2.6 (3.1)	t=9.4; p < 0.001
Sleep	Sleep efficiency (%)	91.7 (16.0)	96.4 (7.9)	t=-2.3; p=0.025
Metabolic Syndrome	Waist circumference (cm)	97.2 (18.1)	88.2 (18.9)	t=2.5; p=0.013
	Triglyceride (mg/dl)	1.6 (1.2)	0.9 (0.5)	t=4.6; p < 0.001
	HDL (mmol/L)	1.1 (0.3)	1.4 (0.3)	t=-6.1; p < 0.001
	Mean arterial pressure (mmHg)	90.4 (11.0)	89.6 (9.7)	t=0.4; p=0.66
	Glycemia (mmol/L)	5.1 (1.0)	4.9 (0.5)	t=1.4; p=0.16
	Number of metabolic syndrome indicators	1.9 (1.3)	0.8 (1.2)	t=4.6; p < 0.001

AUDIT, Alcohol Use Disorder Identification Test; DAST, Drug Abuse Screening Test; HDL, high-density lipoprotein; OEA, oleoylethanolamide; the metabolic syndrome is obtained when the number of syndrome indicators is three or more.

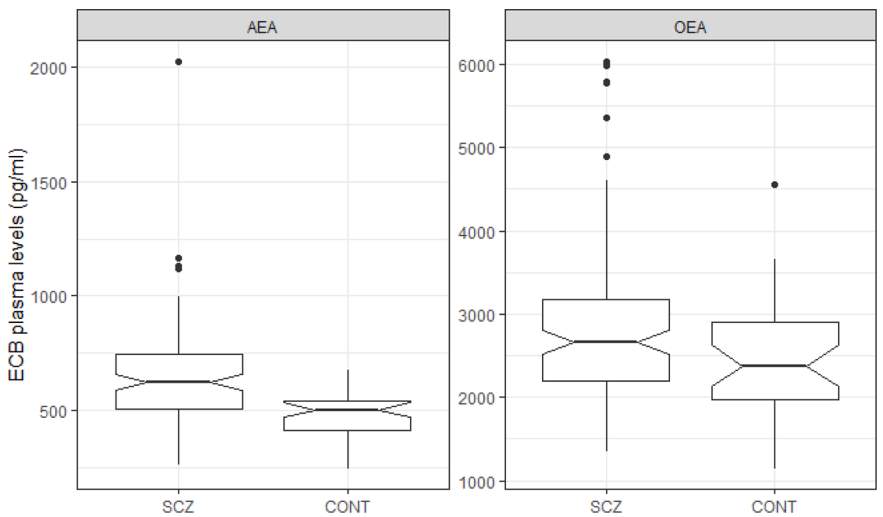

FIGURE 2 | Boxplot of ECB plasma levels by Group (Schizophrenia vs. Controls).

Regression Analyses

Across groups, a significant and positive association was found between anandamide levels and depressive symptoms (p=0.009) (**Table 2** and **Figure 3**). This association remained significant after adjusting for the false-discovery rate (p*=0.043). A smaller negative association was found between anandamide levels and sleep efficiency (p=0.053) (**Table 2**). Across groups, there were no significant correlations between endogenous cannabinoid levels and substance use, anxiety and psychosis (**Table 2**). Positive associations of small magnitude were observed between anandamide levels and waist circumference (p=0.013) and arterial pressure (p=0.018), while a small negative association was found between OEA levels and triglyceride levels (p=0.038) (**Table 2**). These associations were no longer significant after accounting for the FDR adjustment. No association was found between the number of metabolic syndrome indicators and ECB levels.

Paired t Test

The mean length of hospitalization was 26.1 days (± 22.4). For both endocannabinoids, the mean levels decreased between admission (T1) and discharge (T2). In the case of anandamide, we observed a mean decrease of 132.6 pg/ml (p < 0.001) and as for OEA, a decrease of 639.7 pg/ml (p=0.005) (**Table 3**). Aside from endocannabinoids, four clinical outcomes showed significant improvements from T1 to T2, namely psychosis (0.024), anxiety (0.013), depression (p < 0.001), and mean arterial pressure (0.05) (**Table 3**).

Antipsychotic Treatment

No statistically significant associations were found between prescribed antipsychotic dosage (e.g. olanzapine equivalents) and plasma ECB levels (rAEA: r=0.06; p=0.58; OEA: r= −0.02; p=0.89). Between-group differences in anandamide and OEA levels remained significant after controlling for antipsychotic dosage.

TABLE 2 | Linear regression analyses.

Clinical variable		Anandamide					OEA				
		Est.	SE	t	p	p*	Est.	SE	t	p	p*
AUDIT	Int.	2.642	1.787	1.478	0.14	0.14	3.491	1.725	2.024	0.045	0.045
	Group	-0.269	1.270	-0.212	0.83	0.83	-0.725	1.210	-0.599	0.55	0.55
	ECB	0.004	0.003	1.521	0.13	0.28	0.001	0.001	1.058	0.29	0.63
DAST	Int.	2.855	0.817	3.493	<0.001	0.001	2.668	0.784	3.402	<0.001	<0.001
	Group	-2.542	0.580	-4.381	<0.001	<0.001	-2.542	0.550	-4.621	<0.001	<0.001
	ECB	0.000	0.001	0.288	0.77	0.77	0.000	0.000	0.555	0.58	0.63
Psychosis (PSQ)	Int.	1.672	0.347	4.821	<0.001	<0.001	1.637	0.334	4.899	<0.001	<0.001
	Group	-1.539	0.247	-6.238	<0.001	<0.001	-1.509	0.234	-6.456	<0.001	<0.001
	ECB	-0.000	0.000	-0.571	0.57	0.71	-0.000	0.000	-0.483	0.63	0.63
Anxiety (STAI)	Int.	42.579	4.003	10.637	<0.001	<0.001	45.479	3.884	11.71	<0.001	<0.001
	Group	-12.456	2.857	-4.359	<0.001	<0.001	-13.569	2.726	-4.979	<0.001	<0.001
	ECB	0.008	0.006	1.388	0.17	0.28	0.001	0.001	0.642	0.52	0.63
Depression (PHQ-9)	Int.	6.095	1.789	3.407	<0.001	0.001	7.481	1.746	4.285	<0.001	<0.001
	Group	-6.715	1.277	-5.259	<0.001	<0.001	-7.503	1.226	-6.120	<0.001	<0.001
	ECB	0.007	0.003	2.668	**0.009**	**0.043**	0.001	0.001	1.901	0.06	0.30
Sleep efficiency (%)	Int.	99.408	4.179	23.786	<0.001	<0.001	93.817	4.086	22.96	<0.001	<0.001
	Group	2.552	3.019	0.845	0.40	0.40	4.426	2.900	1.526	0.13	0.13
	ECB	-0.012	0.006	-1.950	0.053	0.053	-0.001	0.001	-0.544	0.59	0.59
Waist circumference (cm)	Int.	84.780	5.254	16.135	<0.001	<0.001	97.320	5.183	18.78	<0.001	<0.001
	Group	-5.318	3.751	-1.418	0.16	0.24	-8.814	3.640	-2.421	0.017	0.025
	ECB	0.019	0.008	2.506	**0.013**	0.054	-0.000	0.002	-0.026	0.98	0.98
Triglyceride (mg/dL)	Int.	1.950	0.304	6.420	<0.001	<0.001	2.144	0.292	7.356	<0.001	<0.001
	Group	-0.739	0.219	-3.371	<0.001	0.002	-0.712	0.205	-3.472	<0.001	0.001
	ECB	-0.001	0.000	-1.325	0.19	0.28	-0.000	0.000	-2.097	**0.038**	0.23
HDL (mmol/L)	Int.	1.146	0.083	13.863	<0.001	<0.001	0.955	0.079	12.021	<0.001	<0.001
	Group	0.335	0.060	5.625	<0.001	<0.001	0.372	0.056	6.651	<0.001	<0.001
	ECB	-0.000	0.000	-0.816	0.42	0.50	0.000	0.000	1.706	0.09	0.27
Mean arterial pressure (mmHg)	Int.	83.477	3.074	27.156	<0.001	<0.001	86.851	3.009	28.86	<0.001	<0.001
	Group	0.917	2.194	0.418	0.68	0.68	-0.485	2.112	-0.230	0.82	0.82
	ECB	0.011	0.004	2.393	**0.018**	0.054	0.001	0.001	1.261	0.21	0.42
Glucose (mmol/L)	Int.	5.117	0.263	19.451	<0.001	<0.001	5.249	0.254	20.64	<0.001	<0.001
	Group	-0.185	0.190	-0.972	0.33	0.40	-0.196	0.179	-1.094	0.28	0.33
	ECB	-0.000	0.000	-0.215	0.83	0.83	-0.000	0.000	-0.777	0.44	0.53
# of metabolic syndrome indicators (0-5)	Int.	1.426	0.377	3.789	<0.001	<0.001	2.203	0.366	6.017	<0.001	<0.001
	Group	-0.941	0.272	-3.463	<0.001	0.002	-1.119	0.258	-4.341	<0.001	<0.001
	ECB	0.001	0.001	1.350	0.18	0.28	-0.000	0.000	-0.865	0.39	0.53

AUDIT, Alcohol Use Disorder Identification Test; DAST, Drug Abuse Screening Test; ECB, endocannabinoid; HDL, high-density lipoproteins; OEA, oleoylethanolamide; PHQ-9, Patient Health Questionnaire-9; PSQ, Psychosis Screening Questionnaire; STAI, State Trait Anxiety Inventory; SE, standard error; p= p-value adjusted for the false-discovery rate.*

DISCUSSION

Despite substantial evidence that cannabis is a risk factor for psychosis (1) and growing evidence suggesting that the endocannabinoid system is altered in schizophrenia, studies examining peripheral levels of anandamide have produced inconsistent results thus far. In the current study, we sought to determine the blood levels of anandamide and OEA in schizophrenia patients recruited at the emergency setting. As hypothesized, we found that anandamide levels were robustly increased in patients relative to a group of healthy volunteers; in the case of OEA, there was also an increase at baseline but it was smaller. Importantly, we observed a significant decrease in both biomarkers in a subset of patients after discharge from the emergency setting. As such, these results suggest that anandamide, and OEA to a lesser extent, are potential biomarkers of the stress induced by an acute mental crisis prior to the presentation to the emergency department. In addition, we observed a positive correlation between peripheral levels of anandamide and depressive symptoms. However, there was no association with psychotic symptoms, unlike the previous reports of negative correlations between psychotic symptoms and anandamide levels, as measured in the CSF (23) and the serum (47). Being in a psychiatric inpatient setting is a source of significant stress for patients with schizophrenia, and as mentioned in the introduction, acute stress has been shown to result in increased peripheral anandamide levels in healthy volunteers (29). Moreover, it is well documented that stress is a risk factor for depression (48–50) and that the hypothalamic-pituitary-adrenal (HPA) axis is disturbed in major depressive disorder (51). Taken together, these observations suggest that anandamide alterations are more related to emotional turmoil associated with the psychiatric emergency setting rather than the severity of psychotic symptoms. At the physiological level, this association could be mediated by a dysregulation of the HPA axis.

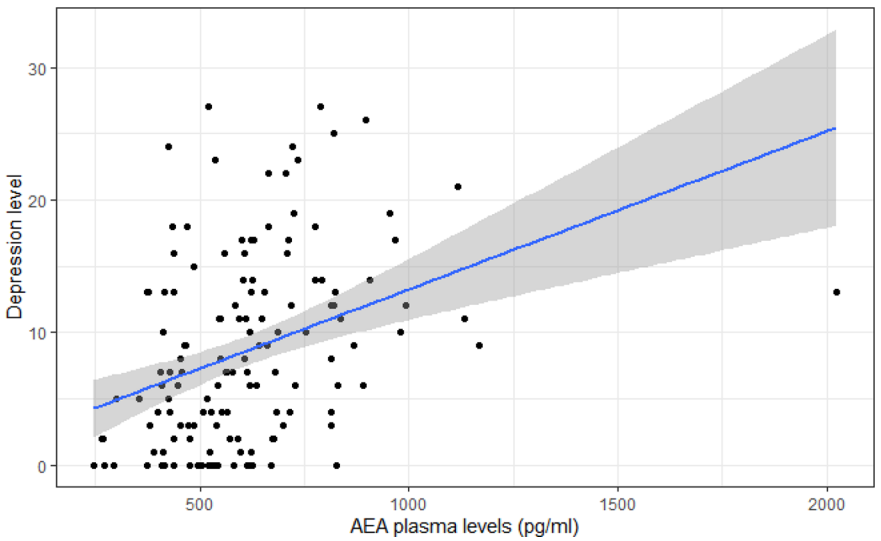

FIGURE 3 | Dispersion plot of depression levels (PHQ-9) on AEA levels.

TABLE 3 | Paired t-test to assess the differences between endocannabinoid levels and clinical variables at admission (T1) and at release (T2).

Endocannabinoids (pg/ml; n=30)	Mean (sd) T1	Mean (sd) T2	Mean Diff. (T1-T2)	t	p	p*
Anandamide	543.5 (171)	410.8 (139)	132.6	3.76	<0.001	0.002
OEA	2287.0 (915)	1647.3 (757)	639.7	3.07	0.005	0.005
AUDIT	4.9 (7.5)	4.6 (7.8)	0.30	0.53	0.60	0.60
DAST	3.0 (3.1)	2.5 (2.8)	0.57	1.32	0.20	0.25
Psychosis (PSQ)	1.5 (1.4)	0.9 (1.0)	0.53	2.39	0.024	0.040
Anxiety (STAI)	45.3(15.0)	38.4 (15.0)	6.89	2.64	0.013	0.033
Depression (PHQ-9)	10.2 (6.2)	6.0 (4.9)	4.23	4.35	<0.001	<0.001
Sleep efficiency (%)	95.4 (8.3)	97.7 (4.1)	-2.30	-1.38	0.18	0.18
Waist circumference (cm)	97.0 (13.5)	98.5 (12.5)	-0.91	-0.78	0.44	0.84
Triglyceride (mg/dL)	1.6 (1.1)	1.6 (1.0)	0.02	0.10	0.92	0.92
HDL (mmol/L)	1.1 (0.3)	1.1 (0.3)	0.01	0.38	0.71	0.85
Mean arterial pressure (mmHg)	93.5 (11.0)	89.2 (8.9)	4.32	2.05	0.050	0.30
Glycemia (mmol/L)	5.1 (0.9)	5.3 (2.0)	-0.16	-0.58	0.56	0.84
# of metabolic syndrome indicators (0-5)	1.8 (1.2)	1.6 (1.5)	0.27	1.35	0.19	0.57

AUDIT, Alcohol Use Disorder Identification Test; DAST, Drug Abuse Screening Test; ECB, endocannabinoid; HDL, high-density lipoproteins; OEA, oleoylethanolamide; PHQ-9, Patient Health Questionnaire-9; PSQ, Psychosis Screening Questionnaire; STAI, State Trait Anxiety Inventory. p= p-value adjusted for the false-discovery rate.*

A secondary objective of the current study was to examine the potential association of peripheral ECB levels and common comorbidities (e.g. substance use disorder and metabolic syndrome) in schizophrenia. In the current study, the prevalence of the metabolic syndrome was increased in schizophrenia patients, relative to controls, consistently with the vast literature on the topic (33). Moreover, small associations were found between metabolic variables and plasma anandamide and OEA levels, which were no longer significant after applying corrections for multiple comparisons. In animal studies, there is strong evidence showing that CB_1 receptor agonists and OEA exert control over food intake *via* central and peripheral mechanisms, including hepatic triglyceride biosynthesis (34, 37). In humans, mounting clinical evidence gathered in populations with no severe mental illness suggests that blood levels of anandamide and OEA are increased in obese individuals (52, 53). Moreover, complex associations between plasma OEA levels and limbic activity (e.g. insula) elicited by food cues have been observed in obese and control individuals (54). In addition, controlled trials of the CB_1 inverse agonist rimonabant for the treatment of obesity have shown significant reductions in body weight, triglyceride levels and the prevalence of the metabolic syndrome (55). Similarly, preliminary evidence suggests that OEA reduces appetite in obese people (56). As mentioned in the introduction, preliminary evidence has linked the ECB system to appetite dysregulation in schizophrenia (35, 36). Overall, the significant associations between anandamide and OEA and metabolic variables are consistent with current evidence. Finally, we found a small and negative relationship between anandamide

and sleep efficiency. To our knowledge, this is the first study describing such an association in schizophrenia. The result is consistent with the increasing evidence on anandamide as a sleep regulator (57).

Unexpectedly, we found no relationships between ECBs (anandamide and OEA) and substance use problems. In the past, two studies have examined the influence of substance use on ECB levels in schizophrenia. Our team found that plasma anandamide and OEA levels were increased in a population of schizophrenia patients with comorbid substance use disorder (mainly alcohol and cannabis) (32). Conversely, another team found that CSF anandamide levels were increased in schizophrenia patients who used cannabis *occasionally*, relative to controls, whereas there were no differences in CSF anandamide levels between controls and schizophrenia patients who used cannabis *frequently* (58). Considering that we found no association, here, between substance use severity and plasma levels of ECBS, the available evidence suggest that the impact of substance use on ECBs in schizophrenia is complex, and that results are influenced by factors such as the pattern of substance use (e.g. use, frequent use, disorder), the type of substance (e.g. cannabis and/or alcohol) and the biological sample used to measure ECBs (e.g. blood versus CSF).

The current study has a few limitations that need to be acknowledged. First, the positive symptoms (delusions, hallucinations) of schizophrenia patients were assessed with the PSQ, a self-report instrument, although interview-based assessments are considered as the gold standard in the field (59). Due to a lack of insight, patients may not self-report psychotic symptoms. This may not only impede the assessment of whether patients are in the acute phase of illness or not, but also impede the investigation of a potential association between ECB levels and psychotic symptoms. Second, schizophrenia patients were treated with antipsychotic medication before blood collection. Although the impact of antipsychotics on peripheral anandamide and OEA levels are currently poorly understood (32, 35), we cannot rule out this confounding effect. However, no significant associations were found between antipsychotic dosage and ECB plasma levels. Moreover, it is worth noting that psychiatric hospitalization is associated with very poor drug compliance in schizophrenia (60). On the other hand, the main strength of the current study is that it investigated peripheral anandamide and OEA levels in the largest sample of schizophrenia patients assessed in the psychiatric emergency setting (at least, to our knowledge), and that a subgroup of these patients were reassessed after release from the emergency when they no longer had acute symptoms.

The results of the current study show that plasma anandamide and OEA levels are significantly increased in schizophrenia patients evaluated in the psychiatric emergency setting. As such, anandamide and OEA are candidate biomarker of this phase. In the future, longitudinal studies will need to be performed in larger samples of schizophrenia patients in both the acute phase of illness and after psychiatric stabilization. Future studies will also need to assess a larger range of endocannabinoid biomarkers that are not restricted to anandamide and OEA, and to examine if changes in ECBs vary according to antipsychotic response. Finally, the potential interactions between ECBs and the HPA axis will need to be investigated in schizophrenia.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because The current study was performed using an institutional databank including genetic information, and the ethics committee has not granted permission to make the dataset of individual studies available to the community. Requests to access the datasets should be directed to stephane.guay.CEMTL@ssss.gouv.qc.ca.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Centre de Recherche de l'Institut Universitaire en Santé Mentale de Montréal. The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

LM and AF performed the biochemical analyses. RG, MC, and SP designed the study. SP and RG provided funding. Statistical analyses were performed by C-EG. SP wrote the manuscript. All authors provided critical comments.

FUNDING

The study was funded by Bell Canada mental health initiatives, Centre de recherche de l'Hôpital Rivière-des-Prairies, the Institut Universitaire en Santé Mentale de Montréal and a grant from the Canadian Institute of Health Research to SP.

ACKNOWLEDGMENTS

SP is holder of the Eli Lilly Canada Chair on schizophrenia research.

REFERENCES

1. Marconi A, Di Forti M, Lewis CM, Murray RM, Vassos E. Meta-analysis of the Association Between the Level of Cannabis Use and Risk of Psychosis. *Schizophr Bull* (2016) 42:1262–9. doi: 10.1093/schbul/sbw003
2. Schoeler T, Petros N, Di Forti M, Klamerus E, Foglia E, Murray R, et al. Poor medication adherence and risk of relapse associated with continued cannabis use in patients with first-episode psychosis: a prospective analysis. *Lancet Psychiatry* (2017) 4:627–33. doi: 10.1016/S2215-0366(17)30233-X
3. D'Souza DC, Perry E, MacDougall L, Ammerman Y, Cooper T, Wu Y, et al. The Psychotomimetic Effects of Intravenous Delta-9-Tetrahydrocannabinol

in Healthy Individuals: Implications for Psychosis. *Neuropsychopharmacology* (2004) 29:1558–72. doi: 10.1038/sj.npp.1300496
4. Sherif M, Radhakrishnan R, D'Souza DC, Ranganathan M. Human Laboratory Studies on Cannabinoids and Psychosis. *Biol Psychiatry* (2016) 79:526–38. doi: 10.1016/j.biopsych.2016.01.011
5. Emrich HM, Leweke FM, Schneider U. Towards a cannabinoid hypothesis of schizophrenia: cognitive impairments due to dysregulation of the endogenous cannabinoid system. *Pharmacol Biochem Behav* (1997) 56:803–7. doi: 10.1016/s0091-3057(96)00426-1
6. Volk DW, Lewis DA. The Role of Endocannabinoid Signaling in Cortical Inhibitory Neuron Dysfunction in Schizophrenia. *Biol Psychiatry* (2016) 79:595–603. doi: 10.1016/j.biopsych.2015.06.015
7. Glass M, Faull RLM, Dragunow M. Cannabinoid receptors in the human brain: a detailed anatomical and quantitative autoradiographic study in the fetal, neonatal and adult human brain. *Neuroscience* (1997) 77:299–318. doi: 10.1016/S0306-4522(96)00428-9
8. Fezza F, Bari M, Florio R, Talamonti E, Feole M, Maccarrone M. Endocannabinoids, related compounds and their metabolic routes. *Mol Basel Switz* (2014) 19:17078–106. doi: 10.3390/molecules191117078
9. Henstridge CM. Off-Target Cannabinoid Effects Mediated by GPR55. *Pharmacology* (2012) 89:179–87. doi: 10.1159/000336872
10. Ryberg E, Larsson N, Sjögren S, Hjorth S, Hermansson N-O, Leonova J, et al. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br J Pharmacol* (2007) 152:1092–101. doi: 10.1038/sj.bjp.0707460
11. Giang DK, Cravatt BF. Molecular characterization of human and mouse fatty acid amide hydrolases. *Proc Natl Acad Sci* (1997) 94:2238–42. doi: 10.1073/pnas.94.6.2238
12. Deutsch DG, Chin SA. Enzymatic synthesis and degradation of anandamide, a cannabinoid receptor agonist. *Biochem Pharmacol* (1993) 46:791–6. doi: 10.1016/0006-2952(93)90486-G
13. Ueda N, Tsuboi K, Uyama T, Ohnishi T. Biosynthesis and degradation of the endocannabinoid 2-arachidonoylglycerol. *BioFactors* (2011) 37:1–7. doi: 10.1002/biof.131
14. Murillo-Rodriguez E, Pastrana-Trejo JC, Salas-Crisóstomo M, de-la-Cruz M. The Endocannabinoid System Modulating Levels of Consciousness, Emotions and Likely Dream Contents. *CNS Neurol Disord Drug Targets* (2017) 16:370–9. doi: 10.2174/1871527316666170223161908
15. Robson PJ, Guy GW, Di VM. Cannabinoids and schizophrenia: therapeutic prospects. *Curr Pharm Des* (2014) 20:2194–204. doi: 10.2174/13816128113199990427
16. Bossong MG, Jansma JM, Bhattacharyya S, Ramsey NF. Role of the endocannabinoid system in brain functions relevant for schizophrenia: An overview of human challenge studies with cannabis or Δ9-tetrahydrocannabinol (THC). *Prog Neuropsychopharmacol Biol Psychiatry* (2014) 52:53–69. doi: 10.1016/j.pnpbp.2013.11.017
17. Eggan SM, Hashimoto T, Lewis DA. Reduced cortical cannabinoid 1 receptor messenger RNA and protein expression in schizophrenia. *Arch Gen Psychiatry* (2008) 65:772–84. doi: 10.1001/archpsyc.65.7.772
18. Urigüen L, García-Fuster MJ, Callado LF, Morentin B, La Harpe R, Casadó V, et al. Immunodensity and mRNA expression of A2A adenosine, D2 dopamine, and CB1 cannabinoid receptors in postmortem frontal cortex of subjects with schizophrenia: effect of antipsychotic treatment. *Psychopharmacol (Berl)* (2009) 206:313–24. doi: 10.1007/s00213-009-1608-2
19. Ceccarini J, De Hert M, Van Winkel R, Peuskens J, Bormans G, Kranaster L, et al. Increased ventral striatal CB1 receptor binding is related to negative symptoms in drug-free patients with schizophrenia. *NeuroImage* (2013) 79:304–12. doi: 10.1016/j.neuroimage.2013.04.052
20. Wong DF, Kuwabara H, Horti AG, Raymont V, Brasic J, Guevara M, et al. Quantification of cerebral cannabinoid receptors subtype 1 (CB1) in healthy subjects and schizophrenia by the novel PET radioligand [11C]OMAR. *NeuroImage* (2010) 52:1505–13. doi: 10.1016/j.neuroimage.2010.04.034
21. Ranganathan M, Cortes-Briones J, Radhakrishnan R, Thurnauer H, Planeta B, Skosnik P, et al. Reduced Brain Cannabinoid Receptor Availability in Schizophrenia. *Biol Psychiatry* (2016) 79:997–1005. doi: 10.1016/j.biopsych.2015.08.021
22. Leweke FM, Giuffrida A, Wurster U, Emrich HM, Piomelli D. Elevated endogenous cannabinoids in schizophrenia. *NeuroReport* (1999) 10:1665–9. Available at: https://journals.lww.com/neuroreport/Fulltext/1999/06030/Elevated_endogenous_cannabinoids_in_schizophrenia.8.aspx [Accessed May 21, 2020].
23. Giuffrida A, Leweke FM, Gerth CW, Schreiber D, Koethe D, Faulhaber J, et al. Cerebrospinal anandamide levels are elevated in acute schizophrenia and are inversely correlated with psychotic symptoms. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol* (2004) 29:2108–14. doi: 10.1038/sj.npp.1300558
24. Reuter AR, Bumb JM, Mueller JK, Rohleder C, Pahlisch F, Hanke F, et al. Association of anandamide with altered binocular depth inversion illusion in schizophrenia. *World J Biol Psychiatry* (2017) 18:483–8. doi: 10.1080/15622975.2016.1246750
25. Koethe D, Giuffrida A, Schreiber D, Hellmich M, Schultze-Lutter F, Ruhrmann S, et al. Anandamide elevation in cerebrospinal fluid in initial prodromal states of psychosis. *Br J Psychiatry* (2009) 194:371–2. doi: 10.1192/bjp.bp.108.053843
26. De Marchi N, De Petrocellis L, Orlando P, Daniele F, Fezza F, Di Marzo V. Endocannabinoid signalling in the blood of patients with schizophrenia. *Lipids Health Dis* (2003) 2:5. doi: 10.1186/1476-511X-2-5
27. Moretti PN, Ota VK, Gouvea ES, Pedrini M, Santoro ML, Talarico F, et al. Accessing Gene Expression in Treatment-Resistant Schizophrenia. *Mol Neurobiol* (2018) 55:7000–8. doi: 10.1007/s12035-018-0876-4
28. Desfossés J, Stip E, Ait Bentaleb L, Lipp O, Chiasson J-P, Furtos A, et al. Plasma Endocannabinoid Alterations in Individuals with Substance Use Disorder are Dependent on the "Mirror Effect" of Schizophrenia. *Front Psychiatry* (2012) 3:85. doi: 10.3389/fpsyt.2012.00085
29. Dlugos A, Childs E, Stuhr KL, Hillard CJ, de Wit H. Acute stress increases circulating anandamide and other N-acylethanolamines in healthy humans. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol* (2012) 37:2416–27. doi: 10.1038/npp.2012.100
30. Desfossés J, Stip E, Bentaleb LA, Potvin S. Endocannabinoids and Schizophrenia. *Pharmaceuticals* (2010) 3:3101–26. doi: 10.3390/ph3103101
31. Khokhar JY, Dwiel LL, Henricks AM, Doucette WT, Green AI. The link between schizophrenia and substance use disorder: A unifying hypothesis. *Schizophr Res* (2018) 194:78–85. doi: 10.1016/j.schres.2017.04.016
32. Potvin S, Kouassi E, Lipp O, Bouchard R-H, Roy M-A, Demers M-F, et al. Endogenous cannabinoids in patients with schizophrenia and substance use disorder during quetiapine therapy. *J Psychopharmacol Oxf Engl* (2008) 22:262–9. doi: 10.1177/0269881107083816
33. Mitchell AJ, Vancampfort D, Sweers K, van Winkel R, Yu W, De Hert M. Prevalence of metabolic syndrome and metabolic abnormalities in schizophrenia and related disorders–a systematic review and meta-analysis. *Schizophr Bull* (2013) 39:306–18. doi: 10.1093/schbul/sbr148
34. Engeli S. Central and peripheral cannabinoid receptors as therapeutic targets in the control of food intake and body weight. *Handb Exp Pharmacol* (2012) 209:357–81. doi: 10.1007/978-3-642-24716-3_17
35. Potvin S, Lungu OV, Stip E. Anandamide is involved in appetite-related amygdala hyperactivations in schizophrenia patients treated with olanzapine: a functional magnetic resonance imaging study. *J Clin Psychopharmacol* (2015) 35:82–3. doi: 10.1097/JCP.0000000000000236
36. Yu W, De Hert M, Moons T, Claes SJ, Correll CU, van Winkel R. CNR1 gene and risk of the metabolic syndrome in patients with schizophrenia. *J Clin Psychopharmacol* (2013) 33:186–92. doi: 10.1097/JCP.0b013e318283925e
37. Laleh P, Yaser K, Alireza O. Oleoylethanolamide: A novel pharmaceutical agent in the management of obesity-an updated review. *J Cell Physiol* (2019) 234:7893–902. doi: 10.1002/jcp.27913
38. World Health Organization. *ICD-10 : international statistical classification of diseases and related health problems : tenth revision*. Geneva: World Health Organization (2004). Available at: https://apps.who.int/iris/handle/10665/42980 [Accessed August 16, 2019].
39. Gache P, Michaud P, Landry U, Accietto C, Arfaoui S, Wenger O, et al. The Alcohol Use Disorders Identification Test (AUDIT) as a Screening Tool for Excessive Drinking in Primary Care: Reliability and Validity of a French Version. *Alcohol Clin Exp Res* (2005) 29:2001–7. doi: 10.1097/01.alc.0000187034.58955.64
40. Yudko E, Lozhkina O, Fouts A. A comprehensive review of the psychometric properties of the Drug Abuse Screening Test. *J Subst Abuse Treat* (2007) 32:189–98. doi: 10.1016/j.jsat.2006.08.002
41. Bebbington P, Nayani T. The Psychosis Screening Questionnaire. *Int J Methods Psychiatr Res* (1995) 5:11–9.

42. Inagaki M, Ohtsuki T, Yonemoto N, Kawashima Y, Saitoh A, Oikawa Y, et al. Validity of the Patient Health Questionnaire (PHQ)-9 and PHQ-2 in general internal medicine primary care at a Japanese rural hospital: a cross-sectional study. *Gen Hosp Psychiatry* (2013) 35:592–7. doi: 10.1016/j.genhosppsych.2013.08.001
43. Tluczek A, Henriques JB, Brown RL. Support for the Reliability and Validity of a Six-Item State Anxiety Scale Derived From the State-Trait Anxiety Inventory. *J Nurs Meas* (2009) 17:19–28.
44. Poulin J, Chouinard S, Pampoulova T, Lecomte Y, Stip E, Godbout R. Sleep habits in middle-aged, non-hospitalized men and women with schizophrenia: A comparison with healthy controls. *Psychiatry Res* (2010) 179:274–8. doi: 10.1016/j.psychres.2009.08.009
45. Leucht S, Samara M, Heres S, Davis JM. Dose Equivalents for Antipsychotic Drugs: The DDD Method. *Schizophr Bull* (2016) 42 Suppl 1:S90–94. doi: 10.1093/schbul/sbv167
46. Alberti KGMM, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation* (2009) 120:1640–5. doi: 10.1161/CIRCULATIONAHA.109.192644
47. Leweke FM, Piomelli D, Pahlisch F, Muhl D, Gerth CW, Hoyer C, et al. Cannabidiol enhances anandamide signaling and alleviates psychotic symptoms of schizophrenia. *Transl Psychiatry* (2012) 2:e94. doi: 10.1038/tp.2012.15
48. Andersen SL, Teicher MH. Stress, sensitive periods and maturational events in adolescent depression. *Trends Neurosci* (2008) 31:183–91. doi: 10.1016/j.tins.2008.01.004
49. Charney DS, Manji HK. Life Stress, Genes, and Depression: Multiple Pathways Lead to Increased Risk and New Opportunities for Intervention. *Sci STKE* (2004) 2004:re5–5. doi: 10.1126/stke.2252004re5
50. Plieger T, Melchers M, Montag C, Meermann R, Reuter M. Life stress as potential risk factor for depression and burnout. *Burn Res* (2015) 2:19–24. doi: 10.1016/j.burn.2015.03.001
51. Vreeburg SA, Hoogendijk WJG, van PJ, RH D, Verhagen JCM, van Dyck R, et al. Major Depressive Disorder and Hypothalamic-Pituitary-Adrenal Axis Activity: Results From a Large Cohort Study. *Arch Gen Psychiatry* (2009) 66:617–26. doi: 10.1001/archgenpsychiatry.2009.50
52. Fanelli F, Mezzullo M, Repaci A, Belluomo I, Ibarra Gasparini D, Di Dalmazi G, et al. Profiling plasma N-Acylethanolamine levels and their ratios as a biomarker of obesity and dysmetabolism. *Mol Metab* (2018) 14:82–94. doi: 10.1016/j.molmet.2018.06.002
53. Martins CJ de M, Genelhu V, Pimentel MMG, Celoria BMJ, Mangia RF, Aveta T, et al. Circulating Endocannabinoids and the Polymorphism 385C>A in Fatty Acid Amide Hydrolase (FAAH) Gene May Identify the Obesity Phenotype Related to Cardiometabolic Risk: A Study Conducted in a Brazilian Population of Complex Interethnic Admixture. *PloS One* (2015) 10:e0142728. doi: 10.1371/journal.pone.0142728
54. Grosshans M, Schwarz E, Bumb JM, Schaefer C, Rohleder C, Vollmert C, et al. Oleoylethanolamide and human neural responses to food stimuli in obesity. *JAMA Psychiatry* (2014) 71:1254–61. doi: 10.1001/jamapsychiatry.2014.1215
55. Christopoulou FD, Kiortsis DN. An overview of the metabolic effects of rimonabant in randomized controlled trials: potential for other cannabinoid 1 receptor blockers in obesity. *J Clin Pharm Ther* (2011) 36:10–8. doi: 10.1111/j.1365-2710.2010.01164.x
56. Laleh P, Yaser K, Abolfazl B, Shahriar A, Mohammad AJ, Nazila F, et al. Oleoylethanolamide increases the expression of PPAR-A and reduces appetite and body weight in obese people: A clinical trial. *Appetite* (2018) 128:44–9. doi: 10.1016/j.appet.2018.05.129
57. Prospéro-García O, Amancio-Belmont O, Becerril Meléndez AL, Ruiz-Contreras AE, Méndez-Díaz M. Endocannabinoids and sleep. *Neurosci Biobehav Rev* (2016) 71:671–9. doi: 10.1016/j.neubiorev.2016.10.005
58. Leweke FM, Giuffrida A, Koethe D, Schreiber D, Nolden BM, Kranaster L, et al. Anandamide levels in cerebrospinal fluid of first-episode schizophrenic patients: impact of cannabis use. *Schizophr Res* (2007) 94:29–36. doi: 10.1016/j.schres.2007.04.025
59. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull* (1987) 13:261–76. doi: 10.1093/schbul/13.2.261
60. Olivares JM, Sermon J, Hemels M, Schreiner A. Definitions and drivers of relapse in patients with schizophrenia: a systematic literature review. *Ann Gen Psychiatry* (2013) 12:32. doi: 10.1186/1744-859X-12-32

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

REVIEW
published: 03 July 2020
doi: 10.3389/fpsyt.2020.00629

Considering the Microbiome in Stress-Related and Neurodevelopmental Trajectories to Schizophrenia

*Kevin W. Hoffman[1], Jakleen J. Lee[1], Cheryl M. Corcoran[1,2], David Kimhy[1,2], Thorsten M. Kranz[3] and Dolores Malaspina[1]**

[1] Department of Psychiatry, Icahn School of Medicine at Mount Sinai, New York, NY, United States, [2] James J. Peters VA Medical Center, Mental Illness Research, Education and Clinical Centers (MIRECC), New York, NY, United States, [3] Department of Psychiatry, Psychosomatic Medicine and Psychotherapy, University Hospital, Goethe University, Frankfurt, Germany

Early life adversity and prenatal stress are consistently associated with an increased risk for schizophrenia, although the exact pathogenic mechanisms linking the exposures with the disease remain elusive. Our previous view of the HPA stress axis as an elegant but simple negative feedback loop, orchestrating adaptation to stressors among the hypothalamus, pituitary, and adrenal glands, needs to be updated. Research in the last two decades shows that important bidirectional signaling between the HPA axis and intestinal mucosa modulates brain function and neurochemistry, including effects on glucocorticoid hormones and brain-derived neurotrophic factor (BDNF). The intestinal microbiome in earliest life, which is seeded by the vaginal microbiome during delivery, programs the development of the HPA axis in a critical developmental window, determining stress sensitivity and HPA function as well as immune system development. The crosstalk between the HPA and the Microbiome Gut Brain Axis (MGBA) is particularly high in the hippocampus, the most consistently disrupted neural region in persons with schizophrenia. Animal models suggest that the MGBA remains influential on behavior and physiology across developmental stages, including the perinatal window, early childhood, adolescence, and young adulthood. Understanding the role of the microbiome on critical risk related stressors may enhance or transform of understanding of the origins of schizophrenia and offer new approaches to increase resilience against stress effects for preventing and treating schizophrenia.

Keywords: schizophrenia, microbiome, brain-derived neurotrophic factor, development, stress, cortisol

OPEN ACCESS

Edited by:
Mary V. Seeman, University of Toronto, Canada

Reviewed by:
Rachel Anne Hill, Monash University, Australia
Eldin Jasarevic, University of Pennsylvania, United States

***Correspondence:**
Dolores Malaspina
dolores.malaspina@mssm.edu

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *20 March 2020*
Accepted: *16 June 2020*
Published: *03 July 2020*

Citation:
Hoffman KW, Lee JJ, Corcoran CM, Kimhy D, Kranz TM and Malaspina D (2020) Considering the Microbiome in Stress-Related and Neurodevelopmental Trajectories to Schizophrenia. Front. Psychiatry 11:629. doi: 10.3389/fpsyt.2020.00629

INTRODUCTION

Schizophrenia presents an enormous burden to individuals, families, communities, and public health, but the mechanisms underlying its pathogenesis, presentation, and course remain largely enigmatic, with no interventions known to prevent or cure the disease. New perspectives are necessary to overcome this roadblock. The microbiome, which broadly refers to the collection of genomes of the commensal microbes inhabiting our bodies, influences our health in broad and

complex ways. The emerging science of the microbiome is a promising new domain that could shed light on crucial disparate features of schizophrenia, including its association with prenatal and life course stressors, neurodevelopmental underpinnings, inflammatory neuropathology, particularly of the hippocampus and its metabolic comorbidity.

The Microbiome

The microbiome comprises a dynamic ecological community of commensal microorganisms that inhabit our body where it interfaces with the environment. These specific microbes, which are collectively referred to as the microbiota, consist of bacteria, viruses, fungi, and protozoa; approximately equal our own cells in number; and combined pose over 200 times the number of genes as the human genome (1, reviewed in 2). Recent advances in high-throughput genetic sequencing and computational abilities reveal the richness, complexity, and essential role of the microbiome in human health. Its composition varies by anatomic region, with the gut microbiome in the distal large intestine considered the most influential for health.

After being seeded at birth by maternal vaginal bacteria in the birth canal, the neonate gut microbiota develops in a phasic manner, largely due to feeding. The gut is initially colonized by microaerophilic *Proteobacteria* and facultative anaerobic *Actinobacteria*, which consume oxygen and create a suitable niche for subsequent obligate anaerobes like *Bacteroides*, *Clostridium*, and *Bifidobacterium* spp. (3, 4) Breast milk stimulates the growth of bifidabacteria, but weaning results in the emergence of *Firmicutes* and *Bacteroidetes* (5). These phyla proliferate with the introduction of solid foods and eventually come to dominate the gut microbiota (5). By 2.5 to 3 years of age, the infant gut microbiota structure stabilizes and resembles the adult gut microbiota, which is also dominated by *Firmicutes* and *Bacteroidetes* (3). The developmental dynamics of the infant gut microbiota are shaped by host genes, host immunity and environmental factors, such as diet, medications, and climate (6–8).

Over the last decade, it has emerged that the human microbiome highly influences the development of the central nervous system (CNS) and the immune system. The microbiome is shaped by stress exposures from early life and, in turn, influences stress responsivity (9). Given this new information, our models of the endocrine modulation of the stress response should be updated to account for the microbiome.

The bidirectional influence of the gut microbiome and CNS occurs through the "gut-brain axis" (GBA), components of which include the vagal nerve, gut hormone signaling, immune system, tryptophan metabolism, and microbial metabolites, such as short-chain fatty acids (reviewed in 10). Activity along the GBA intersects with the HPA axis (**Figure 1**) and may influence many psychiatric disorders, as evidenced by the association of gut dysbiosis with autism, depression, and anxiety disorders as well as functional gastrointestinal disorders (11–16). Given the purported inflammatory underpinnings for schizophrenia and its severe comorbidities with other microbiome-linked metabolic diseases, associations between schizophrenia and the microbiome are of great interest.

Stress Response and the Microbiome

Awareness of the overlap of stress signaling and the microbiome began in 2004 with the report that germ-free mice had an exaggerated hypothalamus-pituitary-adrenal (HPA) responses to stress in comparison to non-germ-free mice (17). The "microbiome-gut-brain axis" (MGBA) refers to bidirectional signaling between the gut flora and CNS. Acute and chronic stressors that activate the HPA axis also influence the microbiome and gut epithelium which participate in behavioral and systemic stress effects. The gut and brain communicate through the vagal (parasympathetic) nerve, which is a cholinergic anti-inflammatory pathway associated with slowed heart and respiratory rates and digestive function. Under stress, the sympathetic nervous system predominates and vagal function is reduced. The gut microbiome produces neurotransmitters that influence behavior, including acetylcholine, catecholamines, γ-aminobutyric acid, histamine, melatonin, and serotonin, all of which are also essential for regulating gastrointestinal peristalsis and sensation. Thus, the HPA axis and GBA are intersecting, co-dependent loops for managing stress and inflammation as part of their physiological function.

In this review, we illuminate aspects of the stress response and the microbiome as the GBA, with respect to schizophrenia. The impact of stress exposures on the brain will almost certainly entail signaling with the microbiome. Some factors that are associated with an increased risk for schizophrenia are considered across developmental stages, including the perinatal window, early childhood, adolescence, and young adulthood.

The HPA Axis, Hippocampus, Neurotrophins, and Schizophrenia

The neurobiology of the stress cascade and its potential for toxicity is well described. The HPA axis is the stress response system through which stress hormones and the CNS interact. Early dysregulation of the HPA axis is associated with adult stress-related disorders, including schizophrenia (18–20). Mechanistically, HPA axis dysregulation is considered to be linked to schizophrenia risk *via* glucocorticoid (GC) overproduction, especially during vulnerable phases of neurodevelopment. Cortisol-releasing hormone (CRH) is released from the paraventricular nucleus of the hypothalamus following physical or psychological stressors. CRH binds receptors on the anterior pituitary gland, driving release of adrenocorticotropic hormone (ACTH). This stimulates the adrenal cortex to release cortisol, the human GC hormone. Under physiological conditions increasing cortisol levels inhibit CRH release, terminating this stress cascade through a negative feedback loop. However, excess and chronic stress hyperactivate the HPA axis and cause abnormally high GC levels (21–24).

The effect of elevated GC levels on the hippocampus, the essential structure for memory and contextualizing new information, may be relevant. The hippocampus is the most commonly abnormal brain region in groups of schizophrenia cases, with progressive hippocampal volume loss a common observation (25). Increased activation, metabolism, and inflammation of the anterior hippocampus are associated with psychotic symptoms (26, 27) (reviewed in 28). The hippocampus possesses a high concentration of GC receptors that promote threat appraisal and help organize the

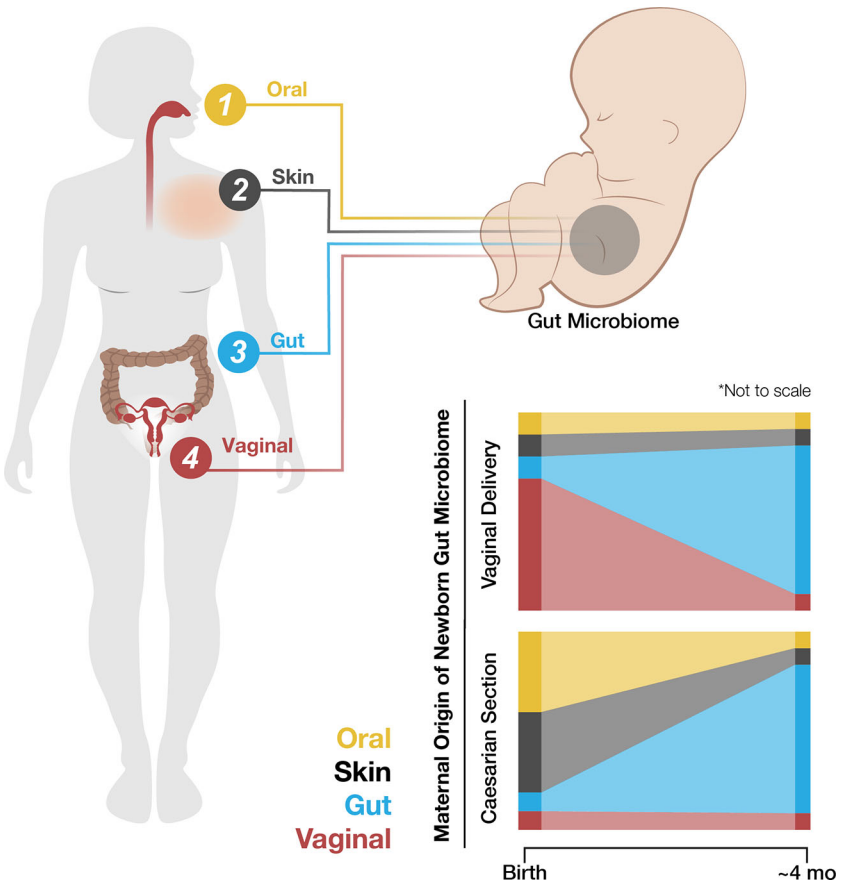

FIGURE 1 | The hypothalamic-pituitary-adrenal (HPA) axis regulates the response to stress (red lines). Stress activates the hypothalamus to secrete cortisol-releasing hormone (CRH), which induces the anterior pituitary gland to release adenocorticotropin hormone (ACTH; solid red lines). ACTH stimulates the adrenal gland cortex to produce cortisol (solid red line), which negatively regulates CRH production to terminate the stress response cascade (dashed red line). Excess or chronic stress can disturb normal HPA axis function *via* altered neuroendocrine signaling and gut dysbiosis (blue). Under excess or chronic stress, the hypothalamus is hyperactivated, leading to upregulation of the anterior pituitary gland and adrenal gland activation (plus signs) as well as downregulation of CRH inhibition (minus sign). Consequently, abnormally high levels of cortisol result in increased hippocampal signaling, which may overactivate the hippocampus, cause inflammation, and alter the crosstalk equilibrium between cortisol and BDNF in the hippocampus. Excess or chronic stress causes gut dysbiosis, which alters gut hormone and microbe metabolite signaling from the gut to the brain through the vagus nerve, i.e., the GBA.

stress response. Increased GC levels may drive overactivation and inflammation of the hippocampus and thereby promote schizophrenia (reviewed in 29–31).

GCs may also influence schizophrenia through interaction with neurotrophin pathways. Neurotrophins are growth factors responsible for neuron growth, differentiation, and formation of new synapses (32). Brain-derived neurotrophic factor (BDNF), the most abundant neurotrophin, is highly active in the hippocampus, cortex, and basal forebrain, where it binds its receptor, tyrosine kinase receptor B (TrkB), to play a key role in synaptic plasticity and long-term memory formation (33). Because GC receptors and TrkB are co-expressed in the hippocampus, important crosstalk between GCs and BDNF occurs here, as threat appraisal relies on both current stress and appropriate context from memory (34). As such, GC and BDNF equilibrium remains crucial for stress response regulation throughout life. Impairment of GC receptors and TrkB in the hippocampus favors vulnerability to stress-related disorders, including schizophrenia (reviewed in 35).

These pathways are influenced by the microbiome. Gut dysbiosis can indirectly influence cortisol release and sensitivity *via* chronic cytokine-mediated inflammation (36–38). This proinflammatory state may be driven by microbes crossing the intestinal barrier, releasing microbial byproducts such as lipopolysaccharide (LPS), or be moderated through bacterial metabolites, such as short-chain fatty acids (39–43) (reviewed in 44). The microbiome further influences the structure and function of the amygdala, which is critical for emotion learning and social behavior, especially responses linked to anxiety and/or fear (45, 46). Studies of germ-free mice show that the absence of the microbiome during early critical developmental windows leads to chronic cortisol elevation and altered hippocampal BDNF levels (17, 47). Depleting the microbiome of previously healthy mice through antibiotics disrupts the HPA axis (36, 48, 49).

Taken together, these findings suggest that a healthy microbiome is an important component of HPA axis development and that early alterations of the microbiome can affect neuroendocrine pathways throughout life.

Resilience in Schizophrenia

Identifying factors to increase resilience against stress is an area of active research that may be addressed through MGBA research. Anxiety and depression-like symptoms in germ free animas as well as the transference of a depression phenotype from a human patient to a rats through fecal microbiota support the feasibility of this approach (50). Mice deficient in the CRH_1 receptor and those with increased GR activity display more resilient behaviors (51–54) and these hormones can be modulating by the gut microbiome (11, 50). Likewise, the expression of serotonergic, glutamatergic, and GABA, which are dysregulated in association with poor resilience (55), are modulated through microbiome effects in animal models (reviewed in 56). A healthy microbiome may also contribute to resilience through emotion regulation that manifests as positive emotions and optimism, cognitive flexibility, and healthy interpersonal function, attributes that are associated with active coping styles (reviewed in 57). There may be treatment role for nutritional supplementation, as stress-related behaviors and HPA dysfunction in socially isolated male mice was remedied by dietary supplementation with DHA (58) and a rat study even demonstrated that stress sensitivity from early life trauma might be remediated through long-term supplementation with an eicosapentaenoic acid (EPA)/DHA mixture (59). The overlap of findings on the M-GBA with neuroendocrine and behavioral measures with those implicated for resilience indicate opportunities to modify the impact of stress exposures and augment resilience by targeting the microbiome.

PERINATAL DEVELOPMENT

Introduction

In 1934, Rosanoff and colleagues published "The Etiology of So-Called Schizophrenic Psychosis" in the American Journal of Psychology (60). This manuscript, which examined 142 pairs of twins either concordant or discordant for schizophrenia, was the first to associate birth complications with schizophrenia. In subsequent decades, schizophrenia risk during pregnancy, birth, and the neonatal period was broadly examined. Many risk factors were identified that occurred in important early developmental stages, including maternal infection, stress, and medical complications during pregnancy, fetal growth restrictions, and hypoxia during pregnancy and birth. Overall, early-life exposures have the greatest impact on the development and function of central neural circuits and the immune system (46).

Missing from this well-developed story is the impact of maternal exposures on her microbiome and the potential for vaginal dysbiosis (61, 62). The newborn's gut microbiome is seeded by the maternal vaginal microbiome during passage through the vaginal canal (8, 63). Disruptions in maternal microbiome may cause the newborn to be seeded with a more inflammatory gut microbiome (64, 65). It is this newborn microbiome that appears to have a strong influence in driving the development of the immune system and directing neurodevelopment (17, 66–70). These important contributions to fetal development must now be included is considering the action of schizophrenia risk factors in the perinatal period.

Maternal Infection

Maternal infection during pregnancy is associated with the risk for schizophrenia and is a maternal stressor. A 1988 study reported an increased rate for persons who were *in utero* during the 1957 influenza epidemic (71). Subsequent studies replicated this finding and suggested the second trimester as the gestational risk period for schizophrenia from influenza infection, although other evidence points to the first trimester (72–74). Other maternal infections associated with the offspring's risk for schizophrenia include rubella, varicella zoster virus, herpes simplex virus, and *Toxoplasma gondii*, known as TORCH agents, which can cross the placental barrier and directly infect the fetus, as can measles, polio, bacterial bronchopneumonias, and infections of the genitals and reproductive tract (75–77). Taken as a whole, infection with this group of pathogens during pregnancy is relatively common and may be an important factor for psychiatric disorder risk.

