



# Elevated Brain Glutamate Levels in Bipolar Disorder and Pyruvate Carboxylase-Mediated Anaplerosis

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*In vivo*  $^1\text{H}$  magnetic resonance spectroscopy studies have found elevated brain glutamate or glutamate + glutamine levels in bipolar disorder with surprisingly high reproducibility. We propose that the elevated glutamate levels in bipolar disorder can be explained by increased pyruvate carboxylase-mediated anaplerosis in brain. Multiple independent lines of evidence supporting increased pyruvate carboxylase-mediated anaplerosis as a common mechanism underlying glutamatergic hyperactivity in bipolar disorder and the positive association between bipolar disorder and obesity are also described.

**Keywords:** glutamate, magnetic resonance spectroscopy, pyruvate carboxylase, bipolar disorder, obesity

## OPEN ACCESS

### Edited by:

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### Specialty section:

This article was submitted to  
Molecular Psychiatry,  
a section of the journal  
Frontiers in Psychiatry

**Received:** 12 December 2020

**Accepted:** 19 January 2021

**Published:** 23 February 2021

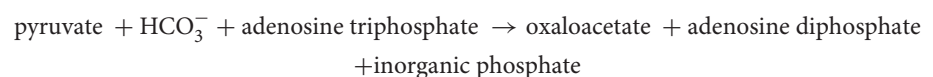
### Citation:

Shen J and Tomar JS (2021) Elevated  
Brain Glutamate Levels in Bipolar  
Disorder and Pyruvate  
Carboxylase-Mediated Anaplerosis.  
Front. Psychiatry 12:640977.  
doi: 10.3389/fpsy.2021.640977

## INTRODUCTION

The etiologic and disease mechanisms of bipolar disorder remain poorly understood. A growing body of evidence indicates a central role of mitochondrial dysfunction in the pathophysiology of bipolar disorder. Post-mortem brain studies have revealed abnormal size, structure and distribution of mitochondria as well as a pronounced and extensive decrease in nuclear gene expression governing oxidative phosphorylation in bipolar disorder (1–3). These post-mortem results are consistent with *in vivo* findings of elevated cerebrospinal fluid pyruvate and lactate levels (4, 5), decreased adenosine triphosphate production and a significant shift from oxidative phosphorylation to glycolysis in brain in bipolar disorder accompanied by elevated brain lactate levels and lowered intracellular pH as reported by *in vivo*  $^{31}\text{P}$  and  $^1\text{H}$  magnetic resonance spectroscopy (MRS) studies (6–10). Paradoxically, despite the impaired mitochondrial function and oxidative metabolism in bipolar disorder *in vivo*  $^1\text{H}$  MRS studies have also reported a highly reproducible pattern of elevated total glutamate or glutamate + glutamine levels (11) (glutamate + glutamine is dominated by glutamate in MRS spectra).

Pyruvate carboxylase is a mitochondrial enzyme. It catalyzes the thermodynamically irreversible carboxylation of pyruvate to oxaloacetate which is a tricarboxylic acid (TCA) cycle intermediate used for various biosynthetic pathways depending on the tissues. The biotin-dependent pyruvate carboxylase employs pyruvate and the polar molecule bicarbonate instead of  $\text{CO}_2$  as its substrates:



Pyruvate carboxylase-mediated anaplerosis is at the metabolic crossroad of carbohydrate and lipid metabolism, playing a key role in gluconeogenesis, lipogenesis, and glutamate homeostasis

(see **Figure 1**). In brain, released neurotransmitter glutamate is replenished by the glutamate-glutamine neurotransmitter cycle and *de novo* glutamate synthesis via pyruvate carboxylase-mediated anaplerosis in astrocytes (12–21). In this work we propose that the elevated brain glutamate levels in bipolar disorder observed by  $^1\text{H}$  MRS with very high consistency can be explained by an increase in pyruvate carboxylase-mediated anaplerosis. Evidence supporting increased pyruvate carboxylase-mediated anaplerosis as a common mechanism underlying glutamatergic hyperactivity in bipolar disorder and the positive association between bipolar disorder and obesity is also discussed. The pyruvate carboxylase-mediated anaplerotic pathway may represent future therapeutic targets for bipolar disorder.

## BRAIN PYRUVATE CARBOXYLATION IN BIPOLAR DISORDER

### Pyruvate Carboxylase-Mediated Anaplerosis Is Essential for Maintaining Glutamate Homeostasis

Presynaptic release of neurotransmitter glutamate is accompanied by its rapid uptake into astrocytes to maintain an extremely low extracellular glutamate level. The released neuronal glutamate is replenished predominantly by astrocytic glutamine supplied by the glutamate