As to mechanism, there are several possibilities. One of these is direct invasion, which is consistent with the very high rate of schizophrenia following prenatal rubella, up to 20%, given rubella's well-known propensity for neural invasion in the developing fetus (76). Supporting invasion, a mouse model of influenza infection showed persistence of influenza RNA in the brains of offspring of infected pregnant mice (78). Another possibility is indirect damage driven by maternal inflammation. During maternal infection, inflammatory cytokine levels are elevated (75) and these may disrupt fetal neurodevelopment and potentially drive schizophrenia risk. For instance, the proinflammatory cytokine IL-1β negatively regulates hippocampal neurogenesis, suggesting a possible mechanism through which chronic inflammation could affect schizophrenia susceptibility (79). Notably, maternal inflammation correlates with later childhood psychiatric symptoms (80). Other potential risk pathways include effects from maternal fever, maternal antibodies crossing the placenta and medications, such as analgesics and anti-inflammatories, taken by the mother during infection, all of which may impact fetal neurodevelopment (81–83).

However, maternal infections also alter her microbiome, potentially leading to increased production of inflammatory products released by her gut, as well as to disrupted seeding of the neonatal microbiome at birth (64, 65). Neonates born to mothers with ongoing HIV infection show decreased gut microbiome diversity including reduced levels of *Prevotella*, a bacterial genus linked to inflammatory regulation of stressor (84). It is possible that dysbiosis secondary to maternal infection sensitizes the neonate to further stress-related injury, including elevated schizophrenia risk. Given the data demonstrating the impact of maternal inflammation on offspring schizophrenia risk and the microbiome's potential contributions to this inflammation, the microbiome may be a key player in schizophrenia pathogenesis.

Maternal Stress

Maternal stressors, such as depression, unwanted pregnancy, death of a partner, and exposure to war and disasters, are associated with schizophrenia in offspring (19, 85–88). For female fetuses, these external stressors are most strongly correlated with schizophrenia when they occur during the first trimester; however, male fetuses demonstrated increased schizophrenia risk through the second trimester, suggesting sex differences in critical periods (87, 88). Importantly, maternal stress during the first six months of postnatal life is associated with worse behavioral outcomes in children, suggesting that disrupted caregiving may also be a component to the schizophrenia risk posed by maternal stress (89). Additionally, prenatal nutritional deficiencies, including gross calorie deficits during famine and micronutrient deficiencies in homocysteine and vitamin D, are associated with both schizophrenia and the above-mentioned stressors (90–94), which certainly impact the microbiome composition. The short-term effects of maternal stress may act through adverse pregnancy outcomes, while the long-term effects on neurodevelopment may involve altered neonatal stress programming and gut dysbiosis (95). Maternal stress increases fetal and neonatal exposure to maternal cortisol, altering growth and behavior in humans and animal models (reviewed in 96). Stress also has well-documented effects on the microbiome, which may in turn alter inflammation and neurodevelopment in a developing neonate (62, 97–103). As an example, maternal perinatal stress increases offspring susceptibility to allergic diseases, which suggests interactivity between maternal GCs, perinatal immune development, and possible maternal dysbiosis (79). In a mouse model, prenatal maternal stress led to dysbiosis in both mother and offspring, increased IL-1β *in utero*, and a corresponding decrease in BDNF in offspring (104). Other experiments have shown antibiotics alter BDNF levels in dysbiotic mice, suggesting that interventions in the gut microbiome may be important in modifying risk (105).

Exploring how the maternal stress influences her microbiome for fetal effects relevant to schizophrenia risk may enhance our understanding of the disease and suggest new treatments or prophylactics through probiotic use (reviewed in 106). Mechanistically, the microbiome-driven effects of stress may manifest through alterations of the HPA axis during key developmental stages (107), impaired development of small intestine immune tissue and IgA production (108, 109), or alterations in gut-metabolites leading to aberrant development (110). Given that many of these downstream events are linked with schizophrenia risk, future work should aim to elicit the microbiome contributions of schizophrenia risk secondary to maternal stress.

Fetal Hypoxia

Many obstetric complications can lead to fetal hypoxia, which carries well-known risks to medial temporal regions. With regards to schizophrenia, fetal hypoxia may be the most significant risk factor among obstetric complications, in addition to maternal infections and fetal growth restriction (111, 112). Multiple studies report increased exposure to fetal hypoxia among persons with schizophrenia (113–115). One study show fetal hypoxia predicts the risk for early onset schizophrenia even after controlling for prenatal infection and fetal growth restriction (116). Further, fetal hypoxia is associated with reduced gray matter and ventricular enlargement in cases with schizophrenia and their non-ill siblings, although not in unrelated controls (117). Mechanistically, hypoxia may have an additive effect with genetic factors hastening the onset of schizophrenia in susceptible individuals (118). Certainly hypoxia may influence the composition and function of the gut microbiome (119, 120). As described above with infection and stress, these alterations increase future susceptibility to stress by influencing systemic inflammation, stress pathways, and BDNF production. Additionally, maternal microbes may invade the fetal brain following a hypoxic episode, as has been shown in sheep (121).

Fetal Growth Restriction

In 1966, a small but significant reduction in birth weight was observed in schizophrenic patients when compared to their siblings (122), prompting consideration that fetal growth restriction was a schizophrenia risk factor. Some, but not all studies associated lower birth weight, reduced head circumference, and congenital malformations with increased schizophrenia risk (123). There are heterogeneous causes of fetal growth restriction, only some of which may be associated with the risk for schizophrenia (124).

Maternal Complications

Other perinatal obstetric complications include maternal bleeding, maternal diabetes, preeclampsia, and caesarean section birth complications (125–127) (reviewed in 128). These perinatal traumas—along with the aforementioned factors of maternal infection, maternal stress, fetal hypoxia, and fetal growth restriction—altogether present a compelling argument for a close connection between the early window of neural development and schizophrenia risk. Recent advances indicate that the vaginal microbiome suggest that it may be a key player in this relationship. After all, these traumas occur during the perinatal period, when initial microbiota seeding of the newborn's gut by the maternal vaginal microbiome occurs during fetal passage through the birth canal.

Neuroendocrine Pathways

Cortisol, the primary human "stress" hormone, is also of central relevance for the developing fetus, promoting the maturation of vital organs, including the lungs, gastrointestinal tract, liver, heart, and brain. As such, the fetal HPA axis is tightly regulated, and is responsive to minute changes in fetal plasma levels of cortisol, which easily crosses the placental barrier (reviewed in 129). Due to their high cortisol sensitivity, developing fetuses rely on the placental enzyme 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) to inactivate maternal cortisol by converting it to less active cortisone, beginning in the second trimester (130). Thus, in early gestation, before placental 11β-HSD2 is induced, maternal hypercortisolemia has potent effects on developmental gene expression. Even after the induction of 11β-HST2, some cortisone can be reactivated through 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2), which converts cortisone back to cortisol (reviewed

in 131). This effect can be heightened by factors like maternal protein malnutrition, which diminish 11β-HSD2 gene expression (132). The detrimental effects of elevated exposure to maternal cortisol go beyond fetal development to influence emotional and behavioral disturbances during infancy and childhood and in later life (85) possibly including the perinatal schizophrenia risk pathways described above (reviewed in 133). Beyond the direct association between maternal stress and schizophrenia, elevated maternal cortisol may enhance other risks. In one study, elevated maternal cortisol during the second trimester enhanced the risk for adolescent onset depression in the offspring of mothers who experienced infections during pregnancy (134).

The neurotrophin BDNF is also critical for neurodevelopment. Elevated levels of BDNF are reported in fetuses with severe growth restriction as well as those with macrosomia in the context of maternal diabetes (135). Mechanistically, it is proposed that BDNF is neuroprotective in the developing fetus through anti-inflammatory mechanisms (136). *In vivo* animal models demonstrate that BDNF can reduce hypoxic brain injury through modulation of inflammatory cytokines and promotion of microglial activation (137). Given BDNF's protective role in the developing brain, it is possible that downregulation of BDNF could exacerbate schizophrenia risk in the perinatal window.

The Microbiome

Colonization of a newborn neonate gut is normally seeded by the vaginal microbiome during birth, as described, along with maternal vaginal, skin, and oral and fecal bacterial strains (8, 63, 138). These vaginal contributions are transient and by four months post-birth, the infant's gut microbiome is more similar to the maternal gut microbiome (**Figure 2**) (139, reviewed in 140). Neonates born *via* caesarean section lack exposure to the maternal vaginal microbiome and demonstrate a higher prevalence of maternal oral and skin microbes. They are also more likely to develop immune-related disorders (8, 64, 65, 141, 142). Disruptions of the maternal vaginal microbiome *via* infection, stress, or other pathways may lead to neonatal dysbiosis (65, reviewed in 143). Pre-term birth, caesarean sections, steroid use, and antibiotic use are also associated with dysbiosis in the newborn infant (144, 145).

The initial development of the microbiome, including its seeding at birth and development through very early childhood, is important for the development of a healthy core microbiome that is resistant to later perturbation. Given that dysregulation of the microbiome can cause pathogenic inflammation, dysbiosis in the perinatal window may lead to long-term inflammatory dysregulation (146–148). Further studies are needed to determine how maternal flora may influence immune development and schizophrenia risk in their offspring.

EARLY CHILDHOOD

Introduction

Childhood onset of schizophrenia is rare, but a number of neurologic and psychiatric features are already present in childhood. Likewise, a

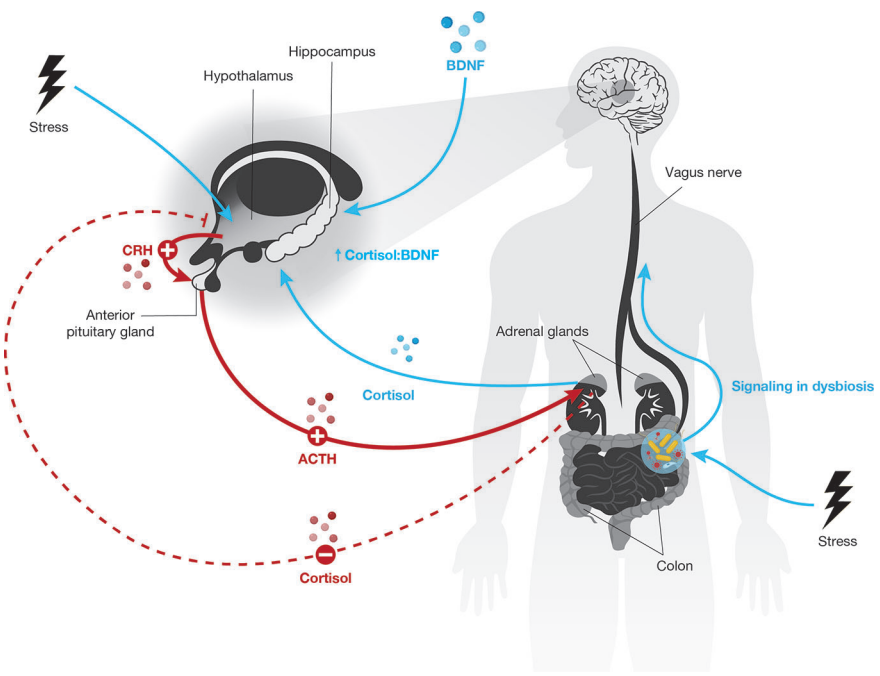

FIGURE 2 | The newborn neonate gut initially contains bacterial strains from the mother's oral, skin, gut, and vaginal microbiomes. The maternal source of initial colonization varies by the method of fetal delivery, i.e., vaginal birth or caesarean section. Although the newborn neonate gut microbiome stabilizes to resemble the mother's gut microbiome by about four months of age, this early and transient variability may have long-term impacts on childhood development.

number of traumatic exposures in childhood are associated with schizophrenia risk. The possibility that these presentations reflect the interactions of stress signaling and neurotrophic effects that may be influenced or modulate by the microbiome effects, which is currently being explored.

Signs Across Neurodevelopment

Schizophrenia is highly heterogeneous and no one developmental trajectory can describe the risk pathway for all cases. However, the literature does note certain clusters of behavioral features, including gross motor dysfunction and lower verbal intelligence (149–153). As children develop, personality traits, social behaviors, and mood symptoms may emerge that are more imminently related to the risk for psychosis (reviewed in 154).

During the first two years of life, infants undergo rapid neurodevelopment achieving important motor milestones, like walking, running, pointing, stacking blocks; language milestones, like simple sentences and phrases from a vocabulary of hundreds of words; and social milestones, like beginning self-sufficiency, responding to requests, recognizing self in photographs (reviewed in 155). As failure to achieve milestones raises concern for neurologic development, this developmental stage has been studied in the offspring of affected mothers, in whom a 10% recurrence risk is anticipated. "Pandysmaturation' was identified as a risk predictor in these "high risk" offspring, which involves a delay in cranial development and visual-motor development and disorganized motor performance (156, 157). Other studies identified passive infants with short attention spans, absence of stranger anxiety, poor communication competence, or abnormal use of language, and lower reactivity as signs of increased schizophrenia risk (158–162).

As children grow into early childhood, they typically become more coordinated, speak fluently, begin to learn reading and writing, and form friendships and social circles. Here again motor difficulties including clumsiness, poor coordination, and poor balance are predictive of higher schizophrenia risk (163, 164). Academically, learning disabilities like dyslexia are associated with higher schizophrenia risk (162). Among children of individuals with schizophrenia, relative decreases in coherence and complexity of language are associated with later schizophrenia risk (165). Socially, isolation, impaired affection, disturbed behavior, hyperactivity, impulsivity, and mood dysregulation including depressive signs and emotional lability are concerning for increased schizophrenia risk (158, 161, 162, 164).

In later childhood before transitioning into adolescence, children continue to improve in athletic, academic, and social behavior. Motor impairment of coordination and balance may become more striking in children with high schizophrenia risk (166–168). Additionally, high risk children may display learning difficulties in attention, concentration, memory, and thought as well as behavioral and mood dysfunctions such as increased aggression, problematic interpersonal relations, social isolation, low self-esteem, offending behaviors, poor affective control, and depression (152, 153, 162, 169–176).

Taken together, childhood impairments in neurologic development, marked by motor, cognitive, and behavioral disturbances, appear be on the trajectory toward schizophrenia, although most children with these features will not become psychotic. Notably, many of these factors entail stress effects on neuroendocrine function and neural plasticity. New research tracking the microbiome over development is showing its role in neurodevelopment and behavioral responses.

Exposure to Trauma

Traumatic experiences, ranging from abuse to accidental injuries, serious infections, and hospitalizations, may increase risk for schizophrenia (reviewed in 177). Trauma that occurs in childhood and adolescence is associated with psychosis and other psychiatric outcomes. Neurobiological studies demonstrate a stress hyporesponsive period in humans during the 6th through 12th postnatal months. Adverse experiences of newborns during this period can have lasting effects on HPA axis modulation (178, 179) from a long term elevation of basal GC secretion. Early life stress (ELS) rodent experiments demonstrate that maternal separation effects on stress sensitivity are mediated through GC-dependent mechanisms (reviewed in 180).

BDNF genetic variants may also influence sensitivity to trauma. There are many variants to the human BDNF gene, however, relatively few common variants fall within coding regions (181). Among these the BDNF^{Val66Met} variant is the most studied overall and has been specifically investigated with regards to schizophrenia risk (reviewed in 182). The BDNF^{Val66Met} polymorphism disrupts episodic memory in humans as a hippocampus-dependent memory function. Extensive studies in both animal models and humans have explored the effects of this polymorphism on numerous psychiatric disorders (reviewed in 183). Regarding schizophrenia risk, the 66Met allele decreases BDNF release probability (184), producing lower efficiency in neurotrophic activity, which is required for neurogenesis and neuroplasticity (185). It is associated with impaired episodic memory and lesser hippocampal activation (186). 66Met carriers with schizophrenia spectrum or bipolar disorders exposed to childhood sexual abuse show reduced grey matter volumes, consistent with the reduced BDNF mRNA levels in 66Met carriers who were exposed to childhood sexual abuse (186).

The higher sensitivity to trauma among 66Met carriers may be explained through the physiopathology of stress-induced changes in neural systems. BDNF plays a key role in neuronal plasticity (32, 187). BDNF-signaling is impaired by ELS; early traumas can evoke significant memory impairments in adulthood in association with reduced BDNF levels (188). This reduction, explained by hypermethylation of the BDNF promoters, can interact with genetic susceptibility, as in the BDNF 66Met carriers (189).

ELS prepares an organism, over the modulation of the HPA axis, for similar adversities during life. This way, a mismatching environment results in an increased susceptibility to psychopathology (131) such as major depression, panic and other co-morbidities. Epigenetics seem to make limbic system structures—mainly the hippocampus and amygdala—more rigid and prone to react depressively and protectively through adulthood. Of clinical significance, a higher occurrence of co-morbidities is usually related to a higher severity of positive and negative symptoms, suicidality, and poorer outcomes (190, 191).

ELS exposure is a negative regulator of BDNF and glucocorticoid receptors (GR) expression in the hippocampus, in the long term, favoring the vulnerability to develop neuropsychiatric disorders, especially upon additional stress exposures (192, 193). An alternative consideration is whether reduced neural capacity leads to a compensatory brain activation that might produce or activate trauma memories. A study of spatial working memory monitored by fMRI found that subjects with schizophrenia had to recruit more cortical regions for the task (194). In this same study, false memory errors were also associated with greater bilateral prefrontal activation. It is plausible that neural strategies to compensate for deficits of perceptual organization, working memory and visuospatial function may lead to a higher recognition of new stimuli as (false) memories. False trauma memory is more frequent among adolescents with posttraumatic stress disorder (PTSD) who experienced childhood sexual abuse (195). Combined, psychosis and childhood sexual abuse may greatly amplify false memories.

It is possible that some of these traumatic experiences are related to PTSD or stress symptoms, as is likely in many cases of abuse. Alternatively, they may be related to direct brain injury, as is likely in many cases of meningitis and encephalitis (196). Most studies examining trauma in schizophrenia risk do not distinguish between events that occur in early childhood versus adolescence, instead identifying events that occur before a determined age (e.g., 16 or 18 years old). However, examination of the timing of trauma suggests that puberty is an important window for distinguishing between anxious and depressive outcomes (197). Future studies examining the timing of traumatic exposures against puberty onset can better elucidate schizophrenia risk in these two populations.

Abuse

History of sexual and physical abuse is strongly correlated with greater psychotic symptom severity among adolescents and young adults in clinical high-risk (CHR) cohorts for schizophrenia. Patients from one such cohort reporting sexual abuse as children or adolescents had increased likelihood of transitioning to psychosis (198). Overall, sexual abuse history is more prevalent in these high-risk individuals than the general population (198–204). Physical abuse is also commonly reported by CHR individuals and may be linked with cognitive defects (205–209). Early physical trauma may lead to hyperarousal of the stress response and chronically elevated cortisol levels (210).

Emotional abuse in childhood, including neglect and maltreatment, has negative effects on mental health (211). Perceived discrimination significantly predicts the transition to psychosis, and emotional trauma and bullying are associated with depression, anxiety, and low self-esteem in CHR individuals (205). These various emotional traumas may impair cognitive function by denying a positive, stimulating environment for the developing brain (212, 213).

Physical and emotional trauma in childhood appears to alter stress response. Adults who reported childhood trauma demonstrate blunted cortisol responses, likely an adaptive response to chronic cortisol elevation (211, 214). In schizophrenia, increased stress sensitivity is a potential causal factor (133, reviewed in 215). Mechanistically, chronically increased cortisol may make the hippocampus vulnerable to injury *via* cortisol-induced dendritic restructuring or altered cortisol receptor levels (216–221). Cytokines like IL-6 and TNF-α are elevated in children exposed to trauma and can alter cortisol responses (222, 223). Additionally, the BDNF pathway may be relatively inhibited from chronically elevated cortisol, further promoting hippocampal injury and schizophrenia risk (224).

The gut microbiome is influenced by early childhood trauma and likely influences schizophrenia risk in turn (225). Gastrointestinal distress is frequently associated with early adversity in children, and the gut microbiome appears to influence stress programming in animal models (226–229) (reviewed in 230). Recent studies describe altered microbial patterns in children subjected to adversity, with elevations in *Lachnospiraceae* spp. suggestive of a potential influence on stress sensitivity (231). Additionally, childhood adversity is associated with altered gut microbiota during pregnancy, and may influence observed alterations in inflammatory and GC response to stress, thus contributing to propagation of schizophrenia risk across generations (232). Mechanistically, microglia have an important role in neuroplasticity and neurogenesis and are also sensitive to peripheral inflammation. Gut dysbiosis may negatively influence neurodevelopment through altered microglia activation (228, 233). Future work examining gut microbiome, inflammation, and effects of probiotics in CHR patients may help further elucidate connections between the microbiome, early trauma, and schizophrenia.

Infections

Childhood infections are another important risk factor for schizophrenia onset, especially viral CNS infections (234–236) implicating the microbiome. Childhood infections increase schizophrenia risk in a dose-dependent manner and familial liability for infection also increases schizophrenia risk (237). Additionally, hospitalization for severe infection and even outpatient antibiotic treatment in children are related to increased risk for future psychiatric hospitalizations, suggesting a broad impact of childhood infections on mental health (238).

Mechanistically, direct CNS damage from infection or indirect inflammatory damage may drive the increased schizophrenia risk following childhood infections (238). Antibiotic use in response to infection may also drive risk. Several antibiotics including fluoroquinolones are associated with neurotoxicity and psychosis risk (239). In addition to neurotoxic effects, infections and antibiotics can elevate cortisol levels, potentially affecting the stress cascade (240).

The microbiome also likely influences infection risk in schizophrenia. Studies of germ-free mice show that the gut microbiome primes microglia, stimulating viral specific immunity and reducing viral-driven demyelination *via* a TLR4-mediated process (241). Dysbiosis driven by antibiotic use or other factors may therefore increase CNS damage from neuroinvasive viruses and thereby increase schizophrenia risk. Interestingly, one study showed antibiotic treatment during adolescence in mice reduced anxiety-like behavior (99). However, cognitive deficits were shown along with reduced hippocampal BDNF and hypothalamic

oxytocin and vasopressin expression so the reduction in anxiety-like behavior is suggestive of negative symptoms.

ADOLESCENCE

Adolescence is the transition from childhood into adulthood that begins with puberty and ends with cessation of physical growth and neural development in the early 20s (242). Puberty broadly impacts mental health, neuroendocrinology, and the microbiome (reviewed in 243). Neurologically, adolescence encompasses improved abstract thinking, reasoning, and knowledge while also seeing a trend toward increased risk-taking behavior. Schizophrenia most frequently develops during adolescence and young adulthood, and the changes that occur during this developmental stage likely participate in shaping schizophrenia risk. As with early childhood, there are concerning signs and exposures during adolescence that are linked to schizophrenia.

Adolescent Signs

As with early childhood, broad impairments in neuromotor development, cognitive function, and behavior often mark individuals at risk for schizophrenia (reviewed in 154). As the adolescent matures, poor coordination, balance, and perceptual-motor and visual-motor functioning may become more apparent in a subgroup of cases (152, 168, 173). Cognitively, lower intelligence and especially a decrease in intellectual function mark schizophrenia risk (151, 153, 169, 244). There is impairment of individual domains including arithmetic and spelling, formal thought disorders, attention difficulties, increased distractibility, poor executive functioning, and general learning and memory difficulties (152, 153, 169, 173, 245). Behaviorally, aggression, withdrawal, and generally poor social competence and peer relations are also concerning, with psychiatric symptoms including affective flattening and anxiety often present (149, 151, 174, 175, 246, 247).

Risk Exposures

As discussed earlier, studies of exposures do not usually distinguish between pre-pubescent children and post-pubescent adolescents. The aforementioned exposures of sexual, physical, and emotional abuse as well as infection similarly convey schizophrenia risk among adolescents. However, trauma may have different long-term outcomes post-puberty, and its potential effect on schizophrenia risk merits further study. Additionally, the increased risk-taking behavior exhibited at this stage may be influenced by early trauma and influence further trauma exposures. New exposures, such as recreational drug use, may also contribute to schizophrenia risk.

Recreational Drug Use

Recreational drugs exploration is frequent in adolescence and many carry a significant risk for psychosis, particularly cannabis. By their first psychotic episode, approximately half of patients will have a history of cannabis use and one-third meet criteria for cannabis use disorder (248). Alcohol use is similarly high among individuals who have experienced their first psychotic episode, and there is elevated use of cocaine, amphetamine, barbiturate, and other drugs. Cause and effect associations of cannabis and psychosis are well described, although some schizophrenia-susceptible individuals may self-medicate to reduce the anxiety surrounding the presentation of schizophrenia symptoms, with this drug-seeking behavior may further exacerbate their risk for the disorder (249). Chronic exposure to tetrahydrocannabinol (THC), an active ingredient in cannabis, can disrupt neurodevelopmental maturation dependent on endocannabinoid pathways and may lead to overactivation of a pro-hallucinogenic pathway of 5-HT2A receptors, which may promote schizophrenia onset in susceptible individuals (250).

Substance abuse can dysregulate the HPA axis. Alcohol and nicotine use induce cortisol production, and long-term use can cause chronic cortisol elevation and dysregulation similarly to previously described trauma (251–254). Additionally, the gut microbiome is dysregulated by psychostimulants, alcohol, and opioids (255–259) (reviewed in 260). Microbiome influences on addiction are an active area of research. Microglial function is shaped by the microbiome and altered by drugs of abuse (233, 261). Likewise, BDNF dysregulation by dysbiosis is associated with altered behavioral response to cocaine and alcohol (256, 262, 263). While more work is needed to establish causal relationships, these findings suggest multiple ways in which the microbiome may influence addiction behaviors.

YOUNG ADULTHOOD

The transition from adolescence to adulthood occurs during the 20s (242). This transition is typically marked by completion of education and transition to complete independence, which can increase stress in a young adult's life. Onset of schizophrenia typically occurs around this life transition, peaking at 18 to 25 years old in men and 25 to 35 years old in women, with 80% of cases initially presenting before 40 years of age (264–266). The age of schizophrenia onset may be related to immune activation and stress. Interestingly, inflammatory diseases including inflammatory bowel disease, multiple sclerosis, and some autoimmune diseases tend to initially present in young adulthood (264–266). Gut dysbiosis and cortisol dysregulation are observed in many autoimmune diseases and disruptions to these systems in early adulthood likely influence schizophrenia onset as well (reviewed in 267, 268). First-episode schizophrenia patients have well-documented inflammatory disturbances, such as cytokine elevations and microglial activation (reviewed in 269).

Metabolic disturbances, including glucose intolerance, insulin resistance, and hyperglycemia, also frequently present in this age group and are more common among antipsychotic and naïve first-episode schizophrenic patients compared to the general population (270, 271). These changes may promote schizophrenia onset through persistent inflammatory effects. Stress-related cortisol elevations and gut dysbiosis both contribute to metabolic disturbances, suggesting alternative pathways that influence schizophrenia risk (272, 273). The microbiota also regulate adult neuroplasticity and microglia activation (233, 274).

Aerobic Exercise: A Potentially Protective Factor

While a number of risk factors for schizophrenia are identified, recent evidence points to protective factors. Specifically, aerobic exercise (AE) is hypothesized to play an important protective role against stress induced effects. AE induces a cascade of molecular and cellular processes that support brain plasticity and growth of new vasculature and trigger the processes through which neurotrophins mediate neural plasticity (reviewed in 275–278). Among neurotrophins, BDNF is the most susceptible to regulation by physical exercise (279–281), with synthesis and release into the blood circulation increasing in a dose-response manner (282, 283). Consistent with these findings, Voss et al. (284, 285) found increased connectivity between the bilateral parahippocampus and the bilateral middle temporal gyrus was linked to BDNF increase in AE subjects. A recent meta-analysis (286) of 29 studies (N = 1111 healthy subjects) examined the effect of exercise on BDNF in three exercise paradigms: 1) a single session of exercise; 2) a session of exercise following a program of regular exercise; and 3) resting BDNF levels following a program of regular exercise. Results demonstrated a moderate effect size for increases in BDNF following a single session of exercise (Hedges' $g = .46$, $p < .001$). Further, regular exercise intensified the effect of a session of exercise on BDNF levels (Hedges' $g = .59$, $p = .02$). Finally, results indicated a small effect of regular exercise on resting BDNF levels (Hedges' $g = .27$, $p = .005$). Examination of moderator effects across paradigms found that subjects' age was not significantly related to changes in BDNF following exercise, but sex significantly moderated the effect of exercise on BDNF levels, such that studies with more women showed less BDNF change resulting from exercise.

Consistent with these reports, findings indicate individuals with schizophrenia tend to have highly sedentary lifestyle characterized by low aerobic fitness which was highly correlated with poor cognitive functioning and symptoms (287). These findings parallel reports among individuals at clinical high risk for psychosis indicating lower levels of fitness, less physical activity, as well as more barriers to exercise (288–292). Yet, a pilot AE RCT indicated engagement in AE led to 11.0% increase aerobic fitness (293) as well as BDNF vs. a 1.9% in the TAU subjects (294) (reviewed in 295). A hierarchical multiple regression analysis indicated that, after controlling for age, sex, changes in anti-psychotic and SSRIs, and changes in menstrual cycle phase, BDNF changes independently predicted changes in cognitive function ($b = .38$, $t = 2.06$, $p = .05$) (296). Notably, improvements in cognitive functioning were associated with intensity of AE activity (294).

Exercise alters the composition and functional capacity of the gut microbiome independent of diet (reviewed by 28). As the effects of AE on BDNF production are further studied in schizophrenia, examination of how the microbiome influences this pathway may be illuminating.

POTENTIAL MECHANISMS

Although some stress exposure is essential for growth and development, stress that overwhelms adaptive capacities has adverse physiological consequences, as initially described in 1938 by Hans Selye (297). The initial stress axis model included direct and feedback interactions among the hypothalamus (release of corticotropin-releasing factor), pituitary (ACTH), and adrenal glands (cortisol), which was then expanded by Sapolsky's "glucocorticoid cascade hypothesis" (298) to encompass catecholamines and other interacting mediators of adaptation in addition to GCs. This model must now be widened to include the central influence of the microbiota on the initial programming of the stress axis and ongoing bidirectional effects that influence stress responding. The communication pathways between the gut and brain includes the vagal nerve, through which some microbial species invoke anxiolytic effects of some species (299). Enteroendocrine cells secrete biologically active peptides, including galanin, which stimulates the central HPA axis leading to increased adrenal cortisol secretion, and ghrelin which has similar effects linked to nutritional and metabolic conditions (300, 301) (reviewed in 302, 303). Reciprocally, even short durations of stress impact the relative proportions of phyla in the microbiota mediated through neuroendocrine and autonomic nervous system activity (304). The neuro-immuno-endocrine pathways linking the gut and brain include afferent and efferent neural pathways, immune effects, bi-directional neuroendocrine signaling and by alterations in intestinal permeability, critically influenced by relative proportions of microbiota species, as shown in **Figure 1**.

Examined as a whole, broad pathways through which the gut may influence stress and schizophrenia risk include cytokine-driven global inflammatory modifications, stress hormone metabolism, microglial activation, neuroplastic regulation, direct infection, and other nervous system activity as described above. Given schizophrenia risks at key developmental stages also coincide with microbiome development and associated changes, examining these pathways across development may be especially poignant. During the perinatal period, as the brain and HPA axis develop, dysbiosis in mother and child is influenced by multiple factors including infection and stress and in turn may influence the brain and HPA axis. As the child continues to grow and develop, the microbiome continues to adapt and change. While stressors including psychic and physical trauma, recreational substance use, inflammatory diseases, metabolic disturbances, and AE have been previously understood in context of neuroendocrine pathways, these events also affect the microbiome which in turn likely feed back into stress and neurodevelopment pathways. When viewed as one interconnected system, the ways microbial, endocrine, and neurological pathways influence each other across development should improve our understanding of schizophrenia risk and perhaps offer novel treatment methods. While current knowledge rests largely on germ free, antibiotic treated or probiotic supplemented animal models, the field is finally advancing to human studies.

CONCLUSION

Our understanding of schizophrenia risk has evolved over the past century as technological improvements have made better

research methods possible. Recent decades demonstrate the profound impact that neuroendocrine pathways have on schizophrenia risk across human development. The microbiome represents one of the newest frontiers in research that is broadly impacting healthcare. Recent work has already demonstrated many interactions between schizophrenia risk, neuroendocrinology, and the microbiome, but there are unexplored areas throughout development where further interactions likely occur. Thus, future work examining schizophrenia risk must continue to incorporate the crosstalk between the neuroendocrine pathways and the microbiome.

AUTHOR CONTRIBUTIONS

KH, JL, CC, DK, TK, and DM all contributed to writing and editing manuscript.

FUNDING

This work is supported by NIMH R01 MH110623 and P50MH115843 (DK); NIMH R01 MH107558 and R01 MH115332 (CC); NIMH R01 MH110418 (DM).

REFERENCES

1. Huttenhower C, Gevers D, Knight R, Abubucker S, Badger JH, Chinwalla AT, et al. Structure, function and diversity of the healthy human microbiome. *Nature* (2012) 486:207–14. doi: 10.1038/nature11234
2. Kamada N, Seo SU, Chen GY, Núñez G. Role of the gut microbiota in immunity and inflammatory disease. *Nat Rev Immunol* (2013) 13:321–35. doi: 10.1038/nri3430
3. Milani C, Duranti S, Bottacini F, Casey E, Turroni F, Mahony J, et al. The First Microbial Colonizers of the Human Gut: Composition, Activities, and Health Implications of the Infant Gut Microbiota. *Microbiol Mol Biol Rev* (2017) 81: e00036–17. doi: 10.1128/MMBR.00036-17
4. Rodríguez JM, Murphy K, Stanton C, Ross RP, Kober OI, Juge N, et al. The composition of the gut microbiota throughout life, with an emphasis on early life. *Microb Ecol Health Dis* (2015) 26:26050. doi: 10.3402/mehd.v26.26050
5. Moore RE, Townsend SD. Temporal development of the infant gut microbiome. *Open Biol* (2019) 9:190128. doi: 10.1098/rsob.190128
6. Yatsunenko T, Rey FE, Manary MJ, Trehan I, Dominguez-Bello MG, Contreras M, et al. Human gut microbiome viewed across age and geography. *Nature* (2012) 486:222–7. doi: 10.1038/nature11053
7. Koenig JE, Spor A, Scalfone N, Fricker AD, Stombaugh J, Knight R, et al. Succession of microbial consortia in the developing infant gut microbiome. *Proc Natl Acad Sci U.S.A.* (2011) 108:4578–85. doi: 10.1073/pnas.1000081107
8. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, Fierer N, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U.S.A.* (2010) 107:11971–5. doi: 10.1073/pnas.1002601107
9. Stewart CJ, Ajami NJ, O'Brien JL, Hutchinson DS, Smith DP, Wong MC, et al. Temporal development of the gut microbiome in early childhood from the TEDDY study. *Nature* (2018) 562:583–8. doi: 10.1038/s41586-018-0617-x
10. Cryan JF, Dinan TG. Mind-altering microorganisms: The impact of the gut microbiota on brain and behaviour. *Nat Rev Neurosci* (2012) 13:701–12. doi: 10.1038/nrn3346
11. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, Dinan TG, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *Proc Natl Acad Sci U.S.A.* (2011) 108:16050–5. doi: 10.1073/pnas.1102999108
12. Jiang H, Ling Z, Zhang Y, Mao H, Ma Z, Yin Y, et al. Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav Immun* (2015) 48:186–94. doi: 10.1016/j.bbi.2015.03.016
13. Naseribafrouei A, Hestad K, Avershina E, Sekelja M, Linløkken A, Wilson R, et al. Correlation between the human fecal microbiota and depression. *Neurogastroenterol Motil* (2014) 26:1155–62. doi: 10.1111/nmo.12378
14. Neufeld KM, Kang N, Bienenstock J, Foster JA. Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol Motil* (2011) 23:255–64. doi: 10.1111/j.1365-2982.2010.01620.x
15. Simreń M, Barbara G, Flint HJ, Spiegel BMR, Spiller RC, Vanner S, et al. Intestinal microbiota in functional bowel disorders: A Rome foundation report. *Gut* (2013) 62:159–76. doi: 10.1136/gutjnl-2012-302167
16. Song Y, Liu C, Finegold SM. Real-time PCR quantitation of clostridia in feces of autistic children. *Appl Environ Microbiol* (2004) 70:6459–65. doi: 10.1128/AEM.70.11.6459-6465.2004
17. Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, Yu XN, et al. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J Physiol* (2004) 558:263–75. doi: 10.1113/jphysiol.2004.063388
18. Daskalakis NP, Cohen H, Cai G, Buxbaum JD, Yehuda R. Expression profiling associates blood and brain glucocorticoid receptor signaling with trauma-related individual differences in both sexes. *Proc Natl Acad Sci U.S.A.* (2014) 111:13529–34. doi: 10.1073/pnas.1401660111
19. Yehuda R, Daskalakis NP, Lehrner A, Desarnaud F, Bader HN, Makotkine I, et al. Influences of maternal and paternal PTSD on epigenetic regulation of the glucocorticoid receptor gene in Holocaust survivor offspring. *Am J Psychiatry* (2014) 171:872–80. doi: 10.1176/appi.ajp.2014.13121571
20. Ruby E, Rothman K, Corcoran C, Goetz RR, Malaspina D. Influence of early trauma on features of schizophrenia. *Early Interv Psychiatry* (2017) 11:322–33. doi: 10.1111/eip.12239
21. Lawrence MS, Sapolsky RM. Glucocorticoids accelerate ATP loss following metabolic insults in cultured hippocampal neurons. *Brain Res* (1994) 646:303–6. doi: 10.1016/0006-8993(94)90094-9
22. Sapolsky RM. Why stress is bad for your brain. *Science (80-)* (1996) 273:749–50. doi: 10.1126/science.273.5276.749
23. Branchi I, Karpova NN, D'Andrea I, Castrén E, Alleva E. Epigenetic modifications induced by early enrichment are associated with changes in timing of induction of BDNF expression. *Neurosci Lett* (2011) 495:168–72. doi: 10.1016/j.neulet.2011.03.038
24. Bremner JD, Vythilingam M, Vermetten E, Southwick SM, McGlashan T, Nazeer A, et al. MRI and PET study of deficits in hippocampal structure and function in women with childhood sexual abuse and posttraumatic stress disorder. *Am J Psychiatry* (2003) 160:924–32. doi: 10.1176/appi.ajp.160.5.924
25. Osimo EF, Beck K, Reis Marques T, Howes OD. Synaptic loss in schizophrenia: a meta-analysis and systematic review of synaptic protein and mRNA measures. *Mol Psychiatry* (2019) 24:549–61. doi: 10.1038/s41380-018-0041-5
26. McHugo M, Talati P, Armstrong K, Vandekar SN, Blackford JU, Woodward ND, et al. Hyperactivity and Reduced Activation of Anterior Hippocampus in Early Psychosis. *Am J Psychiatry* (2019) 176:1030–8. doi: 10.1176/appi.ajp.2019.19020151
27. Velakoulis D, Wood SJ, Wong MTH, McGorry PD, Yung A, Phillips L, et al. Hippocampal and amygdala volumes according to psychosis stage and diagnosis: A magnetic resonance imaging study of chronic schizophrenia, first-episode psychosis, and ultra-high-risk individuals. *Arch Gen Psychiatry* (2006) 63:139–49. doi: 10.1001/archpsyc.63.2.139
28. Mailing LJ, Allen JM, Buford TW, Fields CJ, Woods JA. Exercise and the Gut Microbiome. *Exerc Sport Sci Rev* (2019) 47:75–85. doi: 10.1249/JES.0000000000000183
29. De Kloet ER, Joëls M, Holsboer F. Stress and the brain: From adaptation to disease. *Nat Rev Neurosci* (2005) 6:463–75. doi: 10.1038/nrn1683
30. de Kloet ER, Vreugdenhil E, Oitzl MS, Joëls M. Brain Corticosteroid Receptor Balance in Health and Disease 1. *Endocr Rev* (1998) 19:269–301. doi: 10.1210/edrv.19.3.0331
31. McEWEN BS. Stress, Adaptation, and Disease: Allostasis and Allostatic Load. *Ann N Y Acad Sci* (1998) 840:33–44. doi: 10.1111/j.1749-6632.1998.tb09546.x
32. Ghosh A, Greenberg ME. Distinct roles for bFGF and NT-3 in the regulation of cortical neurogenesis. *Neuron* (1995) 15:89–103. doi: 10.1016/0896-6273(95)90067-5

33. Bekinschtein P, Cammarota M, Katche C, Slipczuk L, Rossato JI, Goldin A, et al. BDNF is essential to promote persistence of long-term memory storage. *Proc Natl Acad Sci U.S.A.* (2008) 105:2711–6. doi: 10.1073/pnas.0711863105
34. Jeanneteau FD, Lambert WM, Ismaili N, Bath KG, Lee FS, Garabedian MJ, et al. BDNF and glucocorticoids regulate corticotrophin-releasing hormone (CRH) homeostasis in the hypothalamus. *Proc Natl Acad Sci U.S.A.* (2012) 109:1305–10. doi: 10.1073/pnas.1114122109
35. Jeanneteau F, Chao MV. Are BDNF and glucocorticoid activities calibrated? *Neuroscience* (2013) 239:173–95. doi: 10.1016/j.neuroscience.2012.09.017
36. Fröhlich EE, Farzi A, Mayerhofer R, Reichmann F, Jačan A, Wagner B, et al. Cognitive impairment by antibiotic-induced gut dysbiosis: Analysis of gut microbiota-brain communication. *Brain Behav Immun* (2016) 56:140–55. doi: 10.1016/j.bbi.2016.02.020
37. Rothhammer V, Mascanfroni ID, Bunse L, Takenaka MC, Kenison JE, Mayo L, et al. Type i interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. *Nat Med* (2016) 22:586–97. doi: 10.1038/nm.4106
38. Serrats J, Schiltz JC, García-Bueno B, van Rooijen N, Reyes TM, Sawchenko PE. Dual Roles for Perivascular Macrophages in Immune-to-Brain Signaling. *Neuron* (2010) 65:94–106. doi: 10.1016/j.neuron.2009.11.032
39. Chang PV, Hao L, Offermanns S, Medzhitov R. The microbial metabolite butyrate regulates intestinal macrophage function via histone deacetylase inhibition. *Proc Natl Acad Sci U.S.A.* (2014) 111:2247–52. doi: 10.1073/pnas.1322269111
40. Farzi A, Reichmann F, Meinitzer A, Mayerhofer R, Jain P, Hassan AM, et al. Synergistic effects of NOD1 or NOD2 and TLR4 activation on mouse sickness behavior in relation to immune and brain activity markers. *Brain Behav Immun* (2015) 44:106–20. doi: 10.1016/j.bbi.2014.08.011
41. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* (2013) 504:446–50. doi: 10.1038/nature12721
42. Mayerhofer R, Fröhlich EE, Reichmann F, Farzi A, Kogelnik N, Fröhlich E, et al. Diverse action of lipoteichoic acid and lipopolysaccharide on neuroinflammation, blood-brain barrier disruption, and anxiety in mice. *Brain Behav Immun* (2017) 60:174–87. doi: 10.1016/j.bbi.2016.10.011
43. Usami M, Kishimoto K, Ohata A, Miyoshi M, Aoyama M, Fueda Y, et al. Butyrate and trichostatin A attenuate nuclear factor κB activation and tumor necrosis factor α secretion and increase prostaglandin E2 secretion in human peripheral blood mononuclear cells. *Nutr Res* (2008) 28:321–8. doi: 10.1016/j.nutres.2008.02.012
44. De Punder K, Pruimboom L. Stress induces endotoxemia and low-grade inflammation by increasing barrier permeability. *Front Immunol* (2015) 6:223. doi: 10.3389/fimmu.2015.00223
45. LeDoux J. The amygdala. *Curr Biol* (2007) 17:PR868–74. doi: 10.1016/j.cub.2007.08.005
46. Stilling RM, Ryan FJ, Hoban AE, Shanahan F, Clarke G, Claesson MJ, et al. Microbes & neurodevelopment - Absence of microbiota during early life increases activity-related transcriptional pathways in the amygdala. *Brain Behav Immun* (2015) 50:209–20. doi: 10.1016/j.bbi.2015.07.009
47. Roceri M, Hendriks W, Racagni G, Ellenbroek BA, Riva MA. Early maternal deprivation reduces the expression of BDNF and NMDA receptor subunits in rat hippocampus. *Mol Psychiatry* (2002) 7:609–16. doi: 10.1038/sj.mp.4001036
48. Ait-Belgnaoui A, Durand H, Cartier C, Chaumaz G, Eutamene H, Ferrier L, et al. Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. *Psychoneuroendocrinology* (2012) 37:1885–95. doi: 10.1016/j.psyneuen.2012.03.024
49. Scheer S, Medina TS, Murison A, Taves MD, Antignano F, Chenery A, et al. Early-life antibiotic treatment enhances the pathogenicity of CD4+ T cells during intestinal inflammation. *J Leukoc Biol* (2017) 101:893–900. doi: 10.1189/jlb.3MA0716-334RR
50. Liu S, Guo R, Liu F, Yuan Q, Yu Y, Ren F. Gut microbiota regulates depression-like behavior in rats through the neuroendocrine-immune-mitochondrial pathway. *Neuropsychiatr Dis Treat* (2020) 16:859–69. doi: 10.2147/NDT.S243551
51. Jochems J, Teegarden SL, Chen Y, Boulden J, Challis C, Ben-Dor GA, et al. Enhancement of stress resilience through histone deacetylase 6-mediated regulation of glucocorticoid receptor chaperone dynamics. *Biol Psychiatry* (2015) 77:345–55. doi: 10.1016/j.biopsych.2014.07.036
52. Hartmann J, Wagner KV, Liebl C, Scharf SH, Wang XD, Wolf M, et al. The involvement of FK506-binding protein 51 (FKBP5) in the behavioral and neuroendocrine effects of chronic social defeat stress. *Neuropharmacology* (2012) 62(1):332–9. doi: 10.1016/j.neuropharm.2011.07.041
53. Ridder S, Chourbaji S, Hellweg R, Urani A, Zacher C, Schmid W, et al. Mice with genetically altered glucocorticoid receptor expression show altered sensitivity for stress-induced depressive reactions. *J Neurosci* (2005) 25:6243–50. doi: 10.1523/JNEUROSCI.0736-05.2005
54. Smith GW, Aubry JM, Dellu F, Contarino A, Bilezikjian LM, Gold LH, et al. Corticotropin releasing factor receptor 1-deficient mice display decreased anxiety, impaired stress response, and aberrant neuroendocrine development. *Neuron* (1998) 20:1093–102. doi: 10.1016/S0896-6273(00)80491-2
55. Faye C, Mcgowan JC, Denny CA, David DJ. Neurobiological Mechanisms of Stress Resilience and Implications for the Aged Population. *Curr Neuropharmacol* (2018) 16:234–70. doi: 10.2174/1570159x15666170818095105
56. Saulnier DM, Ringel Y, Heyman MB, Foster JA, Bercik P, Shulman RJ, et al. The intestinal microbiome, probiotics and prebiotics in neurogastroenterology. *Gut Microbes* (2013) 4:17–27. doi: 10.4161/gmic.22973
57. Southwick S, Vythilingam M, Charney D. The Psychobiology of Depression and Resilience to Stress: Implications for Prevention and Treatment. *Annu Rev Clin Psychol* (2005) 1:255–91. doi: 10.1146/annurev.clinpsy.1.102803.143948
58. Davis DJ, Hecht PM, Jasarevic E, Beversdorf DQ, Will MJ, Fritsche K, et al. Sex-specific effects of docosahexaenoic acid (DHA) on the microbiome and behavior of socially-isolated mice. *Brain Behav Immun* (2017) 59:38–48. doi: 10.1016/j.bbi.2016.09.003
59. Pusceddu MM, El Aidy S, Crispie F, O'Sullivan O, Cotter P, Stanton C, et al. N-3 polyunsaturated fatty acids (PUFAs) reverse the impact of early-life stress on the gut microbiota. *PloS One* (2015) 10:e0139721. doi: 10.1371/journal.pone.0139721
60. Rosanoff AJ, Handy LM, Plesset IR, Brush S. The etiology of so-called schizophrenic psychoses. *Am J Psychiatry* (1934) 91:247–86. doi: 10.1176/ajp.91.2.247
61. Brosnahan AJ, Vulchanova L, Witta SR, Dai Y, Jones BJ, Brown DR. Norepinephrine potentiates proinflammatory responses of human vaginal epithelial cells. *J Neuroimmunol* (2013) 259:8–16. doi: 10.1016/j.jneuroim.2013.03.005
62. Jašarević E, Howerton CL, Howard CD, Bale TL. Alterations in the vaginal microbiome by maternal stress are associated with metabolic reprogramming of the offspring gut and brain. *Endocrinology* (2015) 156:3265–76. doi: 10.1210/en.2015-1177
63. Ferretti P, Pasolli E, Tett A, Asnicar F, Gorfer V, Fedi S, et al. Mother-to-Infant Microbial Transmission from Different Body Sites Shapes the Developing Infant Gut Microbiome. *Cell Host Microbe* (2018) 24:133–145.e5. doi: 10.1016/j.chom.2018.06.005
64. Borre YE, Moloney RD, Clarke G, Dinan TG, Cryan JF. The impact of microbiota on brain and behavior: Mechanisms & therapeutic potential. *Adv Exp Med Biol* (2014) 817:373–403. doi: 10.1007/978-1-4939-0897-4_17
65. Hočevar K, Maver A, Vidmar Šimic M, Hodžić A, Haslberger A, Premru Seršen T, et al. Vaginal Microbiome Signature Is Associated With Spontaneous Preterm Delivery. *Front Med* (2019) 6:201. doi: 10.3389/fmed.2019.00201
66. Ly NP, Ruiz-Pérez B, Onderdonk AB, Tzianabos AO, Litonjua AA, Liang C, et al. Mode of delivery and cord blood cytokines: A birth cohort study. *Clin Mol Allergy* (2006) 4:13. doi: 10.1186/1476-7961-4-13
67. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C, Koga Y. The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. *J Immunol* (1997) 159:1739–45.
68. Bager P, Wohlfahrt J, Westergaard T. Caesarean delivery and risk of atopy and allergic disesase: Meta-analyses. *Clin Exp Allergy* (2008) 38:634–42. doi: 10.1111/j.1365-2222.2008.02939.x
69. Kummeling I, Stelma FF, Dagnelie PC, Snijdersa BEP, Penders J, Huber M, et al. Early life exposure to antibiotics and the subsequent development of eczema, wheeze, and allergic sensitization in the first 2 years of life: The KOALA Birth Cohort Study. *Pediatrics* (2007) 119:e225–31. doi: 10.1542/peds.2006-0896
70. Souza DG, Vieira AT, Soares AC, Pinho V, Nicoli JR, Vieira LQ, et al. The Essential Role of the Intestinal Microbiota in Facilitating Acute Inflammatory Responses. *J Immunol* (2004) 173:4137–46. doi: 10.4049/jimmunol.173.6.4137

71. Mednick SA, Machon RA, Huttunen MO, Bonett D. Adult Schizophrenia Following Prenatal Exposure to an Influenza Epidemic. *Arch Gen Psychiatry* (1988) 45:189–92. doi: 10.1001/archpsyc.1988.01800260109013
72. Byrne M, Agerbo E, Bennedsen B, Eaton WW, Mortensen PB. Obstetric conditions and risk of first admission with schizophrenia: a Danish national register based study. *Schizophr Res* (2007) 97:51–9. doi: 10.1016/j.schres.2007.07.018
73. Machón RA, Mednick SA, Huttunen MO. Adult major affective disorder after prenatal exposure to an influenza epidemic. *Arch Gen Psychiatry* (1997) 54:322–8. doi: 10.1001/archpsyc.1997.01830160040006
74. Brown AS, Begg MD, Gravenstein S, Schaefer CA, Wyatt RJ, Bresnahan M, et al. Serologic evidence of prenatal influenza in the etiology of schizophrenia. *Arch Gen Psychiatry* (2004) 61:774–80. doi: 10.1001/archpsyc.61.8.774
75. Boksa P. Maternal infection during pregnancy and schizophrenia. *J Psychiatry Neurosci* (2008) 33:183–5.
76. Brown AS, Cohen P, Harkavy-Friedman J, Babulas V, Malaspina D, Gorman JM, et al. Prenatal rubella, premorbid abnormalities, and adult schizophrenia. *Biol Psychiatry* (2001) 49:473–86. doi: 10.1016/S0006-3223(01)01068-X
77. Mortensen PB, Nørgaard-Pedersen B, Waltoft BL, Sørensen TL, Hougaard D, Torrey EF, et al. Toxoplasma gondii as a Risk Factor for Early-Onset Schizophrenia: Analysis of Filter Paper Blood Samples Obtained at Birth. *Biol Psychiatry* (2007) 61:688–93. doi: 10.1016/j.biopsych.2006.05.024
78. Aronsson F, Lannebo C, Paucar M, Brask J, Kristensson K, Karlsson H. Persistence of viral RNA in the brain of offspring to mice infected with influenza A/WSN/33 virus during pregnancy. *J Neurovirol* (2002) 8:353–7. doi: 10.1080/13550280290100480
79. Moustaki M, Tsabouri S, Priftis KN, Douros K. Prenatal Stress Enhances Susceptibility to Allergic Diseases of Offspring. *Endocrine Metab Immune Disord Drug Targets* (2017) 17:255–63. doi: 10.2174/1871530317666170912160646
80. Mac Giollabhui N, Breen EC, Murphy SK, Maxwell SD, Cohn BA, Krigbaum NY, et al. Maternal inflammation during pregnancy and offspring psychiatric symptoms in childhood: Timing and sex matter. *J Psychiatr Res* (2019) 111:96–103. doi: 10.1016/j.jpsychires.2019.01.009
81. Khan VR, Brown IR. The effect of hyperthermia on the induction of cell death in brain, testis, and thymus of the adult and developing rat. *Cell Stress Chaperones* (2002) 7:73–90. doi: 10.1379/1466-1268(2002)007<0073:TEOHOT>2.0.CO;2
82. Sørensen HJ, Mortensen EL, Reinisch JM, Mednick SA. Association between prenatal exposure to analgesics and risk of schizophrenia. *Br J Psychiatry* (2004) 185:366–71. doi: 10.1192/bjp.185.5.366
83. Wright P, Takei N, Rifkin L, Murray RM. Maternal influenza, obstetric complications, and schizophrenia. *Am J Psychiatry* (1995) 152:1714–20. doi: 10.1176/ajp.152.12.1714
84. Bender JM, Li F, Martelly S, Byrt E, Rouzier V, Leo M, et al. Maternal HIV infection influences the microbiome of HIV-uninfected infants. *Sci Transl Med* (2016) 8:349ra100. doi: 10.1126/scitranslmed.aaf5103
85. Davis EP, Sandman CA. The timing of prenatal exposure to maternal cortisol and psychosocial stress is associated with human infant cognitive development. *Child Dev* (2010) 81:131–48. doi: 10.1111/j.1467-8624.2009.01385.x
86. Huttunen MO, Niskanen P. Prenatal Loss of Father and Psychiatric Disorders. *Arch Gen Psychiatry* (1978) 35:429–31. doi: 10.1001/archpsyc.1978.01770280039004
87. Malaspina D, Corcoran C, Kleinhaus KR, Perrin MC, Fennig S, Nahon D, et al. Acute maternal stress in pregnancy and schizophrenia in offspring: A cohort prospective study. *BMC Psychiatry* (2008) 8:71. doi: 10.1186/1471-244X-8-71
88. Van Os J, Selten J-P. Prenatal exposure to maternal stress and subsequent schizophrenia. *Br J Psychiatry* (1998) 172:324–6. doi: 10.1192/bjp.172.4.324
89. Meijer A. Child psychiatric sequelae of maternal war stress. *Acta Psychiatr Scand* (1985) 72:505–11. doi: 10.1111/j.1600-0447.1985.tb02647.x
90. McGrath J. Hypothesis: Is low prenatal vitamin D a risk-modifying factor for schizophrenia? *Schizophr Res* (1999) 40:173–7. doi: 10.1016/S0920-9964(99)00052-3
91. Brown AS, Bottiglieri T, Schaefer CA, Quesenberry CP, Liu L, Bresnahan M, et al. Elevated prenatal homocysteine levels as a risk factor for schizophrenia. *Arch Gen Psychiatry* (2007) 64:31–9. doi: 10.1001/archpsyc.64.1.31
92. Xu MQ, Sun WS, Liu BX, Feng GY, Yu L, Yang L, et al. Prenatal malnutrition and adult Schizophrenia: Further evidence from the 1959-1961 chinese famine. *Schizophr Bull* (2009) 35:568–76. doi: 10.1093/schbul/sbn168
93. St Clair D, Xu M, Wang P, Yu Y, Fang Y, Zhang F, et al. Rates of adult schizophrenia following prenatal exposure to the Chinese famine of 1959-1961. *J Am Med Assoc* (2005) 294:557–62. doi: 10.1001/jama.294.5.557
94. Susser E, Neugebauer R, Hoek HW, Brown AS, Lin S, Labovitz D, et al. Schizophrenia after prenatal famine further evidence. *Arch Gen Psychiatry* (1996) 53:25–31. doi: 10.1001/archpsyc.1996.01830010027005
95. Rakers F, Rupprecht S, Dreiling M, Bergmeier C, Witte OW, Schwab M. Transfer of maternal psychosocial stress to the fetus. *Neurosci Biobehav Rev* (Forthcoming 2016). doi: 10.1016/j.neubiorev.2017.02.019
96. Lu A, Petrullo L, Carrera S, Feder J, Schneider-Crease I, Snyder-Mackler N. Developmental responses to early-life adversity: Evolutionary and mechanistic perspectives. *Evol Anthropol* (2019) 28:249–66. doi: 10.1002/evan.21791
97. Bailey MT, Dowd SE, Galley JD, Hufnagle AR, Allen RG, Lyte M. Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. *Brain Behav Immun* (2011) 25:397–407. doi: 10.1016/j.bbi.2010.10.023
98. Clarke G, Grenham S, Scully P, Fitzgerald P, Moloney RD, Shanahan F, et al. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol Psychiatry* (2013) 18:666–73. doi: 10.1038/mp.2012.77
99. Desbonnet L, Clarke G, Traplin A, O'Sullivan O, Crispie F, Moloney RD, et al. Gut microbiota depletion from early adolescence in mice: Implications for brain and behaviour. *Brain Behav Immun* (2015) 48:165–73. doi: 10.1016/j.bbi.2015.04.004
100. Golubeva AV, Crampton S, Desbonnet L, Edge D, O'Sullivan O, Lomasney KW, et al. Prenatal stress-induced alterations in major physiological systems correlate with gut microbiota composition in adulthood. *Psychoneuroendocrinology* (2015) 60:58–74. doi: 10.1016/j.psyneuen.2015.06.002
101. Hyland NP, O'Mahony SM, O'Malley D, O'Mahony CM, Dinan TG, Cryan JF. Early-life stress selectively affects gastrointestinal but not behavioral responses in a genetic model of brain-gut axis dysfunction. *Neurogastroenterol Motil* (2015) 27:105–13. doi: 10.1111/nmo.12486
102. Jašarević E, Howard CD, Morrison K, Misic A, Weinkopff T, Scott P, et al. The maternal vaginal microbiome partially mediates the effects of prenatal stress on offspring gut and hypothalamus. *Nat Neurosci* (2018) 21:1061–71. doi: 10.1038/s41593-018-0182-5
103. Zaneveld JR, McMinds R, Thurber RV. Stress and stability: Applying the Anna Karenina principle to animal microbiomes. *Nat Microbiol* (2017) 2:1–8. doi: 10.1038/nmicrobiol.2017.121
104. Gur TL, Shay L, Palkar AV, Fisher S, Varaljay VA, Dowd S, et al. Prenatal stress affects placental cytokines and neurotrophins, commensal microbes, and anxiety-like behavior in adult female offspring. *Brain Behav Immun* (2017) 64:50–8. doi: 10.1016/j.bbi.2016.12.021
105. Soto M, Herzog C, Pacheco JA, Fujisaka S, Bullock K, Clish CB, et al. Gut microbiota modulate neurobehavior through changes in brain insulin sensitivity and metabolism. *Mol Psychiatry* (2018) 23:2287–301. doi: 10.1038/s41380-018-0086-5
106. Scott LV, Clarke G, Dinan TG. The brain-gut axis: A target for treating stress-related disorders. *Inflammation Psychiatry* (2013) 28:90–9. doi: 10.1159/000343971
107. Heijtz RD, Wang S, Anuar F, Qian Y, Björkholm B, Samuelsson A, et al. Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci U.S.A.* (2011) 108:3047–52. doi: 10.1073/pnas.1010529108
108. Macpherson AJ, Hunziker L, McCoy K, Lamarre A. IgA responses in the intestinal mucosa against pathogenic and non-pathogenic microorganisms. *Microbes Infect* (2001) 3:1021–35. doi: 10.1016/S1286-4579(01)01460-5
109. Moreau MC, Ducluzeau R, Guy-Grand D, Muller MC. Increase in the population of duodenal immunoglobulin A plasmocytes in axenic mice associated with different living or dead bacterial strains of intestinal origin. *Infect Immun* (1978) 21:532–9. doi: 10.1128/iai.21.2.532-539.1978
110. Samuel BS, Shaito A, Motoike T, Rey FE, Backhed F, Manchester JK, et al. Effects of the gut microbiota on host adiposity are modulated by the short-chain fatty-acid binding G protein-coupled receptor, Gpr41. *Proc Natl Acad Sci U.S.A.* (2008) 105:16767–72. doi: 10.1073/pnas.0808567105
111. McNeil TF. Obstetric factors and perinatal injuries. In: Tsuang MF, Simpson JC, editors. *Handbook of Schizophrenia, Vol. 3: Nosology, Epidemiology and Genetics*. New York: Elsevier (1988) p. 319–44.

112. Buka SL, Tsuang MT, Lipsitt LP. Pregnancy/Delivery Complications and Psychiatric Diagnosis: A Prospective Study. *Arch Gen Psychiatry* (1993) 50:151–6. doi: 10.1001/archpsyc.1993.01820140077009
113. Dalman C, Thomas HV, David AS, Gentz J, Lewis G, Allebeck P. Signs of asphyxia at birth and risk of schizophrenia: Population-based case-control study. *Br J Psychiatry* (2001) 179:403–8. doi: 10.1192/bjp.179.5.403
114. Zornberg GL, Buka SL, Tsuang MT. Hypoxic-ischemia-related fetal/neonatal complications and risk of schizophrenia and other nonaffective psychoses: A 19-year longitudinal study. *Am J Psychiatry* (2000) 157:196–202. doi: 10.1176/appi.ajp.157.2.196
115. Geddes JR, Verdoux H, Takei N, Lawrie SM, Bovet P, Eagles JM, et al. Schizophrenia and complications of pregnancy and labor: An individual patient data meta-analysis. *Schizophr Bull* (1999) 25:413–23. doi: 10.1093/oxfordjournals.schbul.a033389
116. Rosso IM, Cannon TD, Huttunen T, Huttunen MO, Lönnqvist J, Gasperoni TL. Obstetric risk factors for early-onset schizophrenia in a Finnish birth cohort. *Am J Psychiatry* (2000) 157:801–7. doi: 10.1176/appi.ajp.157.5.801
117. Cannon TD, Van Erp TGM, Rosso IM, Huttunen M, Lönnqvist J, Pirkola T, et al. Fetal hypoxia and structural brain abnormalities in schizophrenic patients, their siblings, and controls. *Arch Gen Psychiatry* (2002) 59:35–41. doi: 10.1001/archpsyc.59.1.35
118. Van Erp TGM, Saleh PA, Rosso IM, Huttunen M, Lönnqvist J, Pirkola T, et al. Contributions of genetic risk and fetal hypoxia to hippocampal volume in patients with schizophrenia or schizoaffective disorder, their unaffected siblings, and healthy unrelated volunteers. *Am J Psychiatry* (2002) 159:1514–20. doi: 10.1176/appi.ajp.159.9.1514
119. Moreno-Indias I, Torres M, Montserrat JM, Sanchez-Alcoholado L, Cardona F, Tinahones FJ, et al. Intermittent hypoxia alters gut microbiota diversity in a mouse model of sleep apnoea. *Eur Respir J* (2015) 45:1055–65. doi: 10.1183/09031936.00184314
120. Tripathi A, Melnik AV, Xue J, Poulsen O, Meehan MJ, Humphrey G, et al. Intermittent Hypoxia and Hypercapnia, a Hallmark of Obstructive Sleep Apnea, Alters the Gut Microbiome and Metabolome. *mSystems* (2018) 3: e00020–18. doi: 10.1128/msystems.00020-18
121. Zarate MA, Rodriguez MD, Chang EI, Russell JT, Arndt TJ, Richards EM, et al. Post-hypoxia Invasion of the fetal brain by multidrug resistant Staphylococcus. *Sci Rep* (2017) 7:6458–68. doi: 10.1038/s41598-017-06789-6
122. Lane EA, Albee GW. Comparative Birth Weights of Schizophrenics and Their Siblings. *J Psychol Interdiscip Appl* (1966) 64:227–31. doi: 10.1080/00223980.1966.10544847
123. Nilsson E, Stålberg G, Lichtenstein P, Cnattingius S, Olausson PO, Hultman CM. Fetal growth restriction and schizophrenia: A Swedish twin study. *Twin Res Hum Genet* (2005) 8:402–8. doi: 10.1375/1832427054936727
124. Cannon M, Jones PB, Murray RM. Obstetric complications and schizophrenia: Historical and meta-analytic review. *Am J Psychiatry* (2002) 159:1080–92. doi: 10.1176/appi.ajp.159.7.1080
125. Pasamanick B, Rogers ME, Lilienfeld AM. Pregnancy experience and the development of behavior disorders in children. *Am J Psychiatry* (1956) 112:613–8. doi: 10.1176/ajp.112.8.613
126. Schaefer CA, Brown AS, Wyatt RJ, Kline J, Begg MD, Bresnahan MA, et al. Maternal prepregnant body mass and risk of schizophrenia in adult offspring. *Schizophr Bull* (2000) 26:275–86. doi: 10.1093/oxfordjournals.schbul.a033452
127. Verdoux H, Geddes JR, Takei N, Lawrie SM, Bovet P, Eagles JM, et al. Obstetric complications and age at onset in schizophrenia: An international collaborative meta-analysis of individual patient data. *Am J Psychiatry* (1997) 154:1220–7. doi: 10.1176/ajp.154.9.1220
128. Clarke MC, Harley M, Cannon M. The role of obstetric events in schizophrenia. *Schizophr Bull* (2006) 32:3–8. doi: 10.1093/schbul/sbj028
129. Wood CE, Keller-Wood M. The critical importance of the fetal hypothalamus-pituitary-adrenal axis. *F1000Research* (2016) 5:115–22. doi: 10.12688/f1000research.7224.1
130. Shams M, Kilby MD, Somerset DA, Howie AJ, Gupta A, Wood PJ, et al. 11β-hydroxysteroid dehydrogenase type 2 in human pregnancy and reduced expression in intrauterine growth restriction. *Hum Reprod* (1998) 13(4):799–804. doi: 10.1093/humrep/13.4.799
131. Van Bodegom M, Homberg JR, Henckens MJAG. Modulation of the hypothalamic-pituitary-adrenal axis by early life stress exposure. *Front Cell Neurosci* (2017) 11:87–120. doi: 10.3389/fncel.2017.00087
132. Cottrell EC, Holmes MC, Livingstone DE, Kenyon CJ, Seckl JR. Reconciling the nutritional and glucocorticoid hypotheses of fetal programming. *FASEB J* (2012) 26:1866–74. doi: 10.1096/fj.12-203489
133. Corcoran C, Walker E, Huot R, Mittal V, Tessner K, Kestler L, et al. The Stress Cascade and Schizophrenia: Etiology and Onset. *Schizophr Bull* (2003) 29(4):671–92. doi: 10.1093/oxfordjournals.schbul.a007038
134. Murphy S, Breen E, Cohn B, Kringbaum N, Cirillo P, Perez C, et al. F157. Infection and Increased Cortisol During Pregnancy and Risk for Adolescent Depression. *Biol Psychiatry* (2018) 83:S299. doi: 10.1016/j.biopsych.2018.02.771
135. Antonakopoulos N, Iliodromiti Z, Mastorakos G, Iavazzo C, Valsamakis G, Salakos N, et al. Association between brain-derived neurotrophic factor (BDNF) levels in 2 nd trimester amniotic fluid and fetal development. *Mediators Inflammation* (2018) 2018:1–7. doi: 10.1155/2018/8476217
136. Saha RN, Liu X, Pahan K. Up-regulation of BDNF in astrocytes by TNF-α: A case for the neuroprotective role of cytokine. *J Neuroimmune Pharmacol* (2006) 1:212–22. doi: 10.1007/s11481-006-9020-8
137. Jiang Y, Wei N, Lu T, Zhu J, Xu G, Liu X. Intranasal brain-derived neurotrophic factor protects brain from ischemic insult via modulating local inflammation in rats. *Neuroscience* (2011) 172:398–405. doi: 10.1016/j.neuroscience.2010.10.054
138. Yassour M, Jason E, Hogstrom LJ, Arthur TD, Tripathi S, Siljander H, et al. Strain-Level Analysis of Mother-to-Child Bacterial Transmission during the First Few Months of Life. *Cell Host Microbe* (2018) 24:146–154.e4. doi: 10.1016/j.chom.2018.06.007
139. Korpela K, Costea P, Coelho LP, Kandels-Lewis S, Willemsen G, Boomsma DI, et al. Selective maternal seeding and environment shape the human gut microbiome. *Genome Res* (2018) 28:561–8. doi: 10.1101/gr.233940.117
140. Mohammadkhah AI, Simpson EB, Patterson SG, Ferguson JF. Development of the Gut Microbiome in Children, and Lifetime Implications for Obesity and Cardiometabolic Disease. *Children* (2018) 5:160. doi: 10.3390/children5120160
141. Darabi B, Rahmati S, HafeziAhmadi MR, Badfar G, Azami M. The association between caesarean section and childhood asthma: an updated systematic review and meta-analysis. *Allergy Asthma Clin Immunol* (2019) 15:62. doi: 10.1186/s13223-019-0367-9
142. Li H-T, Ye R, Achenbach T, Ren A, Pei L, Zheng X, et al. Caesarean delivery on maternal request and childhood psychopathology: a retrospective cohort study in China. *BJOG Int J Obstet Gynaecol* (2011) 118:42–8. doi: 10.1111/j.1471-0528.2010.02762.x
143. Dunn AB, Jordan S, Baker BJ, Carlson NS. The Maternal Infant Microbiome: Considerations for Labor and Birth. *MCN Am J Matern Nurs* (2017) 42:318–25. doi: 10.1097/NMC.0000000000000373
144. Wandro S, Osborne S, Enriquez C, Bixby C, Arrieta A, Whiteson K. The Microbiome and Metabolome of Preterm Infant Stool Are Personalized and Not Driven by Health Outcomes, Including Necrotizing Enterocolitis and Late-Onset Sepsis. *mSphere* (2018) 3:e00104–18. doi: 10.1128/mSphere.00104-18
145. Chernikova DA, Koestler DC, Hoen AG, Housman ML, Hibberd PL, Moore JH, et al. Fetal exposures and perinatal influences on the stool microbiota of premature infants. *J Matern Neonatal Med* (2016) 29:99–105. doi: 10.3109/14767058.2014.987748
146. De Agüero MG, Ganal-Vonarburg SC, Fuhrer T, Rupp S, Uchimura Y, Li H, et al. The maternal microbiota drives early postnatal innate immune development. *Science (80-)* (2016) 351:1296–302. doi: 10.1126/science.aad2571
147. Fulde M, Sommer F, Chassaing B, van Vorst K, Dupont A, Hensel M, et al. Neonatal selection by Toll-like receptor 5 influences long-term gut microbiota composition. *Nature* (2018) 560:489–93. doi: 10.1038/s41586-018-0395-5
148. Olszak T, An D, Zeissig S, Vera MP, Richter J, Franke A, et al. Microbial exposure during early life has persistent effects on natural killer T cell function. *Science (80-)* (2012) 336:489–93. doi: 10.1126/science.1219328
149. Dworkin RH, Cornblatt BA, Friedmann R, Kaplansky LM, Lewis JA, Rinaldi A, et al. Childhood precursors of affective vs. social deficits in adolescents at risk for schizophrenia. *Schizophr Bull* (1993) 19:563–77. doi: 10.1093/schbul/19.3.563
150. Iacono WG, Clementz BA. A strategy for elucidating genetic influences on complex psychopathological syndromes (with special reference to ocular

motor functioning and schizophrenia). *Prog Exp Pers Psychopathol Res* (1993) 16:11–65.
151. Mednick SA, Schulsinger F. Some premorbid characteristics related to breakdown in children with schizophrenic mothers. *J Psychiatr Res* (1968) 6:267–91. doi: 10.1016/0022-3956(68)90022-8
152. Sohlberg SC, Yaniv S. Social adjustment and cognitive performance of high-risk children. *Schizophr Bull* (1985) 11:61–5. doi: 10.1093/schbul/11.1.61
153. Weintraub S. Risk factors in schizophrenia: The Stony Brook high-risk project. *Schizophr Bull* (1987) 13:439–50. doi: 10.1093/schbul/13.3.439
154. Niemi LT, Suvisaari JM, Tuulio-Henriksson A, Lönnqvist JK. Childhood developmental abnormalities in schizophrenia: Evidence from high-risk studies. *Schizophr Res* (2003) 60:239–58. doi: 10.1016/S0920-9964(02)00234-7
155. Scharf RJ, Scharf GJ, Stroustrup A. Developmental milestones. *Pediatr Rev* (2016) 37:25–38. doi: 10.1542/pir.2014-0103
156. McNeil TF, Fish B, Schubert EW. Prospective study of pandysmaturation and adult mental disorder in high-risk and normal-risk offspring. *J Psychiatr Res* (2011) 45:561–7. doi: 10.1016/j.jpsychires.2010.09.010
157. Fish B, Marcus J, Hans SL, Auerbach JG, Perdue S. Infants at Risk for Schizophrenia: Sequelae of a Genetic Neurointegrative Defect: A Review and Replication Analysis of Pandysmaturation in the Jerusalem Infant Development Study. *Arch Gen Psychiatry* (1992) 49:221–35. doi: 10.1001/archpsyc.1992.01820030053007
158. Sameroff AJ, Barocas R, Seifer R. The early development of children born to mentally ill women. In: *Children at risk for schizophrenia: A longitudinal perspective*. New York, NY, US: Cambridge University Press. (1984) p. 482–514.
159. Parnas J, Schulsinger F, Schulsinger H, Mednick SA, Teasdale TW. Behavioral Precursors of Schizophrenia Spectrum: A Prospective Study. *Arch Gen Psychiatry* (1982) 39:658–64. doi: 10.1001/archpsyc.1982.04290060020005
160. Näslund B, Persson-Blennow I, McNeil T, Kaij L, Malmquist-Larsson A. Offspring of women with nonorganic psychosis: fear of strangers during the first year of life. *Acta Psychiatr Scand* (1984) 69:435–44. doi: 10.1111/j.1600-0447.1984.tb02516.x
161. Goodman SH. Emory University Project on Children of Disturbed Parents. *Schizophr Bull* (1987) 13:411–23. doi: 10.1093/schbul/13.3.411
162. Fish B. Infant predictors of the longitudinal course of schizophrenic development. *Schizophr Bull* (1987) 13:395–409. doi: 10.1093/schbul/13.3.395
163. McNeil TF, Harty B, Blennow G, Cantor-Graae E. Neuromotor deviation in offspring of psychotic mothers: A selective developmental deficiency in two groups of children at heightened psychiatric risk? *J Psychiatr Res* (1993) 27:39–54. doi: 10.1016/0022-3956(93)90048-7
164. Rieder RO, Nichols PL. Offspring of Schizophrenics III: Hyperactivity and Neurological Soft Signs. *Arch Gen Psychiatry* (1979) 36:665–74. doi: 10.1001/archpsyc.1979.01780060055006
165. Gooding DC, Ott SL, Roberts SA, Erlenmeyer-Kimling L. Thought disorder in mid-childhood as a predictor of adulthood diagnostic outcome: Findings from the New York High-Risk Project. *Psychol Med* (2013) 43:1003–12. doi: 10.1017/S0033291712001791
166. Hans SL, Marcus J, Nuechterlein KH, Asarnow RF, Styr B, Auerbach JG. Neurobehavioral deficits at adolescence in children at risk for schizophrenia: The Jerusalem Infant Development Study. *Arch Gen Psychiatry* (1999) 56:741–8. doi: 10.1001/archpsyc.56.8.741
167. Marcus J, Hans SL, Auerbach JG, Auerbach AG. Children at Risk for Schizophrenia: The Jerusalem Infant Development Study: II. Neurobehavioral Deficits at School Age. *Arch Gen Psychiatry* (1993) 50:797–809. doi: 10.1001/archpsyc.1993.01820220053006
168. Marcus J, Hans SL, Lewow E, Wilkinson L, Burack CM. Neurological findings in high-risk children: childhood assessment and 5-year followup. *Schizophr Bull* (1985) 11:85–100. doi: 10.1093/schbul/11.1.85
169. Hodges A, Byrne M, Grant E, Johnstone E. People at risk of schizophrenia. Sample characteristics of the first 100 cases in the Edinburgh high-risk study. *Br J Psychiatry* (1999) 174:547–53. doi: 10.1192/bjp.174.6.547
170. Garmezy N, Devine V. Project Competence: The Minnesota studies of children vulnerable to psychopathology. In: *Children at risk for schizophrenia: A longitudinal perspective*. New York, NY, US: Cambridge University Press. (1984) p. 289–303.
171. Erlenmeyer-Kimling L, Rock D, Roberts SA, Janal M, Kestenbaum C, Cornblatt B, et al. Attention, memory, and motor skills as childhood predictors of schizophrenia-related psychoses: The New York high-risk project. *Am J Psychiatry* (2000) 157:1416–22. doi: 10.1176/appi.ajp.157.9.1416
172. Bolinskey PK, Gottesman II, Nichols DS, Shapiro BM, Roberts SA, Adamo UH, et al. A new MMPI-derived indicator of liability to develop schizophrenia: Evidence from the New York High-Risk Project. *Assessment* (2001) 8:127–43. doi: 10.1177/107319110100800202
173. Lifshitz M, Kugelmass S, Karov M. Perceptual-motor and memory performance of high-risk children. *Schizophr Bull* (1985) 11:74–84. doi: 10.1093/schbul/11.1.74
174. Nagler S, Glueck Z. The clinical interview. *Schizophr Bull* (1985) 11:38–47. doi: 10.1093/schbul/11.1.38
175. Weintraub S, Neale JM. The Stony Brook High-Risk Project. In: . *Children at risk for schizophrenia: A longitudinal perspective*. New York, NY, US: Cambridge University Press. (1984) p. 243–63.
176. Johnstone EC, Abukmeil SS, Byrne M, Clafferty R, Grant E, Hodges A, et al. Edinburgh high risk study–findings after four years: demographic, attainment and psychopathological issues. *Schizophr Res* (2000) 46:1–15. doi: 10.1016/s0920-9964(99)00225-x
177. Redman SL, Corcoran CM, Kimhy D, Malaspina D. Effects of early trauma on psychosis development in clinical high-risk individuals and stability of trauma assessment across studies: a review. *Arch Psychol (Chicago Ill)* (2017) 1:28–49. [Accessed January 22, 2020].
178. Ramsay DS, Lewis M. Developmental Change in Infant Cortisol and Behavioral Response to Inoculation. *Child Dev* (1994) 65:1491–502. doi: 10.1111/j.1467-8624.1994.tb00831.x
179. Gunnar MR, Brodersen L, Krueger K, Rigatuso J. Dampening of Adrenocortical Responses during Infancy: Normative Changes and Individual Differences. *Child Dev* (1996) 67:877–89. doi: 10.1111/j.1467-8624.1996.tb01770.x
180. Daskalakis NP, Kloet ER, De, Yehuda R, Malaspina D, Kranz TM. Early life stress effects on glucocorticoid—BDNF interplay in the hippocampus. *Front Mol Neurosci* (2015) 8:1–13. doi: 10.3389/fnmol.2015.00068
181. Notaras M, van den Buuse M. Neurobiology of BDNF in fear memory, sensitivity to stress, and stress-related disorders. *Mol Psychiatry* (2020) doi: 10.1038/s41380-019-0639-2
182. Notaras M, Hill R, Van den Buuse M. A role for the BDNF gene Val66Met polymorphism in schizophrenia? A comprehensive review. *Neurosci Biobehav Rev* (2015) 51:15–30. doi: 10.1016/j.neubiorev.2014.12.016
183. Notaras M, Hill R, Van Den Buuse M. The BDNF gene Val66Met polymorphism as a modifier of psychiatric disorder susceptibility: Progress and controversy. *Mol Psychiatry* (2015) 20:916–30. doi: 10.1038/mp.2015.27
184. Jing D, Lee FS, Ninan I. The BDNF Val66Met polymorphism enhances glutamatergic transmission but diminishes activity-dependent synaptic plasticity in the dorsolateral striatum. *Neuropharmacology* (2017) 112:84–93. doi: 10.1016/j.neuropharm.2016.06.030
185. Pattwell SS, Bath KG, Perez-Castro R, Lee FS, Chao MV, Ninan I. The BDNF Val66Met polymorphism impairs synaptic transmission and plasticity in the infralimbic medial prefrontal cortex. *J Neurosci* (2012) 32:2410–21. doi: 10.1523/JNEUROSCI.5205-11.2012
186. Aas M, Haukvik UK, Djurovic S, Bergmann Ø, Athanasiu L, Tesli MS, et al. BDNF val66met modulates the association between childhood trauma, cognitive and brain abnormalities in psychoses. *Prog Neuropsychopharmacol Biol Psychiatry* (2013) 46:181–8. doi: 10.1016/j.pnpbp.2013.07.008
187. Lee J, Duan W, Mattson MP. Evidence that brain-derived neurotrophic factor is required for basal neurogenesis and mediates, in part, the enhancement of neurogenesis by dietary restriction in the hippocampus of adult mice. *J Neurochem* (2002) 82:1367–75. doi: 10.1046/j.1471-4159.2002.01085.x
188. Grassi-Oliveira R, Ashy M, Stein LM. Psychobiology of childhood maltreatment: Effects of allostatic load? *Rev Bras Psiquiatr* (2008) 30:60–8. doi: 10.1590/S1516-44462008000100012
189. Elzinga BM, Molendijk ML, Oude Voshaar RC, Bus BAA, Prickaerts J, Spinhoven P, et al. The impact of childhood abuse and recent stress on serum brain-derived neurotrophic factor and the moderating role of BDNF Val 66Met. *Psychopharmacol (Berl)* (2011) 214:319–28. doi: 10.1007/s00213-010-1961-1
190. Bermanzohn PC, Porto L, Arlow PB, Pollack S, Stronger R, Siris SG. At Issue: Hierarchical Diagnosis in Chronic Schizophrenia: A Clinical Study of Co-

occurring Syndromes. *Schizophr Bull* (2000) 26:517–25. doi: 10.1093/oxfordjournals.schbul.a033472
191. Mehl S, Landsberg MW, Schmidt AC, Cabanis M, Bechdolf A, Herrlich J, et al. Why do bad things happen to me? Attributional style, depressed mood, and persecutory delusions in patients with schizophrenia. *Schizophr Bull* (2014) 40:1338–46. doi: 10.1093/schbul/sbu040
192. Grassi-Oliveira R, Stein LM, Lopes RP, Teixeira AL, Bauer ME. Low Plasma Brain-Derived Neurotrophic Factor and Childhood Physical Neglect Are Associated with Verbal Memory Impairment in Major Depression-A Preliminary Report. *Biol Psychiatry* (2008) 64:281–5. doi: 10.1016/j.biopsych.2008.02.023
193. Liu D, Diorio J, Day JC, Francis DD, Meaney MJ. Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nat Neurosci* (2000) 3:799–806. doi: 10.1038/77702
194. Lee J, Folley BS, Gore J, Park S. Origins of spatial working memory deficits in schizophrenia: An event-related fMRI and near-infrared spectroscopy study. *PloS One* (2008) 3:e1760. doi: 10.1371/journal.pone.0001760
195. Goodman GS, Ogle CM, Block SD, Harris LS, Larson RP, Augusti EM, et al. False memory for trauma-related Deese-Roediger-McDermott lists in adolescents and adults with histories of child sexual abuse. *Dev Psychopathol* (2011) 23:423–38. doi: 10.1017/S0954579411000150
196. Strakowski SM, Keck PE, McElroy SL, Lonczak HS, West SA. Chronology of comorbid and principal syndromes in first-episode psychosis. *Compr Psychiatry* (1995) 36:106–12. doi: 10.1016/s0010-440x(95)90104-3
197. Marshall AD. Developmental timing of trauma exposure relative to puberty and the nature of psychopathology among adolescent girls. *J Am Acad Child Adolesc Psychiatry* (2016) 55:25–32.e1. doi: 10.1016/j.jaac.2015.10.004
198. Thompson A, Nelson B, McNab C, Simmons M, Leicester S, McGorry PD, et al. Psychotic symptoms with sexual content in the "ultra high risk" for psychosis population: Frequency and association with sexual trauma. *Psychiatry Res* (2010) 177:84–91. doi: 10.1016/j.psychres.2010.02.011
199. Kraan T, Velthorst E, Smit F, de Haan L, van der Gaag M. Trauma and recent life events in individuals at ultra high risk for psychosis: Review and meta-analysis. *Schizophr Res* (2015) 161:143–9. doi: 10.1016/j.schres.2014.11.026
200. Falukozi E, Addington J. Impact of trauma on attenuated psychotic symptoms. *Psychosis* (2012) 4:203–12. doi: 10.1080/17522439.2011.626867
201. Bechdolf A, Thompson A, Nelson B, Cotton S, Simmons MB, Amminger GP, et al. Experience of trauma and conversion to psychosis in an ultra-high-risk (prodromal) group. *Acta Psychiatr Scand* (2010) 121:377–84. doi: 10.1111/j.1600-0447.2010.01542.x
202. Russo DA, Stochl J, Painter M, Dobler V, Jackson E, Jones PB, et al. Trauma history characteristics associated with mental states at clinical high risk for psychosis. *Psychiatry Res* (2014) 220:237–44. doi: 10.1016/j.psychres.2014.08.028
203. Thompson JL, Kelly M, Kimhy D, Harkavy-Friedman JM, Khan S, Messinger JW, et al. Childhood trauma and prodromal symptoms among individuals at clinical high risk for psychosis. *Schizophr Res* (2009) 108:176–81. doi: 10.1016/j.schres.2008.12.005
204. Thompson A, Marwaha S, Nelson B, Wood SJ, McGorry PD, Yung AR, et al. Do affective or dissociative symptoms mediate the association between childhood sexual trauma and transition to psychosis in an ultra-high risk cohort? *Psychiatry Res* (2016) 236:182–5. doi: 10.1016/j.psychres.2016.01.017
205. Stowkowy J, Liu L, Cadenhead KS, Cannon TD, Cornblatt BA, McGlashan TH, et al. Early traumatic experiences, perceived discrimination and conversion to psychosis in those at clinical high risk for psychosis. *Soc Psychiatry Psychiatr Epidemiol* (2016) 51:497–503. doi: 10.1007/s00127-016-1182-y
206. Stowkowy J, Addington J. Predictors of a clinical high risk status among individuals with a family history of psychosis. *Schizophr Res* (2013) 147:281–6. doi: 10.1016/j.schres.2013.03.030
207. Şahin S, Yüksel Ç, Güler J, Karadayi G, Akturan E, Göde E, et al. The history of childhood trauma among individuals with ultra high risk for psychosis is as common as among patients with first-episode schizophrenia. *Early Interv Psychiatry* (2013) 7:414–20. doi: 10.1111/eip.12022
208. Yung AR, McGorry PD. The initial prodrome in psychosis: Descriptive and qualitative aspects. *Aust N Z J Psychiatry* (1996) 30:587–99. doi: 10.3109/00048679609062654
209. Üçok A, Kaya H, Uğurpala C, Çikrikçili U, Ergül C, Yokuşoğlu Ç, et al. History of childhood physical trauma is related to cognitive decline in individuals with ultra-high risk for psychosis. *Schizophr Res* (2015) 169:199–203. doi: 10.1016/j.schres.2015.08.038
210. Trotman HD, Holtzman CW, Walker EF, Addington JM, Bearden CE, Cadenhead KS, et al. Stress exposure and sensitivity in the clinical high-risk syndrome: Initial findings from the North American Prodrome Longitudinal Study (NAPLS). *Schizophr Res* (2014) 160:104–9. doi: 10.1016/j.schres.2014.09.017
211. Carpenter LL, Tyrka AR, Ross NS, Khoury L, Anderson GM, Price LH. Effect of Childhood Emotional Abuse and Age on Cortisol Responsivity in Adulthood. *Biol Psychiatry* (2009) 66:69–75. doi: 10.1016/j.biopsych.2009.02.030
212. van Dam DS, Korver-Nieberg N, Velthorst E, Meijer CJ, de Haan L. Childhood maltreatment, adult attachment and psychotic symptomatology: a study in patients, siblings and controls. *Soc Psychiatry Psychiatr Epidemiol* (2014) 49:1759–67. doi: 10.1007/s00127-014-0894-0
213. Heins M, Simons C, Lataster T, Pfeifer S, Versmissen D, Lardinois M, et al. Childhood trauma and psychosis: A case-control and case-sibling comparison across different levels of genetic liability, psychopathology, and type of trauma. *Am J Psychiatry* (2011) 168:1286–94. doi: 10.1176/appi.ajp.2011.10101531
214. Suzuki A, Poon L, Papadopoulos AS, Kumari V, Cleare AJ. Long term effects of childhood trauma on cortisol stress reactivity in adulthood and relationship to the occurrence of depression. *Psychoneuroendocrinology* (2014) 50:289–99. doi: 10.1016/j.psyneuen.2014.09.007
215. Ruby E, Polito S, McMahon K, Gorovitz M, Corcoran C, Malaspina D. Pathways Associating Childhood Trauma to the Neurobiology of Schizophrenia. *Front Psychol Behav Sci* (2014) 3:1–17.
216. Kitraki E, Kremmyda O, Youlatos D, Alexis MN, Kittas C. Spatial performance and corticosteroid receptor status in the 21-day restraint stress paradigm. *Ann New York Acad Sci* (2004) 1018(1):323–7. doi: 10.1196/annals.1296.039
217. Magariños AM, McEwen BS. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: Involvement of glucocorticoid secretion and excitatory amino acid receptors. *Neuroscience* (1995) 69:89–98. doi: 10.1016/0306-4522(95)00259-L
218. Sunanda, Rao MS, Raju TR. Effect of chronic restraint stress on dendritic spines and excrescences of hippocampal CA3 pyramidal neurons-a quantitative study. *Brain Res* (1995) 694:312–7. doi: 10.1016/0006-8993(95)00822-8
219. Tata DA, Marciano VA, Anderson BJ. Synapse loss from chronically elevated glucocorticoids: Relationship to neuropil volume and cell number in hippocampal area CA3. *J Comp Neurol* (2006) 498:363–74. doi: 10.1002/cne.21071
220. Vyas A, Mitra R, Shankaranarayana Rao BS, Chattarji S. Chronic stress induces contrasting patterns of dendritic remodeling in hippocampal and amygdaloid neurons. *J Neurosci* (2002) 22:6810–8. doi: 10.1523/jneurosci.22-15-06810.2002
221. Wright RL, Lightner EN, Harman JS, Meijer OC, Conrad CD. Attenuating corticosterone levels on the day of memory assessment prevents chronic stress-induced impairments in spatial memory. *Eur J Neurosci* (2006) 24:595–605. doi: 10.1111/j.1460-9568.2006.04948.x
222. Baumeister D, Akhtar R, Ciufolini S, Pariante CM, Mondelli V. Childhood trauma and adulthood inflammation: A meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-α. *Mol Psychiatry* (2016) 21:642–9. doi: 10.1038/mp.2015.67
223. Slopen N, Kubzansky LD, McLaughlin KA, Koenen KC. Childhood adversity and inflammatory processes in youth: A prospective study. *Psychoneuroendocrinology* (2013) 38:188–200. doi: 10.1016/j.psyneuen.2012.05.013
224. Benedetti F, Ambrée O, Locatelli C, Lorenzi C, Poletti S, Colombo C, et al. The effect of childhood trauma on serum BDNF in bipolar depression is modulated by the serotonin promoter genotype. *Neurosci Lett* (2017) 656:177–81. doi: 10.1016/j.neulet.2017.07.043
225. Hemmings SMJ, Malan-Müller S, Van Den Heuvel LL, Demmitt BA, Stanislawski MA, Smith DG, et al. The Microbiome in Posttraumatic Stress Disorder and Trauma-Exposed Controls: An Exploratory Study. *Psychosom Med* (2017) 79:936–46. doi: 10.1097/PSY.0000000000000512
226. Bradford K, Shih W, Videlock EJ, Presson AP, Naliboff BD, Mayer EA, et al. Association Between Early Adverse Life Events and Irritable Bowel Syndrome. *Clin Gastroenterol Hepatol* (2012) 10:385–90. doi: 10.1016/j.cgh.2011.12.018

227. García-Ródenas CL, Bergonzelli GE, Nutten S, Schumann A, Cherbut C, Turini M, et al. Nutritional approach to restore impaired intestinal barrier function and growth after neonatal stress in rats. *J Pediatr Gastroenterol Nutr* (2006) 43:16–24. doi: 10.1097/01.mpg.0000226376.95623.9f
228. O'Mahony SM, Marchesi JR, Scully P, Codling C, Ceolho AM, Quigley EMM, et al. Early Life Stress Alters Behavior, Immunity, and Microbiota in Rats: Implications for Irritable Bowel Syndrome and Psychiatric Illnesses. *Biol Psychiatry* (2009) 65:263–7. doi: 10.1016/j.biopsych.2008.06.026
229. Park SH, Videlock EJ, Shih W, Presson AP, Mayer EA, Chang L. Adverse childhood experiences are associated with irritable bowel syndrome and gastrointestinal symptom severity. *Neurogastroenterol Motil* (2016) 28:1252–60. doi: 10.1111/nmo.12826
230. Chitkara DK, Van Tilburg MAL, Blois-Martin N, Whitehead WE. Early life risk factors that contribute to irritable bowel syndrome in adults: A systematic review. *Am J Gastroenterol* (2008) 103:765–74. doi: 10.1111/j.1572-0241.2007.01722.x
231. Callaghan BL, Fields A, Gee DG, Gabard-Durnam L, Caldera C, Humphreys KL, et al. Mind and gut: Associations between mood and gastrointestinal distress in children exposed to adversity. *Dev Psychopathol* (2019) 32:309–28. doi: 10.1017/S0954579419000087
232. Hantsoo L, Jašarević E, Criniti S, McGeehan B, Tanes C, Sammel MD, et al. Childhood adversity impact on gut microbiota and inflammatory response to stress during pregnancy. *Brain Behav Immun* (2019) 75:240–50. doi: 10.1016/j.bbi.2018.11.005
233. Erny D, De Angelis ALH, Jaitin D, Wieghofer P, Staszewski O, David E, et al. Host microbiota constantly control maturation and function of microglia in the CNS. *Nat Neurosci* (2015) 18:965–77. doi: 10.1038/nn.4030
234. Rantakallio P, Jones P, Moring J, Von Wendt L. Association between central nervous system infections during childhood and adult onset schizophrenia and other psychoses: A 28-year follow-up. *Int J Epidemiol* (1997) 26:837–43. doi: 10.1093/ije/26.4.837
235. Leask SJ, Done DJ, Crow TJ. Adult psychosis, common childhood infections and neurological soft signs in a national birth cohort. *Br J Psychiatry* (2002) 181:387–92. doi: 10.1192/bjp.181.5.387
236. Dalman C, Allebeck P, Gunnell D, Harrison G, Kristensson K, Lewis G, et al. Infections in the CNS during childhood and the risk of subsequent psychotic illness: A cohort study of more than one million Swedish subjects. *Am J Psychiatry* (2008) 165:59–65. doi: 10.1176/appi.ajp.2007.07050740
237. Debost JC, Larsen JT, Munk-Olsen T, Mortensen PB, Agerbo E, Petersen LV. Childhood infections and schizophrenia: The impact of parental SES and mental illness, and childhood adversities. *Brain Behav Immun* (2019) 81:341–7. doi: 10.1016/j.bbi.2019.06.031
238. Köhler-Forsberg O, Petersen L, Gasse C, Mortensen PB, Dalsgaard S, Yolken RH, et al. A Nationwide Study in Denmark of the Association between Treated Infections and the Subsequent Risk of Treated Mental Disorders in Children and Adolescents. *JAMA Psychiatry* (2019) 76:271–9. doi: 10.1001/jamapsychiatry.2018.3428
239. Bhattacharyya S, Darby RR, Raibagkar P, Castro LNG, Berkowitz AL. Antibiotic-associated encephalopathy. *Neurology* (2016) 86:963–71. doi: 10.1212/WNL.0000000000002455
240. Torpy DJ, Ho JT. Value of free cortisol measurement in systemic infection. *Hormone Metab Res* (2007) 39(6):439–44. doi: 10.1055/s-2007-980200
241. Brown DG, Soto R, Yandamuri S, Stone C, Dickey L, Gomes-Neto JC, et al. The microbiota protects from viral- induced neurologic damage through microglia-intrinsic TLR signaling. *Elife* (2019) 8:e47117. doi: 10.7554/eLife.47117
242. Jaworska N, MacQueen G. Adolescence as a unique developmental period. *J Psychiatry Neurosci* (2015) 40:291–3. doi: 10.1503/jpn.150268
243. McVey Neufeld KA, Luczynski P, Dinan TG, Cryan JF. Reframing the teenage wasteland: Adolescent microbiota-gut-brain axis. *Can J Psychiatry* (2016) 61:214–21. doi: 10.1177/0706743716635536
244. Worland J, Weeks DG, Weiner SM, Schechtman J. Longitudinal, Prospective Evaluations of Intelligence in Children at Risk. *Schizophr Bull* (1982) 8:135–41. doi: 10.1093/schbul/8.1.135
245. Erlenmeyer-Kimling L, Cornblatt BA. A summary of attentional findings in the New York High-Risk Project. *J Psychiatr Res* (1992) 26:405–26. doi: 10.1016/0022-3956(92)90043-n
246. Ayalon M, Merom H. The teacher interview. *Schizophr Bull* (1985) 11:117–20. doi: 10.1093/schbul/11.1.117
247. Kugelmass S, Faber N, Frenkel E, Ingraham LJ, Mirsky AF, Nathan M, et al. Reanalysis of SCOR and anxiety measures in the Israeli high-risk study. *Schizophr Bull* (1995) 21:205–17. doi: 10.1093/schbul/21.2.205
248. Wisdom JP, Manuel JI, Drake RE. Substance Use Disorder Among People With First-Episode Psychosis: A Systematic Review of Course and Treatment. *Psychiatr Serv* (2011) 62:1007. doi: 10.1176/appi.ps.62.9.1007
249. Asher CJ, Gask L. Reasons for illicit drug use in people with schizophrenia: Qualitative study. *BMC Psychiatry* (2010) 10:94. doi: 10.1186/1471-244X-10-94
250. Ibarra-Lecue I, Mollinedo-Gajate I, Meana JJ, Callado LF, Diez-Alarcia R, Urigüen L. Chronic cannabis promotes pro-hallucinogenic signaling of 5-HT2A receptors through Akt/mTOR pathway. *Neuropsychopharmacology* (2018) 43:2028–35. doi: 10.1038/s41386-018-0076-y
251. Al'Absi M. Hypothalamic-pituitary-adrenocortical responses to psychological stress and risk for smoking relapse. *Int J Psychophysiol* (2006) 59:218–27. doi: 10.1016/j.ijpsycho.2005.10.010
252. Mendelson JH, Ogata M, Mello NK. Adrenal function and alcoholism. I. Serum cortisol. *Psychosom Med* (1971) 33:145–57. doi: 10.1097/00006842-197103000-00006
253. Junghanns K, Junghanns K, Tietz U, Dibbelt L, Kuether M, Jurth R, et al. Attenuated salivary cortisol secretion under cue exposure is associated with early relapse. *Alcohol Alcohol* (2005) 40(1):80–5. Available at: http://citeseerx.ist.psu.edu/viewdoc/summary?doi=10.1.1.583.4191 [Accessed May 29, 2020]. doi: 10.1093/alcalc/agh107
254. Junghanns K, Backhaus J, Tietz U, Lange W, Bernzen J, Wetterling T, et al. Impaired serum cortisol stress response is a predictor of early relapse. *Alcohol Alcohol* (2003) 38:189—193. doi: 10.1093/alcalc/agg052
255. de Timary P, Leclercq S, Stärkel P, Delzenne N. A dysbiotic subpopulation of alcohol-dependent subjects. *Gut Microbes* (2015) 6:388–91. doi: 10.1080/19490976.2015.1107696
256. Kiraly DD, Walker DM, Calipari ES, Labonte B, Issler O, Pena CJ, et al. Alterations of the host microbiome affect behavioral responses to cocaine. *Sci Rep* (2016) 6:1–12. doi: 10.1038/srep35455
257. Lee K, Vuong HE, Nusbaum DJ, Hsiao EY, Evans CJ, Taylor AMW. The gut microbiota mediates reward and sensory responses associated with regimen-selective morphine dependence. *Neuropsychopharmacology* (2018) 43:2606–14. doi: 10.1038/s41386-018-0211-9
258. Ning T, Gong X, Xie L, Ma B. Gut microbiota analysis in rats with methamphetamine-induced conditioned place preference. *Front Microbiol* (2017) 8:1620–9. doi: 10.3389/fmicb.2017.01620
259. Peterson VL, Jury NJ, Cabrera-Rubio R, Draper LA, Crispie F, Cotter PD, et al. Drunk bugs: Chronic vapour alcohol exposure induces marked changes in the gut microbiome in mice. *Behav Brain Res* (2017) 323:172–6. doi: 10.1016/j.bbr.2017.01.049
260. Wang F, Roy S. Gut Homeostasis, Microbial Dysbiosis, and Opioids. *Toxicol Pathol* (2017) 45:150–6. doi: 10.1177/0192623316679898
261. Meckel KR, Kiraly DD. A potential role for the gut microbiome in substance use disorders. *Psychopharmacol (Berl)* (2019) 236:1513–30. doi: 10.1007/s00213-019-05232-0
262. Xu Z, Wang C, Dong X, Hu T, Wang L, Zhao W, et al. Chronic alcohol exposure induced gut microbiota dysbiosis and its correlations with neuropsychic behaviors and brain BDNF/Gabra1 changes in mice. *BioFactors* (2019) 45:187–99. doi: 10.1002/biof.1469
263. Xiao Hw, Ge C, Feng Gx, Li Y, Luo D, Dong J, et al. Gut microbiota modulates alcohol withdrawal-induced anxiety in mice. *Toxicol Lett* (2018) 287:23–30. doi: 10.1016/j.toxlet.2018.01.021
264. Galderisi S, Bucci P, Üçok A, Peuskens J. No gender differences in social outcome in patients suffering from schizophrenia. *Eur Psychiatry* (2012) 27:406–8. doi: 10.1016/j.eurpsy.2011.01.011
265. Gureje O. Gender and schizophrenia: age at onset and sociodemographic attributes. *Acta Psychiatr Scand* (1991) 83:402–5. doi: 10.1111/j.1600-0447.1991.tb05564.x
266. Goldstein JM, Tsuang MT, Faraone SV. Gender and schizophrenia: Implications for understanding the heterogeneity of the illness. *Psychiatry Res* (1989) 28:243–53. doi: 10.1016/0165-1781(89)90205-9
267. De Luca F, Shoenfeld Y. The microbiome in autoimmune diseases. *Clin Exp Immunol* (2019) 195:74–85. doi: 10.1111/cei.13158
268. Stojanovich L, Marisavljevich D. Stress as a trigger of autoimmune disease. *Autoimmun Rev* (2008) 7:209–13. doi: 10.1016/j.autrev.2007.11.007

269. Müller N, Weidinger E, Leitner B, Schwarz MJ. The role of inflammation in schizophrenia. *Front Neurosci* (2015) 9:372–81. doi: 10.3389/fnins.2015.00372
270. Ryan MCM, Collins P, Thakore JH. Impaired fasting glucose tolerance in first-episode, drug-naive patients with schizophrenia. *Am J Psychiatry* (2003) 160:284–9. doi: 10.1176/appi.ajp.160.2.284
271. Saddichha S, Manjunatha N, Ameen S, Akhtar S. Metabolic syndrome in first episode schizophrenia - A randomized double-blind controlled, short-term prospective study. *Schizophr Res* (2008) 101:266–72. doi: 10.1016/j.schres.2008.01.004
272. He Y, Wu W, Wu S, Zheng HM, Li P, Sheng HF, et al. Linking gut microbiota, metabolic syndrome and economic status based on a population-level analysis. *Microbiome* (2018) 6:172. doi: 10.1186/s40168-018-0557-6
273. Brunner EJ, Hemingway H, Walker BR, Page M, Clarke P, Juneja M, et al. Adrenocortical, autonomic, and inflammatory causes of the metabolic syndrome: Nested case-control study. *Circulation* (2002) 106:2659–65. doi: 10.1161/01.CIR.0000038364.26310.BD
274. Ogbonnaya ES, Clarke G, Shanahan F, Dinan TG, Cryan JF, O'Leary OF. Adult Hippocampal Neurogenesis Is Regulated by the Microbiome. *Biol Psychiatry* (2015) 78:e7–9. doi: 10.1016/j.biopsych.2014.12.023
275. van Praag H. Exercise and the brain: something to chew on. *Trends Neurosci* (2009) 32:283–90. doi: 10.1016/j.tins.2008.12.007
276. Van Praag H. Neurogenesis and exercise: Past and future directions. *NeuroMolecular Med* (2008) 10:128–40. doi: 10.1007/s12017-008-8028-z
277. Hennigan A, O'Callaghan RM, Kelly ÁM. Neurotrophins and their receptors: Roles in plasticity, neurodegeneration and neuroprotection. *Biochem Soc Trans* (2007) 35(2):424–7. doi: 10.1042/BST0350424
278. Johnston MV. Plasticity in the developing brain: implications for rehabilitation. *Dev Disabil Res Rev* (2009) 15:94–101. doi: 10.1002/ddrr.64
279. Farmer J, Zhao X, Van Praag H, Wodtke K, Gage FH, Christie BR. Effects of voluntary exercise on synaptic plasticity and gene expression in the dentate gyrus of adult male sprague-dawley rats in vivo. *Neuroscience* (2004) 124:71–9. doi: 10.1016/j.neuroscience.2003.09.029
280. Tong L, Shen H, Perreau VM, Balazs R, Cotman CW. Effects of exercise on gene-expression profile in the rat hippocampus. *Neurobiol Dis* (2001) 8:1046–56. doi: 10.1006/nbdi.2001.0427
281. Neeper SA, Góauctemez-Pinilla F, Choi J, Cotman C. Exercise and brain neurotrophins. *Nature* (1995) 373:109. doi: 10.1038/373109a0
282. Seifert T, Brassard P, Wissenberg M, Rasmussen P, Nordby P, Stallknecht B, et al. Endurance training enhances BDNF release from the human brain. *Am J Physiol Regul Integr Comp Physiol* (2010) 298:R372–7. doi: 10.1152/ajpregu.00525.2009
283. Rasmussen P, Brassard P, Adser H, Pedersen MV, Leick L, Hart E, et al. Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Exp Physiol* (2009) 94:1062–9. doi: 10.1113/expphysiol.2009.048512
284. Voss MW, Vivar C, Kramer AF, van Praag H. Bridging animal and human models of exercise-induced brain plasticity. *Trends Cognit Sci* (2013) 17:525–44. doi: 10.1016/j.tics.2013.08.001
285. Voss MW, Erickson KI, Prakash RS, Chaddock L, Kim JS, Alves H, et al. Neurobiological markers of exercise-related brain plasticity in older adults. *Brain Behav Immun* (2013) 28:90–9. doi: 10.1016/j.bbi.2012.10.021
286. Szuhany KL, Bugatti M, Otto MW. A meta-analytic review of the effects of exercise on brain-derived neurotrophic factor. *J Psychiatr Res* (2015) 60:56–64. doi: 10.1016/j.jpsychires.2014.10.003
287. Kimhy D, Vakhrusheva J, Khan S, Chang RW, Hansen MC, Ballon JS, et al. Emotional granularity and social functioning in individuals with schizophrenia: An experience sampling study. *J Psychiatr Res* (2014) 53:141–8. doi: 10.1016/j.jpsychires.2014.01.020
288. Deighton S, Addington J. Exercise practices of young people at their first episode of psychosis. *Schizophr Res* (2014) 152:311–2. doi: 10.1016/j.schres.2013.10.045
289. Hodgekins J, French P, Birchwood M, Mugford M, Christopher R, Marshall M, et al. Comparing time use in individuals at different stages of psychosis and a non-clinical comparison group. *Schizophr Res* (2015) 161:188–93. doi: 10.1016/j.schres.2014.12.011
290. Koivukangas J, Tammelin T, Kaakinen M, Mäki P, Moilanen I, Taanila A, et al. Physical activity and fitness in adolescents at risk for psychosis within the Northern Finland 1986 Birth Cohort. *Schizophr Res* (2010) 116:152–8. doi: 10.1016/j.schres.2009.10.022
291. Newberry RE, Dean DJ, Sayyah MD, Mittal VA. What prevents youth at clinical high risk for psychosis from engaging in physical activity? An examination of the barriers to physical activity. *Schizophr Res* (2018) 201:400–5. doi: 10.1016/j.schres.2018.06.011
292. Mittal VA, Vargas T, Juston Osborne K, Dean D, Gupta T, Ristanovic I, et al. Exercise Treatments for Psychosis: a Review. *Curr Treat Options Psychiatry* (2017) 4:152–66. doi: 10.1007/s40501-017-0112-2
293. Armstrong HF, Bartels MN, Paslavski O, Cain D, Shoval HA, Ballon JS, et al. The impact of aerobic exercise training on cardiopulmonary functioning in individuals with schizophrenia. *Schizophr Res* (2016) 173:116–7. doi: 10.1016/j.schres.2016.03.009
294. Kimhy D, Gill KE, Brucato G, Vakhrusheva J, Arndt L, Gross JJ, et al. The impact of emotion awareness and regulation on social functioning in individuals at clinical high risk for psychosis. *Psychol Med* (2016) 46:2907–18. doi: 10.1017/S0033291716000490
295. Vakhrusheva J, Marino B, Stroup TS, Kimhy D. Aerobic Exercise in People with Schizophrenia: Neural and Neurocognitive Benefits. *Curr Behav Neurosci Rep* (2016) 3:165–75. doi: 10.1007/s40473-016-0077-2
296. Kimhy D, Vakhrusheva J, Bartels MN, Armstrong HF, Ballon JS, Khan S, et al. The Impact of Aerobic Exercise on Brain-Derived Neurotrophic Factor and Neurocognition in Individuals With Schizophrenia: A Single-Blind, Randomized Clinical Trial. *Schizophr Bull* (2015) 41:859–68. doi: 10.1093/schbul/sbv022
297. Selye H. Adaptation energy. *Nature* (1938) 141:926. doi: 10.1038/141926a0
298. Sapolsky RM, Krey LC, McEwen BS. The neuroendocrinology of stress and aging: The glucocorticoid cascade hypothesis. *Endocr Rev* (1986) 7:284–301. doi: 10.1210/edrv-7-3-284
299. Bercik P, Park AJ, Sinclair D, Khoshdel A, Lu J, Huang X, et al. The anxiolytic effect of Bifidobacterium longum NCC3001 involves vagal pathways for gut-brain communication. *Neurogastroenterol Motil* (2011) 23:1132–9. doi: 10.1111/j.1365-2982.2011.01796.x
300. Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone-releasing acylated peptide from stomach. *Nature* (1999) 402:656–60. doi: 10.1038/45230
301. Muccioli G, Papotti M, Locatelli V, Ghigo E, Deghenghi R. Binding of 125I-labeled ghrelin to membranes from human hypothalamus and pituitary gland. *J Endocrinol Invest* (2001) 24:7–9. doi: 10.1007/BF03343831
302. Giordano R, Pellegrino M, Picu A, Bonelli L, Balbo M, Berardelli R, et al. Neuroregulation of the hypothalamus-pituitary-adrenal (HPA) axis in humans: Effects of GABA-, mineralocorticoid-, and GH-secretagogue-receptor modulation. *Sci World J* (2006) 6:1–11. doi: 10.1100/tsw.2006.09
303. Tortorella C, Neri G, Nussdorfer GG. Galanin in the regulation of the hypothalamic-pituitary-adrenal axis (review). *Int J Mol Med* (2007) 19:639–47. doi: 10.3892/ijmm.19.4.639
304. Galley JD, Nelson MC, Yu Z, Dowd SE, Walter J, Kumar PS, et al. Exposure to a social stressor disrupts the community structure of the colonic mucosa-associated microbiota. *BMC Microbiol* (2014) 14:189–202. doi: 10.1186/1471-2180-14-189

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 10 July 2020
doi: 10.3389/fpsyt.2020.00680

Stress, Cortisol and NR3C1 in At-Risk Individuals for Psychosis: A Mendelian Randomization Study

Anton Iftimovici[1,2], *Oussama Kebir*[1,3], *Qin He*[1], *Thérèse M. Jay*[1], *ICAAR Study Group*, *Guy A. Rouleau*[4], *Marie-Odile Krebs*[1,3] *and Boris Chaumette*[1,3,5]*

[1] Institut de Psychiatrie et Neurosciences de Paris, INSERM UMR 1266, Laboratoire de Physiopathologie des Maladies Psychiatriques, Université de Paris, GDR3557-Institut de Psychiatrie, Paris, France, [2] NeuroSpin, Atomic Energy Commission, Gif-sur-Yvette, France, [3] GHU Paris Psychiatrie et Neurosciences, Paris, France, [4] Department of Neurology and Neurosurgery, Montreal Neurological Institute, McGill University, Montreal, QC, Canada, [5] Department of Psychiatry, McGill University, Montreal, QC, Canada

OPEN ACCESS

Edited by:
Jerome Brunelin,
INSERM U1028 Centre de Recherche en Neurosciences de Lyon, France

Reviewed by:
Romain Rey,
Centre Hospitalier Le Vinatier, France
Alexis E. Cullen,
King's College London, United Kingdom

***Correspondence:**
Boris Chaumette
boris.chaumette@inserm.fr

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: 01 May 2020
Accepted: 29 June 2020
Published: 10 July 2020

Citation:
Iftimovici A, Kebir O, He Q, Jay TM, ICAAR Study Group, Rouleau GA, Krebs M-O and Chaumette B (2020) Stress, Cortisol and NR3C1 in At-Risk Individuals for Psychosis: A Mendelian Randomization Study. Front. Psychiatry 11:680. doi: 10.3389/fpsyt.2020.00680

Introduction: The emergence of psychosis in at-risk individuals results from interactions between genetic vulnerability and environmental factors, possibly involving dysregulation of the hypothalamic-pituitary-adrenal axis. Hypercorticism was indeed described in schizophrenia and ultra-high-risk states, but its association with clinical outcome has yet to be demonstrated. The impact of stress through cortisol may vary depending on the expression level of genes related to the stress pathway.

Methods: To test this hypothesis, we selected *NR3C1*, the gene encoding the glucocorticoid receptor, and modeled through logistic regression how its peripheral expression could explain some of the risk of psychosis, independently of peripheral cortisol levels, in a French longitudinal prospective cohort of 133 at-risk individuals, adjusted for sex, age, cannabis, and antipsychotic medication intake. We then performed a genome-wide association analysis, stratified by sex (55 females and 78 males), to identify *NR3C1* expression quantitative trait loci to be used as instrumental variables in a Mendelian randomization framework.

Results: *NR3C1* expression was significantly associated with a higher risk of conversion to psychosis (OR = 2.03, p = 0.03), independently of any other factor. Cortisol was not associated with outcome nor correlated with *NR3C1*. In the female subgroup, rs6849528 was associated both with *NR3C1* mRNA levels (p = 0.015, Effect-Size = 2.7) and conversion (OR = 8.24, p = 0.03).

Conclusions: For the same level of cortisol, *NR3C1* expression increases psychotic risk, independently of sex, age, cannabis, and antipsychotic intake. In females, Mendelian randomization confirmed *NR3C1*'s effect on outcome to be unbiased by any environmental confounder.

Keywords: ultra-high risk of psychosis, stress, cortisol, hypothalamic–pituitary–adrenal axis, Mendelian randomization, expression quantitative trait locus, genome-wide analysis study

INTRODUCTION

The concept of schizophrenia has moved from a chronic to a progressive illness that typically emerges during late adolescence and goes through several stages: early vulnerability, at-risk mental state, first episode of psychosis (FEP), and finally, chronic disease (1, 2). The at-risk state includes subjects with psychotic symptoms that are either attenuated or not frequent enough to allow a diagnosis of FEP, or who have a genetic risk and present with nonspecific functional decline. Only up to a third of at-risk subjects might convert to FEP after 3 years and the reasons for this differential outcome are yet to be understood (3). According to the main hypothesis in the field, the emergence of psychotic symptoms could imply an interaction between genes and environment, and could be mediated through epigenetic (4) and transcriptomic processes (5). The biological response to stress has been hypothesized to play a role in pathophysiology. Abnormal cortisol levels have indeed been suggested at each stage: increased basal cortisol in subjects at-risk (6) or with schizophrenia (7), a possibly blunted cortisol awakening response in FEP and in schizophrenia (8), and an attenuated cortisol response to acute psychosocial stress in at-risk subjects (9). Cortisol levels also have been proposed to predict the prognosis in the at-risk individuals (10). However, relatively small sample sizes and heterogeneous measures and outcomes mean that these findings need to be interpreted with caution. Moreover, our recent meta-analysis did not confirm the association between morning salivary cortisol levels and conversion to psychosis (6). The frequency of stress-related dysregulation in the at-risk group as a whole might indeed not allow cortisol by itself to discriminate between future converters and non-converters.

Further investigations of the stress pathway, at a molecular and genetic levels, are therefore warranted. To this purpose, confounders such as sex or environmental factors need to be controlled, as they might account for cortisol's observed weak predictive power. Cortisol awakening response could be significantly lower in males with FEP compared to male controls and affected females (11). Sex differences have also been suggested in morning cortisol levels in the at-risk stage (12). This moderating effect of sex might further be seen at the molecular level, on the glucocorticoid nuclear receptor *NR3C1*, which acts as a transcription factor that binds to glucocorticoid response elements and regulates gene expression upon stress. Sex-specific effects of negative environment on *NR3C1* regulatory regions' methylation have been observed (13), as well as sex-specific upregulation of *NR3C1* transcription under acute stress in animal models (14). In humans, it has recently been suggested that epigenetic mechanisms, including *NR3C1* regulation, might differ between males and females (15), with a sex-dependent role of *NR3C1*'s methylation in depression, but also a sex-specific effect of allelic variation of the *NR3C1* gene (16, 17).

Moreover, *NR3C1* expression could be of interest in psychosis, not only through its relationship with cortisol, but also as a possible direct marker. One study, although limited in sample size, suggested that *NR3C1* mRNA might be decreased in the dorso-lateral prefrontal cortex of schizophrenia cases, relative to controls, while among schizophrenia cases, it might be increased in suicide-positive vs suicide-negative subjects (18). Furthermore, anxiety and depression are important prodromal symptoms in the emergence of psychosis (2), and allelic variations in the *NR3C1* gene have been found associated with depression, with or without psychotic features (19, 20), as well as with cognitive deterioration, independently of cortisol levels (20). However, there is not, to our knowledge, any *NR3C1* gene expression (mRNA or protein) study in the context of emergence of psychosis.

In this context, we hypothesized that, in at-risk individuals, *NR3C1* peripheral expression may explain some of the risk of psychosis, independently of cortisol levels, and adjusted by sex, age, cannabis and antipsychotic medication intake. To shore up this argument, we considered a Mendelian randomization analysis in order to account for all possible unknown environmental factors. In this analysis, the effect of an "exposure" variable (here, the level of gene expression) on an "outcome" variable (here, conversion to psychosis) is tested through the use of an "instrument" that explains exposure independently of confounding factors (e.g. environmental biases): stratifying a population using the instrumental variable thus allows to test the unbiased effect of the exposure on the outcome (21). A genetic variant representing an expression quantitative trait locus (eQTL) of this gene could serve as such an instrumental variable, because it does not depend on the environment (**Figure 1**) (22). In our data, if an eQTL explained the variation of *NR3C1* expression, and if this expression level was associated with conversion to psychosis, then stratifying at-risk individuals on this eQTL could highlight the differences in conversion rates that are only due to *NR3C1* expression levels, independently of any non-genetic confounders.

Using a French longitudinal prospective cohort of individuals at-risk for psychosis, presenting attenuated or prodromal symptoms [ICAAR, previously described (23)], with data on cortisol levels, *NR3C1* expression levels, and whole-genome genotyping, we thus tested the following hypotheses in a Mendelian randomization framework (**Figure 2**). i) At the hormonal and molecular level, are basal cortisol and *NR3C1* expression reliable biomarkers of conversion to psychosis? ii) At the genetic level, does a genome-wide analysis study (GWAS),

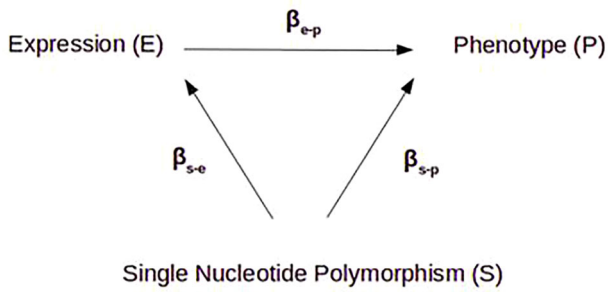

FIGURE 1 | Principle of Mendelian randomization. In a purely causal model, the variation of gene expression (e) is fully determined by an eQTL SNP (s), which itself has no effect (β) on phenotype (p) except through that gene's expression, so βs-e x βe-p = βs-p. By identifying the different β, we can estimate the effect of gene expression on the phenotype (explained variance), free of other potential confounding factors.

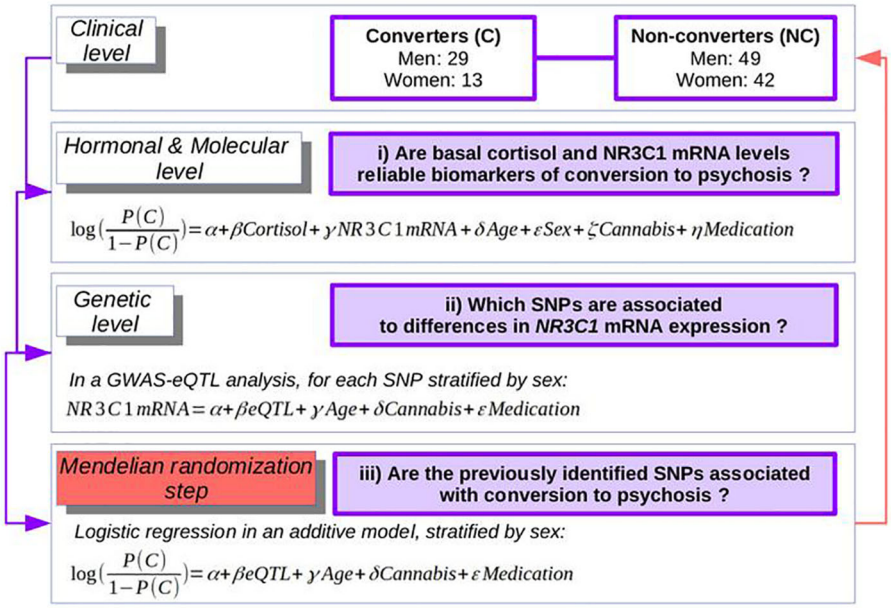

FIGURE 2 | The strategy of the analyses: from clinical to hormonal, molecular, and genetics levels.

stratified by sex to account for genotype-by-sex interactions (16, 17), identify any eQTL that can explain *NR3C1* mRNA levels? iii) Finally, would such an eQTL be associated with psychosis, thus confirming the environmentally unbiased effect of *NR3C1* on risk of psychosis? Last, as secondary outcomes, we tested if *NR3C1* expression is associated with functional or depressive outcomes or more specifically with psychotic features.

MATERIAL AND METHODS

Population

Participants were recruited through the French ICAAR cohort (PHRC AOM-07-118, promoted by Hôpital Sainte-Anne) among help-seeking individuals (16 to 30 years old) consecutively referred to the Adolescent and Young Adult Assessment Centre (Service Hospitalo-Universitaire, Hôpital Sainte-Anne, Paris, France) between 2009 and 2014 (23). All help-seeking individuals were examined at baseline and after 1 year follow-up with the Comprehensive Assessment of at-risk mental state, CAARMS (24), in its translated version (25), by specifically trained psychiatrists. After baseline assessment, a consensus meeting for best estimated diagnosis was held, and help-seekers were classified as at-risk for psychosis stage IA or stage IB according to the staging model distinction (26). Were included in the analysis all at-risk stage IA and stage IB individuals, for which clinical, biological and genetic data was available. Stage IA included patients with mild or non-specific symptoms of psychosis or severe mood disorder, and mild functional change. Stage IB included patients with moderate subthreshold symptoms and moderate functional change. Inclusion criteria were alterations in global functioning (Social and Occupational Functioning Assessment Scale score < 70) during the past year, which were associated with psychiatric symptoms and/or subjective cognitive complaints. Exclusion criteria included manifest symptoms of psychosis (fulfilling DSM-IV criteria), or other established psychiatric diagnoses (pervasive developmental disorder, bipolar disorder, obsessive compulsive disorder), serious or non-stabilized somatic and neurological disorders, head injury and IQ below 70. Psychotic conversion was characterized using the CAARMS-defined psychosis onset threshold (i.e., supra-threshold psychotic symptoms—thought content, perceptual abnormalities and/or disorganized speech—present for more than 1 week). Individuals who reached the threshold during the follow-up were considered as converters and individuals who recovered or displayed persistent subthreshold symptoms were called non-converters. Additionally, each individual underwent clinical assessments including the Social and Occupational Functioning Assessment Scale (SOFAS), the Positive And Negative Syndrome Scale (PANSS) and the Montgomery-Åsberg depression rating scale (MADRS), cannabis intake in the last month, and antipsychotic treatment summarized by the chlorpromazine equivalent doses (references for the computation of chlorpromazine equivalent doses are available in **Supplementary Table 1**). The population demographic and clinical characteristics at baseline are described in **Table 1**. We used the conversion status available after 1-year follow-up in order to have an outcome that was closest to biological sampling.

Cortisol Measures

Salivary cortisol was collected using a synthetic swab at four time-points during the day after initial enrollment. Cortisol was

TABLE 1 | Demographic, clinical characteristics, and cortisol measures of the male and female datasets at baseline.

Measure	Males			Females		
	Converters mean ± std	Non-Converters mean ± std	P-value	Converters mean ± std	Non-Converters mean ± std	P-value
Number of subjects	29	49		13	42	
Age at baseline	19.9 ± 2.5	21.4 ± 3.4	0.112	22.1 ± 3.7	21.1 ± 4.1	0.340
Symptoms						
PANSS total	75.9 ± 15.6	67.3 ± 20.2	**0.041**	66.7 ± 22.0	61.7 ± 13.7	0.307
MADRS	21.8 ± 8.4	18.2 ± 9.9	**0.038**	21.4 ± 8.9	22.9 ± 9.8	0.368
SOFAS	43.9 ± 8.8	48.1 ± 11.6	1.0	51.9 ± 9.4	48.7 ± 8.4	0.156
Treatment						
Antipsychotic use: % of patients	34.5 (10/29)	22.5 (11/49)	0.371	7.7 (1/13)	19 (8/42)	0.590
Chlorpromazine equivalent (mg)*	75.1 ± 37.6	138 ± 73.1	0.071	462 ± 0.0	77.4 ± 44.4	0.217
Substance use (last month)						
Tobacco use: % of patients	48.3 (14/29)	30.6 15/49	**0.046**	54 (7/13)	38 (16/42)	0.095
Cannabis use: % of patients	34.5 (10/29)	16.3 (8/49)	**0.01**	61.5 (8/13)	12 (5/42)	**$7.7 * 10^{-9}$**
Cortisol measures						
at 7:00 am (C1)	9.0 ± 6.3	8.4 ± 4.8	0.984	8.1 ± 4.2	9.7 ± 5.7	1.0
at 9:00 am (C2)	7.4 ± 3.9	9.5 ± 4.4	0.894	8.4 ± 1.5	10.4 ± 6.8	1.0
at 12:00 pm (C3)	5.3 ± 3.0	5.2 ± 3.6	0.941	5.3 ± 5.7	4.6 ± 2.5	1.0
at 5:00 pm (C4)	3.6 ± 2.9	4.3 ± 2.6	0.989	2.1 ± 1.2	2.3 ± 1.1	1.0

PANSS, Positive And Negative Syndrome Scale.
MADRS, Montgomery-Åsberg depression rating scale.
SOFAS, Social and Occupational Functioning Assessment Scale.
C1 to C4 are the four times of cortisol measures: 7am, 9am, 12pm, 5pm, respectively.
References for the computation of each chlorpromazine equivalent are available in **Supplementary Table 1.*
P-values < 0.05 are in bold.

measured at 7:00 am (C1), 9:00 am (C2), 12:00 pm (C3), and 5:00 pm (C4). A range of ± 1 h was accepted for each time of sampling. None of the subjects worked night shifts. The saliva samples were stored at -20°C until analysis. After thawing, saliva samples were centrifuged at 2000 g for 10 min, which resulted in a clear supernatant of low viscosity; 100 µl of saliva were used for duplicate analysis of each sample. Cortisol measurement was done using a competitive solid phase time-resolved fluorescence immunoassay with fluorometric end point detection (DELFIA) conducted by Cortisollabor, University of Trier, Department of Clinical and Physiological Psychology, Trier, Germany (27). The inter-assay coefficient of variation was 8.6% and intra-assay coefficient of variation was 4.3% as previously reported (6).

Gene Expression

Total RNA was extracted and purified from blood samples (PAXgene tubes) using a QIAcube robot and PAXgene Blood RNA kit (QIAGEN) according to the manufacturer's protocol. Quality control was done using LabChip GX (Perkin Elmer, Waltham USA). The full quantitative PCR (qPCR) protocol has been described in Chaumette et al. (5). Briefly, complementary DNA (cDNA) synthesis was performed using Reverse Transcription Master Mix from Fluidigm® according to the manufacturer's protocol with random primers using a Nexus thermocycler (Eppendorf). Specific target pre-amplification was performed using a Fluidigm® PreAmp Master Mix at 12 cycles. Real time PCR was performed on the qPCR-HD-Genomic Paris Centre platform, using BioMark™ HD System, GE Dynamic Arrays (Fluidigm) and TaqMan® Gene Expression assays (Life Technologies, ThermoFisher). Thermal conditions for qPCR were: 25°C for 30 min and 70°C for 60 min for thermal mix; 50°C for 2 min and 95°C for 10 min for hot start; 40 cycles at 95°C for 15 s and 60°C for 1 min. Data were processed by automatic threshold, with linear derivative baseline correction using BioMark Real-Time PCR Analysis Software 4.0.1 (Fluidigm). The quality threshold was set at the default setting of 0.65. Normalization was done using the GAPDH rate followed by a livak normalization with a transformation by the 2ΔΔCT method (28) providing the relative mRNA expression level of *NR3C1* in each sample. Moreover, we checked on the Genevestigator platform (https://genevestigator.com), using the RefGenes tool (29), that *GAPDH* was among the top 10 genes with an expression that was both stable, and in *NR3C1* ranges, for it to be a good reference for normalization (**Supplementary Figure 1**).

Genotyping Data

In the ICAAR cohort, 102 Caucasian individuals have been genotyped using the Infinium PsychArray-24 v1.2 BeadChip (Illumina). This chip was designed by the Psychiatric Genomic Consortium and is enriched for polymorphisms relevant for psychiatric diseases. Single Nucleotide Polymorphisms (SNPs) annotation was given by the Illumina annotation file. Plink v2.0 (www.cog-genomics.org/plink/2.0/) was used for quality control and association analyses. The quality control excludes samples with less than 90% genotyping rate (mind > 0.1), SNPs with a minor allele frequency less than 1% (maf < 0.01) and SNPs that were not genotyped in at least 60% of the sample (geno > 0.4). No sample was excluded during the quality control; after filtering, 306,841 SNPs remained to be analyzed in male samples and

300,732 in female samples. Linkage disequilibrium and haplotype blocks were analyzed with Haploview.

Statistical Analysis

Statistical analyses of basal cortisol level and gene expression in ICAAR cohort were performed using Python 3.7.2 and R 3.6.2. Group distributions of quantitative values were compared with a non-parametric Mann-Whitney Wilcoxon rank sum test. Comparisons of multiple ordinal categorical groups were made with a Chi-squared test for proportions. Correlations were calculated using Spearman's test. For demographic and clinical comparisons between groups, the Bonferroni corrected threshold was at 0.004. However, to be more stringent in detecting the potential confounders at baseline, we considered the uncorrected p-value threshold of 0.05 in order not to ignore any clinical difference that could bias the association between *NR3C1* expression and conversion, and for which Mendelian randomization would be needed. All measures were standardized using the mean and standard deviation in all the cohort. The reported effect-sizes (ES) were calculated with Hedge's g. In our cohort of 133 subjects, we first regressed the odds of conversion, OR, as follows (**Figure 2**):

$$\mathrm{OR} = \alpha + \beta\mathrm{Cortisol} + \gamma NR3C1\mathrm{mRNA} + \delta\mathrm{Age} + \varepsilon\mathrm{Sex} + \zeta\mathrm{Cannabis} + \eta\mathrm{Medication} \quad \text{(I)}$$

A second model using a linear regression on the same combination of explanatory variables was applied to test their association with depression (MADRS scale) and functional outcomes (SOFAS scale). Then for each SNP, stratified by sex, we applied a linear regression as follows:

$$NR3C1\mathrm{mRNA} = \alpha + \beta\mathrm{eQTL} + \gamma\mathrm{Age} + \delta\mathrm{Cannabis} + \varepsilon\mathrm{Medication}\,. \quad \text{(II)}$$

Finally, we applied a logistic regression to model the odds of conversion in the female group as follows:

$$\mathrm{OR} = \alpha + \beta\mathrm{eQTL} + \gamma\mathrm{Age} + \delta\mathrm{Cannabis} + \varepsilon\mathrm{Medication}\,. \quad \text{(III)}$$

Random Permutation Analysis and Bootstrapping

In order to derive robust non-parametric p-values for each Wilcoxon test, we randomly permuted the assignment of values to the groups, and repeated the statistical test 10,000 times. We then computed how many times a p-value was smaller or equal to the observed one. The reported p-value was calculated as the ratio of this number to the total number of tests done (10,000). The 95% confidence intervals (95% CI) were computed by bootstrapping, where the variance of means from each group was estimated by random sampling with replacement. This prevents any inference on the statistical distribution of the population.

Mendelian Randomization Analysis

The Mendelian randomization analysis follows the steps of a two-stage least squares (2SLS) regression analysis. First, the effect of gene expression (e) on phenotype (p) was estimated by >logistically regressing the log-odds of conversion on cortisol levels, *NR3C1* mRNA levels, age, sex, cannabis and antipsychotic intake. This gives a βe-p parameter for the effect of *NR3C1* mRNA on conversion. Second, using Plink v2.0 (30), we performed a GWAS eQTL analysis stratified by sex. *NR3C1* mRNA levels were used as the quantitative trait, linearly regressed on the alleles of each SNP in the GWAS, giving a βs-e parameter that estimates the effect of SNP eQTLs on expression (e). The usual false discovery rate (FDR) threshold of 0.05 was lowered to 0.025 to account for the sex stratification. Last, we measured the effect of the SNP we found (s) on phenotype (p) by applying logistic regression to explain the log-odds of conversion by the alleles of the SNP, giving a βs-p parameter (**Figure 1**). Its significance would confirm the effect of mRNA levels on the risk of conversion, independently from non-genetic confounders (**Figure 2**). An additive model was applied for the SNPs' alleles.

RESULTS

NR3C1 Expression but not Cortisol Levels are Associated with Conversion to Psychosis

At baseline, converters and non-converters were comparable in age, antipsychotic treatment and clinical scales, except for male converters who exhibited higher total PANSS and MADRS scores than male non-converters (p = 0.041 and 0.038, respectively). In both male and female groups, converters showed a higher cannabis use than non-converters (p = 0.010 and p = $7.7*10^{-9}$ respectively). Male converters smoked more tobacco than male non-converters (p = 0.046). None of the cortisol levels at any 4 times of the day were significantly different between converters and non-converters in either group (**Table 1**). None of the cortisol levels were significantly associated with *NR3C1* expression (**Supplementary Figure 2**).

In model (I), an increase in *NR3C1* mRNA levels significantly increased the odds of conversion, independently of cortisol, age, sex, cannabis use, and antipsychotic intake (OR = 2.03, p = 0.03), with an explained variance of 11.6% (pseudo-R^2) (**Table 2**, **Figure 3**, **Supplementary Figure 3**).

When applying the same combination of variables from model (I) to explain dimensional outcomes, no significant association was found between depression, or functional outcome, and *NR3C1* mRNA or cortisol, adjusted for age, sex, cannabis use, and antipsychotic intake.

TABLE 2 | NR3C1 expression explains the risk of conversion independently of cortisol, age, sex, cannabis, and antipsychotic intake.

Variable	Odds Ratio	95% Confidence Interval	P-value
Intercept	0.52	[0.13-2.19]	0.37
Sexe	0.41	[0.11-1.49]	0.18
***NR3C1* mRNA**	**2.03**	**[1.08-3.82]**	**0.03**
Cortisol at M0	0.97	[0.86-1.08]	1.08
Age	0.85	[0.44-1.63]	0.63
Antipsychotic use	0.95	[0.41-2.19]	0.90
Cannabis in the last month	1.42	[0.85-2.36]	0.18

P-values < 0.05 are in bold.

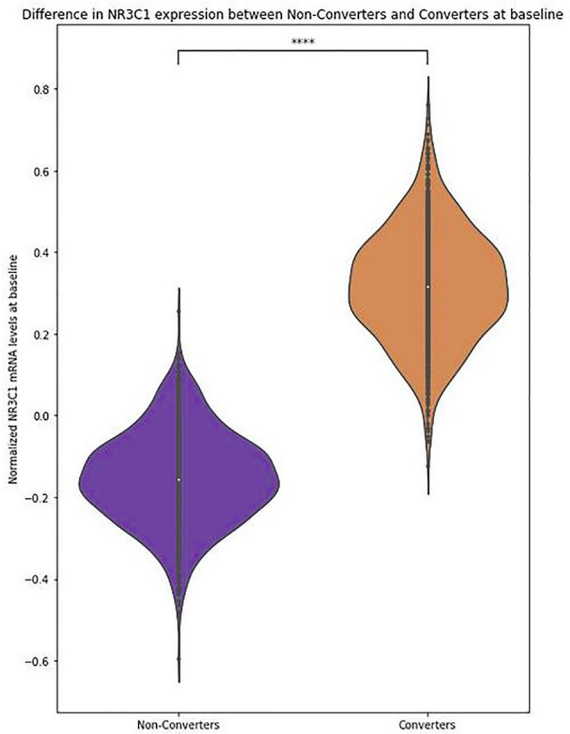

FIGURE 3 | NR3C1 expression levels are higher in converters than in non-converters.

Genome-Wide Analysis Study of eQTL for *NR3C1* Expression, Stratified by Sex

After FDR correction, correction for stratification, and adjustment for age, cannabis, and antipsychotic intake, only one SNP, rs6849528, remained associated with *NR3C1* expression (p = 0.015, ES = 2.7) and explained 43% of its variance in the female group. The mean of *NR3C1* mRNA levels was robustly higher in subjects with at least one allele A (genotype AG or AA, 95% CI = [0.12-0.15]) than in subjects with genotype GG (95%CI = [0.07 – 0.09]), with a significant non-parametric p-value = 2*10-4 (**Supplementary Figures 4** and **5**). No significant eQTL was found in males with the same correction and adjustments.

eQTL-Based Mendelian Randomization

rs6849528 could therefore be used as an instrumental variable for Mendelian randomization in the female group. Because the probability of distribution of its alleles is not related to environmental factors such as cannabis or tobacco use, randomizing the female cohort on rs6849528 would account for any unknown environmental confounder that could bias the association between *NR3C1* expression and conversion. Accounting for age, cannabis, and antipsychotic intake, the risk of conversion to psychosis was significantly associated with rs6849528, in an additive model, with an odds-ratio of 8.24 (p = 0.03) (**Figure 4**).

DISCUSSION

We applied multiple levels of analysis (hormonal, molecular and genetic) to test the association of conversion to psychosis with potential biomarkers from the biological stress pathway (*NR3C1* expression level and cortisol levels at different times of the day), while adjusting for age, sex, cannabis use, and antipsychotic intake, in a longitudinal cohort of 133 subjects at-risk for psychosis. Both stage IA and stage IB were included to be representative of daily clinical practice.

First, we showed that after adjusting for cortisol levels, an increase in *NR3C1* expression was significantly associated with a higher risk of conversion to psychosis. Cortisol itself was not associated with conversion, which is in line with our previous meta-analysis assessing the morning levels of cortisol (6). This negative result was extended to other times of the day (waking time, noon, afternoon). As cortisol levels were neither correlated with *NR3C1* expression, this may suggest cortisol's effects in psychosis might be conditional to the underlying biological and genetic background.

Second, to strengthen the validity of *NR3C1* mRNA's association with psychosis, we performed a Mendelian randomization analysis. This allowed to account for any possibly uncontrolled environmental confounding factor that could have biased the association. Because of reported *NR3C1* genotype-by-sex effects, we performed this GWAS eQTL analysis stratifying by sex. In females, we identified a SNP (rs6849528) strongly associated with *NR3C1* expression, and the odds of conversion to psychosis appeared significantly increased in the group with the minor allele A. Because the probability of distribution of alleles is independent of environmental factors, this SNP's association with psychosis confirmed the involvement of *NR3C1* expression in the risk of psychosis, independently of any non-genetic confounder (**Figure 4**).

Whereas we initially postulated that the environment regulated gene expression, through the mediation of biological stress, we found that *NR3C1* expression, one of the genes most implicated in the stress pathway, was not dependent on cortisol levels, but rather on genetic variability, in females. Given the same level of cortisol, presuming the same level of stress, female individuals with higher *NR3C1* expression levels appeared thus more vulnerable than others to the risk of conversion.

The GWAS analysis did not find any significant eQTL associated with *NR3C1* expression in males, so Mendelian randomization could not be applied to bolster the association between *NR3C1* and psychosis in males. The absence of significant eQTL in males might either be due to a lack of power in a sample not large enough to detect the small effect-sizes of common polymorphisms, either to the aforementioned genotype-by-sex specificity of *NR3C1* regulation (16, 17).

In this Mendelian randomization framework, we used rs6849528 as an independent instrumental variable for randomization. This SNP, located in an intron of the acyl-CoA oxidase 3 gene (*ACOX3*) on chromosome 4, acted as a trans-eQTL regulator of *NR3C1*, located on chromosome 5. We wish to highlight the fact that we relied on its association with *NR3C1* expression, regardless of any actual pathophysiological mechanism, and with the unique purpose of randomizing the

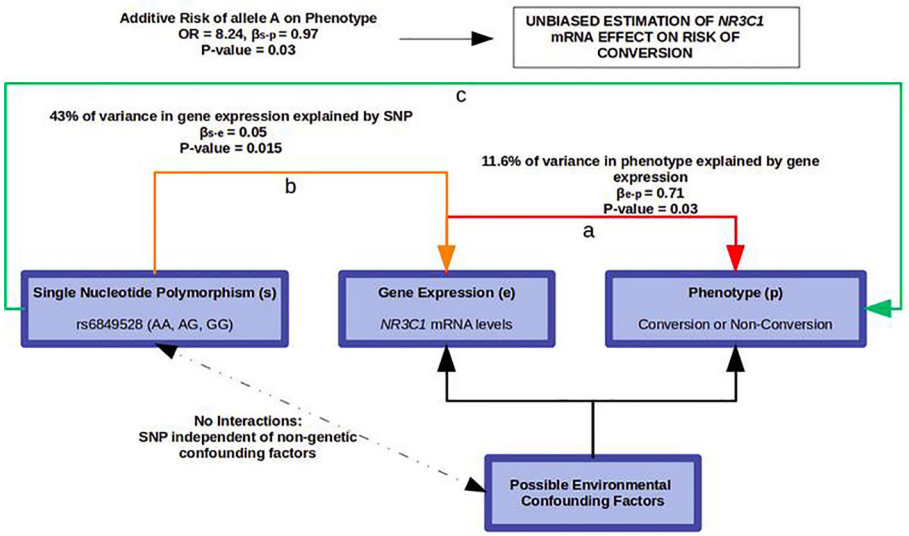

FIGURE 4 | Mendelian Randomization using rs6849528 as an instrumental variable. a) NR3C1 mRNA levels are associated with psychosis. b) rs6849528 alleles lead to differential NR3C1 expression. c) Because the probability of distribution of allele A is independent of environmental factors, its association with psychosis is in favour of the involvement of NR3C1 mRNA in this risk, independently of non-genetic confounding factors.

cohort in an environmentally unbiased way. It is indeed not possible to draw any conclusion regarding rs6849528's real biological effect, especially as it may be associated with psychosis through a pleiotropic effect rather than causal association. ACOX3 participates in peroxisomal fatty acid beta-oxidation. Peroxisomal dysregulation has been described in relation with psychotic symptoms, in the context of inborn errors of metabolism (31), so a possible direct effect of ACOX3 gene on conversion to psychosis could not be ruled out. rs6849528 might affect *NR3C1* gene expression on the one hand, and phenotype, independently of *NR3C1* expression, on the other. In a purely causal model, the variation of gene expression (e) is fully determined by an eQTL SNP (s), which itself has no effect on phenotype (p) except through that gene's expression, and $\beta s\text{-}e \times \beta e\text{-}p = \beta s\text{-}p$ (22). However, in our study, $\beta s\text{-}e \times \beta e\text{-}p << \beta s\text{-}p$ ($0.04 < 0.97$) suggesting pleiotropy. This was expected as psychosis is a complex phenotype resulting from the effect of many genetic and environmental factors. This also explained the limited variance of risk of conversion explained by *NR3C1* gene expression (11.6%) compared to the variance of gene expression determined by the eQTL (43%) (**Figure 4**).

Moreover, the causal biological effect may be driven by other SNPs in linkage disequilibrium with rs6849528, which could act as a proxy for its entire haplotype block in our eQTL analysis. None of the SNPs in rs6849528 haplotype block have been associated with schizophrenia in a GWAS. However, the haplotype block around rs6849528 comprises several SNPs acting as eQTLs of transfer RNA methyltransferase 44 (TRMT44), according to the GTEx portal (https://www.gtexportal.org/home/). One of these eQTL variants, rs6845969, impacts the expression of *TRMT44* in regions such as the hypothalamus or the pituitary gland, involved in the stress pathway. Other variants in this haplotype act as eQTLs for expression of *TRMT44* in the nucleus accumbens, relevant to motivation through reward and reinforcement (rs2386223; rs12503034, rs1880025). None of these SNPs were available for testing in our eQTL GWAS, but they could constitute pathophysiologically relevant factors; tRNAs have a major role in translation, decoding mRNA sequence into protein, and methylation of tRNA contributes to its stabilization (32). Conversely, hypomethylation leads to cleavage of tRNAs into tRNA-derived small RNA fragments, like microRNAs, which have been shown to activate stress pathways (33). This might explain how genetic variants could be associated with the expression levels of a distant mRNA (trans eQTL). Through their eQTL effect on TRMT44 activity, they might indirectly lead to an increase in specific miRNAs which in turn regulate gene expression. For instance, *NR3C1* has been reported to contain in its 3' untranslated region a target of miR-124 (34).

Our study has several limitations. First, sampling times varied up to 1 h around the theoretical times for cortisol sampling. This could explain why cortisol measurements did not correlate with outcome. Second, a limitation inherent to Mendelian randomization analysis is that it is not possible to account for genetic confounders. In the same haplotype block, one SNP might only be associated with *NR3C1* expression level, while another might only correlate with phenotype. Their linkage disequilibrium would therefore act as a genetic confounding factor that cannot be disentangled by such analysis (35). Third, we only considered the variance of gene expression explained by the eQTL, while other non-genetic factors are also known to regulate *NR3C1* expression. For instance, CpG-specific methylation of *NR3C1* promoter regions has been robustly implicated in its expression and correlated with psychosocial stress (36). However, this restriction to genetic factors was necessary in order to ensure

the unbiasedness of Mendelian randomization. Fourth, the eQTL analysis did not find any of the previously reported SNPs that are in the *NR3C1* gene (19). However, such studies used a candidate gene approach to analyze SNPs, while we looked for eQTL genome-wide. Any eQTL we found had to have therefore an important effect-size strengthening its interest for Mendelian randomization. Also, all the data came from peripheral measures: gene expression in blood and cortisol level in saliva. These data may imperfectly reflect the levels in brain tissue. However, blood-brain correlation has been reported to range from 0.25 to 0.64 and to be greater for genes highly expressed in both tissues (37). Finally, we cannot exclude that some non-converters at 1 year did convert later, but the conversion rate is maximal during the first year (3).

Further work is required to replicate our results, but our multimodal approach, using phenotypic, hormonal, transcriptomic and genetic data, illustrates how the implementation of statistical methods, e.g. with Mendelian randomization analyses, could help to detect biomarkers in psychiatry by adjusting for environmental factors. This also suggests that the impact of stress should be investigated in a comprehensive way, where cortisol levels would only be one of these factors.

DATA AVAILABILITY STATEMENT

The datasets presented in this article are not readily available because of an ethical issue. The consent form signed by the participants to the ICAAR study did not indicate that the genetic data would be shared or deposited in a repository.

ETHICS STATEMENT

This study involved human participants and was reviewed and approved by Comité de protection des personnes, Ile-de-France III, Paris, France. Written informed consent to participate in this study was provided by the participants or their legal guardians.

AUTHOR CONTRIBUTIONS

AI, BC and M-OK designed the study. AI, OK, M-OK, GR, and BC obtained the funding and supervised the study. AI, OK, the ICAAR study group and BC collected the data. AI and BC analyzed the data. AI, OK, QH, TJ, GR, M-OK, and BC interpreted the data. AI, OK, and BC drafted the manuscript. All authors contributed to the article and approved the submitted version.

FUNDING

This work has been supported by the French government's "Investissements d'Avenir" programme, which is managed by the Agence Nationale de la Recherche (ANR), under the reference ANR-18-RHUS-0014 ("Project PsyCARE"). This work was also supported by the French Ministry grant PHRC AOM07-118 (for the ICAAR cohort), Institut National de la Santé et de la Recherche Médicale (INSERM), Université Paris Descartes (recurrent funding), the Canadian Institutes of Health research (GR), the Fondation Bettencourt Schueller (BC), and the Fondation pour la Recherche Médicale (AI). The Centre Hospitalier Sainte-Anne promoted the study. The sponsors had no role in the design and conduct of the study, in the collection, management, analysis or interpretation of the data, in the preparation, review or approval of the manuscript, or in the decision to submit the manuscript for publication. All the authors declare they have no competing interests related to this work.

ACKNOWLEDGMENTS

We would like to thank all the patients and parents who participated in the ICAAR study, the staff from the C'JAAD team and the staff from the Clinical Evaluation and Research Center at the Service Hospitalo-Universitaire, Centre Hospitalier Sainte-Anne. A special thanks to Yannick Morvan for his help with the data management and his statistical advice. We would like to thank the contributors of the ICAAR Study Group: Isabelle Amado, Julie Bourgin, Claire Daban Huard, Célia Jantac Mam-Lam-Fook, Marion Plaze, Fabrice Rivollier.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.00680/full#supplementary-material

REFERENCES

1. Nelson B, McGorry PD, Wichers M, Wigman JTW, Hartmann JA. Moving From Static to Dynamic Models of the Onset of Mental Disorder: A Review. *JAMA Psychiatry* (2017) 74(5):528. doi: 10.1001/jamapsychiatry.2017.0001
2. Howes OD, Murray RM. Schizophrenia: An Integrated Sociodevelopmental-Cognitive Model. *Lancet* (2014) 383:1677–87. doi: 10.1016/S0140-6736(13)62036-X
3. Fusar-Poli P, Borgwardt S, Bechdolf A, Addington J, Riecher-Rössler A, Schultze-Lutter F, et al. The Psychosis High-Risk State: A Comprehensive State-of-the-Art Review. *JAMA Psychiatry* (2013) 70(1):107. doi: 10.1001/jamapsychiatry.2013.269
4. Kebir O, Chaumette B, Rivollier F, Miozzo F, Lemieux Perreault LP, Barhdadi A, et al. Methylomic Changes during Conversion to Psychosis. *Mol Psychiatry* (2017) 22(4):512–18. doi: 10.1038/mp.2016.53
5. Chaumette B, Kebir O, Pouch J, Ducos B, Selimi F, ICAAR study group, et al. Longitudinal Analyses of Blood Transcriptome During Conversion to Psychosis. *Schizophr Bull* (2019) 45(1):247–55. doi: 10.1093/schbul/sby009
6. Chaumette B, Kebir O, Mam-Lam-Fook C, Morvan Y, Bourgin J, Godsil BP, et al. Salivary Cortisol in Early Psychosis: New Findings and Meta-Analysis.

Psychoneuroendocrinology (2016) 63:262–70. doi: 10.1016/j.psyneuen.2015.10.007
7. Girshkin L, Matheson SL, Shepherd AM, Green MJ. Morning Cortisol Levels in Schizophrenia and Bipolar Disorder: A Meta-Analysis. *Psychoneuroendocrinology* (2014) 49:187–206. doi: 10.1016/j.psyneuen.2014.07.013
8. Berger M, Kraeuter AK, Romanik D, Malouf P, Amminger GP, Sarnyai Z. Cortisol Awakening Response in Patients with Psychosis: Systematic Review and Meta-Analysis. *Neurosci Biobehav Rev* (2016) 68:157–66. doi: 10.1016/j.neubiorev.2016.05.027
9. Pruessner M, Béchard-Evans L, Boekestyn L, Iyer SN, Pruessner JC, Malla AK. Attenuated Cortisol Response to Acute Psychosocial Stress in Individuals at Ultra-High Risk for Psychosis. *Schizophr Res* (2013) 146:79–86. doi: 10.1016/j.schres.2013.02.019
10. Walker EF, Trotman HD, Pearce BD, Addington J, Cadenhead KS, Cornblatt BA, et al. Cortisol Levels and Risk for Psychosis: Initial Findings From the North American Prodrome Longitudinal Study. *Biol Psychiatry* (2013) 74(6):410–7. doi: 10.1016/j.biopsych.2013.02.016
11. Pruessner M, Vracotas N, Joober R, Pruessner JC, Malla AK. Blunted Cortisol Awakening Response in Men with First Episode Psychosis: Relationship to Parental Bonding. *Psychoneuroendocrinology* (2013) 38:229–40. doi: 10.1016/j.psyneuen.2012.06.002
12. Carol EE, Spencer RL, Mittal VA. Sex Differences in Morning Cortisol in Youth at Ultra-High-Risk for Psychosis. *Psychoneuroendocrinology* (2016) 72:87–93. doi: 10.1016/j.psyneuen.2016.06.013
13. Jaric I, Rocks D, Cham H, Herchek A, Kundakovic M. Sex and Estrous Cycle Effects on Anxiety- and Depression-Related Phenotypes in a Two-Hit Developmental Stress Model. *Front Mol Neurosci* (2019) 12:74. doi: 10.3389/fnmol.2019.00074
14. Bourke CH, Raees MQ, Malviya S, Bradburn CA, Binder EB, Neigh GN. Glucocorticoid Sensitizers Bag1 and Ppid Are Regulated by Adolescent Stress in a Sex-Dependent Manner. *Psychoneuroendocrinology* (2013) 38:84–93. doi: 10.1016/j.psyneuen.2012.05.001
15. Hill J, Pickles A, Wright N, Quinn JP, Murgatroyd C, Sharp H. Mismatched Prenatal and Postnatal Maternal Depressive Symptoms and Child Behaviours: A Sex-Dependent Role for NR3C1 DNA Methylation in the Wirral Child Health and Development Study. *Cells* (2019) 8:943. doi: 10.3390/cells8090943
16. Sarubin N, Hilbert S, Naumann F, Zill P, Wimmer AM, Nothdurfter C, et al. The Sex-Dependent Role of the Glucocorticoid Receptor in Depression: Variations in the NR3C1 Gene Are Associated with Major Depressive Disorder in Women but Not in Men. *Eur Arch Psychiatry Clin Neurosci* (2017) 267:123–33. doi: 10.1007/s00406-016-0722-5
17. Kumsta R, Entringer S, Koper JW, van Rossum EFC, Hellhammer DH, Wüst S. Sex Specific Associations between Common Glucocorticoid Receptor Gene Variants and Hypothalamus-Pituitary-Adrenal Axis Responses to Psychosocial Stress. *Biol Psychiatry* (2007) 62:863–69. doi: 10.1016/j.biopsych.2007.04.013
18. Sinclair D, Fullerton JM, Webster MJ, Weickert CS. Glucocorticoid Receptor 1B and 1C MRNA Transcript Alterations in Schizophrenia and Bipolar Disorder, and Their Possible Regulation by GR Gene Variants. *PloS One* (2012) 7:e31720. doi: 10.1371/journal.pone.0031720
19. Schatzberg AF, Keller J, Tennakoon L, Lembke A, Williams G, Kraemer FB, et al. HPA Axis Genetic Variation, Cortisol and Psychosis in Major Depression. *Mol Psychiatry* (2014) 19:220–27. doi: 10.1038/mp.2013.129
20. Keller J, Gomez R, Williams G, Lembke A, Lazzeroni L, Murphy GM, et al. HPA Axis in Major Depression: Cortisol, Clinical Symptomatology and Genetic Variation Predict Cognition. *Mol Psychiatry* (2017) 22:527–36. doi: 10.1038/mp.2016.120
21. Davey Smith G, Ebrahim S. 'Mendelian Randomization': Can Genetic Epidemiology Contribute to Understanding Environmental Determinants of Disease? *Int J Epidemiol* (2003) 32:1–22. doi: 10.1093/ije/dyg070
22. Zhu Z, Zhang F, Hu H, Bakshi A, Robinson MR, Powell JE, et al. Integration of Summary Data from GWAS and EQTL Studies Predicts Complex Trait Gene Targets. *Nat Genet* (2016) 48:481–87. doi: 10.1038/ng.3538
23. Oppetit A, Bourgin J, Martinez G, Kazes M, Mam-Lam-Fook C, Gaillard R, et al. The C'JAAD: A French Team for Early Intervention in Psychosis in Paris: An Early Intervention Team in Paris. *Early Interv Psychiatry* (2018) 12:243–49. doi: 10.1111/eip.12376
24. Yung AR, Yuen HP, Mcgorry PD, Phillips LJ, Kelly D, Dell'Olio M, et al. Mapping the Onset of Psychosis: The Comprehensive Assessment of At-Risk Mental States. *Aust N Z J Psychiatry* (2005) 39:964–71. doi: 10.1080/j.1440-1614.2005.01714.x
25. Krebs MO, Magaud E, Willard D, Elkhazen C, Chauchot F, Gut A, et al. Évaluation des états mentaux à risque de transition psychotique : validation de la version française de la CAARMS. *L'Encéphale* (2014) 40:447–56. doi: 10.1016/j.encep.2013.12.003
26. McGorry PD, Purcell R, Hickie IB, Yung AR, Pantelis C, Jackson HJ. Clinical staging: a heuristic model for psychiatry and youth mental health. *Med J Aust* (2007) 187(S7):S40–2. doi: 10.5694/j.1326-5377.2007.tb01335.x
27. Dressendörfer RA, Kirschbaum C, Rohde W, Stahl F, Strasburger CJ. Synthesis of a Cortisol-Biotin Conjugate and Evaluation as a Tracer in an Immunoassay for Salivary Cortisol Measurement. *J Steroid Biochem Mol Biol* (1992) 43:683–92. doi: 10.1016/0960-0760(92)90294-S
28. Livak KJ, Schmittgen TD. Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2–ΔΔCT Method. *Methods* (2001) 25:402–8. doi: 10.1006/meth.2001.1262
29. Hruz T, Wyss M, Docquier M, Pfaffl MW, Masanetz S, Borghi L, et al. RefGenes: identification of reliable and condition specific reference genes for RT-qPCR data normalization. *BMC Genomics* (2011) 12:156. doi: 10.1186/1471-2164-12-156
30. Chang CC, Chow CC, Tellier LCAM, Vattikuti S, Purcell SM, Lee JJ. Second-Generation PLINK: Rising to the Challenge of Larger and Richer Datasets. *GigaScience* (2015) 4:7. doi: 10.1186/s13742-015-0047-8
31. Trakadis YJ, Fulginiti V, Walterfang M. Inborn errors of metabolism associated with psychosis: literature review and case-control study using exome data from 5090 adult individuals. *J Inherit Metab Dis* (2018) 41:613–21. doi: 10.1007/s10545-017-0023-9
32. Swinehart WE, Jackman JE. Diversity in Mechanism and Function of TRNA Methyltransferases. *RNA Biol* (2015) 12:398–411. doi: 10.1080/15476286.2015.1008358
33. Blanco S, Dietmann S, Flores JV, Hussain S, Kutter C, Humphreys P, et al. Aberrant Methylation of t RNA s Links Cellular Stress to Neuro-developmental Disorders. *EMBO J* (2014) 33:2020–39. doi: 10.15252/embj.201489282
34. Kozuka T, Omori Y, Watanabe S, Tarusawa E, Yamamoto H, Chaya T, et al. MiR-124 Dosage Regulates Prefrontal Cortex Function by Dopaminergic Modulation. *Sci Rep* (2019) 9:3445. doi: 10.1038/s41598-019-38910-2
35. Bandres-Ciga S, Noyce AJ, Traynor BJ. Mendelian Randomization—A Journey From Obscurity to Center Stage With a Few Potholes Along the Way. *JAMA Neurol* (2019) 77(1):7–8. doi: 10.1001/jamaneurol.2019.3419
36. Palma-Gudiel H, Córdova-Palomera A, Tornador C, Falcón C, Bargalló N, Deco G, et al. Increased Methylation at an Unexplored Glucocorticoid Responsive Element within Exon 1 D of NR3C1 Gene Is Related to Anxious-Depressive Disorders and Decreased Hippocampal Connectivity. *Eur Neuropsychopharmacol* (2018) 28:579–88. doi: 10.1016/j.euroneuro.2018.03.015
37. Tylee DS, Kawaguchi DM, Glatt SJ. On the Outside, Looking in: A Review and Evaluation of the Comparability of Blood and Brain '-Omes.'. *Am J Med Genet B Neuropsychiatr Genet* (2013) 162:595–603. doi: 10.1002/ajmg.b.32150

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

MINI REVIEW
published: 15 July 2020
doi: 10.3389/fpsyt.2020.00693

Schizophrenia and Sex Hormones: What Is the Link?

Noa A. Brzezinski-Sinai[1] *and Amnon Brzezinski*[2*]

[1] Department of Obstetrics and Gynecology, Rabin Medical Center, Beilinson Hospital, Petah Tikva, Israel, [2] Departments of Obstetrics & Gynecology, Hadassah-Hebrew-University Medical Center, Jerusalem, Israel

The involvement of gonadal hormones in the pathogenesis of schizophrenia has long been suspected because the psychosis differs in women and men and the illness first makes its appearance shortly after puberty. Changes in sex hormones have been linked with increased vulnerability to mood disorders in women, while testosterone have been associated with increased sexual drive and aggressiveness in men as well as women. Some studies have found abnormal levels of estrogens and testosterone in schizophrenia patients, but the results have been inconsistent and sometimes attributed to the hyperprolactinemia effect of antipsychotics, which may interfere with sex hormones production. The purpose of this review is to present the current knowledge on the link between blood levels of sex-hormones in women during the various stages of the female reproductive life (i.e. puberty, menstrual cycle, pregnancy, contraception, and menopause) and the course of schizophrenia. We also attempt to optimize the clinical approach to women with schizophrenia at these different stages.

Keywords: schizophrenia, gonadal hormones, estrogen, progesterone, puberty, pregnancy, menstrual-cycle, menopause

OPEN ACCESS

Edited by:
Grazia Rutigliano,
University of Pisa, Italy

Reviewed by:
Andrea Gogos,
University of Melbourne, Australia
Krzysztof Krysta,
Medical University of Silesia, Poland
Natalie Paige Thomas,
Monash Alfred Psychiatry Research Centre, Australia

***Correspondence:**
Amnon Brzezinski
amnonbrz@gmail.com

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *22 February 2020*
Accepted: *01 July 2020*
Published: *15 July 2020*

Citation:
Brzezinski-Sinai NA and Brzezinski A (2020) Schizophrenia and Sex Hormones: What Is the Link? Front. Psychiatry 11:693. doi: 10.3389/fpsyt.2020.00693

INTRODUCTION

During the fertile life and reproductive aging there are significant roles for gonadal hormones in the regulation of several CNS processes, especially mood and cognitive functions. The sex hormones help to organize and activate structural connections within the brain (1). Animal studies suggest that sex steroid hormones play an essential role in myelination [e.g. (2)]. In humans, sex steroids play a developmental role in gray matter and white matter structures in the brain and may continue to exert effects in adult life and into old age (3). Cellular morphology in the brain changes throughout the life span in response to environmental stimuli, and these effects are partially mediated, levels of circulating gonadal hormones (4).

There are sex differences in the prevalence, onset, symptom profiles, and disease outcome that are evident in schizophrenia (5). There are also differences between the sexes in the association of some commonly considered risk genes for schizophrenia, such as DISC1 (6). Since sex steroids have both genomic and non-genomic actions, other factors may also be responsible for the sex differences, (e.g. the influence of sex steroids on the expression patterns of genes).

Higher brain functions, such as cognition, mood, and memory, are modulated by gonadal hormones (7). Their action is accompanied by alterations in neuron and synapse numbers, as well as in dendritic and synaptic morphology (8, 9). Although the determined sex difference in schizophrenia is relatively small, (male: female ratio 58:42 (the age of onset is earlier in males

and is accompanied by more severe negative symptoms (10, 11). These observations lead many scientists to investigate a putative co-regulation between schizophrenia and gonadal steroids. As nicely described by Markham (12), the development of the central nervous system in the embryo is strongly affected by sex steroids. They modulate the interconnections between neurons. They also control the function of glial cells. Different receptor isoforms, different interactions between receptors and co-regulators, chains of events originating at the cell membrane and leading to effects in the nucleus all interact to determine selective modulations of brain cells. All these actions affect brain function which change through adolescence, pregnancy, adulthood, up to menopause and ageing (12).

In this review we summarize the current knowledge on the relationship between schizophrenia and gonadal hormones (progesterone, estrogens, and testosterone) and its clinical significance and implications. The data and recommendations are based on literature derived from searching PubMed, and PsychINFO, with appropriate search terms (schizophrenia, gonadal hormones, estrogen, progesterone, testosterone, puberty, pregnancy, menstrual-cycle, contraception menopause) for all years subsequent to the year 2000.

SCHIZOPHRENIA AND ESTROGEN

There are well-established differences in the expression of schizophrenia in women and men (13–16) many of which have been attributed to the action of estrogen (17). Psychotic episodes are more often during periods of estrogen withdrawal, (e.g. the menstrual phase of the menstrual cycle, post-partum, following cessation of estrogen therapy, and postmenopause). Reduced relapse rates have been observed in women during pregnancy, when plasma estrogen levels are high (12, 18). Estrogens, mainly 17b-estradiol (E2), are known to exert many genomic and non-genomic effects in the CNS (19). These effects are mediated by two types of estrogen receptors, ER alpha and ER beta, and they influence neuronal development, dendritogenesis, synaptic plasticity, and neuronal excitability. The neuroprotective effects are the ones most relevant for schizophrenia. These are achieved through co-operation between membrane and genomic signals, through epigenetic mechanisms such as histone acetylation and DNA methylation, and through regulation of synaptic function, synaptic plasticity, and neurogenesis. Estrogens also promote cell survival, protecting neurons against cytotoxic insults and other forms of stress and injury (19, 20). It has been suggested that cognitive deficits in people with schizophrenia may be especially responsive to circulating estrogen levels and that cognitive performance in women with schizophrenia may be improved by estrogen (21, 22). Selective ER modulators (SERMs), such as tamoxifen, raloxifene, and bazedoxifene, bind to the ligand-binding domain of classic ERs and may act either as agonists or antagonists, depending on the tissue. In several animal models, not only estrogens but also SERMs have been shown to exert neuroprotective effects (19, 23).

Schizophrenia and Progesterone

The role of progesterone in schizophrenia was given less consideration in the literature than estrogen. As Sun et al. (24) well described it, "existing data on progesterone in relation to schizophrenia is inconsistent, with some studies suggesting a neuroprotective role for the hormone (e.g. animal models of cognitive dysfunction and positive symptoms), while other studies posit a disruptive impact of the hormone (e.g. negative correlations with symptom modulation in patients). Based on the clinical studies available there appears to be a link between lower symptom scores and the mid-luteal phase of the menstrual cycle, which is associated with high progesterone/high estradiol levels" (24). There are some animal studies which support the inhibiting effect of progesterone on hyperactive behavior (25).

Recently, it was reported that baseline levels of progesterone were significantly higher in first-episode antipsychotic-naïve patients with schizophrenia than in normal controls. It was speculated that lower levels of progesterone at baseline may predict better therapeutic outcome of antipsychotic treatment (26).

Schizophrenia and Testosterone

The earlier age of onset and greater incidence of schizophrenia among males might be partially explained (apart from the "estrogen hypothesis") by testosterone exposure (12). However, Most of the information about testosterone and schizophrenia is in reference to males. Elevated levels of testosterone have been associated with increased psychiatric symptoms (27). Very few studies examined testosterone serum levels in men with schizophrenia. Some of them reported either lower testosterone levels compared to healthy controls (28) or no difference between these groups (29).

Hyperprolactinemia often follows long-term antipsychotic drug use, especially the typical antipsychotics (e.g. Risperidone), so the studies of gonadal hormone levels might be affected by the abnormal prolactin levels (30). It should be pointed out that hyperprolactinemia is less common with the newer antipsychotics (e.g., clozapine and aripiprazole), and thus does not occur so often anymore.

While one study has reported normal testosterone levels for unmedicated, first episode patients (31), others (32) reported that gonadal hormone levels among men acutely admitted (for symptom exacerbation or at first episode) were significantly lower than controls (both testosterone and estrogen). It has also been reported that circulating testosterone levels are negatively correlated with negative symptoms (33).

Regarding women, it was recently reported (34) that there were statistically significantly higher levels of serum DHEA-S in schizophrenic women than in normal controls. No statistically significant difference was determined between the groups regarding serum testosterone and cortisol levels. It was suggested that DHEA-S (and not testosterone) might be a potential biologic marker for schizophrenia in women. However, further research with greater patient numbers is required to verify this theory.

CLINICAL IMPLICATIONS

Our cumulative knowledge about the effects of gonadal hormones on brain functions has significant clinical implications in every stage of the schizophrenic woman's reproductive life-cycle. The following is an attempt to optimize the approach to these women at each of these stages (i.e., puberty, menstrual cycle, pregnancy, contraception, and menopause).

Puberty

In women, but not man, there is significant inverse relation between puberty and age at onset of schizophrenia. This difference led to the theory that female hormones act on the developing brain to protect its function and delay the expression of psychosis (35). Nevertheless, it should be remembered that the start of puberty is associated with more than hormonal changes. It is the beginning of increasingly divergent psychosocial pathways that differentiate women and men at this important time in their lives. Therefore, special attention should be made by the family physicians as well as family members and teachers, to possible early signs of schizophrenic behavior. It should be taken into account that stress in adolescent girls has been implicated in increased prevalence of depression and anxiety disorders. Integrative clinical approach is suggested while examining pubertal psychiatric complaints and genetical and psychosocial aspects should be taken into consideration (36).

The Menstrual Cycle

The severity of psychotic symptoms in pre-menopausal women with schizophrenia increases in phases of the cycle with low estrogen (37–41). Also, a negative correlation has been reported between estrogen levels and the required dose of antipsychotics in menstruating women (42). Levels of estrogen are typically peaking around the time of ovulation (midcycle) and declining before the start of menses. The estrogen protection hypothesis predicts that psychotic disorders worsen at times in the cycle when estrogen is low, around menstruation (43) and several publications support this assumption. A recent meta-analysis of studies with women with psychiatric diagnoses demonstrated worse mental health outcomes around the time of menstruation (44).

Psychiatric admission rates are higher than expected during the perimenstrual phase. This is in agreement with the observation that a worsening of psychotic symptoms occurs during this phase (45). Most of the studies about the menstrual cycle and psychotic symptoms lack measurements of hormonal fluctuations throughout the menstrual cycle. Therefore, further research with more precise measurements of the menstrual cycle and symptomatology is required.

Pregnancy

Typically the age of onset for schizophrenia in women is during the childbearing years so many of these women become pregnant. Pregnancy reportedly appears to worsen mental symptoms in women with schizophrenia. Psychotic denial of pregnancy is a symptom that poses especially high risks for poor outcomes if not addressed. Up to now, little research has been done into interventions for psychotic disorders in pregnancy and in particular, few studies have been done into use of antipsychotic medication (46, 47). Research on the safety of medication during pregnancy and breastfeeding is limited. Nevertheless, it is still necessary to make treatment recommendations based on the accumulated current information. It is generally accepted that there is a greater risk for the mother and the fetus in not treating schizophrenia during pregnancy and postpartum than in providing antipsychotic treatment (48).

The following are the main recommendations to care providers suggested in the literature:

"Take a sexual history and initiate discussion about intimate relationships and contraception with all women diagnosed with schizophrenia. During pregnancy, adjust antipsychotic dose to clinical status, link the patient with prenatal care services, and help her prepare for childbirth. There are pros and cons to breastfeeding while on medication, and these need thorough discussion. During the postpartum period, mental health home visits should be provided. Parenting support is critical" (46, 47).

"Psychoeducation can apparently reduce pregnancy complications for women with schizophrenia. Short-term, focused psychotherapy can be helpful for some pregnant women with schizophrenia. Some modifications need to be made in the inpatient treatment of pregnant patients with schizophrenia. In the postpartum period, women can be especially susceptible for acute exacerbation of their schizophrenia" (49).

Contraception

It has been reported that mental illness is a risk factor for inconsistent contraceptive use (50). The prevalence of sexually transmitted infections is high in this population. The overall rate of pregnancy in women with schizophrenia of child-bearing age is lower than in the general population, but the percentage of unwanted pregnancies is higher than that in the general population. Contraceptive counseling to women and their partners should be part of the care for women with schizophrenia. Women with schizophrenia, who smoke, are overweight, have diabetes, migraine, cardiovascular disease or thrombophilia, and should be offered non-hormonal contraception. Women with more than one sexual partner should be advised on barrier methods in addition to any other contraceptive measures they are using. Long-acting contraceptives, such as intrauterine devices and progesterone-depot injections, are reasonable options for schizophrenic women. Women who completed their family planning might be offered tubal ligation (or more recently salpingectomy) [for further information and discussion see (51)].

Menopause

Women with schizophrenia may have the same climacteric complaints as healthy women (e.g. vasomotor, physical, cognitive, sexual, and psychosocial symptoms) (52, 53). These symptoms are sometimes aggravated by factors associated with schizophrenia such as lack of occupation, poverty, substance abuse, loneliness, and side-effects of antipsychotic medications. Moreover, the psychotic symptoms of schizophrenia, such as hallucinations and delusions,

worsen as women approach the menopause, while in men, at the same age, these symptoms generally improve (52, 53).

It has been reported that many schizophrenia women (and their relatives) perceive menopause as being associated with increased psychiatric symptoms (52, 54) and a decreased quality of life (52, 55). The main symptoms that worsen in these women are depression, anxiety, fatigue, and poor memory (53).

Apart from vasomotor symptoms many women with schizophrenia suffer also from associated symptoms, such as insomnia, irritability, and subsequently reduced quality of life. Estrogen therapy with or without a progestogen is the proven most effective treatment (56–58). Treatment of moderate to severe vasomotor symptoms remains the primary indication for HT (hormonal treatment). Almost all systemic HT products have government approval for this indication. Selective estrogen receptor modulators (SERM's) such as tamoxifen, raloxifene, or bazedoxifene, on the other hand can make flushing worse. As with women in the general population, HT should be offered early after the start of menopause, and potential hazards, such as breast and cardiovascular changes, need to be monitored.

Women with schizophrenia at menopause may require increased antipsychotic doses. The need for higher doses may continue and become more marked the longer the period since menopause (59, 60). Drugs that raise prolactin levels are best avoided.

CONCLUSIONS

The data presented above indicate that the gonadal hormones are involved in the pathogenesis of schizophrenia and affect its course. A clear link between sex hormones and schizophrenia is based on numerous studies and clinical observations of later onset of schizophrenia associated with early puberty in girls, lower relapse of psychiatric symptoms during pregnancy, high relapse postpartum, fluctuation of the symptoms across the menstrual cycle, and exacerbation of psychotic symptoms in women with schizophrenia during the menopausal transition. However, the exact mechanism by which sex hormones affect the appearance, course and outcome of schizophrenia is still not entirely understood.

A medical and social approach to women with schizophrenia should be based on our knowledge about the inter relationship between these women and their sex hormones in every stage of their reproductive life.

AUTHOR CONTRIBUTIONS

The authors contributed equally to the literature search and the writing of the manuscript.

REFERENCES

1. van der Leeuw C, Habets P, Gronenschild E, Domen P, Michielse S, van Kroonenburgh M, et al. Testing the Estrogen Hypothesis of Schizophrenia: Associations Between Cumulative Estrogen Exposure and Cerebral Structural Measures. *Schizophr Res* (2013) 150(1):114–20. doi: 10.1016/j.schres.2013.07.033
2. Darling JS, Daniel JM. Pubertal hormones mediate sex differences in levels of myelin basic protein in the orbitofrontal cortex of adult rats. *Neuroscience* (2019) 406:487–95. doi: 10.1016/j.neuroscience.2019.03.041
3. Peper JS, Brouwer RM, Schnack HG, van Baal GC, van Leeuwen M, van den Berg SM, et al. Sex steroids and brain structure in pubertal boys and girls. *Psychoneuroendocrinology* (2009) 34(3):332–42. doi: 10.1016/j.psyneuen.2008.09.012
4. Parducz A, Hajszan T, Maclusky NJ, Hoyk Z, Csakvari E, Kurunczi A, et al. Synaptic Remodeling Induced by Gonadal Hormones: Neuronal Plasticity as a Mediator of Neuroendocrine and Behavioral Responses to Steroids. *Neuroscience* (2006) 138(3):977–85. doi: 10.1016/j.neuroscience.2005.07.008
5. Gogos A, Ney LJ, Seymour N, Van Rheenen TE, Felmingham KL. Sex differences in schizophrenia, bipolar disorder, and post-traumatic stress disorder: Are gonadal hormones the link? *Br J Pharmacol* (2019) 176 (21):4119–35. doi: 10.1111/bph.14584
6. Hennah W, Varilo T, Kestilä M, Paunio T, Arajärvi R, Haukka J, et al. Haplotype Transmission Analysis Provides Evidence of Association for DISC1 to Schizophrenia and Suggests Sex-Dependent Effects. *Hum Mol Genet* (2003) 12(23):3151–9. doi: 10.1093/hmg/ddg341
7. McEwen BS, Milner TA. Understanding the Broad Influence of Sex Hormones and Sex Differences in the Brain. *J Neurosci Res* (2017) 95(1-2):24–39. doi: 10.1002/jnr.23809
8. Bollinger JL, Salinas I, Fender E, Sengelaub DR, Wellman CL. Gonadal hormones differentially regulate sex-specific stress effects on glia in the medial prefrontal cortex. *J Neuroendocrinol.* (2019) 31(8):E12762. doi: 10.1111/jne.12762
9. Waters EM, Mazid S, Dodos M, Puri R, Janssen WG, Morrison JH, et al. Milner TA Effects of estrogen and aging on synaptic morphology and distribution of phosphorylated Tyr1472 NR2B in the female rat hippocampus. *Neurobiol Aging.* (2019) 73:200–10. doi: 10.1016/j.neurobiolaging.2018.09.025
10. Saha S, Chant D, Welham J, McGrath J. A systematic review of the prevalence of schizophrenia. *PLoS Med* (2005) 2(5):e141. doi: 10.1371/journal.pmed.0020141
11. Abel KM, Drake R, Goldstein JM. Sex differences in schizophrenia. *Int Rev Psychiatry* (2010) 22(5):417–28. doi: 10.3109/09540261.2010.515205
12. Markham JA. Sex steroids and schizophrenia. *Rev Endocr Metab Disord* (2012) 13:187–207. doi: 10.1007/s11154-011-9184-2
13. Bao AM, Swaab DF. Sex differences in the brain, behavior, and neuropsychiatric disorders. *Neuroscientist* (2010) 16(5):550–65. doi: 10.1177/1073858410377005
14. Maric N, Krabbendam L, Vollebergh W, de Graaf R, van Os J. Sex differences in symptoms of psychosis in a non-selected, general population sample. *Schizophr Res* (2003) 63(1-2):89–95. doi: 10.1016/S0920-9964(02)00380-8
15. Krysta K, Murawiec S, Klasik A, Wiglusz MS, Krupka-Matuszczyk I. Sex-specific differences in cognitive functioning among schizophrenic patients. *Psychiatr Danub.* (2013) 25 Suppl 2:S244–6.
16. Gogos A, Sbisa AM, Sun J, Gibbons A, Udawela M, Dean B. A Role for Estrogen in Schizophrenia: Clinical and Preclinical Findings. *Int J Endocrinol* (2015) 61:53–6. doi: 10.1155/2015/615356
17. Weickert TW, Allen KM, Weickert CS. Potential role of oestrogen modulation in the treatment of neurocognitive deficits in schizophrenia. *CNS Drugs* (2016) 30(2):125–33. doi: 10.1007/s40263-016-0312-0
18. Kendell RE, Chalmers JC, Platz C. Epidemiology of puerperal psychoses. *Br J Psychiatry* (1987) 150:662–73. doi: 10.1192/bjp.150.5.662
19. Cersosimo MG, Benarroch EE. Estrogen actions in the nervous system: Complexity and clinical implications. *Neurology* (2015) 85(3):263–73. doi: 10.1212/WNL.0000000000001776
20. Ji E, Weickert CS, Lenroot R, Kindler J, Skulleter AJ, Vercamlin A, et al. Adjunctive selective estrogen receptor modulator increases neural activity in the hippocampus and inferior frontal gyrus during emotional face recognition in schizophrenia. *Transl Psychiatry* (2016) 6:e795. doi: 10.1038/tp.2016.59
21. Hoff AL, Kremen WS, Wieneke MH, Lauriello HM, Blankfeld WO, Faustman JG, et al. Association of estrogen levels with neuropsychological performance in women with schizophrenia. *Am J Psychiatry* (2001) 158(7):1134–9. doi: 10.1176/appi.ajp.158.7.1134
22. Ko YH, Joe SH, Cho W, Park JH, Lee JJ, Jung IK, et al. Effect of hormone replacement therapy on cognitive function in women with chronic

schizophrenia. *Int J Psychiatry Clin Pract* (2006) 10(2):97–104. doi: 10.1080/13651500500526235
23. Kulkarni J, Butler S, Riecher-Rössler A. Estrogens and SERMS as adjunctive treatments for schizophrenia. *Front Neuroendocrinol.* (2019) 53:100743. doi: 10.1016/j.yfrne.2019.03.002
24. Sun J, Walker AJ, Dean B, van den Buuse M, Gogos A. Progesterone: The neglected hormone in schizophrenia? A focus on progesterone-dopamine interactions. *Psychoneuroendocrinology* (2016) 74:126–40. doi: 10.1016/j.psyneuen.2016.08.019
25. Fryea CA, Sorab I. Progesterone reduces hyperactivity of female and male dopamine transporter knockout mice. *Behav Brain Res* (2010) 209(1):59–65. doi: 10.1016/j.bbr.2010.01.015
26. Cai H, Zhou X, Dougherty GG, Reddy RD, Haas GL, Montrose DM, et al. Pregnenolone-progesterone-allopregnanolone pathway as a potential therapeutic target in first-episode antipsychotic-naïve patients with schizophrenia. *Psychoneuroendocrinology* (2018) 90:43–51. doi: 10.1016/j.psyneuen.2018.02.004
27. Talih F, Fattal O, Malone D Jr. Anabolic steroid abuse: psychiatric and physical costs. *Cleve Clin J Med* (2007) 74(5):341–4. doi: 10.3949/ccjm.74.5.341
28. Rinieris P, Hatzimanolis J, Markianos M, Stefanis C. Effects of 4 weeks treatment with chlorpromazine and/or trihexyphenidyl on the pituitary-gonadal axis in male paranoid schizophrenics. *Eur Arch Psychiatry Neurol Sci* (1988) 237(4):189–93. doi: 10.1007/BF00449905
29. Tourney G, Erb JL. Temporal variations in androgens and stress hormones in control and schizophrenic subjects. *Biol Psychiatry* (1979) 14(2):395–404.
30. Brown AS, Hembree WC, Friedman JH, Kaufmann CA, Gorman JM. The gonadal axis in men with schizophrenia. *Psychiatry Res* (1995) 57(3):231–9. doi: 10.1016/0165-1781(95)02643-B
31. Ceskova E, Prikryl R, Kasparek T. Testosterone in first-episode schizophrenia. *Neuro Endocrinol Lett* (2007) 28(6):811–4.
32. Huber TJ, Tettenborn C, Leifke E, Emrich HM. Sex hormones in psychotic men. *Psychoneuroendocrinology* (2005) 30(1):111–4. doi: 10.1016/j.psyneuen.2004.05.010
33. Akhondzadeh S, Rezaei F, Larijani B, Nejatisafa AA, Kashani L, Abbasi SH. Correlation between testosterone, gonadotropins and prolactin and severity of negative symptoms in male patients with chronic schizophrenia. *Schizophr Res* (2006) 84(2–3):405–10. doi: 10.1016/j.schres.2006.02.008
34. Bulut SD, Bulut S, Gundogmus AG, Aydemir C, Serum DHEA-S. Testosterone and Cortisol Levels in Female Patients With Schizophrenia. *Endocr Metab Immune Disord Drug Targets* (2018) 18(4):348–54. doi: 10.2174/1871530318666180212102128
35. Cohen RZ, Seeman MV, Gotowiec A, Kopala L. Earlier Puberty as a Predictor of Later Onset of Schizophrenia in Women. *Am J Psychiatry* (1999) 156:1059–64. doi: 10.1176/ajp.156.7.1059
36. Yazici E1, Bursalioglu FS, Aydin N, Yazici AB. Menarche, puberty and psychiatric disorders. *Gynecol. Endocrinol* (2013) 29(12):1055–8. doi: 10.3109/09513590.2013.829447
37. Bergemann N, Parzer P, Nagl I, Salbach B, Runnebaum B, Mundt C, et al. Acute psychiatric admission and menstrual cycle phase in women with schizophrenia. *Arch Wom Ment Health* (2002) 5(3):119–26. doi: 10.1007/s00737-002-0004-2
38. Hallonquist JD, Seeman MV, Lang M, Rector NA. Variation in symptom severity over the menstrual cycle of schizophrenics. *Biol Psychiatry* (1993) 33 (3):207–9. doi: 10.1016/0006-3223(93)90141-Y
39. Riecher-Rossler A, Hafner H, Stumbaum M, Maurer K, Schmidt R. Can estradiol modulate schizophrenic symptomatology? *Schizophr Bull* (1994) 20 (1):203–14. doi: 10.1093/schbul/20.1.203
40. Rubin LH, Carter CS, Drogos L, Pournajafi-Nazarloo H, Sweeney JA, Maki PM. Peripheral oxytocin is associated with reduced symptom severity in schizophrenia. *Schizophr Res* (2010) 124(1–3):13–21. doi: 10.1016/j.schres.2010.09.014
41. Bergemann N, Parzer P, Runnebaum B, Resch F, Mundt C. Estrogen, menstrual cycle phases, and psychopathology in women suffering from schizophrenia. *Psychol Med* (2007) 37(10):1427–36. doi: 10.1017/S0033291707000578
42. Gattaz WF, Vogel P, Riecher-Rossler A, Soddu G. Influence of the menstrual cycle phase on the therapeutic response in schizophrenia. *Biol Psychiatry* (1994) 36(2):137–9. doi: 10.1016/0006-3223(94)91195-9
43. Riecher-Rossler A, Kulkarni J. Estrogens and gonadal function in schizophrenia and related psychoses. *Curr Top Behav Neurosci* (2011) 8:155–71. doi: 10.1007/7854_2010_100
44. Jang D, Elfenbein HA. Menstrual cycle effects on mental health outcomes: a meta-analysis. *Arch Suicide Res* (2019) 23(2):312–32. doi: 10.1080/13811118.2018.1430638
45. Reilly TJ, Sagnay de la Bastida VC, Joyce DW, Cullen AE, McGuire P. Exacerbation of Psychosis During the Perimenstrual Phase of the Menstrual Cycle: Systematic Review and Meta-analysis. *Schizophr Bull* (2020) 46(1):78–90. doi: 10.1093/schbul/sbz030
46. Webb RT, Howard L, Abel KM. Antipsychotic drugs for non-affective psychosis during pregnancy and postpartum. *Cochrane Database Syst Rev* (2004) 2:CD004411. doi: 10.1002/14651858.CD004411.pub2
47. Jones I, Chandra PS, Dazzan P, Howard LM. Bipolar disorder, affective psychosis, and schizophrenia in pregnancy and the post-partum period. *Lancet* (2014) 384(9956):1789–99. doi: 10.1016/S0140-6736(14)61278-2
48. Robinson GE. Treatment of Schizophrenia in Pregnancy and Postpartum. *J Popul. Ther Clin Pharmacol* (2012) 19(3):e380–6.
49. Solari H, Dickson KE, Miller L. Understanding and Treating Women With Schizophrenia During Pregnancy and postpartum–Motherisk Update 2008. *Can J Clin Pharmacol* (2009) 16(1):e23–32.
50. Callegari LS, Zhao X, Nelson KM, Borrero S. Contraceptive adherence among women Veterans with mental illness and substance use disorder. *Contraception* (2015) 91(5):386–92. doi: 10.1016/j.contraception.2015.01.013
51. Seeman MV, Ross R. Prescribing contraceptives for women with schizophrenia. *J Psychiatr Pract* (2011) 17(4):258–69. doi: 10.1097/01.pra.0000400263.52913.dc
52. Friedman SH, Sajatovic M, Schuermeyer IN, Safavi R, Hays RW, West J, et al. Menopause-related quality of life in chronically mentally ill women. *Int J Psychiatry Med* (2005) 35(3):259–71. doi: 10.2190/BR03-8GYD-5L9J-LU17
53. Sajatovic M, Friedman SH, Schuermeyer IN, Safavi R, Ignacio RV, Hays RW, et al. Menopause knowledge and subjective experience among peri- and postmenopausal women with bipolar disorder, schizophrenia and major depression. *J Nerv Ment Dis* (2006) 194(3):173–8. doi: 10.1097/01.nmd.0000202479.00623.86
54. Brzezinski A, Brzezinski-Sinai NA. Seeman MV Treating schizophrenia during menopause. *Menopause* (2017) 24(5):582–8. doi: 10.1097/GME.0000000000000772
55. Sajatovic M, Rosenthal MB, Plax MS, Meyer ML, Bingham CR. Mental illness and menopause: A patient and family perspective. *J Gend Specif Med* (2003) 6 (2):31–4.
56. Barnabei VM, Cochrane BB, Aragaki AK, Nygaard I, Wiliams RS, McGovern PG, et al. Menopausal symptoms and treatment-related effects of estrogen and progestin in the women's health initiative. *Obstet. Gynecol.* (2005) 105(5 Pt 1):1063–73. doi: 10.1097/01.AOG.0000158120.47542.18
57. North American Menopause Society. Position statement: The 2017 hormone therapy position statement of the North American Menopause Society. *Menopause* (2017) 24:7. doi: 10.1097/GME.0000000000000921
58. Sarri G, Davies M, Lumsden MA. Guideline Development Group. Diagnosis and management of menopause: summary of NICE guidance. *BMJ* (2015) 12:351–6. doi: 10.1136/bmj.h5746
59. González-Rodríguez A, Catalán R, Penadés R. Antipsychotic response worsens with postmenopausal duration in women with schizophrenia. *J Clin Psychopharmacol.* (2016) 36(6):580–7. doi: 10.1097/JCP.0000000000000571
60. Shi S, Klotz U. Age-related changes in pharmacokinetics. *Curr Drug Metab* (2011) 12(7):601–10. doi: 10.2174/138920011796504527

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 05 August 2020
doi: 10.3389/fpsyt.2020.00775

Glucose Metabolism, Thyroid Function, and Prolactin Level in Adolescent Patients With First Episode of Schizophrenia and Affective Disorders

Maria Giuseppina Petruzzelli[1], Lucia Marzulli[2], Orazio Valerio Giannico[2], Flora Furente[2], Mariella Margari[2], Emilia Matera[3] and Francesco Margari[1]*

[1] Department of Basic Medical Sciences, Neuroscience and Sense Organs, University of Bari "Aldo Moro", Bari, Italy, [2] Department of Biomedical Sciences and Human Oncology, University of Bari "Aldo Moro", Bari, Italy, [3] Child Neuropsychiatry Unit, Azienda Ospedaliero-Universitaria Policlinico di Bari, Bari, Italy

OPEN ACCESS

Edited by:
Grazia Rutigliano, University of Pisa, Italy

Reviewed by:
Jaakko Keinänen, National Institute for Health and Welfare, Finland
Kirsten Wedervang-Resell, Oslo University Hospital, Norway

***Correspondence:**
Maria Giuseppina Petruzzelli
maria.petruzzelli@uniba.it

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *03 May 2020*
Accepted: *21 July 2020*
Published: *05 August 2020*

Citation:
Petruzzelli MG, Marzulli L, Giannico OV, Furente F, Margari M, Matera E and Margari F (2020) Glucose Metabolism, Thyroid Function, and Prolactin Level in Adolescent Patients With First Episode of Schizophrenia and Affective Disorders. Front. Psychiatry 11:775. doi: 10.3389/fpsyt.2020.00775

Schizophrenia and affective spectrum disorders (ASD) typically begin in adolescence or early adulthood. The pathophysiological mechanisms underlying these disorders are still not fully understood, and recent studies have suggested an involvement of dysfunctions in cardiometabolic and neuroendocrine systems at the onset of both disorders. In this context, we aimed to assess thyroid function, prolactin level, glucose metabolism, and lipid profile in drug naive adolescents, comparing patients with first episode of schizophrenia spectrum disorders (SSD) and patients with ASD. We performed a retrospective chart review from inpatients aged from ten to eighteen years, referred to Child and Adolescent Psychiatric Unit of University of Bari "Aldo Moro" over a period of 4 years, with diagnosis of SSD (n=30) or ASD (n=22), according to Diagnostic and Statistical Manual for Mental Disorders-fifth edition (DSM-5) criteria. Data on serum prolactin, glucose, insulin, total cholesterol, high density lipoprotein cholesterol, low density lipoprotein cholesterol, triglycerides, thyroid stimulating hormone, free triiodothyronin, and free thyroxin were collected, and the insulin resistance (IR) indexes "HOMA1-IR" and "HOMA2-IR" were calculated. The multivariable linear regression models, adjusting for potential confounding factors (age, sex, and BMI), showed HOMA1-IR (p=0.001), HOMA2-IR (p=0.002), glucose (p=0.004), insulin (p=0.004) and free thyroxin (p<0.001) values higher in the SSD group than in ASD. No others significant differences were found. Our findings suggest the need for a metabolic and endocrine screening at the onset of SSD and ASD, particularly for indexes of IR, that is a testable and treatable risk factor for cardiometabolic diseases. Further studies are required to better understand the role of endocrinological and metabolic dysfunctions at the onset of severe mental illness also considering influencing factors as age, gender, and BMI.

Keywords: early onset psychopathology, neuroendocrine dysregulation, metabolic syndrome risk factors, insulin resistance, mood disorders, first episode of psychosis (FEP)

INTRODUCTION

Schizophrenia and bipolar spectrum disorders are considered as part of the psychosis continuum, with similar clinical features such as psychotic and mood symptoms as well as neurocognitive impairments of varying degrees (1). Similarities and differences between neurodevelopmental trajectories in patients with early onset schizophrenia and early onset bipolar disorder have been described with regard to genetic, neurobiological, and environmental risk factors as well as premorbid developmental impairments (2–4). The etiopathological mechanisms underlying both disorders are still not fully understood, although the hypothesis of complex and multifactorial interactions between genetic and environmental risk factors is now widely accepted (2, 3).

Epidemiological studies have clarified that both disorders typically begins in adolescence or early adulthood (5, 6). The relationship between typical changes in the adolescent maturational brain and the full onset of psychopathology is not a unitary phenomenon and one of the fields of greatest interest in this topic is the potential role of hormones in modulating neuronal activity. A lot of evidence supported the association between abnormal gonadal and adrenal hormones levels and different psychopathological conditions (7), anyway, other hormones are thought to have a role in the development and correct functioning of the central nervous system. Recent studies showing dysfunctions in cardiometabolic and neuroendocrine systems, suggested that both psychotic and affective disorders may involve multiple systems at different stage of their clinical course (8, 9).

An increasing number of observational studies on antipsychotic-naïve patients suggested the existence of a pre-diabetic condition at the onset of the psychotic illness, while data from patients with chronic course of schizophrenia showed a higher rate of comorbid metabolic syndrome and type 2 diabetes (10–12). Studies on glucose and lipid metabolism deregulation at the onset of depressive and bipolar disorders are fewer and less agree (13–15). Anyway a bidirectional relationship between major depressive disorder, bipolar disorders, and cardiovascular disease has been proposed (16, 17). Insulin signaling is suggested to play a central role in the mechanisms underlying the association between schizophrenia spectrum disorders (SSD)/ affective spectrum disorders (ASD) and cardiovascular risk factors, also considering the potential action of insulin as neuropeptide (18, 19).

Moreover the regulation of glucose homeostasis and insulin sensitivity could be influenced by prolactin (PRL) and thyroid hormones actions (20, 21), with a likely age-dependent variability (22–24). We know that PRL, beside the lactogenic activity, is involved in appetite regulation and plays metabolic actions in both pancreatic and adipose tissue (25), so that hyperprolactinemia (HPRL) could take part in metabolic disorders. Moreover, higher PRL levels have been found in first episode drug-naïve psychotic patients compared to healthy controls; further researches are needed to clarify the relationship between stress, HPRL, and emergence of the psychotic symptoms, also considering the role of confounding factors as age, sex, body mass index (BMI), and thyroid stimulating hormone (26–28). Thyroid dysfunctions are frequently associated in clinical practice with metabolic syndrome (29, 30) as well as a relationship exists between HPRL and hypothyroidism, also in children (31). In addition, altered hypothalamic-pituitary-thyroid system's function has been described in schizophrenia, bipolar and depressive disorders (32–34), but very few studies have been conducted at the onset of these illnesses (1, 35).

The study of cardiometabolic and neuroendocrine dysfunctions occurring in the acute phase of psychopathological onset may be very informative of their implications in the pathogenesis of SSD/ ASD, since some confounding factors related to the chronicity, as persistent negative symptoms, long-term antipsychotic treatment or unhealthy lifestyle, are minimized. Moreover, subjects in adolescent age may be considered at lower risk of cardiovascular disease and endocrine disorders than adult subjects, therefore more suitable to verify the hypothesis of an intrinsic relationship between endocrine-metabolic dysfunctions and psychiatric disorders, despite the limit of larger diagnostic instability and stress-related hormonal variability than in adult patients.

Starting from the hypothesis of a co-shared vulnerability between impaired glucose tolerance and SSD, detectable in subclinical form even in patients with adolescent onset of psychosis, in our previous study we found higher level of PRL and increase in Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) in a sample of drug naïve adolescents in the acute phase of first episode psychosis compared to subjects at clinical high risk of developing psychosis (36). The purpose of the present paper was to extend our previous finding exploring glucose and lipid metabolism as well as PRL regulation and thyroid status, to verify the hypothesis that a greater impairment of the parameters under study may be associated to the adolescent onset of SSD more than of ASD.

Therefore, the aims of the present study were 1. to perform a baseline evaluation of glucose metabolism and lipid profile, PRL level and thyroid function in two samples of drug naïve adolescents in the acute phase of first episode ASD and SSD; 2. to compare the parameters of study between these two different diagnostic groups, adjusting for age, sex and BMI.

METHODS

Subjects

We performed a retrospective chart review from inpatients of both sexes aged between 10 and 18, referred to Child and Adolescent Psychiatric Unit, Department of Basic Medical Sciences, Neurosciences and Sense Organs over a period of 4 years. According to the purpose of this study, we selected patients that, at the time of admission, had received diagnosis of first episode of SSD or first episode of ASD and had undergone biochemical evaluation of glucose and lipid profiles, PRL level,

Abbreviations: SSD, schizophrenia spectrum disorders; ASD, affective spectrum disorders.

and thyroid function. Diagnoses of early onset first-episode SSD (schizophrenia, schizophreniform disorder, schizoaffective disorder, psychosis not otherwise specified) were made in accordance to Diagnostic and Statistical Manual for Mental Disorders-fifth edition (DSM-5) criteria (37). The validated Italian version of the Positive and Negative Syndrome Scale (PANSS) (38), was performed within the first 72 h after the admission to assess the severity of psychotic symptoms (positive, negative and general symptoms). Diagnoses of early onset ASD (Bipolar I disorder, Bipolar II disorder, Cyclothymic Disorder, Disruptive Mood Dysregulating Disorder, Major Depressive disorder, Dysthymia) were made in accordance to DSM-5 criteria (37). The validated Italian version of the Hamilton Depression Rating Scale (HAM-D) (39) and Young Mania Rating Scale (YMRS) (40) were performed within the first 72 h after the admission to assess the severity of affective symptoms (depressive, manic, and hypomanic symptoms). Parents and patients were interviewed by two experienced psychiatrists belonging to the research group and the evaluations were discussed in regular reliability meetings, under the supervision of a senior researcher. All the procedures above described were conducted at the time of admission, as part of a more general clinical and laboratory assessment needed for diagnostic evaluation. Patients were excluded: if they were younger than 10 years or older than 18 years; if they had an history of antipsychotics, antidepressants or mood stabilizing assumption; if medical history, physical examination, laboratory, and instrumental findings had revealed that psychopathological symptoms were substance induced or due to another medical condition; if there were any evidences of medical causes of HPRL (such as pituitary/hypothalamic disorders, primary hypothyroidism, renal, and liver insufficiency), abnormal thyroid function and insulin resistance (IR). For each study participant, body weight (kg) and height (m) were measured simultaneously with the blood test; the BMI was obtained by dividing weight by height squared (kg/m^2). Electrocardiogram, electroencephalogram, and brain magnetic resonance have been used when indicated. Written informed consent from the parents of all participants was obtained during hospitalization so the clinical and laboratory data collected could be used for the research purposes. The approval of methodology of the study was obtained from the independent ethical committee of the University-Hospital Policlinico of Bari.

Biochemical Measurements

Glucose Metabolism Parameters

Peripheral blood samples from all participants were collected between 7,30 and 9 AM, following an overnight fast. Serum glucose was determined using an enzymatic method; levels between 3.33 and 5.55 mmol/L were considered normal for both males and females. Serum insulin was estimated by chemiluminescence. Hyperinsulinemia was defined as values higher than 113.2 pmol/L, for both sexes. HOMA1-IR was calculated using the homeostatic model of assessment as the product of the fasting plasma insulin level (μU/ml) and the fasting plasma glucose level (mmol/L), divided by 22.5. A HOMA1-IR value higher than 2.6 was considered indicative of increased risk of IR, according to references on normal weight adolescents (41–43). We performed the evaluation of HOMA2-IR using the HOMA-2 calculator, version 2.2.3, provided by Oxford University (free download is available from the website www.dtu.ox.ac.uk. No defined thresholds for "normal" vs "abnormal" values are reported).

Lipid Profile

Total cholesterol levels were measured through a standardized method traceable to the International Federation of Clinical Chemistry Working Group (IFCC-WG) Reference Method. Levels of 4.03± 0.13 mmol/L for males and 4.47 ± 0.12 mmol/L for females were considered normal. High-density lipoprotein cholesterol (HDLc) was estimated by clearance assay. Levels between 1.01 ± 0.04 mmol/L for males and 1.16 ± 0.03 mmol/L for females were considered normal. Fasting plasma levels of low-density lipoprotein cholesterol (LDLc) were determined using Friedewald formula (44). LDLc levels of 2.61 ± 0.12 mmol/L for males and 2.79 ± 0.1 mmol/L for females were considered normal. Triglycerides (Tg) levels were measured using a traceable IFCC standardized method. Levels within the range 0.25–1.56 mmol/L were considered normal for both male and female patients. Cut point values for acceptable, borderline-high, and high plasma lipid have been considered according to the 2011 Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (45).

Serum PRL and Thyroid Status

Serum PRL levels were estimated by an immunoassay system. HPRL was defined as PRL levels higher than 0.87 nmol/L in male patients and 1.09 nmol/L in female patients. Thyroid stimulating hormone (TSH) was determined by chemiluminescence; levels between 0.36–3.74 mUI/L were considered normal for both sexes. Free triiodothyronine (fT3) levels were determined by chemiluminescence. Levels between 2.6–8 pmol/L were considered normal for both males and females. Free thyroxin (fT4) was evaluated by chemiluminescence. Levels between 9.78–18.79 pmol/L were considered normal for both sexes.

Statistical Analyses

Statistical analysis was performed using R 3.5.2 (released on 2018-12-20). Statistical significance α was fixed to 0.05. Categorical variables were reported as absolute and relative frequencies (%) and compared through chi-square test. Numerical variables were reported as mean ± standard deviation and compare through Welch t-test. In order to account for non-normality, evaluated through Shapiro Wilk test, right-skewed numerical variables were transformed in their natural logarithm. To analyze the association between the SSD or ASD and the logarithmic transformation of the 12 parameters, adjusting for potential confounding factors (age, sex, and BMI), 12 multivariable linear regression models were fitted with estimation of the ß coefficients. For each model, a global validation linear model assumption significance test was performed in order to verify the linearity assumption of the dependent variable and the normality and homoskedasticity assumptions of the residuals. We reported all p values and

confidence intervals. Considering the features of the working hypotheses, no correction for multiple testing were applied. However, this should be considered in the interpretation of the statistical significance.

RESULTS

The two samples of study were composed by 30 patients for the SSD group and 22 patients for the ASD group. No significant differences emerged for age, gender and BMI and the mean value of BMI was within normal weight range in both study groups. Demographic and clinical features of the two groups are summarized in **Table 1**. When we performed the multivariate regression analysis, adjusting for potential confounding factors (age, sex, and BMI) (**Table 2**), in order to study the association between the SSD or ASD and the hormonal and metabolic parameters, we found a significant differences concerning HOMA1-IR and HOMA2-IR index, fasting glucose, insulin and fT4 (p values 0.001, 0.002, 0.004, 0.004, <0.001 respectively). Specifically, the HOMA1-IR was significantly higher in the SSD group (3.1 ± 2.0) rather than in ASD group (1.9 ± 1.2), with a mean value indicative of increase risk of IR; also the HOMA2-IR was higher in the SSD group (1.9 ± 1.0) rather than in the ASD group (1.3 ± 0.7). The mean value of fasting glucose, although within the normal range, was significantly higher in SSD group (85.1 ± 12.1) rather than in ASD group (75.9 ± 6.9). In the same way, the mean value of insulin was within the normal range for both groups, but significantly higher in SSD group (14.6 ± 8.7) rather than in ASD group (10.3 ± 6.1). No differences were found between the two groups of study regarding the comparison of lipid profile. Lower mean value of fT4 was found in ASD group (1.0 ± 0.1), with a significant difference when compared with SSD group (1.2 ± 0.2). No significant differences were found for the mean serum level of fT3 and TSH, within the range of normality in both groups. No difference was found between the two groups for the mean serum level of PRL, even if patients with SSD tend to have higher PRL values (24.5 ± 27.6) than patients with ASD (17 ± 12.7), with a mean level near to a condition of HPRL.

TABLE 1 | Demographic and clinical features of ASD and SSD patients.

	ASD (n = 22)	SSD (*n* = 30)	P value
Age [years] mean (S.D.)	15.4 (1.7)	15.8 (1.3)	0.362
Gender			0.235
Male n (%)	6 (27.3)	13 (43.3)	–
Female n (%)	16 (72.7)	17 (56.7)	–
Residence	South of Italy	South of Italy	–
BMI [kg/m^2] mean (S.D.)	23.2 (5.3)	20.7 (2.7)	0.052
PANSS mean (S.D.)	–	91.14 (8.7)	
total	–	19.28 (2.9)	
positive	–	23 (3.4)	
negative	–	48.85 (4.4)	
general			
YMRS mean (S.D.)	32.7 (8)	–	–
HAMD mean (S.D.)	18.58 (6.6)	–	–

ASD, affective spectrum disorder; BMI, body mass index; SSD, schizophrenia spectrum disorder.

DISCUSSION

Glucose and Lipid Metabolism

The main findings of this study were a significant increase in fasting glucose, fasting insulin, and HOMA-IR indexes in drug naïve adolescents with first episode of SSD compared to ones with first episode of ASD, adjusted for age, sex, and BMI. Similar lipid profile was found between the two groups, with mean values including in the acceptable range according to the 2011 Expert Panel on Integrated Guidelines for Cardiovascular Health and Risk Reduction in Children and Adolescents (45). These findings would indicate that adolescents with first episode of SSD might

TABLE 2 | Results of the multivariate regression analysis (p-value), with adjustment by age, sex, and BMI.

	ASD (n = 22)		SSD (*n* = 30)		ß Coefficient	[95%CI]	p value (<0,05)
	Mean value (SD)	Natural logarithmic transformation	Mean value (SD)	Natural logarithmic transformation			
PRL (nmol/L)	0.7 (0.6)	–0.5 (0.7)	1.1 (1.2)[a]	–0.4 (0.9)	0.18	[–0.26;0.63]	0.407
TSH (mUI/L)	2.2 (1.3)	0.6 (0.6)	2.0 (1.1)	0.6 (0.5)	0.01	[–0.33;0.35]	0.940
fT3 (pmol/L)	4.6(0.7)	1.5 (0.1)	4.7 (0.8)	1.5 (0.2)	0.04	[–0.04:0.13]	0.329
fT4 (pmol/L)	12.6 (1.7)	2.5 (0.1)	15.3 (2.6)	2.7 (0.2)	0.20	[0.10;0.30]	**<0.001**
Cholester (mmol/L)	3.7 (0.9)[b]	1.3 (0.2)	3.8 (0.7)	1.3 (0.2)	0.03	[–0.1;0.16]	0.629
LDLc (mmol/L)	2.0 (0.8)[b]	0.6 (0.4)	2.1 (0.6)[a]	0.7 (0.3)	0.1	[–0.15;0.31]	0.499
HDLc (mmol/L)	1.3 (0.3)[b]	0.2 (0.2)	1.4 (0.3)[a]	0.3 (0.2)	0.11	[–0.02;0.23]	0.086
Tg (mmol/L)	0.9 (0.4)[b]	–0.3 (0.5)	0.7 (0.3)	–0.4 (0.4)	–0.19	[–0.47;0.09]	0.189
HOMA2-IR	1.3 (0.7)	0.1 (0.5)	1.9 (1.0)	0.5 (0.6)	0.53	[0.21;0.85]	**0.002**
HOMA1-IR	1.9 (1.2)	0.5 (0.6)	3.1 (2.0)	0.9 (0.7)	0.65	[0.27;1.03]	**0.001**
Glucose mmol/L	4.2 (0.4)	1.4 (0.1)	4.7 (0.7)	1.5 (0.1)	0.11	[0.04;0.19]	**0.004**
Insulin (pmol/L)	70.5 (43.0)	4.1 (0.7)	101.1 (60.2)	4.4 (0.7)	0.57	[0.22;0.92]	**0.002**

[a]*Missing values for one participant,* [b]*Missing values for two participants.*

ASD, affective spectrum disorder; fT3, free triiodothyronine; fT4, free thyroxin; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; PRL, prolactin; SSD, schizophrenia spectrum disorder; Tg, triglycerides; TSH, thyroid stimulating hormone.

have an increased risk of developing IR and diabetes mellitus compared to adolescents with first episode of ASD, regardless to antipsychotic treatment.

To our knowledge most of the studies on cardiovascular risk factors at the onset of psychiatric illness involved adult patients, with substantial numbers regarding the first episode of SSD more than ASD. Two recent papers comparing cardiovascular risk factors between adolescent samples with first episode of psychosis (FEP) and healthy controls, found that abnormalities in lipid profile resulted associated with early-onset psychosis more than alterations of glucose homeostasis, regardless of antipsychotic treatment. Wedervang-Resell K. et al. reported significantly higher TC/HDLc and Tg values in patients with early onset psychosis, with and without antipsychotic exposure, than healthy subjects; significantly increased in HOMA-IR scores were found only in antipsychotic-exposed patients (46). In the study of Jensen K.G. et al. youth patients with FEP had higher cholesterol and LDLc than matched controls, while increased in insulin and HOMA-IR were found in early onset patients with dyslipidemia or family history of type 2 diabetes mellitus (T2DM) (47). Cardiometabolic risk assessment performed in a sample of recent onset bipolar disorder ranged between 12 and 35 years of age, showed higher triglyceride levels than healthy controls (14).

The variability of these results among studies on adolescent samples may be related to a lot of confounding factors, including dietary intake, sedentary lifestyle, substance use, ethnicity as well as to small samples size and age and sex distribution across different samples of study. Furthermore, an age-related diagnostic heterogeneity could explain some discrepancy between different data, so that longitudinal studies will be more useful to understand the trajectories of changes in glucose tolerance and lipid profile over the time in adolescent population.

Anyway, our hypothesis that adolescents in acute phase of first episode of SSD may have an intrinsic risk of IR appears in accordance with data emerged by systematic review and meta-analysis performed by A.M. Greenhalgh et al. to assess glucose tolerance, insulin and IR in early adulthood antipsychotic-naïve patients with non-affective psychosis (10). Their results showed that, at the time of the onset of psychosis, patients have a slight increase in fasting glucose, usually in the normal range, despite a small increase in IR, by secreting additional insulin (10). In addition, a systematic review and meta-analysis examining lipid parameters in adult patients found that FEP was associated with decreased total and LDLc levels but increased triglyceride levels compared with healthy control groups, with no difference in HDLc levels. The authors suggested that hypertriglyceridemia may be added to the evidence for glucose dysregulation in this cohort, considering it as a feature of T2DM (8). On the other hand IR has been reported in more than half of all bipolar patients and some authors supported the hypothesis that it is associated with the chronic course of illness rather than to early stage (19).

We know that a lot of physiological conditions and disease states, involving neuroendocrine response to stress, were found to be accompanied by IR (48). Increasing evidence showed that a co-shared genetic pathway partially explain the comorbidity of schizophrenia, major depressive disorder, type 2 diabetes, and metabolic syndrome (49, 50) and recent evidence supported the hypothesis that intrinsic dysfunction in central nervous system insulin signaling might represent the final common pathway of interaction between metabolic syndrome schizophrenia and mood disorders (18). As a result, considering that IR may be a reversible condition, the use of HOMA-IR index at the onset of psychosis may be a useful instrument to assess a latent risk of later development of cardiovascular disorders, also in normal weight adolescents (11, 51).

The calculation of HOMA-IR index is a good sensitive and specific method for assessing insulin sensitivity, well accepted by researchers, and used in epidemiological studies in adults, adolescents, and children. The successful application of HOMA-IR index in a given population is related to the use of specific cutoffs for gender, ethnicity, age, and/or sexual maturation level. There is no consensus regarding the reference value of HOMA-IR for the diagnosis of IR in the pediatric age group and several cutoff points have been reported in the literature (43, 52). Moreover, the HOMA index may be assessed using different methods, not exactly comparable, as the original model for the HOMA1-IR or the update HOMA computer model, with some physiological adjustment, for the HOMA 2- IR. It is generally accepted that a value of HOMA1-IR ≥ 2.6 accurately classify normal-weight adolescents at increased cardiovascular and metabolic risk (41–43). According to this indication, the mean value of HOMA1-IR we found in the group of SSD (3.1 ± 2.0) suggested a condition of risk of IR at the onset of psychosis, despite the normal value of other metabolic parameters and BMI. No clear cutoff points have been identified for the use of HOMA2-IR in adolescents, anyway also when we performed the comparison of HOMA2-IR between the two groups of study we found a significant higher mean value in SSD group, suggesting a higher risk of IR in adolescent with SSD rather than with ASD.

PRL Levels

Although no significant difference emerged comparing patients with SSD and patients with ASD, we observed a higher mean value of PRL in SSD group, close enough to the cutoff for HPRL. Previous studies have found PRL levels above the physiological limits in first episode drug-naïve psychotic patients (26, 36, 53–55). Riecher-Rossler has suggested that stress may induce HPRL and that both inflammation and deregulation in the serotoninergic system could contribute to the HPRL observed in first episode psychotic patients who have not previously received antipsychotic treatment (54). Future studies evaluating the levels of PRL in drug-naïve patients are needed also considering the role of other factors as hormonal influence, age and gender.

Thyroid Status

Regarding evaluation of thyroid function, first of all we must consider that values compatible with good functionality have emerged in both study groups. We know that thyroid disease can always be ruled out when the serum TSH level is normal without

drug administration or in the absence of obvious hypothalamic-pituitary disease (56). Anyway, we found a significant lower level of fT4 in ASD group, compared with SSD group and the meaning of this data is not easy to explain. One recent study investigated the association of thyroid function and suicide attempt in major depressive disorder (MDD) patients, showing a lower serum fT4 level in suicide attempters than non-attempters, but without significant differences in TSH and fT3 levels (57). Further studies are needed to clarify the association between thyroid dysfunction and onset of psychotic and affective disorders (56).

Limitations

Some methodological limitations should be recognized. The small sample size as well as the retrospective design of the study limit the statistical power of the study and, consequently, the generalizability of our results. Moreover, a comparison with a healthy sample would give more value to these findings. Further researches with larger sample size allow us to better characterize abnormalities of hormonal and metabolic parameters with more specific association with diagnostic subgroups. It is important to note that because of the cross-sectional design of the study we cannot infer information about the causality of the relationship between glucose metabolism abnormalities and early onset SSD. Despite these limitations, in our knowledge this is one of the very few studies in this field carried out on a sample of patients under 18.

CONCLUSIONS

In conclusion this study showed an increase in HOMA-IR and higher glucose, insulin levels and free thyroxin in drug naive adolescents with first episode of SSD rather than in first episode of ASD, suggesting the need to perform a metabolic and endocrine screening at the onset of serious mental illness. IR is a testable and treatable modifying factor and early identification may be very important for prevention and management of the progression of cardiometabolic diseases. Further studies with larger sample size and with longitudinal design are needed to better understand the role of endocrinological and metabolic dysfunctions at the onset of severe mental illness also considering influencing factors as age, gender and BMI.

DATA AVAILABILITY STATEMENT

All datasets presented in this study are included in the article/ **Supplementary Material**.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Comitato Etico Interregionale, University of Bari "Aldo Moro". Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

MP designed the study and drafted the manuscript. LM contributed in the literature searches and analyses and in the enrolment of the patients. FM contributed in the literature searches and in revising critically of the manuscript. OG performed the statistical analysis. FF contributed in the enrolment and assessment of the patients. MM contributed in the literature searches and in critical revising of the manuscript. EM coordinated the study group and has been involved in revising critically the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpsyt.2020.00775/full#supplementary-material

REFERENCES

1. Vedal TSJ, Steen NE, Birkeland KI, Dieset I, Reponen EJ, Laskemoen JF, et al, et al. Free thyroxine and thyroid-stimulating hormone in severe mental disorders: A naturalistic study with focus on antipsychotic medication. *J Psychiatr Res* (2018) 106:74-81. doi: 10.1016/j.jpsychires.2018.09.014
2. Arango C, Fraguas D, Parellada M. Differential neurodevelopmental trajectories in patients with early-onset bipolar and schizophrenia disorders. *Schizophr Bull* (2014) 40 Suppl 2(Suppl 2):S138–46. doi: 10.1093/schbul/sbt198
3. Parellada M, Gomez-Vallejo S, Burdeus M, Arango C. Developmental Differences between Schizophrenia and Bipolar Disorder. *Schizophr Bull* (2017) 43(6):1176–89. doi: 10.1093/schbul/sbx126
4. Kloiber S, Rosenblat JD, Husain MI, Ortiz A, Berk M, Quevedo J, et al, et al. Neurodevelopmental pathways in bipolar disorder. *Neurosci Biobehav Rev* (2020) 112:213–226. doi: 10.1016/j.neubiorev.2020.02.005
5. Paus T, Keshavan M, Giedd JN. Why do many psychiatric disorders emerge during adolescence? *Nat Rev Neurosci* (2008) 9(12):947–57. doi: 10.1038/nrn2513
6. Miguel-Hidalgo JJ. Brain structural and functional changes in adolescents with psychiatric disorders. *Int J Adolesc Med Health* (2013) 25(3):245–56. doi: 10.1515/ijamh-2013-0058
7. Trotman HD, Holtzman CW, Ryan AT, Shapiro DI, MacDonald AN, Goulding SM, et al. The development of psychotic disorders in adolescence: A potential role for hormones. *Horm Behav* (2013) 64(2):411–9. doi: 10.1016/j.yhbeh.2013.02.018
8. Pillinger T, D'Ambrosio E, McCutcheon R, Howes OD. Is psychosis a multisystem disorder? A meta-review of central nervous system, immune, cardiometabolic, and endocrine alterations in first-episode psychosis and perspective on potential models. *Mol Psychiatry* (2019) 24(6):776–94. doi: 10.1038/s41380-018-0058-9
9. Benedetti F, Aggio V, Pratesi ML, Greco G, Furlan R. Neuroinflammation in Bipolar Depression. *Front Psychiatry* (2020) 11:71. doi: 10.3389/fpsyt.2020.00071

10. Greenhalgh AM, Gonzalez-Blanco L, Garcia-Rizo C, Fernandez-Egea E, Miller B, Arroyo MB, et al. Meta-analysis of glucose tolerance, insulin, and insulin resistance in antipsychotic-naïve patients with nonaffective psychosis. *Schizophr Res* (2017) 179: 57–63. doi: 10.1016/j.schres.2016.09.026
11. Garcia-Rizo C, Fernandez-Egea E, Oliveira C, Meseguer A, Cabrera B, Mezquida G, et al, et al. Metabolic syndrome or glucose challenge in first episode of psychosis? *Eur Psychiatry* (2017) 41:42–6. doi: 10.1016/j.eurpsy.2016.10.001
12. Mitchell AJ, Vancampfort D, Sweers K, Van Winkel R, Yu W, De Hert M. Prevalence of Metabolic Syndrome and Metabolic Abnormalities in Schizophrenia and Related Disorders-A Systematic Review and Meta-Analysis. *Schizophr Bull* (2013) 39:306–18. doi: 10.1093/schbul/sbr148
13. Kucukgoncu S, Kosir U, Zhou E, Sullivan E, Srihari VH, Tek C. Glucose metabolism dysregulation at the onset of mental illness is not limited to first episode psychosis: A systematic review and meta-analysis. *Early Interv. Psychiatry* (2019) 13(5):1021–31. doi: 10.1111/eip.12749
14. Wulsin LR, Blom TJ, Durling M, Welge JA, DelBello MP, Adler CM, et al. Cardiometabolic risks and omega-3 index in recent-onset bipolar I disorder. *Bipolar Disord* (2018) 20(7):658–65. doi: 10.1111/bdi.12633
15. Sylvia LG, Shelton RC, Kemp DE, Bernstein EE, Friedman ES, Brody BD, et al, et al. Medical burden in bipolar disorder: Findings from the Clinical and Health Outcomes Initiative in Comparative Effectiveness for Bipolar Disorder study (Bipolar CHOICE). *Bipolar Disord* (2015) 17(2):212–23. doi: 10.1111/bdi.12243
16. Nasca C, Watson-Lin K, Bigio B, Robakis TK, Myoraku A, Wroolie TE, et al. Childhood trauma and insulin resistance in patients suffering from depressive disorders. *Exp Neurol* (2019) 315:15–20. doi: 10.1016/j.expneurol.2019.01.005
17. Goldstein BI, Carnethon MR, Matthews KA, McIntyre RS, Miller GE, Raghuveer G, et al. Major Depressive Disorder and Bipolar Disorder Predispose Youth to Accelerated Atherosclerosis and Early Cardiovascular Disease: A Scientific Statement from the American Heart Association. *Circulation* (2015) 132:965–86. doi: 10.1161/CIR.0000000000000229
18. Agarwal SM, Caravaggio F, Costa-Dookhan KA, Castellani L, Kowalchuk C, Asgariroozbehani R, et al. Brain insulin action in schizophrenia: Something borrowed and something new. *Neuropharmacology* (2020) 163:107633. doi: 10.1016/j.neuropharm.2019.05.010
19. Calkin CV. Insulin resistance takes center stage: a new paradigm in the progression of bipolar disorder. *Ann Med* (2019) 51(5–6):281–93. doi: 10.1080/07853890.2019.1659511
20. Bastemir M, Akin F, Emral R, Alkis E. Impact of insulin sensitivity in relationship with prolactin and thyroid stimulating hormone. *Exp Clin Endocrinol Diabetes* (2007) 115:257–60. doi: 10.1055/s-2007-960492
21. Li J, Rice MS, Huang T, Hankinson SE, Clevenger CV, Hu FB, et al. Circulating prolactin concentrations and risk of type 2 diabetes in US women. *Diabetologia* (2018) 61:2549–60. doi: 10.1007/s00125-018-4733-9
22. Wagner R, Heni M, Linder K, Ketterer C, Peter A, Böhm A, et al, et al. Age-dependent association of serum prolactin with glycaemia and insulin sensitivity in humans. *Acta Diabetol.* (2014) 51:71–8. doi: 10.1007/s00592-013-0493-7
23. Le TN, Celi FS, Wickham EP. Thyrotropin Levels Are Associated with Cardiometabolic Risk Factors in Euthyroid Adolescents. *Thyroid* (2016) 26:1441–9. doi: 10.1089/thy.2016.0055
24. Lundbäck V, Ekbom K, Hagman E, Dahlman I, Marcus C. Thyroid-Stimulating Hormone, Degree of Obesity, and Metabolic Risk Markers in a Cohort of Swedish Children with Obesity. *Horm Res Paediatr* (2017) 88:140–6. doi: 10.1159/000475993
25. Grattan DR. The hypothalamo-prolactin axis. *J Endocrinol* (2015) 226:T101–22. doi: 10.1530/JOE-15-0213
26. Lally J, Ajnakina O, Stubbs B, Williams HR, Colizzi M, Carra E, et al. Hyperprolactinaemia in first episode psychosis - A longitudinal assessment. *Schizophr Res* (2017) 189:117–25. doi: 10.1016/j.schres.2017.07.037
27. Del Cacho N, Butjosa A, Vila-Badia R, Cuadras D, Kaplan M, Rubio-Abadal E, et al, et al. Prolactin levels in drug-naïve first episode nonaffective psychosis patients compared with healthy controls. Sex differences. *Psychiatry Res* (2019) 276:218–22. doi: 10.1016/j.psychres.2019.03.027
28. Reeves KW, Okereke OI, Qian J, Tworoger SS, Rice MS, Hankinson SE. Antidepressant use and circulating prolactin levels. *Cancer Causes Control* (2016) 27(7):853–61. doi: 10.1007/s10552-016-0758-x
29. Mullur R, Liu YY, Brent GA. Thyroid hormone regulation of metabolism. *Physiol Rev* (2014) 94:355–82. doi: 10.1152/physrev.00030.2013
30. Teixeira P de F dos S, dos Santos PB, Pazos-Moura CC. The role of thyroid hormone in metabolism and metabolic syndrome. *Ther Adv Endocrinol Metab* (2020) 11:2042018820917869. doi: 10.1177/2042018820917869
31. Sharma N, Dutta D, Sharma LK. Hyperprolactinemia in children with subclinical hypothyroidism. *JCRPE J Clin Res Pediatr Endocrinol* (2017) 9:350–4. doi: 10.4274/jcrpe.4536
32. Bičíková M, Hampl R, Hill M, Řípová D, Mohr P, Putz Z, et al. Neuro- and immunomodulatory steroids and other biochemical markers in drug-naive schizophrenia patients and the effect of treatment with atypical antipsychotics. *Neuroendocrinol. Lett* (2011) 32(2):141–7.
33. Othman SS, Kadir KA, Hassan J, Hong GK, Singh BB, Raman N. High prevalence of thyroid function test abnormalities in chronic schizophrenia. *Australas Psychiatry* (1994) 28(4):620–4. doi: 10.3109/00048679409080785
34. Santos NC, Costa P, Ruano D, MacEdo A, Soares MJ, Valente J, et al. Revisiting thyroid hormones in schizophrenia. *J Thyroid Res* (2012) 2012:569147. doi: 10.1155/2012/569147
35. Barbero JD, Palacín A, Serra P, Solé M, Ortega L, Cabezas Á, et al. Association between anti-thyroid antibodies and negative symptoms in early psychosis. *Early Interv. Psychiatry* (2019) 14(4):470–75. doi: 10.1111/eip.12873
36. Petruzzelli MG, Margari M, Peschechera A, de Giambattista C, De Giacomo A, Matera E, et al. Hyperprolactinemia and insulin resistance in drug naive patients with early onset first episode psychosis. *BMC Psychiatry* (2018) 18 (1):246. doi: 10.1186/s12888-018-1827-3
37. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders* (Fifth ed.). Arlington, VA: American Psychiatric Publishing (2013) pp. 5–25. doi: 10.1176/appi.books.9780890425596.744053
38. Kay SR, Fiszbein A, Opler LA. The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull* (1987) 13(2):261–76. doi: 10.1093/schbul/13.2.261
39. Hamilton MC. Hamilton Depression Rating Scale (HAM-D). *Redloc* (1960) 23:56–62. doi: 10.1111/j.1600-0447.1986.tb10903.x
40. Young RC, Biggs JT, Ziegler VE, Meyer DA. Young Mania Rating Scale. In: *Handbook of Psychiatric Measures*. Washington, DC: American Psychiatric Association (2004) 2000:540–42.
41. Burrows R, Correa-Burrows P, Reyes M, Blanco E, Albala C, Gahagan S. Healthy chilean adolescents with HOMA-IR ≥ 2.6 have increased cardiometabolic risk: Association with genetic, biological, and environmental factors. *J Diabetes Res* (2015) 2015:783296. doi: 10.1155/2015/783296
42. Shashaj B, Luciano R, Contoli B, Morino GS, Spreghini MR, Rustico C, et al. Reference ranges of HOMA-IR in normal-weight and obese young Caucasians. *Acta Diabetol.* (2016) 53(2):251–60. doi: 10.1007/s00592-015-0782-4
43. de Andrade MIS, Oliveira JS, Leal VS, da Lima NMS, Costa EC, de Aquino NB, et al. Identification of cutoff points for Homeostatic Model Assessment for Insulin Resistance index in adolescents: Systematic review. *Rev Paul Pediatr* (2016) 34(2):234–42. doi: 10.1016/j.rppede.2016.01.004
44. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* (1972) 18(6):499–502. doi: 10.1093/clinchem/18.6.499
45. De Jesus JM. Expert panel on integrated guidelines for cardiovascular health and risk reduction in children and adolescents: Summary report. *Pediatrics* (2011) 128:S213. doi: 10.1542/peds.2009-2107C
46. Wedervang-Resell K, Friis S, Lonning V, Smelror RE, Johannessen C, Agartz I, et al. Lipid alterations in adolescents with early-onset psychosis may be independent of antipsychotic medication. *Schizophr Res* (2020) 216:295–301. doi: 10.1016/j.schres.2019.11.039
47. Jensen KG, Correll CU, Rudå D, Klauber DG, Stentebjerg-Olesen M, Fagerlund B, et al. Pretreatment cardiometabolic status in youth with early-onset psychosis: Baseline results from the TEA trial. *J Clin Psychiatry* (2017) 78:e1035–46. doi: 10.4088/JCP.15m10479
48. Straub RH. Insulin resistance, selfish brain, and selfish immune system: An evolutionarily positively selected program used in chronic inflammatory diseases. *Arthritis Res Ther* (2014) 16:S4. doi: 10.1186/ar4688
49. Li Z, Chen P, Chen J, Xu Y, Wang Q, Li X, et al. Glucose and Insulin-Related Traits, Type 2 Diabetes and Risk of Schizophrenia: A Mendelian

Randomization Study. *EBioMedicine* (2018) 34:182–8. doi: 10.1016/j.ebiom.2018.07.037
50. Postolache TT, del Bosque-Plata L, Jabbour S, Vergare M, Wu R, Gragnoli C. Co-shared genetics and possible risk gene pathway partially explain the comorbidity of schizophrenia, major depressive disorder, type 2 diabetes, and metabolic syndrome. *Am J Med Genet Part B Neuropsychiatr Genet* (2019) 180:186–203. doi: 10.1002/ajmg.b.32712
51. Keinänen J, Mantere O, Kieseppä T, Mäntylä T, Torniainen M, Lindgren M, et al. Early insulin resistance predicts weight gain and waist circumference increase in first-episode psychosis - A one year follow-up study. *Schizophr Res* (2015) 169:458–63. doi: 10.1016/j.schres.2015.11.002
52. Nogueira-de-Almeida CA, de Mello ED. Different criteria for the definition of insulin resistance and its relation with Dyslipidemia in overweight and obese children and adolescents. *Pediatr Gastroenterol Hepatol. Nutr* (2018) 21 (1):59–67. doi: 10.5223/pghn.2018.21.1.59
53. Aston J, Rechsteiner E, Bull N, Borgwardt S, Gschwandtner U, Riecher-Rössler A. Hyperprolactinaemia in early psychosis-not only due to antipsychotics. *Prog Neuropsychopharmacol Biol Psychiatry* (2010) 34 (7):1342–4. doi: 10.1016/j.pnpbp.2010.02.019
54. Riecher-Rössler A, Rybakowski JK, Pflueger MO, Beyrau R, Kahn RS, Malik P, et al. Hyperprolactinemia in antipsychotic-naive patients with first-episode psychosis. *Psychol Med* (2013) 43(12):2571–82. doi: 10.1017/S0033291713000226
55. Ittig S, Studerus E, Heitz U, Menghini-Müller S, Beck K, Egloff L, et al. Sex differences in prolactin levels in emerging psychosis: Indication for enhanced stress reactivity in women. *Schizophr Res* (2017) 189:111–16. doi: 10.1016/j.schres.2017.02.010
56. Li H, Yuan X, Liu L, Zhou J, Li C, Yang P, et al. Clinical evaluation of various thyroid hormones on thyroid function. *Int J Endocrinol* (2014) 2014:618572. doi: 10.1155/2014/618572
57. Peng R, Dai W, Li Y. Low serum free thyroxine level is correlated with lipid profile in depressive patients with suicide attempt. *Psychiatry Res* (2018) 266:111–15. doi: 10.1016/j.psychres.2018.05.059

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

MINI REVIEW
published: 21 August 2020
doi: 10.3389/fpsyt.2020.00852

Maternal Immune Activation and the Development of Dopaminergic Neurotransmission of the Offspring: Relevance for Schizophrenia and Other Psychoses

Argel Aguilar-Valles, Brandon Rodrigue and Edna Matta-Camacho*

Department of Neuroscience, Carleton University, Ottawa, ON, Canada

Prenatal infections have been linked to the development of schizophrenia (SCZ) and other neurodevelopmental disorders in the offspring, and work in animal models indicates that this is to occur through the maternal inflammatory response triggered by infection. Several studies in animal models demonstrated that acute inflammatory episodes are sufficient to trigger brain alterations in the adult offspring, especially in the mesolimbic dopamine (DA) system, involved in the pathophysiology of SCZ and other disorders involving psychosis. In the current review, we synthesize the literature on the clinical studies implicating prenatal infectious events in the development of SCZ. Then, we summarize evidence from animal models of maternal immune activation (MIA) and the behavioral and molecular alterations relevant for the function of the DAergic system. Furthermore, we discuss the evidence supporting the involvement of maternal cytokines, such as interleukin 6 (IL-6) and leptin (a hormone with effects on inflammation) in mediating the effects of MIA on the fetal brain, leading to the long-lasting effects on the offspring. In particular, IL-6 has been involved in mediating the effects of MIA animal models in the offspring through actions on the placenta, induction of IL-17a, or triggering the decrease in non-heme iron (hypoferremia). Maternal infection is very likely interacting with additional genetic and environmental risk factors in the development of SCZ; systematically investigating how these interactions produce specific phenotypes is the next step in understanding the etiology of complex psychiatric disorders.

Keywords: maternal infection, schizophrenia, dopamine, animal models, cytokines, IL-6, iron, leptin

OPEN ACCESS

Edited by:
Grazia Rutigliano,
University of Pisa, Italy

Reviewed by:
Urs Meyer,
ETH Zürich, Switzerland
Felice Iasevoli,
University of Naples Federico II, Italy

****Correspondence:***
Argel Aguilar-Valles
argel.aguilavalles@carleton.ca

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *06 May 2020*
Accepted: *04 August 2020*
Published: *21 August 2020*

Citation:
Aguilar-Valles A, Rodrigue B and Matta-Camacho E (2020) Maternal Immune Activation and the Development of Dopaminergic Neurotransmission of the Offspring: Relevance for Schizophrenia and Other Psychoses. Front. Psychiatry 11:852. doi: 10.3389/fpsyt.2020.00852

INTRODUCTION

We are currently undergoing a SARS-CoV-2 pandemic, which like previous viral outbreaks [e.g., Zika (1)] can leave behind sequelae of health complications, including direct effects in the nervous system (2) and alterations of brain development if infections occur during perinatal stages.

Indeed, maternal infection has been identified as a risk factor for several neurodevelopmental disorders such as cerebral palsy, intellectual disability, autism spectrum disorder (ASD), bipolar disorder

(BD), and schizophrenia (SCZ) (3–9). We will focus on reviewing the effects of maternal infection on the dopaminergic neurotransmitter system and the link with psychosis, particularly SCZ.

SCZ is one of the top leading causes of disability worldwide (10) and the seventh most costly medical illness in modern society (11, 12). SCZ is characterized by psychotic symptoms such as delusions and hallucinations (also known as the positive symptom dimension); alterations in drive and volition, including lack of motivation, blunted affect, social withdrawal, and reduction in spontaneous speech (the negative symptom dimension) and alterations in neurocognition, including difficulties in memory, attention, and executive functioning (the cognitive symptom dimension) (13–15).

The positive symptoms of SCZ overlap with different psychiatric disorders. Indeed, psychosis is also frequent during mood episodes in BD, severe depression, substance use disorder and neurodegenerative disorders (16–18). Intriguingly, some SCZ-like psychopathological abnormalities (i.e., paranoid delusional thinking and auditory hallucinations) are expressed in an attenuated form in 5–8% of the otherwise healthy population, especially in individuals with schizotypal or schizoid personality traits (13, 19). This extensive overlapping of symptoms and genetic risk factors with other psychiatric and neurological conditions is suggestive of a common underlying neuropathophysiology for these disorders, which, rather than discrete diagnoses, may represent a continuum that extends to the general population (13, 19, 20).

THE DOPAMINE THEORY OF SCZ AND PSYCHOSIS

The classical dopamine (DA) hypothesis of SCZ (21) states that the hyperactivity of the DA system is responsible for the symptoms of the disorder. More recently, this hypothesis was elaborated to include the proposal that the hyperactivity of the mesolimbic DA system (**Figure 1A**) contributes to positive symptoms in SCZ. Meanwhile, impaired function of the DA system in the prefrontal cortex (PFC, **Figure 1A**) contributes to the cognitive symptom dimension (22, 23).

The DA hypothesis of SCZ derives, in part, from the identification of the mechanisms of action of antipsychotics, many of which act as DA receptor 2 (D2 receptor) blockers (15). Furthermore, pharmacological studies show that a single exposure to amphetamine (AMPH), a stimulant drug that increases extracellular levels of DA in striatal and cortical regions *via* release and reverse transport (24, 25), evokes or exacerbates positive symptoms in SCZ patients at doses which do not induce psychosis in healthy subjects (26–28). Imaging studies demonstrate that a significant number of non-medicated SCZ patients show marked elevation of AMPH-induced striatal dopamine release in comparison to healthy volunteers (29–31). This response correlates significantly with the emergence or worsening of positive symptoms (31–35).

Understanding the etiology of SCZ is an active area of research. However, evidence accumulated in the last three decades on environmental risk factors that affect early

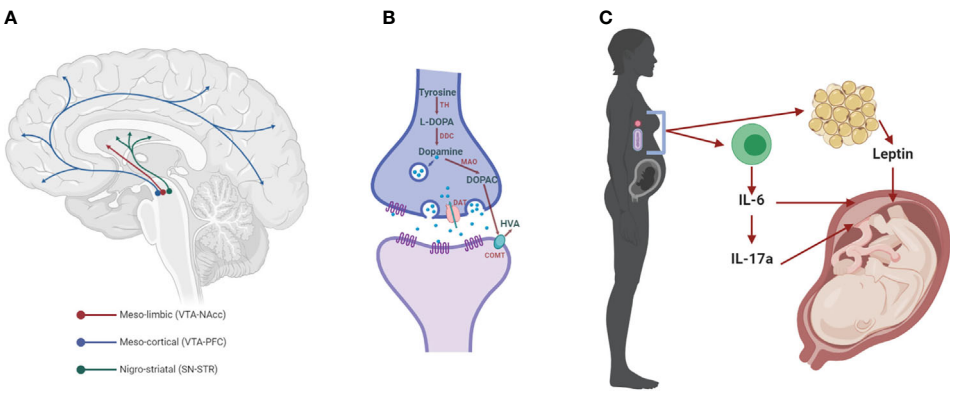

FIGURE 1 | The dopaminergic system and mediators of maternal immune activation. **(A)** The meso-limbic DA neurons have their cell bodies in the ventral tegmental area (VTA) and terminals innervate the nucleus accumbens (NAcc). Other VTA neurons project to the prefrontal cortex (PFC), constituting the meso-cortical system. The nigro-striatal DA neurons lie in the substantia nigra (SN) and project to the dorsal striatum (STR). **(B)** Dopaminergic synapse, where dopamine is synthesized by the conversion of tyrosine into L-3,4-dihydroxyphenylalanine (L-DOPA) by the enzyme tyrosine hydroxylase (TH). L-DOPA is then converted to dopamine by the L-DOPA decarboxylase (DDC). Once packaged in synaptic vesicles and released to the extracellular space, dopamine can act on its receptors (DRs) on the post- and pre-synaptic membrane. Dopamine neurotransmission is terminated when the dopamine transporter (DAT) reuptakes the neurotransmitter to the pre-synaptic side, where it can be metabolized into 3,4-dihydroxyphenylacetic acid (DOPAC) by the monoamine oxidase (MAO) and homovanillic acid (HVA) by the catechol-O-methyl transferase (COMT). **(C)** Maternal immune activation (MIA) with bacteria or viruses leads to the activation of immune cells that release cytokines, including interleukin 6 (IL-6) and, in turn, IL-17a. Both of these cytokines affect brain development in the fetus, increasing the risk for neurodevelopmental disorders, such as SCZ (SCZ). These cytokines can act indirectly on the placenta, or in the case of IL-6 through the induction of hypoferremia, a reduction in circulating non-heme iron. Adipose tissue can also release hormones such as leptin, which affects fetal development.

neurodevelopment during pregnancy has led to the proposal of the neurodevelopmental hypothesis for SCZ (36–38). In this sense, accumulating evidence suggests that perinatal insults also contribute to an increase risk of developing BD (9), particularly those cases with psychoses (8).

NEURODEVELOPMENTAL ETIOLOGY OF SCZ AND OTHER PSYCHIATRIC DISORDERS

SCZ has been hypothesized to have a neurodevelopmental origin (22): an outcome of an aberration in developmental processes within the brain, which begins long before the onset of the clinical symptoms (36, 39). There are numerous independent lines of evidence supporting this hypothesis. For example, there is a conspicuous absence of gross physical damage or signs of progressive neurodegeneration in SCZ (22, 39). Besides, children that go on to develop SCZ present behavioral, physical and brain morphological alterations, before the clinical onset of psychosis (15, 36, 39–41).

Finally, individuals who develop SCZ are more likely to have experienced pre- or perinatal adverse events (22, 42), or adolescent disturbances in brain development, compared to control individuals (36, 39, 41, 43). These adverse events include intrauterine growth retardation, pregnancy and birth complications (44), nutritional deficiencies (45, 46) maternal stress (47), and maternal infections (48).

There is also mounting evidence for the role of neurodevelopmental disturbances in the etiology of BD, as thoroughly reviewed by (9). In this regard, there is high co-morbidity between BD and other developmental disorders such as attention-deficit/hyperactivity disorder (ADHD) and ASD (9). Remarkably, there are increased rates of BD due to obstetric complications, cesarean section birth and perinatal infection (8, 9, 49, 50).

MATERNAL INFECTION, SCZ, AND OTHER NEURODEVELOPMENTAL DISORDERS

Ecological studies, including those based on the subjective report of illness, suggest that SCZ is more prevalent in the offspring of women that were pregnant during periods of influenza epidemics (51, 52), as well as other types of infections, including diphtheria, pneumonia, measles, varicella zoster, mumps and poliovirus (4, 53–56). Similarly, SCZ is more prevalent among individuals born to pregnancies that occur during winter, a season associated with an increased frequency of respiratory infections (36, 51, 57). The main limitation of these studies is that "exposure to infection" was defined solely by the fact that the individual was pregnant during the time of the epidemic (i.e., based on the date of birth of the offspring).

It was later shown that SCZ in the offspring is significantly associated with maternal infections using individual biomarkers of illness in the maternal serum or clinical diagnoses (4). These included respiratory infections (58), influenza (59, 60), rubella (61, 62), *Toxoplasma gondii* (63, 64), herpes simplex virus-2 (HSV-2) (65, 66), maternal genital or reproductive infections (67), and maternal bacterial infections (68). Some of these studies used a broad definition of psychosis, where both non-affective (e.g., SCZ) and affective (e.g., major depression or BDs with psychotic features) psychiatric disorders were included (62, 66, 69). This suggests that maternal infection may be involved in the development of psychotic features that may not be necessarily restricted to those that characterize SCZ, but several other disorders as well. Indeed, MIA involving influenza has been linked to BD (9) [and Toxoplasma gondii infections to a lesser extent (70)], especially for those patients that also develop psychotic features (8).

What remains unclear from these studies is the critical stage (s) of gestation during which the developing brain may be more vulnerable to this prenatal insult. Indeed, those studies that have tried to dissect a specific trimester of gestation where vulnerability to MIA may be increased, have provided evidence for all three trimesters (58, 59, 68, 69, 71, 72). Overall, effect sizes of prenatal infection across gestation and development of SCZ in the offspring range from 1.5 to 7 for different infections (73), suggesting the existence of additional factors that confer vulnerability or resilience (6).

The wide variety of infections associated with SCZ and BD with psychosis suggests that there may be a common factor underlying increased susceptibility (74). Therefore, it has been hypothesized that maternal immune activation (MIA), and the inflammatory mediators released following all types of infections (4), may be fundamentally involved. Epidemiological studies have provided some evidence supporting this hypothesis. Increased levels of maternal pro-inflammatory cytokines, specifically interleukin (IL)-8 (72), tumor necrosis factor (TNF)α (69, 71, 75, 76), IL-6 (71, 75, 76), C-reactive protein (77) are associated with a higher risk of psychosis or SCZ in the offspring. Several animal models have been developed to investigate the immunological and neurobiological link between MIA and altered behavior in the offspring, with heavy emphasis in behavioral alterations.

ANIMAL MODELS OF MATERNAL INFECTION

Initial approaches used prenatal infection with an influenza virus [at gestational day (GD) 9 in mice], followed by the application of a battery of behavioral tests relevant to SCZ in the adult offspring (78). These studies showed that the adult offspring of infected mothers presented, compared to the offspring of control dams, decreased social interaction, reduced exploration in the open field, impaired performance in the novel object test, indicative of impaired working memory as observed in SCZ and diminished PPI of acoustic startle (78). These behavioral alterations are analogous to aspects of SCZ.

Viral Mimetic Poly I:C

Further studies investigated the consequences of MIA using molecular immunogens in rats and mice. The viral mimic polyinosinic:polycytidylic acid (poly I:C) has been used to stimulate the maternal immune system (with one or multiple injections), at several stages of pregnancy in mice or rats, ranging from GD 8.5 until GD 18.5. The effects of these prenatal treatments have been extensively reviewed elsewhere (6, 7, 79–81); thus, we will focus on those consequences more closely relevant for psychosis. Prenatal stimulation with poly I:C induced deficits in an operational measure of sensorimotor gating (82), pre-pulse inhibition of acoustic startle and increased sensitivity to the locomotor activating effects of cocaine, AMPH and methamphetamine, whose locomotor effects depend on the mesolimbic DA system (83–105).

An overall trend regarding these two phenotypes is one where PPI deficits are more consistently observed when MIA occurs at gestational stages earlier than GD 16 in both mice and rats. At the same time, hyper-responsiveness to activators of the mesolimbic dopaminergic system appears when challenging the mothers at any developmental age [reviewed in (79)].

Models of Bacterial Infections

The role of bacterial infection has also been investigated by using the Gram-negative bacterial cell wall component, lipopolysaccharide (LPS). In rats, injections at several stages of gestation, ranging from GD 9 until birth, induced, in the offspring, impairments in PPI, and increases in sensitivity to the locomotor effects of AMPH (106–117). LPS has also been administered either in alternate days (118) or daily throughout pregnancy (119, 120). Similarly to acute LPS administration, these chronic prenatal treatments also induced impairments in PPI (118–120).

Other Models of Inflammation

Turpentine (TURP) is an inflammatory agent whose injection [intramuscular (i.m.)] produces localized necrotic damage (121) and the sequential induction of TNFα and IL-1β at the site of injury, which trigger IL-6 release into the circulation (122, 123). Using TURP at GD 15 or 18 in rats, we found that an earlier challenge with TURP induces greater maternal inflammatory response compared to later in gestation (124). Furthermore, this difference in the inflammatory response during pregnancy correlates with the effect on the offspring, such that treatment at GD 15 induces impairment in PPI and hyper-responsiveness to AMPH, while the same treatment at GD 18 does not affect any of these behaviors (124).

Overall, some of the alterations in behavior induced by either polyI:C or LPS, have been shown to appear in the adult but not in the juvenile offspring (84, 93, 120, 125), as occurs in SCZ patients. Also supporting the validity of the models toward the disorder is the observation that a number of these alterations, including deficits in PPI, were shown to be reversed by either acute or chronic treatment with several antipsychotic drugs in adult or adolescent animals [i.e., haloperidol, chlorpromazine, olanzapine, risperidone or clozapine, which constitute the primary pharmacological treatment for psychotic illness (78, 83–85, 89, 119, 126–132)].

EFFECTS OF MATERNAL INFECTION ON DOPAMINE NEUROTRANSMISSION IN MOUSE MODELS

Given the central role of DA neurotransmission in SCZ, the findings on the exaggerated locomotor response to AMPH and other drugs that stimulate DAergic neurotransmission following MIA, and the effectiveness of antipsychotic treatments to reverse MIA effects, several studies investigated the effects of prenatal immune activation on this neurotransmitter system. One often used approach is the measurement of tissue DA content and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA, **Figure 1B**).

Prenatal poly I:C treatment at GD 15 induces enhanced release of DA from striatal explants in the adult offspring (83). In addition, poly I:C treatment at GD 9 results in increases DA and DOPAC levels in the PFC and the globus pallidus (GP) and HVA in the nucleus accumbens (NAcc) and GP of adult mice (133). Increases in DA are also found in the NAcc following poly I:C treatment at GD 9 (134). Similarly, several injections of poly I:C (GD 12-17) result in elevated levels of DOPAC and HVA in the adult STR (84).

Prenatal LPS treatment has been shown to have somewhat variable effects on DA. For example, daily administration of LPS throughout the entire pregnancy results in increased DA levels in the NAcc of adult animals (P 120, 170, or 400), but lower DA levels in younger animals (P 39) (119, 120). Interestingly, a single LPS administration at GD 10, results in a decrease of DA in the dSTR (135–140). Decreased DA is also found in the NAcc, PFC, amygdala, hippocampus, and hypothalamus, accompanied by decreased levels of HVA in the NAcc and amygdala (P 120) (140). Similarly, decreased DA levels in the NAcc at P 83 were found when escalating doses of LPS were administered daily from GD 15 until 19 (141).

Using MIA with TURP, we found increases in DA, DOPAC, and HVA in the NAcc, but not in the dorsal STR or the PFC of the male offspring in rats (142, 143).

Prenatal poly I:C treatment (GD 9) results in increased tyrosine hydroxylase (TH) immunoreactivity, the rate-limiting enzyme for the synthesis of DA, in the mesencephalon of embryonic mice at GD 13 and 17 (92), as well as in the NAcc and SN of adult mice (P 120) (93). In the NAcc, TH immunoreactivity was decreased at P 35 (93) but increased at P 70 (90, 93). DAT immunoreactivity is also found to be increased in the fetal mesencephalon (GD 17) (92), but decreased in the dSTR at P 35, as well as in the NAcc at GD 19 and P 35 (93). Immunoreactivity of DA receptors, D1 and D2, is reduced in the adult mice's PFC (90, 144) and increased in the NAcc (for both D1 and D2) and dSTR (only D1) (93). In contrast, Ozawa et al. reported that DA D2 receptor's binding is reduced in the STR of adult mice (84). Most of these data are consistent with a scenario of increased synthesis of DA in the adult brain of MIA

offspring, particularly in the meso-limbic, but not in the meso-cortical pathway.

The effects of prenatal LPS administration on these markers of DA neurotransmission are rather conflicting. Borrell et al. (118) found increased TH immunoreactivity in the NAcc and bed nucleus of the stria terminalis in adult rats whose mothers were treated with LPS on alternate days during the entire pregnancy. In contrast, Ling et al. reported in several studies that a single dose of LPS at GD 10 leads to a significant decrease in TH immunoreactivity, which was significant in the SN, at several postnatal ages (P 21, 120, 210, 420, 510) as well as in the VTA of post-weanling rats (135–140). As above, these results support the idea that models of bacterial MIA have different outcomes compared to those involving viral mimetics.

Finally, prenatal TURP administration at GD 15 leads to an increase of TH levels in the NAcc, but not in other DA terminal areas such as the dorsal STR or the PFC, nor in the VTA or SN (124, 142, 143).

Overall, poly I:C and TURP induce molecular changes consistent with hyperactivity of mesolimbic DA neurotransmission, which may underlie the hyperactivity in response to AMPH and other behavioral alterations that can be corrected by administration of antipsychotics.

ROLE OF MATERNAL CYTOKINES IN INDUCING MIA ALTERATIONS

A more causal role for elevated maternal cytokines in SCZ-related alterations has been established through the administration of exogenous cytokines to pregnant rats or mice. These manipulations have been shown to be sufficient to induce several molecular and behavioral effects in the offspring. For example, prenatal administration of IL-6 in mice (at GD 9, 5 μg, i.p.) results in impairments in PPI and other behaviors in the adult offspring, whereas a similar treatment with IFNγ or TNFα does not affect the offspring (86). Significantly, the effect of an influenza virus and poly I:C treatments on the fetal brain transcriptome overlapped to those of IL-6 administration in utero, supporting the idea that many effects of poly I:C are mediated by this cytokine (145).

Importantly, functional inhibition of poly I:C-induced IL-6 in pregnant mice prevented several of the behavioral effects of prenatal poly I:C in the offspring, including impaired PPI (86). Also, the offspring of IL-6 "knock-out" mothers treated with poly I:C, do not present these alterations (86). Similarly, knock-out of IL-6 receptor in the placental trophoblasts prevented several effects of prenatal poly I:C treatment (146), indicating a crucial role of this organ in mediating the effects of MIA.

We also observed that co-treatment with an anti–IL-6 antibody during gestation and TURP prevented the development of a hyper-active DAergic system (143). This prenatal treatment effectively rescued the exaggerated AMPH-induced hyperlocomotion and behavioral sensitization, elevated DA, and TH in the NAcc in the offspring of TURP-treated mothers (143).

IL-6 can, in turn, act in more than one way to affect neurodevelopment (**Figure 1C**). One such mechanism is hypoferremia, a reduction in maternal circulating non-heme iron, which characterizes the acute phase response and is triggered by all types of infection (147, 148). Proper iron homeostasis is fundamental for healthy brain development, especially for the DAergic neurons (149). Indeed, we demonstrated that maternal iron supplementation, which counteracts inflammation-induced hypoferremia, prevented the development of exacerbated responses to a single AMPH injection and enhanced behavioral sensitization following repeated exposure to this drug in the offspring (142). Furthermore, maternal iron supplementation during MIA also reversed the increased levels of TH, DA and its metabolites in the NAcc found in the offspring of mothers treated with TURP (142). Notably, iron levels in the placenta were reduced by MIA (but not in the fetal brain), which were rescued by maternal iron supplementation (142), supporting a role for this organ in mediating the effects of MIA in the development of the brain.

Another potential mediator of MIA, downstream of IL-6, is IL-17a (**Figure 1C**), since blocking the latter cytokine with anti–IL-17a antibodies prevented cortical malformations and the emergence of abnormal behaviors in adult MIA offspring, including impaired social interaction and increases marble-burying behavior (150, 151). Meanwhile, overexpression of the anti-inflammatory cytokine IL-10 in maternal macrophages prevented the MIA-induced deficits in PPI, although in itself, elevated IL-10 also induced other behavioral alterations (91). In addition to IL-6, the hormone leptin has also been implicated on the effects of MIA in the DAergic system.

Leptin

Leptin is the product of *ob* gene (152), a hormone that regulates food intake and energy expenditure (153–155). Leptin is primarily produced by adipose tissue and secreted into the circulation, where levels correlate positively with body fat mass (156, 157). Leptin has a multitude of physiological roles, including regulation of inflammatory processes (158, 159). For example, leptin treatment induces pro-inflammatory cytokines, including TNFα, IL-1β, IL-6, and IFN-γ (160–162). Inflammatory stimuli (e.g., TNFα, IL-1β, LPS, and TURP) in turn increase leptin synthesis (163–167). During the acute inflammatory response, leptin is involved in the induction of several sickness-type responses, such as anorexia and fever (168–173).

Despite its clear involvement in several aspects of the inflammatory response to infection, the role of leptin in brain development has not yet been extensively studied. We demonstrated that neutralization of leptin during MIA was effective in curtailing several alterations induced by prenatal TURP, including the hype-sensitized locomotor response to AMPH, and increases in DA in the NAcc (**Figure 1C**) (143). Intriguingly, leptin could affect the development of the dopaminergic system, as constitutive leptin mutant mice have impaired locomotor response to AMPH, and diminished DA release in the NAcc (174). Leptin can also exert impairing effects or the control of cytokines expression in the placenta (175).

CONCLUDING REMARKS

MIA alters the development of the dopaminergic system and many other neurotransmitter systems and brain regions (5, 6, 79, 80). Maternal cytokines, particularly IL-6, are central in mediating these effects (5). However, other neuroendocrine factors, such as the adipokine leptin, are potentially involved and deserve further investigation.

Maternal infections and other environmental risk factors for SCZ and neurodevelopmental disorders may independently account for a few clinical cases since exposure to them does not always generate the disorder or are implicated in several psychiatric illnesses (6, 39, 79, 80, 176, 177). In this regard, heterogeneity of response characterizes all known environmental risk factors for psychopathology, including the most overwhelming of traumas (176). Such response heterogeneity is associated with pre-existing genetic (175) or epigenetic (i.e., chromatin modifications) differences (178).

This hypothesis implies that in any given population, individual predisposition is directly responsible for the vulnerability or resilience to the environmental causes of many psychiatric conditions (176), including SCZ (39, 179). Regarding vulnerability, there is a significant interaction between maternal HSV-2 seropositivity and *GRIN2B* genetic variation (*GRIN2B* encodes for a NMDA glutamate receptor) (180). Also, exposure to maternal infection has been reported to increase the risk of SCZ only in cases with a family history of psychiatric disorders (181, 182). Animal models of MIA support this notion, as the effects of poly I:C are enhanced when they occur in mice mutant for genes linked to SCZ and other disorders (183–185). Furthermore, interaction with other environmental risk factors, such as maternal diet, gut microbiota, or experiences of peripubertal trauma, can have a synergistic effect with maternal infection or prevent its detrimental effect (6, 79, 186). Therefore, systematically generating translational models of the interaction between genetic and environmental (or environmental and environmental) risk factors for SCZ and other neurodevelopmental and psychiatric disorders appears to be the next step in understanding the etiology of mental illnesses.

AUTHOR CONTRIBUTIONS

AA-V, BR, and EM-C wrote and edited the manuscript.

REFERENCES

1. Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika Virus and Birth Defects–Reviewing the Evidence for Causality. *N Engl J Med* (2016) 374 (20):1981–7. doi: 10.1056/NEJMsr1604338
2. De Felice FG, Tovar-Moll F, Moll J, Munoz DP, Ferreira ST. Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) and the Central Nervous System. *Trends Neurosci* (2020) 43(6):355–7. doi: 10.1016/j.tins.2020.04.004
3. Deverman BE, Patterson PH. Cytokines and CNS development. *Neuron* (2009) 64 (1):61–78. doi: 10.1016/j.neuron.2009.09.002 S0896-6273(09)00680-1
4. Brown AS, Derkits EJ. Prenatal Infection and Schizophrenia: A Review of Epidemiologic and Translational Studies. *Am J Psychiatry* (2010) 167(3):261–80. doi: 10.1176/appi.ajp.2009.09030361
5. Estes ML, McAllister AK. Maternal immune activation: Implications for neuropsychiatric disorders. *Science* (2016) 353(6301):772–7. doi: 10.1126/science.aag3194
6. Meyer U. Neurodevelopmental Resilience and Susceptibility to Maternal Immune Activation. *Trends Neurosci* (2019) 42(11):793–806. doi: 10.1016/j.tins.2019.08.001
7. Knuesel I, Chicha L, Britschgi M, Schobel SA, Bodmer M, Hellings JA, et al. Maternal immune activation and abnormal brain development across CNS disorders. *Nat Rev Neurol* (2014) 10(11):643–60. doi: 10.1038/nrneurol.2014.187
8. Canetta SE, Bao Y, Co MD, Ennis FA, Cruz J, Terajima M, et al. Serological documentation of maternal influenza exposure and bipolar disorder in adult offspring. *Am J Psychiatry* (2014) 171(5):557–63. doi: 10.1176/appi.ajp.2013.13070943
9. Kloiber S, Rosenblat JD, Husain MII, Ortiz A, Berk M, Quevedo J, et al. Neurodevelopmental pathways in bipolar disorder. *Neurosci Biobehav Rev* (2020) 112:213–26. doi: 10.1016/j.neubiorev.2020.02.005
10. G. B. D. 2017-Disease-Injury-Incidence-Prevalence-Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet (Lond Engl)* (2018) 392(10159):1789–858. doi: 10.1016/S0140-6736(18)32279-7
11. Freedman R. Schizophrenia. *N Engl J Med* (2003) 349(18):1738–49. doi: 10.1056/NEJMra035458349/18/1738
12. Chong HY, Teoh SL, Wu DB, Kotirum S, Chiou CF, Chaiyakunapruk N. Global economic burden of schizophrenia: a systematic review. *Neuropsychiatr Dis Treat* (2016) 12:357–73. doi: 10.2147/NDT.S96649
13. van Os J, Kapur S. Schizophrenia. *Lancet* (2009) 374(9690):635–45. doi: 10.1016/S0140-6736(09)60995-8
14. Walker E, Kestler L, Bollini A, Hochman KM. Schizophrenia: etiology and course. *Annu Rev Psychol* (2004) 55:401–30. doi: 10.1146/annurev.psych.55.090902.141950
15. Ross CA, Margolis RL, Reading SA, Pletnikov M, Coyle JT. Neurobiology of schizophrenia. *Neuron* (2006) 52(1):139–53. doi: 10.1016/j.neuron.2006.09.015
16. Charney AW, Mullins N, Park YJ, Xu J. On the diagnostic and neurobiological origins of bipolar disorder. *Transl Psychiatry* (2020) 10 (1):118. doi: 10.1038/s41398-020-0796-8
17. Butala A, Shepard M, Pontone G. Neuropsychiatric aspects of Parkinson disease psychopharmacology: Insights from circuit dynamics. *Handb Clin Neurol* (2019) 165:83–121. doi: 10.1016/B978-0-444-64012-3.00007-1
18. Deardorff WJ, Grossberg GT. Behavioral and psychological symptoms in Alzheimer's dementia and vascular dementia. *Handb Clin Neurol* (2019) 165:5–32. doi: 10.1016/B978-0-444-64012-3.00002-2
19. Taylor JH, Calkins ME, Gur RE. Markers of Psychosis Risk in the General Population. *Biol Psychiatry* (2020) 88(4):337–48. doi: 10.1016/j.biopsych.2020.02.002
20. Geschwind DH, Flint J. Genetics and genomics of psychiatric disease. *Science* (2015) 349(6255):1489–94. doi: 10.1126/science.aaa8954
21. Carlsson A, Lindqvist M. Effect of Chlorpromazine or Haloperidol on Formation of 3methoxytyramine and Normetanephrine in Mouse Brain. *Acta Pharmacol Toxicol (Copenh)* (1963) 20:140–4. doi: 10.1111/j.1600-0773.1963.tb01730.x
22. Weinberger DR. Implications of normal brain development for the pathogenesis of schizophrenia. *Arch Gen Psychiatry* (1987) 44(7):660–9. doi: 10.1001/archpsyc.1987.01800190080012
23. Davis KL, Kahn RS, Ko G, Davidson M. Dopamine in schizophrenia: a review and reconceptualization. *Am J Psychiatry* (1991) 148(11):1474–86. doi: 10.1176/ajp.148.11.1474
24. Sulzer D, Chen TK, Lau YY, Kristensen H, Rayport S, Ewing A. Amphetamine redistributes dopamine from synaptic vesicles to the cytosol and promotes reverse transport. *J Neurosci* (1995) 15(5 Pt 2):4102–8. doi: 10.1523/JNEUROSCI.15-05-04102.1995

25. Erreger K, Grewer C, Javitch JA, Galli A. Currents in response to rapid concentration jumps of amphetamine uncover novel aspects of human dopamine transporter function. *J Neurosci* (2008) 28(4):976–89. doi: 10.1523/JNEUROSCI.2796-07.2008
26. Lieberman JA, Kane JM, Alvir J. Provocative tests with psychostimulant drugs in schizophrenia. *Psychopharmacol (Berl)* (1987) 91(4):415–33. doi: 10.1007/BF00216006
27. Lieberman JA, Sheitman BB, Kinon BJ. Neurochemical sensitization in the pathophysiology of schizophrenia: deficits and dysfunction in neuronal regulation and plasticity. *Neuropsychopharmacology* (1997) 17(4):205–29. doi: 10.1016/S0893-133X(97)00045-6
28. Yui K, Goto K, Ikemoto S, Ishiguro T, Angrist B, Duncan GE, et al. Neurobiological basis of relapse prediction in stimulant-induced psychosis and schizophrenia: the role of sensitization. *Mol Psychiatry* (1999) 4(6):512–23. doi: 10.1038/sj.mp.4000575
29. Laruelle M, Abi-Dargham A, van Dyck CH, Gil R, D'Souza CD, Erdos J, et al. Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects. *Proc Natl Acad Sci U.S.A.* (1996) 93(17):9235–40. doi: 10.1073/pnas.93.17.9235
30. Abi-Dargham A, van de Giessen E, Slifstein M, Kegeles LS, Laruelle M. Baseline and amphetamine-stimulated dopamine activity are related in drug-naive schizophrenic subjects. *Biol Psychiatry* (2009) 65(12):1091–3. doi: 10.1016/j.biopsych.2008.12.007
31. Abi-Dargham A, Gil R, Krystal J, Baldwin RM, Seibyl JP, Bowers M, et al. Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort. *Am J Psychiatry* (1998) 155(6):761–7. doi: 10.1176/ajp.155.6.761
32. Abi-Dargham A, Rodenhiser J, Printz D, Zea-Ponce Y, Gil R, Kegeles LS, et al. Increased baseline occupancy of D2 receptors by dopamine in schizophrenia. *Proc Natl Acad Sci U.S.A.* (2000) 97(14):8104–9. doi: 10.1073/pnas.97.14.8104
33. Laruelle M, Abi-Dargham A, Gil R, Kegeles L, Innis R. Increased dopamine transmission in schizophrenia: relationship to illness phases. *Biol Psychiatry* (1999) 46(1):56–72. doi: 10.1016/S0006-3223(99)00067-0
34. Breier A, Su TP, Saunders R, Carson RE, Kolachana BS, de Bartolomeis A, et al. Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: evidence from a novel positron emission tomography method. *Proc Natl Acad Sci U.S.A.* (1997) 94(6):2569–74. doi: 10.1073/pnas.94.6.2569
35. Meyer-Lindenberg A, Miletich RS, Kohn PD, Esposito G, Carson RE, Quarantelli M, et al. Reduced prefrontal activity predicts exaggerated striatal dopaminergic function in schizophrenia. *Nat Neurosci* (2002) 5(3):267–71. doi: 10.1038/nn804
36. Fatemi SH, Folsom TD. The neurodevelopmental hypothesis of schizophrenia, revisited. *Schizophr Bull* (2009) 35(3):528–48. doi: 10.1093/schbul/sbn187
37. Murray RM, Bhavsar V, Tripoli G, Howes O. 30 Years on: How the Neurodevelopmental Hypothesis of Schizophrenia Morphed Into the Developmental Risk Factor Model of Psychosis. *Schizophr Bull* (2017) 43(6):1190–6. doi: 10.1093/schbul/sbx121
38. Owen MJ, O'Donovan MC, Thapar A, Craddock N. Neurodevelopmental hypothesis of schizophrenia. *Br J Psychiatry* (2011) 198(3):173–5. doi: 10.1192/bjp.bp.110.084384
39. Rapoport JL, Addington AM, Frangou S, Psych MR. The neurodevelopmental model of schizophrenia: update 2005. *Mol Psychiatry* (2005) 10(5):434–49. doi: 10.1038/sj.mp.4001642
40. Danielyan A, Nasrallah HA. Neurological disorders in schizophrenia. *Psychiatr Clin North Am* (2009) 32(4):719–57. doi: 10.1016/j.psc.2009.08.004S0193-953X(09)00075-6
41. Mathalon DH, Rapoport JL, Davis KL, Krystal JH. Neurotoxicity, neuroplasticity, and magnetic resonance imaging morphometry. *Arch Gen Psychiatry* (2003) 60(8):846–8; author reply 848–9. doi: 10.1001/archpsyc.60.8.846
42. Gilmore JH, van Tol J, Kliewer MA, Silva SG, Cohen SB, Hertzberg BS, et al. Mild ventriculomegaly detected in utero with ultrasound: clinical associations and implications for schizophrenia. *Schizophr Res* (1998) 33(3):133–40. doi: 10.1016/S0920-9964(98)00073-5
43. Feinberg I. Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence? *J Psychiatr Res* (1982) 17(4):319–34. doi: 10.1016/0022-3956(82)90038-3
44. Brown AS. The environment and susceptibility to schizophrenia. *Prog Neurobiol* (2011) 93(1):23–58. doi: 10.1016/j.pneurobio.2010.09.003
45. Eyles DW, Trzaskowski M, Vinkhuyzen AAE, Mattheisen M, Meier S, Gooch H, et al. The association between neonatal vitamin D status and risk of schizophrenia. *Sci Rep* (2018) 8(1):17692. doi: 10.1038/s41598-018-35418-z
46. McGrath JJ, Eyles DW, Pedersen CB, Anderson C, Ko P, Burne TH, et al. Neonatal vitamin D status and risk of schizophrenia: a population-based case-control study. *Arch Gen Psychiatry* (2010) 67(9):889–94. doi: 10.1001/archgenpsychiatry.2010.110
47. Khashan AS, Abel KM, McNamee R, Pedersen MG, Webb RT, Baker PN, et al. Higher risk of offspring schizophrenia following antenatal maternal exposure to severe adverse life events. *Arch Gen Psychiatry* (2008) 65(2):146–52. doi: 10.1001/archgenpsychiatry.2007.20
48. Brown AS, Patterson PH. Maternal infection and schizophrenia: implications for prevention. *Schizophr Bull* (2011) 37(2):284–90. doi: 10.1093/schbul/sbq146
49. Martelon M, Wilens TE, Anderson JP, Morrison NR, Wozniak J. Are obstetrical, perinatal, and infantile difficulties associated with pediatric bipolar disorder? *Bipolar Disord* (2012) 14(5):507–14. doi: 10.1111/j.1399-5618.2012.01027.x
50. Chudal R, Sourander A, Polo-Kantola P, Hinkka-Yli-Salomaki S, Lehti V, Sucksdorff D, et al. Perinatal factors and the risk of bipolar disorder in Finland. *J Affect Disord* (2014) 155:75–80. doi: 10.1016/j.jad.2013.10.026
51. Machon RA, Mednick SA, Schulsinger F. The interaction of seasonality, place of birth, genetic risk and subsequent schizophrenia in a high risk sample. *Br J Psychiatry* (1983) 143:383–8. doi: 10.1192/bjp.143.4.383
52. Mednick SA, Machon RA, Huttunen MO, Bonett D. Adult schizophrenia following prenatal exposure to an influenza epidemic. *Arch Gen Psychiatry* (1988) 45(2):189–92. doi: 10.1001/archpsyc.1988.01800260109013
53. Watson CG, Kucala T, Tilleskjor C, Jacobs L. Schizophrenic birth seasonality in relation to the incidence of infectious diseases and temperature extremes. *Arch Gen Psychiatry* (1984) 41(1):85–90. doi: 10.1001/archpsyc.1984.01790120089011
54. Torrey EF. Stalking the schizovirus. *Schizophr Bull* (1988) 14(2):223–9. doi: 10.1093/schbul/14.2.223
55. O'Callaghan E, Sham PC, Takei N, Murray G, Glover G, Hare EH, et al. The relationship of schizophrenic births to 16 infectious diseases. *Br J Psychiatry* (1994) 165(3):353–6. doi: 10.1192/bjp.165.3.353
56. Suvisaari J, Haukka J, Tanskanen A, Hovi T, Lonnqvist J. Association between prenatal exposure to poliovirus infection and adult schizophrenia. *Am J Psychiatry* (1999) 156(7):1100–2. doi: 10.1176/ajp.156.7.1100
57. Hare EH, Price JS, Slater E. Schizophrenia and season of birth. *Br J Psychiatry* (1972) 120(554):124–5. doi: 10.1192/bjp.120.554.124-a
58. Brown AS, Schaefer CA, Wyatt RJ, Goetz R, Begg MD, Gorman JM, et al. Maternal exposure to respiratory infections and adult schizophrenia spectrum disorders: a prospective birth cohort study. *Schizophr Bull* (2000) 26(2):287–95. doi: 10.1093/oxfordjournals.schbul.a033453
59. Brown AS, Begg MD, Gravenstein S, Schaefer CA, Wyatt RJ, Bresnahan M, et al. Serologic evidence of prenatal influenza in the etiology of schizophrenia. *Arch Gen Psychiatry* (2004) 61(8):774–80. doi: 10.1001/archpsyc.61.8.774
60. Susser ES, Schaefer CA, Brown AS, Begg MD, Wyatt RJ. The design of the prenatal determinants of schizophrenia study. *Schizophr Bull* (2000) 26(2):257–73. doi: 10.1093/oxfordjournals.schbul.a033451
61. Brown AS, Cohen P, Greenwald S, Susser E. Nonaffective psychosis after prenatal exposure to rubella. *Am J Psychiatry* (2000) 157(3):438–43. doi: 10.1176/appi.ajp.157.3.438
62. Brown AS, Cohen P, Harkavy-Friedman J, Babulas V, Malaspina D, Gorman JM, et al. A.E. Bennett Research Award. Prenatal rubella, premorbid abnormalities, and adult schizophrenia. *Biol Psychiatry* (2001) 49(6):473–86. doi: 10.1016/S0006-3223(01)01068-X
63. Brown AS, Schaefer CA, Quesenberry, Jr. CP, Liu L, Babulas VP, Susser ES. Maternal exposure to toxoplasmosis and risk of schizophrenia in adult offspring. *Am J Psychiatry* (2005) 162(4):767–73. doi: 10.1176/appi.ajp.162.4.767

64. Mortensen PB, Norgaard-Pedersen B, Waltoft BL, Sorensen TL, Hougaard D, Torrey EF, et al. Toxoplasma gondii as a risk factor for early-onset schizophrenia: analysis of filter paper blood samples obtained at birth. *Biol Psychiatry* (2007) 61(5):688–93. doi: 10.1016/j.biopsych.2006.05.024
65. Buka SL, Cannon TD, Torrey EF, Yolken RH. Maternal exposure to herpes simplex virus and risk of psychosis among adult offspring. *Biol Psychiatry* (2008) 63(8):809–15. doi: 10.1016/j.biopsych.2007.09.022
66. Buka SL, Tsuang MT, Torrey EF, Klebanoff MA, Bernstein D, Yolken RH. Maternal infections and subsequent psychosis among offspring. *Arch Gen Psychiatry* (2001) 58(11):1032–7 doi: 10.1001/archpsyc.58.11.1032
67. Babulas V, Factor-Litvak P, Goetz R, Schaefer CA, Brown AS. Prenatal exposure to maternal genital and reproductive infections and adult schizophrenia. *Am J Psychiatry* (2006) 163(5):927–9. doi: 10.1176/appi.ajp.163.5.927
68. Sorensen HJ, Mortensen EL, Reinisch JM, Mednick SA. Association between prenatal exposure to bacterial infection and risk of schizophrenia. *Schizophr Bull* (2009) 35(3):631–7. doi: 10.1093/schbul/sbn121
69. Buka SL, Tsuang MT, Torrey EF, Klebanoff MA, Wagner RL, Yolken RH. Maternal cytokine levels during pregnancy and adult psychosis. *Brain Behav Immun* (2001) 15(4):411–20. doi: 10.1006/brbi.2001.0644
70. Bortolato B, Kohler CA, Evangelou E, Leon-Caballero J, Solmi M, Stubbs B, et al. Systematic assessment of environmental risk factors for bipolar disorder: an umbrella review of systematic reviews and meta-analyses. *Bipolar Disord* (2017) 19(2):84–96. doi: 10.1111/bdi.12490
71. Mac Giollabhui N, Breen EC, Murphy SK, Maxwell SD, Cohn BA, Krigbaum NY, et al. Maternal inflammation during pregnancy and offspring psychiatric symptoms in childhood: Timing and sex matter. *J Psychiatr Res* (2019) 111:96–103. doi: 10.1016/j.jpsychires.2019.01.009
72. Brown AS, Hooton J, Schaefer CA, Zhang H, Petkova E, Babulas V, et al. Elevated maternal interleukin-8 levels and risk of schizophrenia in adult offspring. *Am J Psychiatry* (2004) 161(5):889–95. doi: 10.1176/appi.ajp.161.5.889
73. Khandaker GM, Zimbron J, Lewis G, Jones PB. Prenatal maternal infection, neurodevelopment and adult schizophrenia: a systematic review of population-based studies. *Psychol Med* (2013) 43(2):239–57. doi: 10.1017/S0033291712000736
74. Gilmore JH, Jarskog LF. Exposure to infection and brain development: cytokines in the pathogenesis of schizophrenia. *Schizophr Res* (1997) 24 (3):365–7. doi: 10.1016/S0920-9964(96)00123-5
75. Allswede DM, Buka SL, Yolken RH, Torrey EF, Cannon TD. Elevated maternal cytokine levels at birth and risk for psychosis in adult offspring. *Schizophr Res* (2016) 172(1-3):41–5. doi: 10.1016/j.schres.2016.02.022
76. Goldstein JM, Cherkerzian S, Seidman LJ, Donatelli JA, Remington AG, Tsuang MT, et al. Prenatal maternal immune disruption and sex-dependent risk for psychoses. *Psychol Med* (2014) 44(15):3249–61. doi: 10.1017/S0033291714000683
77. Canetta S, Sourander A, Surcel HM, Hinkka-Yli-Salomaki S, Leiviska J, Kellendonk C, et al. Elevated maternal C-reactive protein and increased risk of schizophrenia in a national birth cohort. *Am J Psychiatry* (2014) 171 (9):960–8. doi: 10.1176/appi.ajp.2014.13121579
78. Shi L, Fatemi SH, Sidwell RW, Patterson PH. Maternal influenza infection causes marked behavioral and pharmacological changes in the offspring. *J Neurosci* (2003) 23(1):297–302. doi: 10.1523/JNEUROSCI.23-01-00297.2003
79. Haddad F, Patel S, Schmid S. Maternal Immune Activation by Poly I:C as a preclinical Model for Neurodevelopmental Disorders: A focus on Autism and Schizophrenia. *Neurosci Biobehav Rev* (2020) 113:546–67. doi: 10.1016/j.neubiorev.2020.04.012
80. Bergdolt L, Dunaevsky A. Brain changes in a maternal immune activation model of neurodevelopmental brain disorders. *Prog Neurobiol* (2019) 175:1–19. doi: 10.1016/j.pneurobio.2018.12.002
81. Meyer U. Prenatal Poly(I:C) Exposure and Other Developmental Immune Activation Models in Rodent Systems. *Biol Psychiatry* (2013) 75(4):307–15. doi: 10.1016/j.biopsych.2013.07.011
82. Swerdlow NR, Geyer MA, Braff DL. Neural circuit regulation of prepulse inhibition of startle in the rat: current knowledge and future challenges. *Psychopharmacol (Berl)* (2001) 156(2-3):194–215. doi: 10.1007/s002130100799
83. Zuckerman L, Rehavi M, Nachman R, Weiner I. Immune activation during pregnancy in rats leads to a postpubertal emergence of disrupted latent inhibition, dopaminergic hyperfunction, and altered limbic morphology in the offspring: a novel neurodevelopmental model of schizophrenia. *Neuropsychopharmacology* (2003) 28(10):1778–89. doi: 10.1038/sj.npp.1300248
84. Ozawa K, Hashimoto K, Kishimoto T, Shimizu E, Ishikura H, Iyo M. Immune activation during pregnancy in mice leads to dopaminergic hyperfunction and cognitive impairment in the offspring: a neurodevelopmental animal model of schizophrenia. *Biol Psychiatry* (2006) 59(6):546–54. doi: 10.1016/j.biopsych.2005.07.031
85. Zuckerman L, Weiner I. Maternal immune activation leads to behavioral and pharmacological changes in the adult offspring. *J Psychiatr Res* (2005) 39 (3):311–23. doi: 10.1016/j.jpsychires.2004.08.008
86. Smith SE, Li J, Garbett K, Mirnics K, Patterson PH. Maternal immune activation alters fetal brain development through interleukin-6. *J Neurosci* (2007) 27(40):10695–702. doi: 10.1523/JNEUROSCI.2178-07.2007
87. Meyer U, Schwendener S, Feldon J, Yee BK. Prenatal and postnatal maternal contributions in the infection model of schizophrenia. *Exp Brain Res* (2006) 173(2):243–57. doi: 10.1007/s00221-006-0419-5
88. Meyer U, Nyffeler M, Engler A, Urwyler A, Schedlowski M, Knuesel I, et al. The time of prenatal immune challenge determines the specificity of inflammation-mediated brain and behavioral pathology. *J Neurosci* (2006) 26(18):4752–62. doi: 10.1523/JNEUROSCI.0099-06.2006
89. Meyer U, Spoerri E, Yee BK, Schwarz MJ, Feldon J. Evaluating Early Preventive Antipsychotic and Antidepressant Drug Treatment in an Infection-Based Neurodevelopmental Mouse Model of Schizophrenia. *Schizophr Bull* (2008) 36(3):607–23. doi: 10.1093/schbul/sbn131
90. Meyer U, Nyffeler M, Schwendener S, Knuesel I, Yee BK, Feldon J. Relative prenatal and postnatal maternal contributions to schizophrenia-related neurochemical dysfunction after in utero immune challenge. *Neuropsychopharmacology* (2008) 33(2):441–56. doi: 10.1038/sj.npp.1301413
91. Meyer U, Murray PJ, Urwyler A, Yee BK, Schedlowski M, Feldon J. Adult behavioral and pharmacological dysfunctions following disruption of the fetal brain balance between pro-inflammatory and IL-10-mediated anti-inflammatory signaling. *Mol Psychiatry* (2008) 13(2):208–21. doi: 10.1038/sj.mp.4002042
92. Meyer U, Engler A, Weber L, Schedlowski M, Feldon J. Preliminary evidence for a modulation of fetal dopaminergic development by maternal immune activation during pregnancy. *Neuroscience* (2008) 154(2):701–9. doi: 10.1016/j.neuroscience.2008.04.031
93. Vuillermot S, Weber L, Feldon J, Meyer U. A longitudinal examination of the neurodevelopmental impact of prenatal immune activation in mice reveals primary defects in dopaminergic development relevant to schizophrenia. *J Neurosci* (2010) 30(4):1270–87. doi: 10.1523/JNEUROSCI.5408-09.2010
94. Luan W, Hammond LA, Vuillermot S, Meyer U, Eyles DW. Maternal Vitamin D Prevents Abnormal Dopaminergic Development and Function in a Mouse Model of Prenatal Immune Activation. *Sci Rep* (2018) 8(1):9741. doi: 10.1038/s41598-018-28090-w
95. Borcoi AR, Patti CL, Zanin KA, Hollais AW, Santos-Baldaia R, Ceccon LM, et al. Effects of prenatal immune activation on amphetamine-induced addictive behaviors: Contributions from animal models. *Prog Neuropsychopharmacol Biol Psychiatry* (2015) 63:63–9. doi: 10.1016/j.pnpbp.2015.05.015
96. Missault S, Van den Eynde K, Vanden Berghe W, Fransen E, Weeren A, Timmermans JP, et al. The risk for behavioural deficits is determined by the maternal immune response to prenatal immune challenge in a neurodevelopmental model. *Brain Behav Immun* (2014) 42:138–46. doi: 10.1016/j.bbi.2014.06.013
97. Willi R, Harmeier A, Giovanoli S, Meyer U. Altered GSK3beta signaling in an infection-based mouse model of developmental neuropsychiatric disease. *Neuropharmacology* (2013) 73:56–65. doi: 10.1016/j.neuropharm.2013.05.012
98. Piontkewitz Y, Arad M, Weiner I. Risperidone administered during asymptomatic period of adolescence prevents the emergence of brain structural pathology and behavioral abnormalities in an animal model of schizophrenia. *Schizophr Bull* (2011) 37(6):1257–69. doi: 10.1093/schbul/sbq040
99. Piontkewitz Y, Arad M, Weiner I. Abnormal trajectories of neurodevelopment and behavior following in utero insult in the rat. *Biol Psychiatry* (2011) 70(9):842–51. doi: 10.1016/j.biopsych.2011.06.007

100. Lins BR, Hurtubise JL, Roebuck AJ, Marks WN, Zabder NK, Scott GA, et al. Prospective Analysis of the Effects of Maternal Immune Activation on Rat Cytokines during Pregnancy and Behavior of the Male Offspring Relevant to Schizophrenia. *eNeuro* (2018) 5(4):ENEURO.0249-18.2018. doi: 10.1523/ENEURO.0249-18.2018
101. Chou S, Jones S, Li M. Adolescent olanzapine sensitization is correlated with hippocampal stem cell proliferation in a maternal immune activation rat model of schizophrenia. *Brain Res* (2015) 1618:122–35. doi: 10.1016/j.brainres.2015.05.036
102. Bitanihirwe BK, Peleg-Raibstein D, Mouttet F, Feldon J, Meyer U. Late prenatal immune activation in mice leads to behavioral and neurochemical abnormalities relevant to the negative symptoms of schizophrenia. *Neuropsychopharmacology* (2010) 35(12):2462–78. doi: 10.1038/npp.2010.129
103. da Silveira VT, Medeiros DC, Ropke J, Guidine PA, Rezende GH, Moraes MF, et al. Effects of early or late prenatal immune activation in mice on behavioral and neuroanatomical abnormalities relevant to schizophrenia in the adulthood. *Int J Dev Neurosci* (2017) 58:1–8. doi: 10.1016/j.ijdevneu.2017.01.009
104. Labouesse MA, Langhans W, Meyer U. Abnormal context-reward associations in an immune-mediated neurodevelopmental mouse model with relevance to schizophrenia. *Transl Psychiatry* (2015) 5:e637. doi: 10.1038/tp.2015.129
105. Richetto J, Calabrese F, Meyer U, Riva MA. Prenatal versus postnatal maternal factors in the development of infection-induced working memory impairments in mice. *Brain Behav Immun* (2013) 33:190–200. doi: 10.1016/j.bbi.2013.07.006
106. Fortier ME, Joober R, Luheshi GN, Boksa P. Maternal exposure to bacterial endotoxin during pregnancy enhances amphetamine-induced locomotion and startle responses in adult rat offspring. *J Psychiatr Res* (2004) 38(3):335–45. doi: 10.1016/j.jpsychires.2003.10.001
107. Fortier ME, Luheshi GN, Boksa P. Effects of prenatal infection on prepulse inhibition in the rat depend on the nature of the infectious agent and the stage of pregnancy. *Behav Brain Res* (2007) 181(2):270–7. doi: 10.1016/j.bbr.2007.04.016
108. Liu X, Lee JG, Yee SK, Bresee CJ, Poland RE, Pechnick RN. Endotoxin exposure in utero increases ethanol consumption in adult male offspring. *Neuroreport* (2004) 15(1):203–6. doi: 10.1097/00001756-200401190-00039
109. Girard S, Tremblay L, Lepage M, Sebire G. IL-1 receptor antagonist protects against placental and neurodevelopmental defects induced by maternal inflammation. *J Immunol* (2010) 184(7):3997–4005. doi: 10.4049/jimmunol.0903349
110. Girard S, Kadhim H, Beaudet N, Sarret P, Sebire G. Developmental motor deficits induced by combined fetal exposure to lipopolysaccharide and early neonatal hypoxia/ischemia: a novel animal model for cerebral palsy in very premature infants. *Neuroscience* (2009) 158(2):673–82. doi: 10.1016/j.neuroscience.2008.10.032
111. Lante F, Meunier J, Guiramand J, De Jesus Ferreira MC, Cambonie G, Aimar R, et al. Late N-acetylcysteine treatment prevents the deficits induced in the offspring of dams exposed to an immune stress during gestation. *Hippocampus* (2008) 18(6):602–9. doi: 10.1002/hipo.20421
112. Lante F, Meunier J, Guiramand J, Maurice T, Cavalier M, de Jesus Ferreira MC, et al. Neurodevelopmental damage after prenatal infection: role of oxidative stress in the fetal brain. *Free Radic Biol Med* (2007) 42(8):1231–45. doi: 10.1016/j.freeradbiomed.2007.01.027
113. Wang H, Meng XH, Ning H, Zhao XF, Wang Q, Liu P, et al. Age- and gender-dependent impairments of neurobehaviors in mice whose mothers were exposed to lipopolysaccharide during pregnancy. *Toxicol Lett* (2010) 192(2):245–51. doi: 10.1016/j.toxlet.2009.10.030
114. Golan H, Stilman M, Lev V, Huleihel M. Normal aging of offspring mice of mothers with induced inflammation during pregnancy. *Neuroscience* (2006) 141(4):1909–18. doi: 10.1016/j.neuroscience.2006.05.045
115. Golan HM, Lev V, Hallak M, Sorokin Y, Huleihel M. Specific neurodevelopmental damage in mice offspring following maternal inflammation during pregnancy. *Neuropharmacology* (2005) 48(6):903–17. doi: 10.1016/j.neuropharm.2004.12.023
116. Hava G, Vered L, Yael M, Mordechai H, Mahoud H. Alterations in behavior in adult offspring mice following maternal inflammation during pregnancy. *Dev Psychobiol* (2006) 48(2):162–8. doi: 10.1002/dev.20116
117. Basta-Kaim A, Szczesny E, Leskiewicz M, Glombik K, Slusarczyk J, Budziszewska B, et al. Maternal immune activation leads to age-related behavioral and immunological changes in male rat offspring - the effect of antipsychotic drugs. *Pharmacol Rep* (2012) 64(6):1400–10. doi: 10.1016/s1734-1140(12)70937-4
118. Borrell J, Vela JM, Arevalo-Martin A, Molina-Holgado E, Guaza C. Prenatal immune challenge disrupts sensorimotor gating in adult rats. Implications for the etiopathogenesis of schizophrenia. *Neuropsychopharmacology* (2002) 26(2):204–15. doi: 10.1016/S0893-133X(01)00360-8
119. Romero E, Ali C, Molina-Holgado E, Castellano B, Guaza C, Borrell J. Neurobehavioral and Immunological Consequences of Prenatal Immune Activation in Rats. Influence of Antipsychotics. *Neuropsychopharmacology* (2006) 32(8):1791–804. doi: 10.1038/sj.npp.1301292
120. Romero E, Guaza C, Castellano B, Borrell J. Ontogeny of sensorimotor gating and immune impairment induced by prenatal immune challenge in rats: implications for the etiopathology of schizophrenia. *Mol Psychiatry* (2008) 15(4):372–83 . doi: 10.1038/mp.2008.44
121. Wusteman M, Wight DG, Elia M. Protein metabolism after injury with turpentine: a rat model for clinical trauma. *Am J Physiol* (1990) 259(6 Pt 1):E763–9. doi: 10.1152/ajpendo.1990.259.6.E763
122. Turnbull AV, Prehar S, Kennedy AR, Little RA, Hopkins SJ. Interleukin-6 is an afferent signal to the hypothalamo-pituitary-adrenal axis during local inflammation in mice. *Endocrinology* (2003) 144(5):1894–906. doi: 10.1210/en.2002-220964
123. Aguilar-Valles A, Poole S, Mistry Y, Williams S, Luheshi GN. Attenuated fever in rats during late pregnancy is linked to suppressed interleukin-6 production after localized inflammation with turpentine. *J Physiol* (2007) 583 (Pt 1):391–403. doi: 10.1113/jphysiol.2007.132829
124. Aguilar-Valles A, Luheshi GN. Alterations in cognitive function and behavioral response to amphetamine induced by prenatal inflammation are dependent on the stage of pregnancy. *Psychoneuroendocrinology* (2011) 36(5):634–48. doi: 10.1016/j.psyneuen.2010.09.006
125. Zuckerman L, Weiner I. Post-pubertal emergence of disrupted latent inhibition following prenatal immune activation. *Psychopharmacol (Berl)* (2003) 169(3-4):308–13. doi: 10.1007/s00213-003-1461-7
126. Seeman P, Kapur S. Schizophrenia: more dopamine, more D2 receptors. *Proc Natl Acad Sci U.S.A.* (2000) 97(14):7673–5. doi: 10.1073/pnas.97.14.7673
127. MacDowell KS, Munarriz-Cuezva E, Caso JR, Madrigal JL, Zabala A, Meana JJ, et al. Paliperidone reverts Toll-like receptor 3 signaling pathway activation and cognitive deficits in a maternal immune activation mouse model of schizophrenia. *Neuropharmacology* (2017) 116:196–207. doi: 10.1016/j.neuropharm.2016.12.025
128. Meyer U, Knuesel I, Nyffeler M, Feldon J. Chronic clozapine treatment improves prenatal infection-induced working memory deficits without influencing adult hippocampal neurogenesis. *Psychopharmacol (Berl)* (2010) 208(4):531–43. doi: 10.1007/s00213-009-1754-6
129. Meyer U, Spoerri E, Yee BK, Schwarz MJ, Feldon J. Evaluating early preventive antipsychotic and antidepressant drug treatment in an infection-based neurodevelopmental mouse model of schizophrenia. *Schizophr Bull* (2010) 36(3):607–23. doi: 10.1093/schbul/sbn131
130. Patrich E, Piontkewitz Y, Peretz A, Weiner I, Attali B. Maternal immune activation produces neonatal excitability defects in offspring hippocampal neurons from pregnant rats treated with poly I:C. *Sci Rep* (2016) 6:19106. doi: 10.1038/srep19106
131. Piontkewitz Y, Bernstein HG, Dobrowolny H, Bogerts B, Weiner I, Keilhoff G. Effects of risperidone treatment in adolescence on hippocampal neurogenesis, parvalbumin expression, and vascularization following prenatal immune activation in rats. *Brain Behav Immun* (2012) 26(2):353–63. doi: 10.1016/j.bbi.2011.11.004
132. Richtand NM, Ahlbrand R, Horn P, Stanford K, Bronson SL, McNamara RK. Effects of risperidone and paliperidone pre-treatment on locomotor response following prenatal immune activation. *J Psychiatr Res* (2011) 45(9):1194–201. doi: 10.1016/j.jpsychires.2011.02.007
133. Winter C, Djodari-Irani A, Sohr R, Morgenstern R, Feldon J, Juckel G, et al. Prenatal immune activation leads to multiple changes in basal neurotransmitter levels in the adult brain: implications for brain disorders of neurodevelopmental origin such as schizophrenia. *Int J Neuropsychopharmacol* (2009) 12(4):513–24. doi: 10.1017/S1461145708009206

134. Giovanoli S, Engler H, Engler A, Richetto J, Voget M, Willi R, et al. Stress in puberty unmasks latent neuropathological consequences of prenatal immune activation in mice. *Science* (2013) 339(6123):1095–9. doi: 10.1126/science.1228261
135. Ling Z, Gayle DA, Ma SY, Lipton JW, Tong CW, Hong JS, et al. In utero bacterial endotoxin exposure causes loss of tyrosine hydroxylase neurons in the postnatal rat midbrain. *Mov Disord* (2002) 17(1):116–24. doi: 10.1002/mds.10078
136. Ling ZD, Chang Q, Lipton JW, Tong CW, Landers TM, Carvey PM. Combined toxicity of prenatal bacterial endotoxin exposure and postnatal 6-hydroxydopamine in the adult rat midbrain. *Neuroscience* (2004) 124 (3):619–28. doi: 10.1016/j.neuroscience.2003.12.017
137. Ling Z, Chang QA, Tong CW, Leurgans SE, Lipton JW, Carvey PM. Rotenone potentiates dopamine neuron loss in animals exposed to lipopolysaccharide prenatally. *Exp Neurol* (2004) 190(2):373–83. doi: 10.1016/j.expneurol.2004.08.006
138. Ling Z, Zhu Y, Tong C, Snyder JA, Lipton JW, Carvey PM. Progressive dopamine neuron loss following supra-nigral lipopolysaccharide (LPS) infusion into rats exposed to LPS prenatally. *Exp Neurol* (2006) 199 (2):499–512. doi: 10.1016/j.expneurol.2006.01.010
139. Ling Z, Zhu Y, Tong CW, Snyder JA, Lipton JW, Carvey PM. Prenatal lipopolysaccharide does not accelerate progressive dopamine neuron loss in the rat as a result of normal aging. *Exp Neurol* (2009) 216(2):312–20. doi: 10.1016/j.expneurol.2008.12.004
140. Wang S, Yan JY, Lo YK, Carvey PM, Ling Z. Dopaminergic and serotoninergic deficiencies in young adult rats prenatally exposed to the bacterial lipopolysaccharide. *Brain Res* (2009) 1265:196–204. doi: 10.1016/j.brainres.2009.02.022
141. Bakos J, Duncko R, Makatsori A, Pirnik Z, Kiss A, Jezova D. Prenatal immune challenge affects growth, behavior, and brain dopamine in offspring. *Ann N Y Acad Sci* (2004) 1018:281–7. doi: 10.1196/annals.1296.033
142. Aguilar-Valles A, Flores C, Luheshi GN. Prenatal inflammation-induced hypoferremia alters dopamine function in the adult offspring in rat: relevance for schizophrenia. *PloS One* (2010) 5(6):e10967. doi: 10.1371/journal.pone.0010967
143. Aguilar-Valles A, Jung S, Poole S, Flores C, Luheshi GN. Leptin and interleukin-6 alter the function of mesolimbic dopamine neurons in a rodent model of prenatal inflammation. *Psychoneuroendocrinology* (2012) 37(7):956–69. doi: 10.1016/j.psyneuen.2011.11.003
144. Meyer U, Nyffeler M, Yee BK, Knuesel I, Feldon J. Adult brain and behavioral pathological markers of prenatal immune challenge during early/middle and late fetal development in mice. *Brain Behav Immun* (2008) 22(4):469–86. doi: 10.1016/j.bbi.2007.09.012
145. Garbett KA, Hsiao EY, Kalman S, Patterson PH, Mirnics K. Effects of maternal immune activation on gene expression patterns in the fetal brain. *Transl Psychiatry* (2012) 2:e98. doi: 10.1038/tp.2012.24
146. Wu WL, Hsiao EY, Yan Z, Mazmanian SK, Patterson PH. The placental interleukin-6 signaling controls fetal brain development and behavior. *Brain Behav Immun* (2017) 62:11–23. doi: 10.1016/j.bbi.2016.11.007
147. Grieger TA, Kluger MJ. Fever and survival: the role of serum iron. *J Physiol* (1978) 279:187–96. doi: 10.1113/jphysiol.1978.sp012339
148. Kluger MJ, Rothenburg BA. Fever and reduced iron: their interaction as a host defense response to bacterial infection. *Science* (1979) 203(4378):374–6. doi: 10.1126/science.760197
149. Beard JL, Connor JR. Iron status and neural functioning. *Annu Rev Nutr* (2003) 23:41–58. doi: 10.1146/annurev.nutr.23.020102.075739
150. Choi GB, Yim YS, Wong H, Kim S, Kim H, Kim SV, et al. The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science* (2016) 351(6276):933–9. doi: 10.1126/science.aad0314
151. Kim S, Kim H, Yim YS, Ha S, Atarashi K, Tan TG, et al. Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. *Nature* (2017) 549(7673):528–32. doi: 10.1038/nature23910
152. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. *Nature* (1994) 372(6505):425–32. doi: 10.1038/372425a0
153. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science* (1995) 269(5223):546–9. doi: 10.1126/science.7624778
154. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, et al. Weight-reducing effects of the plasma protein encoded by the obese gene. *Science* (1995) 269(5223):543–6. doi: 10.1126/science.7624777
155. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, et al. Effects of the obese gene product on body weight regulation in ob/ob mice. *Science* (1995) 269(5223):540–3. doi: 10.1126/science.7624776
156. Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB, Flier JS. Leptin Levels Reflect Body Lipid-Content in Mice - Evidence for Diet-Induced Resistance to Leptin Action. *Nat Med* (1995) 1(12):1311–4. doi: 10.1038/nm1295-1311
157. Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. *Nat Med* (1995) 1(11):1155–61. doi: 10.1038/nm1195-1155
158. Jung UJ, Choi MS. Obesity and its metabolic complications: the role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. *Int J Mol Sci* (2014) 15(4):6184–223. doi: 10.3390/ijms15046184
159. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. *Nat Rev Immunol* (2011) 11(2):85–97. doi: 10.1038/nri2921
160. Zarkesh-Esfahani H, Pockley G, Metcalfe RA, Bidlingmaier M, Wu Z, Ajami A, et al. High-dose leptin activates human leukocytes via receptor expression on monocytes. *J Immunol* (2001) 167(8):4593–9. doi: 10.4049/jimmunol.167.8.4593
161. Dixit VD, Schaffer EM, Pyle RS, Collins GD, Sakthivel SK, Palaniappan R, et al. Ghrelin inhibits leptin- and activation-induced proinflammatory cytokine expression by human monocytes and T cells. *J Clin Invest* (2004) 114(1):57–66. doi: 10.1172/JCI200421134
162. Maedler K, Sergeev P, Ehses JA, Mathe Z, Bosco D, Berney T, et al. Leptin modulates beta cell expression of IL-1 receptor antagonist and release of IL-1beta in human islets. *Proc Natl Acad Sci U.S.A.* (2004) 101(21):8138–43. doi: 10.1073/pnas.0305683101
163. Sarraf P, Frederich RC, Turner EM, Ma G, Jaskowiak NT, Rivet DJ, et al. Multiple cytokines and acute inflammation raise mouse leptin levels: potential role in inflammatory anorexia. *J Exp Med* (1997) 185(1):171–5. doi: 10.1084/jem.185.1.171
164. Grunfeld C, Zhao C, Fuller J, Pollock A, Moser A, Friedman J, et al. Endotoxin and cytokines induce expression of leptin, the ob gene product, in hamsters. A role for leptin in the anorexia of infection. *J Clin Invest* (1996) 97(9):2152–7. doi: 10.1172/JCI118653
165. Faggioni R, Fantuzzi G, Fuller J, Dinarello CA, Feingold KR, Grunfeld C. IL-1 beta mediates leptin induction during inflammation. *Am J Physiol* (1998) 274 (1 Pt 2):R204–8. doi: 10.1152/ajpregu.1998.274.1.R204
166. Gualillo O, Eiras S, Lago F, Dieguez C, Casanueva FF. Elevated serum leptin concentrations induced by experimental acute inflammation. *Life Sci* (2000) 67(20):2433–41. doi: 10.1016/S0024-3205(00)00827-4
167. Mastronardi CA, Yu WH, Srivastava VK, Dees WL, McCann SM. Lipopolysaccharide-induced leptin release is neurally controlled. *Proc Natl Acad Sci U States America* (2001) 98(25):14720–5. doi: 10.1073/pnas.251543598
168. Sachot C, Poole S, Luheshi GN. Circulating leptin mediates lipopolysaccharide-induced anorexia and fever in rats. *J Physiol* (2004) 561(Pt 1):263–72. doi: 10.1113/jphysiol.2004.074351
169. Harden LM, du Plessis I, Poole S, Laburn HP. Interleukin-6 and leptin mediate lipopolysaccharide-induced fever and sickness behavior. *Physiol Behav* (2006) 89(2):146–55. doi: 10.1016/j.physbeh.2006.05.016
170. Luheshi GN, Gardner JD, Rushforth DA, Loudon AS, Rothwell NJ. Leptin actions on food intake and body temperature are mediated by IL-1. *Proc Natl Acad Sci U.S.A.* (1999) 96(12):7047–52. doi: 10.1073/pnas.96.12.7047
171. Turek VF, Olster DH, Gililland KR, Sheehy M, Ettenberg A, Carlisle HJ. The effects of melanocortin agonists and antagonists on leptin-induced fever in rats. *J Thermal Biol* (2004) 29(7-8):423–30. doi: 10.1016/j.jtherbio.2004.08.011
172. Phillips MS, Liu Q, Hammond HA, Dugan V, Hey PJ, Caskey CJ, et al. Leptin receptor missense mutation in the fatty Zucker rat. *Nat Genet* (1996) 13 (1):18–9. doi: 10.1038/ng0596-18
173. Inoue W, Poole S, Bristow AF, Luheshi GN. Leptin induces cyclooxygenase-2 via an interaction with interleukin-1beta in the rat brain. *Eur J Neurosci* (2006) 24(8):2233–45. doi: 10.1111/j.1460-9568.2006.05105.x

174. Fulton S, Pissios P, Manchon RP, Stiles L, Frank L, Pothos EN, et al. Leptin regulation of the mesoaccumbens dopamine pathway. *Neuron* (2006) 51 (6):811–22. doi: 10.1016/j.neuron.2006.09.006
175. Lappas M, Permezel M, Rice GE. Leptin and adiponectin stimulate the release of proinflammatory cytokines and prostaglandins from human placenta and maternal adipose tissue via nuclear factor-kappaB, peroxisomal proliferator-activated receptor-gamma and extracellularly regulated kinase 1/2. *Endocrinology* (2005) 146(8):3334–42. doi: 10.1210/en.2005-0406
176. Caspi A, Moffitt TE. Gene-environment interactions in psychiatry: joining forces with neuroscience. *Nat Rev Neurosci* (2006) 7(7):583–90. doi: 10.1038/nrn1925
177. DiCicco-Bloom E, Lord C, Zwaigenbaum L, Courchesne E, Dager SR, Schmitz C, et al. The developmental neurobiology of autism spectrum disorder. *J Neurosci* (2006) 26(26):6897–906. doi: 10.1523/JNEUROSCI.1712-06.2006
178. Richetto J, Meyer U. Epigenetic Modifications in Schizophrenia and Related Disorders: Molecular Scars of Environmental Exposures and Source of Phenotypic Variability. *Biol Psychiatry* (2020). doi: 10.1016/j.biopsych.2020.03.008
179. Dean B, Boer S, Gibbons A, Money T, Scarr E. Recent advances in postmortem pathology and neurochemistry in schizophrenia. *Curr Opin Psychiatry* (2009) 22(2):154–60. doi: 10.1097/YCO.0b013e328323d52e
180. Demontis D, Nyegaard M, Buttenschon HN, Hedemand A, Pedersen CB, Grove J, et al. Association of GRIN1 and GRIN2A-D with schizophrenia and genetic interaction with maternal herpes simplex virus-2 infection affecting disease risk. *Am J Med Genet B Neuropsychiatr Genet* (2011) 156B(8):913–22. doi: 10.1002/ajmg.b.31234
181. Borglum AD, Demontis D, Grove J, Pallesen J, Hollegaard MV, Pedersen CB, et al. Genome-wide study of association and interaction with maternal cytomegalovirus infection suggests new schizophrenia loci. *Mol Psychiatry* (2014) 19(3):325–33. doi: 10.1038/mp.2013.2
182. Clarke MC, Tanskanen A, Huttunen M, Whittaker JC, Cannon M. Evidence for an interaction between familial liability and prenatal exposure to infection in the causation of schizophrenia. *Am J Psychiatry* (2009) 166 (9):1025–30. doi: 10.1176/appi.ajp.2009.08010031
183. Abazyan B, Nomura J, Kannan G, Ishizuka K, Tamashiro KL, Nucifora F, et al. Prenatal interaction of mutant DISC1 and immune activation produces adult psychopathology. *Biol Psychiatry* (2010) 68(12):1172–81. doi: 10.1016/j.biopsych.2010.09.022
184. Lipina TV, Zai C, Hlousek D, Roder JC, Wong AH. Maternal immune activation during gestation interacts with Disc1 point mutation to exacerbate schizophrenia-related behaviors in mice. *J Neurosci* (2013) 33(18):7654–66. doi: 10.1523/JNEUROSCI.0091-13.2013
185. Hemmerle AM, Ahlbrand R, Bronson SL, Lundgren KH, Richtand NM, Seroogy KB. Modulation of schizophrenia-related genes in the forebrain of adolescent and adult rats exposed to maternal immune activation. *Schizophr Res* (2015) 168(1-2):411–20. doi: 10.1016/j.schres.2015.07.006
186. Debost JP, Larsen JT, Munk-Olsen T, Mortensen PB, Meyer U, Petersen L. Joint Effects of Exposure to Prenatal Infection and Peripubertal Psychological Trauma in Schizophrenia. *Schizophr Bull* (2017) 43(1):171–9. doi: 10.1093/schbul/sbw083

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

frontiers in Psychiatry

ORIGINAL RESEARCH
published: 18 September 2020
doi: 10.3389/fpsyt.2020.533872

Dopaminergic, Noradrenergic, Adrenal, and Thyroid Abnormalities in Psychotic and Affective Disorders

Fabrice Duval, Marie-Claude Mokrani, Alexis Erb, Vlad Danila, Felix Gonzalez Lopera and Ludovic Jeanjean*

Pôle 8/9-APF2R, Centre Hospitalier, Rouffach, France

Background: This study aimed to assess hypothalamic-pituitary dopaminergic (DA), noradrenergic (NA), thyroid (HPT), and adrenal (HPA) activity in schizophrenia, in schizoaffective disorder, and in bipolar disorder.

Method: We investigated a combined approach of hormone responses to (1) apomorphine (APO), a short-acting DA receptor agonist which decreases prolactin secretion (PRL), and stimulates secretion of growth hormone (GH), adrenocorticotropin (ACTH), and cortisol; (2) clonidine (CLO), an alpha 2-adrenoceptor agonist which stimulates GH secretion; (3) 8 AM and 11 PM protirelin (TRH) which stimulates thyrotropin (TSH) secretion; and (4) dexamethasone which suppresses cortisol secretion, in 13 hospitalized healthy male controls and 39 untreated male inpatients: 13 with DSM-IV paranoid schizophrenia, 13 with DSM-IV schizoaffective disorder (bipolar subtype, depressed at the time of the study), and 13 with DSM-IV bipolar disorder (depressed).

Results: Compared to controls, paranoid schizophrenic patients showed (1) lower APO-induced ACTH and cortisol stimulation, and (2) higher post-dexamethasone cortisol values. Compared to controls, schizoaffective and bipolar patients showed (1) lower ΔΔTSH values (i.e., difference between 11 PM and 8 AM TRH-TSH responses), (2) lower APO-induced PRL suppression, (3) lower CLO-induced GH stimulation, and (4) higher post-dexamethasone cortisol values.

Conclusions: Although results must be interpreted with caution because of the small sample, this preliminary study suggests that depressed bipolar and schizoaffective patients share common biological dysregulations, distinct from that of paranoid schizophrenic patients. From a pathophysiological viewpoint, paranoid schizophrenic patients can be characterized by hyposensitivity of the hypothalamic DA receptors (possibly resulting from an increase in presynaptic DA release) associated with increased HPA axis activity, while depressed bipolar and schizoaffective patients can be characterized by hyposensitivity of the pituitary TRH and DA-D_2 receptors (possibly linked

OPEN ACCESS

Edited by:
Boris Chaumette, INSERM U1266 Institut de Psychiatrie et Neurosciences de Paris, France

Reviewed by:
Jean-Louis Charli, National Autonomous University of Mexico, Mexico
Javier Labad, Parc Taulí Foundation, Spain

****Correspondence:***
Fabrice Duval
f.duval@ch-rouffach.fr

Specialty section:
This article was submitted to Schizophrenia, a section of the journal Frontiers in Psychiatry

Received: *10 February 2020*
Accepted: *03 September 2020*
Published: *18 September 2020*

Citation:
Duval F, Mokrani M-C, Erb A, Danila V, Gonzalez Lopera F and Jeanjean L (2020) Dopaminergic, Noradrenergic, Adrenal, and Thyroid Abnormalities in Psychotic and Affective Disorders. Front. Psychiatry 11:533872. doi: 10.3389/fpsyt.2020.533872

to the activation of the hypothalamic TRH and tuberoinfundibular DA neurons, respectively), together with subsensitive postsynaptic α_2-adrenoreceptors at the hypothalamic level (possibly secondary to an erratic release of NA) and increased HPA axis activity.

Keywords: schizophrenia, bipolar disorder, schizoaffective disorder, apomorphine challenge, clonidine challenge, TRH test, dexamethasone suppression test

INTRODUCTION

It is now well established that the secretion of the hypothalamic hypophysiotropic hormones is controlled by neurotransmitters posited to play a preeminent role in the pathophysiology of major psychiatric disorders such as schizophrenia (SCH), schizoaffective disorder (SAD), and bipolar disorder (BD) (1, 2). Moreover, significant progress over the last decades has also demonstrated that neuropeptides and neurohormones may be directly involved in numerous mental illnesses [for a review, see (3)]. Thus, the neuroendocrine strategy can characterize the hypothalamic-pituitary dysfunction of affective and psychotic diseases, and assess the functionality of some neurotransmitter systems by using suitable pharmacological stimuli. To evaluate the DA function in psychiatric patients, several studies have used subcutaneous administration of apomorphine (APO), a non-selective short acting dopamine (DA) agonist (4). APO inhibits prolactin (PRL) secretion and stimulates adrenocorticotropic hormone (ACTH), cortisol, and growth hormone (GH) release (4–6). In drug-free SCHs, it has been consistently found blunted hypothalamic-pituitary-adrenal (HPA) axis responses to APO compared to controls (5–8); this blunting may reflect a hyposensitivity of the hypothalamic DA receptors in SCHs. Lower responsiveness of cortisol to APO has also been found in SADs (5); but not in depressed BDs (9). Regarding GH and PRL responses to APO, contradictory results have been reported in SCHs and SADs (4–11). However, some studies found lower APO induced-PRL suppression in depressed BDs compared to healthy controls and unipolar depressed patients (9, 12). Interestingly, it has been reported in patients with major unipolar depressive disorder with HPA axis overactivity and melancholic and psychotic features altered ACTH/cortisol and GH responses to APO (13). These latter findings are in line with the hypothesis that hypercortisolemia by increasing DA release may induce a hyposensitivity of hypothalamic DA receptors (14).

Measurement of GH levels following administration of clonidine (CLO)—a partial α_2-adrenoceptor agonist—has been widely used in the evaluation of noradrenergic (NA) α_2-receptor function in psychiatric patients (15). In depressed patients and in SADs, GH response to CLO is often blunted (9, 15, 16) suggesting a hyposensitivity of hypothalamic α_2-adrenoceptors (15). In SCH, GH response to CLO differs from study to study: increased, decreased, or unchanged responses have been reported [for review, see (3)].

Overactivity of the HPA axis, and increased levels of cortisol, is one of the most replicated biological findings in severe depressed patients (17). However, hyperactivity of the HPA axis is not specific to depression since it has also been found in SCH and SAD (18, 19). Although, the mechanisms underlying this abnormality are not fully understood, the most striking feature is that type II glucocorticoid receptor (GR)-mediated feed back inhibition is impaired—as reflected by a nonsuppression or an early escape of serum cortisol levels in response to the dexamethasone suppression test (DST) (20).

Many euthyroid major depressed inpatients display a chronobiological HPT axis dysregulation (i.e., loss of the nocturnal surge of thyrotropin [TSH], blunted 11 PM TSH response to protirelin [TRH] test, and reduced difference between 11 PM and 8 AM TRH-TSH responses [ΔΔTSH] (21), possibly associated with abnormal morning TRH-TSH response and/or alterations in total and/or free thyroxine (T_4) and triiodothyronine (T_3) serum concentrations (22). Chronobiological dysregulation of the HPT axis (as reflected by reduced ΔΔTSH values) has rarely been found in SCHs, while it has been reported quite comparable rates of reduced ΔΔTSH values in SADs, unipolar, and BD depressed patients (9).

In the present study, we used a series of five neuroendocrine challenges (APO test, CLO test, 8 AM and 11 PM TRH tests, overnight DST) and examined nine hormonal responses in a population of 52 male drug-free hospitalized subjects. Our aim was to identify response patterns in order to provide some indication of altered central nervous system function in patients with psychotic and affective diseases.

MATERIAL AND METHODS

Participants

Thirty-nine drug-free male inpatients, without a history of suicidal behavior, and 13 healthy male hospitalized control (HC) subjects participated in this study. Patients were recruited from the inpatient units of the Pole 8/9 of the Centre Hospitalier of Rouffach (France). All subjects underwent a standard clinical interview and a semi-structured diagnostic interview [Schedule for Affective Disorder and Schizophrenia-Lifetime Version (23)]. Patients were independently classified according to the Diagnostic and Statistical Manual of Mental

Abbreviations: ACTH, adrenocortcotropic hormone; APO, apomorphine; BD, bipolar disorder; CLO, clonidine; DA, dopamine; DST, dexamethasone suppression test; GH, growth hormone; HPA, hypothalamic-pituitary adrenal (axis); HPT, hypothalamic-pituitary thyroid (axis); 5-HT, serotonin (5-hydroxytryptamine); NA, noradrenaline; PRL, prolactin; SAD, schizoaffective Disorder; SCH, schizophrenia; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone (thyrotropin).

Disorders (DSM-IV) (24) criteria by two psychiatrists, blind to the results of neuroendocrine investigations. The patient group consisted of 13 paranoid SCHs, 13 SADs (bipolar subtype, depressed at the time of the study), and 13 BDs (type II, depressed at the time of the study). Before testing, inpatients were medication-free for at least 2 weeks. The intensity of clinical symptoms was evaluated with the Brief Psychiatric Rating Scale (BPRS, 18-item). The control group consisted of 13 hospitalized normal male volunteers without a personal or family history of major psychiatric illness; none of them met criteria for Axis I diagnostic or had been previously treated with psychotropic medications. This study was approved by the local ethical committee (Rouffach Hospital Review Board), and was conducted in accordance with the Declaration of Helsinki. All subjects gave their informed consent prior to participation.

Routine physical examination and laboratory tests were performed in all subjects. None had a history of endocrinopathy, major medical illness, acute weight change (all were within 15% of ideal body weight), alcohol, or substance abuse. All subjects had basal PRL, TSH, FT_4, and FT_3 values within the normal range. No patient had received long-acting neuroleptics, electroconvulsive therapy, lithium salts, fluoxetine, or monoamine oxidase inhibitor antidepressants within 2 years of testing. All subjects were on a caffeine-restricted diet for at least three days before testing and their environment was synchronized, with diurnal activity from 8 AM to 11 PM, and nocturnal rest (sleep).

Procedures

To reduce bias due to interferences between the tests, the order of the tests was carefully determined. Two TRH-TSH stimulation tests were carried out at 8 AM and 11 PM (day 1), using 200 μg of synthetic TRH IV (Stimu-TSH, Laboratoires Roussel, Paris, France) (25). This procedure has the advantage to take into account the circadian activity of the HPT axis, which is maximal during night. After an overnight fast, subjects were awoken at 7 AM. An indwelling cannula was inserted into an antecubital arm vein and kept open with a slow infusion of 0.9% saline. Baseline blood samples for levels of TSH were collected at -15 and 0 min. The first TRH-TSH stimulation test was carried out at 8 AM, and blood samples were taken after 15, 30, and 60 min. The second TRH-TSH test was performed at 11 PM, on the same day, using the same procedure; subjects were awake during the sampling and fasting from 6 PM. The DST was carried out at midnight with oral ingestion of 1 mg of dexamethasone (Dectancyl, Laboratoires Roussel, Paris, France), followed by blood samples drawn for the assay of serum cortisol at 8 AM, 4 PM, and 11 PM the next day (day 2) (26).

On day 4, an APO test (SC injection of 0.75 mg Apokinon, Laboratoires Aguettant, France) (10) and on day 8, a CLO test (0.375 mg of Catapressan®, given orally, Laboratoires Boehringer Ingelheim, France) (27) were carried out at 9 AM, after an overnight fasting, according to the same sampling procedure. Subjects were awoken at 7 AM, and a cannula was inserted into an anterior forearm vein. Blood was drawn at -30, -15, and 0 min before APO or CLO administration and further samples for the assay of GH (following APO and CLO), and PRL, ACTH, cortisol (following APO) were collected at 15, 30, 60, 90, 120, and 150 min. Throughout the tests subjects were in bed and did not smoke.

Assays

Blood samples were centrifuged at 3,000 rpm and 4°C, and the serum separated and stored at -20°C until assay. All hormone concentrations were determined by immunoassay techniques based on enhanced luminescence (13). The ACTH assay (Nichols Advantage® ACTH, Nichols Institute Diagnostics, San Juan Capistrano, CA) had intra-assay and inter-assay coefficients of variation of 2.7%–7.9% respectively; the sensitivity was 1 ng/l. The GH assay (Nichols Advantage® hGH, same supplier) had intra-assay and inter-assay coefficients of variation of 3.9%–7.5% respectively; the sensitivity was 0.1 μg/l. The TSH assay (Amerlite TSH-60 Assay, Amersham International plc, Amersham, UK) had intra-assay and inter-assay coefficients of variation of 5.1%–7% respectively; the sensitivity was less than 0.04 mU/l. The FT4 assay (Amerlite FT4 Assay, same supplier) had intra-assay and inter-assay coefficients of variation of 5.1%–5.3% respectively; the sensitivity was 0.5 pmol/l. The FT3 assay (Amerlite FT3 Assay, same supplier) had intra-assay and inter-assay coefficients of variation of 6.0%–8.0% respectively; the sensitivity was less than 0.5 pmol/l. The prolactin assay (Amerlite Prolactin Assay, same supplier) had intra-assay and inter-assay coefficients of variation of 5.5%–6%, respectively; the sensitivity was less than 1.3 μg/l.

The cortisol assay (Amerlite Cortisol Assay, same supplier) had intra-assay and inter-assay coefficients of variation of 6.2%–8.9%; the sensitivity was less than 3 nmol/l.

Statistical Analysis

Hormonal concentrations at 0 min, immediately before SC injection of APO, were used to define baseline values of PRL, ACTH, and cortisol (i.e., PRL*BL*, ACTH*BL*, and cortisol*BL*) (5). ACTH and cortisol responses were determined for each subject by subtracting the baseline level from the peak level after APO (i.e., ΔACTH and Δcortisol). The PRL response to APO was expressed as percentage of change from baseline according to the formula: PRL_S = (PRL_S*AUC*/PRL*BLAUC*)x100 (10) in which PRL*BLAUC* is the basal PRL area under the curve (calculated as follows: PRL*BL* x 150 min), and PRL_S*AUC* is the PRL suppression area (defined as the difference between PRL*BLAUC* and PRL*AUC* after APO). GH values from time points -30, -15, and 0 min were averaged to obtain a single baseline value before APO (GH_{APO}*BL*) and CLO (GH_{CLO}*BL*) stimulation tests. To be included in this research, subjects had to have, before APO and CLO, a GH*BL* value < 2 μg/l. The maximum GH responses to APO and CLO (ΔGH_{APO} and ΔGH_{CLO}, respectively) were determined for each subject by subtracting the baseline GH level from the peak GH level. The mean of the two TSH values, at -15 and 0 min, was calculated to give baseline TSH (TSH*BL*) value. The maximum TSH response (ΔTSH) was determined by subtracting TSH*BL* level from the peak TSH level after TRH injection; ΔΔTSH was defined as the difference between 11 PM-ΔTSH and 8 AM-ΔTSH values. To evaluate the cortisol response to DST we used the maximum

cortisol level after DST in any blood sample obtained at 8 AM, 4 PM, and 11 PM on day 2 (26).

Analyses were performed using StatView software version 5.0 (SAS Institute Inc, Cary NC, USA). Given the small sample size, non-parametric statistical methods were employed. The comparisons between different patient groups and the control group were performed using the Mann-Whitney two-tailed test (U test)—formal corrections for multiple comparisons were not needed since we made planned comparisons. Within-group differences were tested by the Wilcoxon two-tailed signed rank test (T test) for paired data. Correlations between quantitative variables were estimated using the Spearman rank coefficient (Δ). We used receiver operating characteristic (ROC) curves to determine thresholds of abnormal results (28). Categorical data were analyzed with either Fisher's exact test (two-tailed) or Yates' χ^2-test. Results were considered significant when $p \leq 0.05$.

Results

Table 1 displays the demographic data and the main results of the DST, TRH, APO, and CLO tests for patients and HCs. Patients and HCs were comparable for age. Basal hormone values were not different across diagnostic groups of subjects. BDs had lower BPRS scores (mean ± SD, 44.6 ± 12.1) than SCHs (54.9 ± 14.9) and SADs (52.7 ± 15.6) ($p < 0.05$ by U test).

Apomorphine Test

PRL Levels

There was no age effect for PRL*BL* and PRLs values. Compared with HCs, PRL_S values were lower in SADs and BDs, while in SCHs the difference was not significant. PRLs values were neither influenced by PRL*BL* levels nor by HPA axis activity (i.e., cortisol at baseline and following DST). As illustrated in **Figure 1A**, 3 SCHs (23%), 7 SADs (54%), 8 BDs (61%), and 1 HC (8%) exhibited a PRLs value below 25%. SADs and BDs showed more frequently blunted PRLs values than HCs ($p = 0.03$ and $p = 0.01$, respectively, by Fisher's exact test). The distribution was not significantly different between SCHs and HCs ($p > 0.30$ by Fisher's exact test).

ACTH Levels

ACTH values were not related to age. The ACTH response to APO was not correlated with ACTH*BL* levels. Compared with HCs, ΔACTH values (**Figure 1B**) were lower in SCHs, while in SADs and BDs ΔACTH values were not significantly different. Owing to a wide variation of ΔACTH values, no meaningful threshold for a blunted response could be defined.

Cortisol Levels

Cortisol*BL* and ΔCortisol values were not influenced by age. ΔCortisol values were lower in SCHs than in HCs. In SADs and BDs, ΔCortisol levels were not significantly altered. Cortisol response to APO was unrelated to the HPA axis activity, as evaluated by cortisol values at baseline and following DST. We found a positive correlation between ΔCortisol and ΔACTH values in the overall population ($\rho = 0.75$; $n = 52$; $p < 0.00001$), in SCHs ($\rho = 0.78$; $n = 13$; $p = 0.006$), in SADs ($\rho = 0.68$; $n = 13$; $p = 0.01$), in BDs ($\rho = 0.69$; $n = 13$; $p = 0.01$), and in HCs ($\rho = 0.88$; $n = 13$; $p = 0.002$). As shown in **Figure 1C**, 7

TABLE 1 | Demographic characteristics and biological data for normal controls and patients.

	Control subjects(n =13)	Schizophrenic patients(paranoid subtype)(n=13)	Schizoaffective patients(bipolar subtype)(n=13)	Bipolar patients (depressed)(n=13)
Age, years[a]	33.2 ± 9.2	31.1 ± 10.3	32.3 ± 10.8	34.3 ± 10.8
Apomorphine test				
PRL*BL* (µg/l)	9.2 ± 5.2	7.0 ± 3.5	8.7 ± 4.4	7.7 ± 3.3
PRL_S (%)	40 ± 16	35 ± 15	24 ± 18*	19 ± 10**
ACTH*BL* (ng/l)	27.5 ± 18.9	25.7 ± 16.1	28.1 ± 15.5	27.2 ± 11.2
ΔACTH (ng/l)	50 ± 74	12.5 ± 26*	51 ± 50	23 ± 38
Cortisol*BL* (nmol/l)	257 ± 75	343 ± 150	332 ± 103	243 ± 81
ΔCortisol (nmol/l)	154 ± 160	26 ± 112*	126 ± 155	107 ± 106
GH*BL* (µg/l)	0.4 ± 0.3	0.7 ± 0.5	1.0 ± 0.8	0.5 ± 0.4
ΔGH (µg/l)	16.6 ± 9.4	15.9 ± 21.0	20.2 ± 17.0	14.3 ± 8.7
Clonidine test				
GH*BL* (µg/l)	0.4 ± 0.3	0.6 ± 0.3	0.5 ± 0.4	0.4 ± 0.3
ΔGH (µg/l)	17.4 ± 7.8	15.5 ± 19.4	7.5 ± 10.3**	9.2 ± 8.3*
TRH tests				
8 AM-FT4*BL* (pmol/l)	14.9 ± 3.9	15.1 ± 4.2	14.6 ± 4.0	14.3 ± 4.1
8 AM-FT3*BL* (pmol/l)	5.1 ± 0.8	5.2 ± 0.9	5.3 ± 0.8	5.5 ± 0.7
8 AM-TSH*BL* (mU/l)	1.13 ± 0.45	1.30 ± 0.69	1.27 ± 0.50	1.25 ± 0.58
8 AM-ΔTSH (mU/l)	6.6 ± 3.3	6.6 ± 3.8	5.7 ± 2.5	7.4 ± 3.2
11PM-TSH*BL* (mU/l)	1.23 ± 0.72	1.39 ± 0.80	1.18 ± 0.52	0.99 ± 0.55
11 PM-ΔTSH (mU/l)	10.4 ± 4.1	10.4 ± 4.8	7.2 ± 2.4*	8.2 ± 3.4
ΔΔTSH (mU/l)	3.8 ± 1.4	3.8 ± 2.3	1.4 ± 1.3**	0.7 ± 1.4***
Post-dexamethasone				
Maximum Cortisol (nmol/l)	26 ± 15	86 ± 124*	64 ± 67*	71 ± 91**

[a]*Values are mean ± SD. PRL indicates, prolactin; ACTH, adrenocorticotropin hormone; GH, growth hormone; TSH, thyrotropin; BL, basal concentration; PRLs, prolactin suppression; Δ, peak concentration minus basal concentration; ΔΔTSH, 11-ΔTSH minus 8 AM-ΔTSH.*

*Comparisons between control and patient groups were tested by U test (two-tailed): *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.*

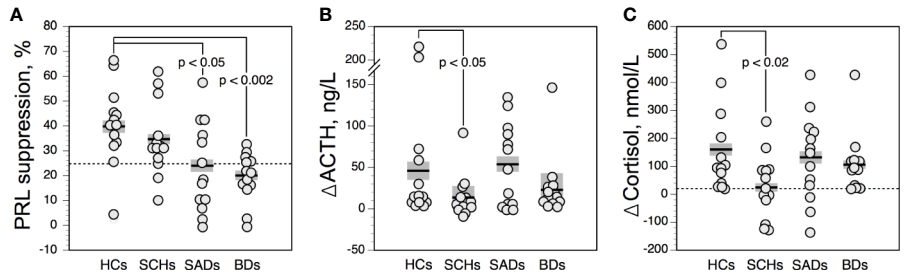

FIGURE 1 | Prolactin suppression **(A)**, and maximum increment in serum adrenocorticotropic hormone (ACTH) **(B)**, and cortisol **(C)** above baseline after 0.75 mg SC of apomorphine in controls and patients. The solid horizontal lines indicate the group mean; the shaded areas represent ± SEM. HCs, healthy control subjects; SCHs, patients with paranoid schizophrenia; SADs, patients with schizoaffective disorder; BDs, patients with bipolar depression.

SCHs (54%), 3 SADs (23%), 3 BDs (23%), and 1 HC (8%) exhibited a ΔCortisol value below 20 nmol/l. While the distribution of blunted cortisol responses was similar in SADs and BDs and did not differ significantly from HCs, blunted cortisol responses were more frequent in SCHs than in HCs ($p = 0.03$ by Fisher's exact test).

GH Levels

ΔGH_{APO} values did not differ across patients and controls, and were unrelated to age. APO-GH responses were not significantly correlated with GH*BL* levels. Interestingly, ΔGH_{APO} values were positively correlated with ΔACTH and ΔCortisol values in the whole population ($\rho = 0.44$; $n = 52$; $p = 0.001$ and $\rho = 0.31$; $n = 52$; $p = 0.02$, respectively), whereas such a correlation was not found significantly in HCs or in patients. When using a ΔGH_{APO} value of less than 6 μg/l to define a blunted response, 6 SCH (46%), 4 SADs (31%), 2 BDs (15%), and 1 HC (8%) showed blunted responses. Compared to HCs, there was a trend towards increased frequency of blunted GH_{APO} response in SCHs ($p = 0.07$ by Fisher's exact test).

Clonidine Test

The GH responses to CLO were not influenced by GH*BL* values. GH*BL* and ΔGH_{CLO} values were not significantly correlated with age in our population. **Figure 2A** shows the time courses of serum GH in the 4 diagnostic groups. ΔGH_{CLO} values were lower in SADs and BDs than in HCs (**Figure 2B**). No such difference was observed between SCHs and HCs. ΔGH_{CLO} and ΔGH_{APO} values were not significantly correlated in the total sample, in patients and in HCs. When using a value of less than 8 μg/l to define a blunted ΔGH_{CLO}, 4 SCH (31%), 8 SADs (61%) and 7 BDs (54%) had blunted responses; none were noted in HCs. Blunted GH_{CLO} response was more frequent in SADs and BDs than in HCs ($p = 0.01$ and $p = 0.03$ respectively, by Fisher's exact test), in SCHs the frequency did not reach statistical significance ($p = 0.09$ by Fisher's exact test).

Protirelin (TRH) Tests

The effect of age was not significant for FT4, FT3, and TSH values (TSH*BL*, ΔTSH, ΔΔTSH). As illustrated in **Figure 3A**, ΔTSH values were higher in the evening than in the morning in HCs, SCHs and SADs (all $p < 0.005$ by T test). In BDs, this increment was not significant ($p = 0.09$ by T test). TRH-TSH responses (i.e., 8 AM-ΔTSH and 11 PM-ΔTSH), when compared with HCs, were not different in SCHs and BDs. In SADs, however, 11PM-ΔTSH values were lower than in HCs. When using an 11PM-ΔTSH value below 6.5 mU/l to define a blunted response, 6 SADs (46%) and 6 BDs (46%) (both $p = 0.07$ by Fisher's exact test, when compared with HCs); 3 SCHs (23%) and 1 HC (8%) exhibited a blunted response. As shown in **Figure 3B**, ΔΔTSH values were reduced in SADs and BDs, while SCH showed similar ΔΔTSH values than HCs. Moreover, 12 SADS (92%) and 13 BDs (100%)—while only 2 SCHs (15%) and 1 HC (8%)—exhibited a ΔΔTSH value below 2,5 mU/l. Rates of reduced ΔΔTSH values were comparable in SADs and BDs and were higher than in HCs and SCHs (all $p <$ 0.0003 by Fisher's exact test).

Dexamethasone Suppression Test

Post-DST cortisol values were not influenced by age. Compared with HCs, post-DST cortisol levels were higher in patients. However the incidence of nonsuppression of cortisol after dexamethasone [i.e., highest post-DST cortisol level > 130 nmol/l (13)] was rather low: DST nonsuppression was

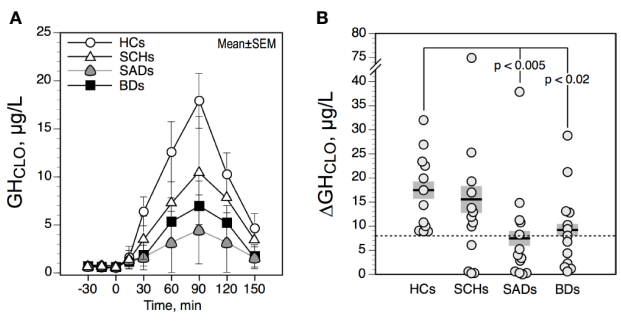

FIGURE 2 | Time course **(A)** and maximum increment **(B)** in serum growth hormone (GH) above baseline after 0.375 mg of clonidine PO in controls and patients. The solid horizontal lines indicate the group mean; the shaded areas represent ± SEM. HCs, healthy control subjects; SCHs, patients with paranoid schizophrenia; SADs, patients with schizoaffective disorder; BDs, patients with bipolar depression.

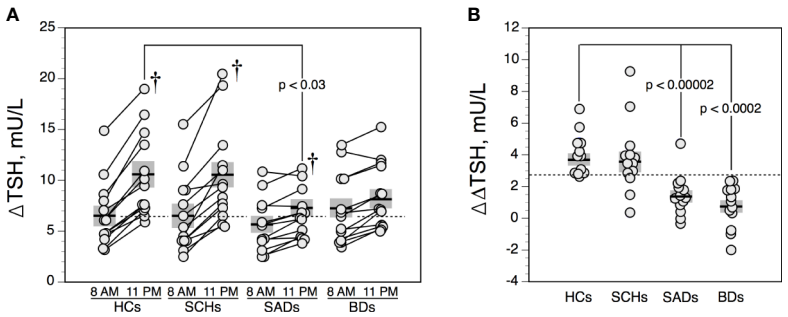

FIGURE 3 | Maximum increment in serum thyrotropin (TSH) level above baseline (ΔTSH) after 200 μg IV of protirelin (TRH) **(A)**, and difference between 11 PM-ΔTSH and 8 A-ΔTSH (ΔΔTSH) **(B)** in controls and patients. The solid horizontal lines indicate the group mean; the shaded areas represent ± SEM. HCs, healthy control subjects; SCHs, patients with paranoid schizophrenia; SADs, patients with schizoaffective disorder; BDs, patients with bipolar depression. †p < 0.005 by T test (comparison between 8 AM-ΔTSH and 11 PM-ΔTSH values).

observed in 2 SCHs and 2 BDs (both 15%); 1 SAD (8%); and none HC (**Figure 4**).

Frequency of Abnormal Test Responses Among Patients and Control Subjects

Figure 5 summarizes the number of abnormal test responses in patients and HCs. When analyzing the frequency of normal/abnormal test responses (i.e., APO-PRLs, APO-ΔCortisol, CLO-ΔGH, TRH-ΔΔTSH), SADs and BDs displayed a similar pattern of abnormalities, significantly different from SCHs (Yates' χ^2 = 28.43, df =7, p < 0.0002).

DISCUSSION

Our study clearly demonstrates that multihormonal responses to a series of neuroendocrine test battery (APO test, CLO test, 8 AM and 11 PM TRH tests, and DST) vary according to diagnostic categories. In unmedicated paranoid SCH inpatients, pituitary-adrenal response to APO (i.e., ΔACTH and ΔCortisol) was reduced, while hormone responses to CLO and TRH tests were not significantly altered. The patterns of abnormality of hormonal responses of unmedicated depressed SAD and BD inpatients were very close and were characterized by a reduced APO-induced PRL suppression, a reduced CLO-induced GH stimulation, and a chronobiological alteration of the HPT axis (as reflected by reduced ΔΔTSH values). It should be noted that in the affective groups, BDs showed weaker psychotic symptoms than SADs (as reflected by lower BPRS scores), while their hormonal profile was quite comparable. Hence, this would suggest that the biological correlates of psychotic symptoms depend on the nosographical context. Increased HPA axis activity (as evidenced by higher post-DST cortisol values compared to HCs) was observed in SCHs as well as SADs and BDs—although overt hyperactivity of this axis (i.e., DST non-suppression) was rather infrequent in patients of our sample. In addition, the differences observed in test responses between patients and HCs did not seem to be an artifact of factors known to influence serum hormone levels (such as age, gender, medication) since we investigated a population of middle-aged male drug-free subjects.

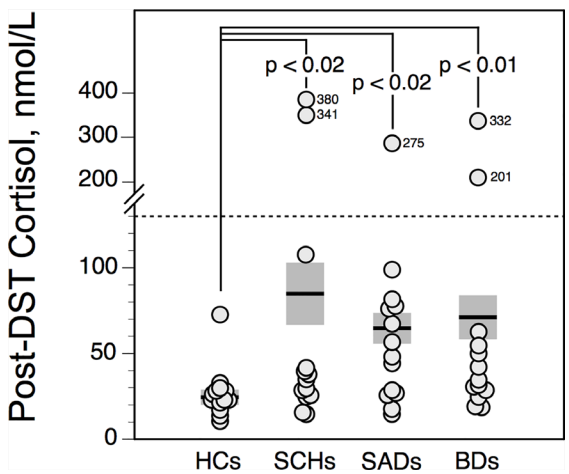

FIGURE 4 | Highest serum cortisol value following dexamethasone suppression test (DST) in controls and patients. The solid horizontal lines indicate the group mean; the shaded areas represent ± SEM. HCs, healthy control subjects; SCHs, patients with paranoid schizophrenia; SADs, patients with schizoaffective disorder; BDs, patients with bipolar depression.

Apomorphine Test

Confirming our previous studies (5, 7, 8), ACTH and cortisol responses to APO are strongly correlated. This suggests that cortisol stimulation by APO, despite localization of DA-D_2 receptors in the adrenal gland (29), is secondary to that of ACTH. Blunted ACTH/cortisol to APO response has consistently been found in schizophrenia (5–8). This blunting appears independent of HPA axis activity (6) and DST status (7, 8). Moreover, it seems unlikely that decrease APO-induced ACTH/cortisol stimulation is due to decreased reserve of pituitary ACTH (8) or residual antipsychotic effect, given that, in our study, baseline PRL levels are similar between SCHs and HC (antipsychotics *via* a D_2 blocking effect can increase prolactinemia). From a pathophysiological viewpoint, the mechanisms underlying a reduced ACTH/cortisol response to

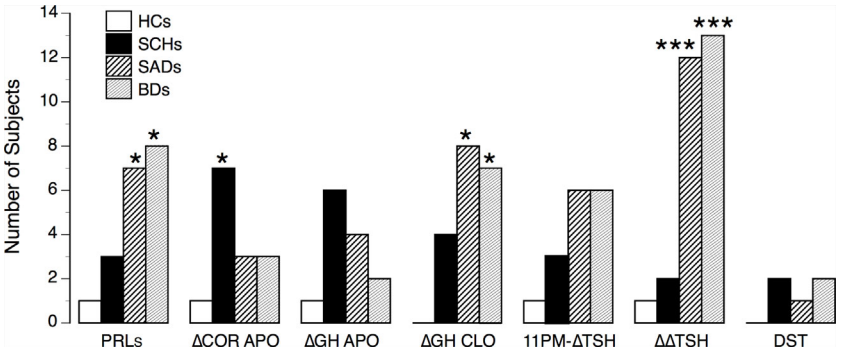

FIGURE 5 | Number of abnormal test responses in controls and patients. HCs, healthy control subjects; SCHs, patients with paranoid schizophrenia; SADs, patients with schizoaffective disorder; BDs, patients with bipolar depression. PRLs, prolactin suppression following apomorphine (APO); ΔCOR APO, maximum increment in serum cortisol level above baseline after APO; ΔGH APO, maximum increment in serum growth hormone (GH) level above baseline after APO; ΔGH CLO, maximum increment in serum growth hormone (GH) level above baseline after clonidine (CLO); 11 PM-ΔTSH, maximum increment in serum thyrotropin level above baseline (ΔTSH) after protirelin (TRH); ΔΔTSH, difference between 11 PM-ΔTSH and 8 A-ΔTSH values. Comparisons between HCs and patients: *$p < 0.05$; ***$p < 0.001$ (by Fisher's exact test).

APO are not completely understood. It is known that APO binds the D_2-like (D_2, D_3, D_4) receptor and the D_1-like receptor (D_1, D_5) subtypes (30). Since D_2 and D_1 receptors are involved in the regulation of CRH (31, 32)—and therefore ACTH release—one may hypothesize that the blunted ACTH/cortisol response to APO reflects reduced hypothalamic DA receptor sensitivity. Interestingly, D_2 receptors are also expressed in the pituitary corticotroph cells but their role is thought to be inhibitory on ACTH secretion (33). Therefore, the blunted ACTH/cortisol response to APO in paranoid SCHs is compatible with a hyposensitivity (or persistent down-regulation) of the DA-D_2 and/or D_1 receptors connected with the regulation of HPA axis possibly secondary to increased presynaptic DA activity at the hypothalamic level. Given the DA abnormality in SCH is thought to primarily involve synthesis and release activity (34), our results are in line with the hypothesis of an increased DA activity in the mesolimbic-hypothalamic pathway in paranoid SCHs. However, APO has also affinity for serotonin receptors (5-HT_{1A}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}), and α-adrenergic receptors (α_{1B}, α_{1D}, α_{2A}, α_{2B}, and α_{2C}) (30, 35). Most of these receptors have been involved to different degrees in the regulation of CRH activity [for a review, see (3)]. Consequently, the blunted APO-induced ACTH/cortisol stimulation might also reflect in part 5-HT and α-adrenergic receptor dysfunction, although this hypothesis needs further investigation in schizophrenic patients.

The GH and ACTH/cortisol responses to APO are correlated in the whole sample, but not in the diagnostic groups of subjects. Despite there is a trend towards blunting in SCHs, ΔGH_{APO} values are not significantly different across patients and HCs. This latter result is in agreement with previous published reports (5–7, 36, 37) but not all (38, 39). As previously discussed (8), the effect of APO on GH involves different pathways from those mediating ACTH/cortisol response, since this response requires the participation of GH-releasing-hormone (GHRH) neurons and acetylcholine, and other neurotransmitters/hormones such as NA, 5-HT, GABA, ghrelin, and cholecystokinin are probably involved in the GH response to APO. These confounding factors consequently may limit the value of the GH response to APO in the investigation of DA function in psychiatry.

In agreement with several studies (5, 9, 12, 40) APO induced-PRL suppression is altered in our population of BDs and SADs. The lack of significant difference in the PRL response to APO between SCHs and HCs is also consistent with prior reports (5, 7, 9) but not all (41). The release of PRL is inhibited by the tuberoinfundibular (TI) DA neurons *via* D_2 receptors (4). Our findings suggest hyposensitivity of the D_2 receptors of the lactotrophs in BDs and SADs, possibly secondary to the activation of the TIDA neurons. However, it is also possible that PRLs blunting might be due to functional alteration of lactotrophs cells. This hypothesis is not confirmed by a previous study (40), in which 8 AM and 11 PM PRL responses to TRH stimulation tests were comparable between unipolar (UP) and bipolar depressed patients, while BDs, unlike UPs, exhibited blunted APO-induced PRLs values (12).

Clonidine Test

CLO induces a robust GH response *via* activation of postsynaptic α_2-adrenoceptors, which increase the secretion of GHRH and inhibit the secretion of somatostatin (42). The blunted GH response to CLO is well documented in depression (16, 26, 43) and in SAD (9, 16). Such a response may be due to decreased postsynaptic α_2-receptor responsiveness linked to an erratic release of presynaptic NA (43). Thus, the comparable ΔGH values found in depressed BDs and SADs suggest a possible biological link between these two diseases (i.e., NA dysregulation). In agreement with a previous study of our group (16), ΔGH_{CLO} values in paranoid SCHs are not altered (suggesting normal sensitivity of hypothalamic α_2-adrenoreceptors in these patients), although in disorganized SCHs it has been found greater CLO-induced GH responses (37) (suggesting hypersensitivity of α_2-adrenoreceptors in these patients). However, this latter finding has not been replicated (16).

Protirelin (TRH) Tests

Results obtained from the morning TRH-TSH challenge agree with those of previous published reports [for review, see (21)]. Morning TRH-TSH responses are not significantly different across the patient and control groups. In the evening, TRH-TSH responses at 11 PM are higher than at 8 AM (albeit not significant in BDs). Consistent with a previous study, ΔΔTSH values are reduced in depressed SADs and BDs, while they are unaltered in SCHs (9). We have already discussed that the ΔΔTSH test is a chronobiological refinement of the TRH test (25, 44). Pathophysiological components involved in an abnormal ΔΔTSH test may be synthesized as follows (21):

1. A chronobiological component involving the determinants of circadian TSH secretion [i.e., a weaker output of the hypothalamic suprachiasmatic nuclei (45)], since reduced ΔΔTSH values are associated with decreased 24-h TSH mesor and amplitude levels in depression (25).
2. A chronesthesic component involving TRH receptor sensitivity, since altered sensitivity of TRH receptors is more evidenced at 11 PM than at 8 AM (25). TRH receptor hyposensitivity may be adaptive to prolonged hypersecretion of endogenous TRH (46).
3. A self-regulating component, since the ΔΔTSH test takes into account the negative feedback of thyroid hormones on TSH secretion. Indeed, the TRH test performed at 8 AM stimulates thyroid hormone secretion, increasing, therefore, the negative feedback of thyroid hormones on TSH secretion in the evening (44).
4. A dynamic component, since 11 PM-ΔTSH blunting could also be related to a reduced TSH resynthesis in the thyrotrophs during the hours following the 8 AM TRH test (given that TRH stimulates preformed TSH). Decreased TSH synthesis could involve a hyposensitivity of the pituitary TRH receptors and/or an increased negative feedback of thyroid hormones, or a decreased central TRH activity [especially in recent suicide attempters in whom FT_4 levels are also reduced (44)]. In our population, no patient had a history of suicidal behavior; therefore reduced ΔΔTSH values in SADs and BDs are unlikely to be due to a decrease in the central activity of TRH.

Dexamethasone Suppression Test

In our sample, SCHs, SADs and BDs exhibit significant higher post-DST cortisol values than HCs, indicating a weaker suppressing effect of dexamethasone. This finding, which could reflect decreased type II GR function, converges with the growing literature on HPA axis dysregulation in psychotic and affective diseases (18, 19, 47). However in our study, DST nonsuppression occurs only in a low proportion of patients. This non-expected low incidence—especially in depressed SADs and BDs—is nonetheless in accordance with some previous but not all reports [for review, see (3)]. We could presume that the sensitivity in detecting HPA axis overactivity would be better using the combined dexamethasone/corticotropin-releasing hormone (DEX/CRH) test (48–50), although all studies do not agree (51, 52). It has been hypothesized that the hyperactivity of the HPA axis is primarily a reflection of abnormal limbic-hypothalamic activation, with increased secretion of hypothalamic CRH and consequent excessive adrenal cortisol secretion (17). Given the high rate of reduced ΔΔTSH values in BDs and SADs—possibly reflecting endogenous TRH hypersecretion—one may hypothesize that increased TRH secretion (both from hypophysiotropic and non-hypophysiotropic neurons) could decrease glucocorticoid secretion by impairing the last steps of 11ß-hydroxylation without affecting the earlier steps (53). In such case, the GR function would be only partially attenuated, despite CRH overdrive, explaining therefore why SAD and BD patients with reduced ΔΔTSH values are often DST suppressors.

Limitations

Some shortcomings in this present study require discussion. First, our results concern only a specific group of drug-free male inpatients; they do not appear at present transposable to outpatients, and consequently they cannot be generalizable to affective and psychotic patients. Second, given the exploratory nature of our research, and the drastic inclusion criteria, we studied a rather small sample of psychiatric inpatients. This may have reduced the statistical power of our analyses (performed with nonparametric methods). Thus, our findings must be considered preliminary until replicated in a larger patient population. Third, among the confounding factors in assessing neurotransmitter function, insufficient washout period could be a major bias. However, our exclusion criteria and the length of the wash-out period (minimum 2 weeks for the APO test and 3 weeks for the CLO test) seem sufficient to avoid biases induced by drugs on the systems studied (5, 54). Finally, we did not measure serum dexamethasone. However, it has been argued that the concentration of dexamethasone bound to the receptors in the pituitary is the relevant physiologic parameter rather than the dexamethasone concentration in plasma (55, 56).

In conclusion, the multivariate neuroendocrine approach used in this study was able to identify patterns of hormonal response abnormalities in drug-free hospitalized patients with psychotic and affective symptoms. From a pathophysiological viewpoint, our results suggest that depressed bipolar and schizoaffective patients share common biological dysregulations, clearly distinct from that of paranoid schizophrenic patients. Future studies are needed to determine whether these findings could be relevant in managing psychiatric treatments.

DATA AVAILABILITY STATEMENT

The datasets generated for this study are available on request to the corresponding author.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Centre Hospitalier Rouffach. The patients/

participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

FD designed the study, wrote the protocol, and wrote the first draft of the manuscript. M-CM undertook the statistical analysis and interpreted the results. AE made clinical assessments. FG made clinical assessments. VD made clinical assessments. LJ managed the literature searches. All authors contributed to the article and approved the submitted version.

FUNDING

Funding of this study was provided by inner hospital sources (Centre Hospitalier, Rouffach). No outside parties had any role in study design; in the collection, analysis, and interpretation of data; in the writing of the report and in the decision to submit the paper for publication.

ACKNOWLEDGMENTS

The authors express their gratitude to the physicians and the nurses of the Pole 8/9, Psychiatric Hospital of Rouffach (France).

REFERENCES

1. Ashok AH, Marques TR, Jauhar S, Nour MM, Goodwin GM, Young AH, et al. The dopamine hypothesis of bipolar affective disorder: the state of the art and implications for treatment. *Mol Psychiatr* (2017) 22:666–79. doi: 10.1038/mp.2017.16
2. Yang AC, Tsai SJ. New targets for schizophrenia treatment beyond the dopamine hypothesis. *Int J Mol Sci* (2017) 18:E1689. doi: 10.3390/ijms18081689
3. Duval F. Endocrinologie et Psychiatrie [Article in French]. *EMC Psychiatr* (2016) 13(4):1–27. doi: 10.1016/S0246-1072(16)75332-6
4. Lal S. Apomorphine in the evaluation of dopaminergic function in man. *Prog Neuro-Psychopharmacol Biol Psychiatry* (1988) 12:117–64. doi: 10.1016/0278-5846(88)90033-4
5. Mokrani MC, Duval F, Crocq MA, Bailey PE, Macher JP. Multihormonal responses to apomorphine in mental illness. *Psychoneuroendocrinology* (1995) 20:365–75. doi: 10.1016/0306-4530(94)00065-4
6. Meltzer HY, Lee MA, Jayathilake K. The blunted plasma cortisol response to apomorphine and its relationship to treatment response in patients with schizophrenia. *Neuropsychopharmacology* (2001) 24:278–90. doi: 10.1016/S0893-133X(00)00201-3
7. Duval F, Mokrani MC, Crocq MA, Bailey P, Diep TS, Correa H, et al. Dopaminergic function and the cortisol response to dexamethasone in psychotic depression. *Prog Neuro-Psychopharmacol Biol Psychiat* (2000) 24:207–25. doi: 10.1016/S0278-5846(99)00098-6
8. Duval F, Mokrani MC, Monréal J, Bailey P, Valdebenito M, Crocq MA, et al. Dopamine and serotonin function in untreated schizophrenia: clinical correlates of the apomorphine and d-fenfluramine tests. *Psychoneuroendocrinology* (2003) 28:627–42. doi: 10.1016/S0306-4530(02)00047-1
9. Duval F, Mokrani MC, Crocq MA, Jautz M, Bailey PE, Diep TS, et al. Multihormonal reponses to a series of neuroendocrine challenges in psychiatry: a multivariate approach. In: Macher JP, Crocq MA, Nedelec JF, editors. *New Prospects in Psychiatry: The Bio-clinical Interface*. Paris, F: John Libbey Eurotext (1995). p. 77–90.
10. Meltzer HY, Kolakowska T, Fang VS, Fogg L, Robertson A, Lewine R, et al. Growth hormone and prolactin response to apomorphine in schizophrenia and the major affective disorders. *Arch Gen Psychiatry* (1984) 41:512–9. doi: 10.1001/archpsyc.1984.01790160098013
11. Muller-Spahn F, Modell S, Ackenheil M, Brachner A, Kurtz G. Elevated response of growth hormone to graded doses of apomorphine in schizophrenic patients. *J Psychiatr Res* (1998) 32:265–71. doi: 10.1016/S0022-3956(98)00005-3
12. Monreal JA, Duval F, Mokrani MC, Fattah S, Palao D. Differences in multihormonal responses to the dopamine agonist apomorphine between unipolar and bipolar depressed patients. *J Psychiatr Res* (2019) 112:18–22. doi: 10.1016/j.jpsychires.2019.02.009
13. Duval F, Mokrani MC, Monréal J, Fattah S, Champeval C, Schulz P, et al. Cortisol hypersecretion in unipolar major depression with melancholic and psychotic features : dopaminergic, noradrenergic and thyroid correlates. *Psychoneuroendocrinology* (2006) 31:876–88. doi: 10.1016/j.psyneuen.2006.04.003
14. Schatzberg AF, Rothschild AJ, Langlais PJ, Bird ED, Cole JO. A corticosteroid/dopamine hypothesis for psychotic depression and related states. *J Psychiatr Res* (1985) 19:57–64. doi: 10.1016/0022-3956(85)90068-8
15. Siever LJ, Uhde TW. New studies and perspectives on the noradrenergic receptor system in depression: effect of the α2-adrenergic agonist clonidine. *Biol Psychiatry* (1984) 19:131–56.
16. Mokrani MC, Duval F, Diep TS, Bailey PE, Macher JP. Multihormonal response to clonidine in patients with affective and psychotic symptoms. *Psychoneuroendocrinology* (2000) 25:741–52. doi: 10.1016/S0306-4530(00)00024-X
17. Gillespie CF, Nemeroff CB. Hypercortisolemia and depression. *Psychosom Med* (2005) 67 Suppl 1:S26–28. doi: 10.1097/01.psy.0000163456.22154.d2
18. Naughton M, Dinan TG, Scott LV. Corticotropin-releasing hormone and the hypothalamic-pituitary-adrenal axis in psychiatric disease. *Handb Clin Neurol* (2014) 124:69–91. doi: 10.1016/B978-0-444-59602-4.00005-8
19. Cherian K, Schatzberg AF, Keller J. HPA axis in psychotic major depression and schizophrenia spectrum disorders: cortisol, clinical symptomatology, and cognition. *Schizophr Res* (2019) 213:72–9. doi: 10.1016/j.schres.2019.07.003
20. APA Task force on laboratory tests. The dexamethasone suppression test: an overview of its current status in psychiatry. *Am J Psychiatry* (1987) 14:1253–62. doi: 10.1176/ajp.144.10.1253
21. Duval F, Mokrani MC. Thyroid Axis Activity in Depression. *Ann Thyroid Res* (2018) 4(3):166–71.
22. Jackson IM. The thyroid axis and depression. *Thyroid* (1998) 8:951–6. doi: 10.1089/thy.1998.8.951
23. Endicott J, Spitzer RL. [Schedule for Affective Disorders and Schizophrenia (SADS)] [Article in French]. *Acta Psychiatr Belg* (1987) 87(4):361–516.
24. American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders. 4th ed.* Washington, DC: American Psychiatric Press (1994). p. 886.
25. Duval F, Macher JP, Mokrani MC. Difference between evening and morning thyrotropin responses to protirelin in major depressive episode. *Arch Gen Psychiatry* (1990) 47:443–8. doi: 10.1001/archpsyc.1990.01810170043007
26. Carroll BJ, Feinberg M, Greden JF, Tarika J, Albala AA, Haskett RF, et al. A specific laboratory test for the diagnosis of melancholia. Standardization, validation, and clinical utility. *Arch Gen Psychiatry* (1981) 8:15–22. doi: 10.1001/archpsyc.1981.01780260017001
27. Valdivieso S, Duval F, Mokrani MC, Schaltenbrandt N, Oliveira Castro J, Crocq MA, et al. Growth hormone response to clonidine and the cortisol response to dexamethasone in depressive patients. *Psychiatry Res* (1996) 60:23–32. doi: 10.1016/0165-1781(95)02606-1
28. Metz CE. Basic principles of ROC analysis. *Semin Nucl Med* (1978) 8:283–98. doi: 10.1016/S0001-2998(78)80014-2
29. Pivonello R, Ferone D, de Herder WW, de Krijger RR, Waaijers M, Mooij DM, et al. Dopamine receptor expression and function in human normal adrenal gland and adrenal tumors. *J Clin Endocrinol Metab* (2004) 89:4493–502. doi: 10.1210/jc.2003-031746

30. Millan MJ, Maiofiss L, Cussac D, Audinot V, Boutin JA, Newman-Tancredi A. Differential actions of antiparkinson agents at multiple classes of monoaminergic receptor. I. A multivariate analysis of the binding pro les of 14 drugs at 21 native and cloned human receptor subtypes. *J Pharmacol Exp Therapeut* (2002) 303:791–804. doi: 10.1124/jpet.102.039867
31. Borowski B, Kuhn C. D1 and D2 dopamine receptors stimulate hypothalamo-pituitary-adrenal activity in rats. *Neuropharmacology* (1992) 31:671–8. doi: 10.1016/0028-3908(92)90145-F
32. Eaton MJ, Cheung S, Moore KE, Lookingland KJ. Dopamine receptor-mediated regulation of corticotropin-releasing hormone neurons in the hypothalamic paraventricular nucleus. *Brain Res* (1996) 738:60–6. doi: 10.1016/0006-8993(96)00765-2
33. Pivonello R, Waaijers M, Kros JM, Pivonello C, de Angelis C, Cozzolino A, et al. Dopamine D2 receptor expression in the corticotroph cells of the human normal pituitary gland. *Endocrine* (2017) 57:314–25. doi: 10.1007/s12020-016-1107-2
34. Howes OD, Kambeitz J, Kim E, Stahl D, Slifstein M, Abi-Dargham A, et al. The nature of dopamine dysfunction in schizophrenia and what this means for treatment. *Arch Gen Psychiatry* (2012) 69:776–86. doi: 10.1001/archgenpsychiatry.2012.169
35. Kvernmo T, Hartter S, Burger E. A review of the receptor-binding and pharmacokinetic properties of dopamine agonists. *Clin Ther* (2006) 28:1065–78. doi: 10.1016/j.clinthera.2006.08.004
36. Malas KL, van Kammen DP, de Fraites EA, Brown GM, Gold PW. Reduced growth hormone response to apomorphine in schizophrenic patients with poor premorbid social functioning. *J Neural Transm* (1987) 69:319–24. doi: 10.1007/BF01244352
37. Brambilla F, Marini S, Saito A, Fassone G, Picardi A, Nerozzi D, et al. Noradrenergic and dopaminergic interrelation in schizophrenia. *Psychiatry Res* (1994) 53(3):231–42. doi: 10.1016/0165-1781(94)90052-3
38. Cleghorn JM, Brown GM, Brown PJ, Kaplan RD, Mitton J. Longitudinal instability of hormone responses in schizophrenia. *Prog Neuropsychopharmacol Biol Psychiatry* (1983) 7:545–9. doi: 10.1016/0278-5846(83)90023-4
39. Zemlan FP, Hirschowitz J, Garver DL. Relation of clinical symptoms to apomorphine-stimulated growth hormone release in mood-incongruent psychotic patients. *Arch Gen Psychiatry* (1986) 43:1162–7. doi: 10.1001/archpsyc.1986.01800120048010
40. Monreal J, Duval F, Mokrani MC, Pinault G, Macher JP. Exploration de la fonction dopaminergique dans les depressions bipolares et unipolares. *Ann Med Psychol* (2005) 163:399–404. doi: 10.1016/j.amp.2005.04.011
41. Tamminga CA, Smith RC, Pandey G, Frohman LA, Davis JM. A neuroendocrine study of supersensitivity in tardive dyskinesia. *Arch Gen Psychiatry* (1977) 34:1199–203. doi: 10.1001/archpsyc.1977.01770220081009
42. Al-Damluji S. Adrenergic control of the secretion of anterior pituitary hormones. *Baillieres Clin Endocrinol Metab* (1993) 7:355–92. doi: 10.1016/S0950-351X(05)80180-6
43. Siever LJ, Davis KL. Overview: toward a dysregulation hypothesis of depression. *Am J Psychiatry* (1985) 142:1017–31. doi: 10.1176/ajp.142.9.1017
44. Duval F, Mokrani MC, Erb A, Gonzalez Lopera FG, Calleja C, Paris V. Relationship between chronobiological thyrotropin and prolactin responses to protirelin (TRH) and suicidal behavior in depressed patients. *Psychoneuroendocrinology* (2017) 85:100–9. doi: 10.1016/j.psyneuen.2017.07.488
45. Duval F, Mokrani MC, Erb A, Gonzalez F, Danila V, Raverot V, et al. Relationship between melatonergic and thyroid systems in depression. *Endocrinol Diabetes Metab J* (2019) 3:1–4. doi: 10.1016/j.euroneuro.2018.11.554
46. Loosen PT, Prange AJJr. Serum thyrotropin response to thyrotropin-releasing hormone in psychiatric patients: a review. *Am J Psychiatry* (1982) 139:405–16. doi: 10.1176/ajp.139.4.405
47. Jacobson L. Hypothalamic-pituitary-adrenocortical axis: neuropsychiatric aspects. *Compr Physiol* (2014) 4:715–38. doi: 10.1002/cphy.c130036
48. Heuser I, Yassouridis A, Holsboer F. The combined dexamethasone/CRH test: a refined laboratory test for psychiatric disorders. *J Psychiatr Res* (1994) 28:341–56. doi: 10.1016/0022-3956(94)90017-5
49. Watson S, Gallagher P, Smith MS, Ferrier IN, Young AH. The dex/CRH test is it better than the DST? *Psychoneuroendocrinology* (2006) 31:889–94. doi: 10.1016/j.psyneuen.2006.03.001
50. Mokhtari M, Arfken C, Boutros N. The DEX/CRH test for major depression: a potentially useful diagnostic test. *Psychiatry Res* (2013) 208:131–9. doi: 10.1016/j.psychres.2012.09.032
51. Schüle C, Baghai TC, Eser D, Häfner S, Born C, Herrmann S, et al. The combined dexamethasone/CRH Test (DEX/CRH test) and prediction of acute treatment response in major depression. *PloS One* (2009) 4(1):e4324. doi: 10.1371/journal.pone.0004324
52. Kinoshita S, Kanazawa T, Kikuyama H, Yoneda H. Clinical application of DEX/CRH test and multi-channel NIRS in patients with depression. *Behav Brain Funct* (2016) 12(1):25. doi: 10.1186/s12993-016-0108-x
53. Neri G, Malendowicz LK, Andreis P. Nussdorfer GG Thyrotropin-releasing hormone inhibits glucocorticoid secretion of rat adrenal cortex: in vivo and in vitro studies. *Endocrinology* (1993) 133:511–4. doi: 10.1210/endo.133.2.8393765
54. Schittecatte M, Charles G, Machowski R, Wilmotte J. Tricyclic wash-out and growth hormone response to clonidine. *Brit J Psychiatry* (1989) 154:858–63. doi: 10.1192/bjp.154.6.858
55. Wiedemann K, Holsboer F. The effect of dexamethasone dosage upon plasma cortisol and dexamethasone during the DST. *J Affect Disord* (1990) 19:133–7. doi: 10.1016/0165-0327(90)90018-4
56. Young EA, Kotun J, Haskett RF, Grunhaus L, Greden JF, Watson SJ, et al. Dissociation between pituitary and adrenal suppression to dexamethasone in depression. *Arch Gen Psychiatry* (1993) 50:395–403. doi: 10.1001/archpsyc.1993.01820170073010

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Advantages of publishing in Frontiers

OPEN ACCESS
Articles are free to read for greatest visibility and readership

FAST PUBLICATION
Around 90 days from submission to decision

HIGH QUALITY PEER-REVIEW
Rigorous, collaborative, and constructive peer-review

TRANSPARENT PEER-REVIEW
Editors and reviewers acknowledged by name on published articles

Frontiers
Avenue du Tribunal-Fédéral 34
1005 Lausanne | Switzerland

Visit us: www.frontiersin.org
Contact us: info@frontiersin.org | +41 21 510 17 00

REPRODUCIBILITY OF RESEARCH
Support open data and methods to enhance research reproducibility

DIGITAL PUBLISHING
Articles designed for optimal readership across devices

FOLLOW US
@frontiersin

IMPACT METRICS
Advanced article metrics track visibility across digital media

EXTENSIVE PROMOTION
Marketing and promotion of impactful research

LOOP RESEARCH NETWORK
Our network increases your article's readership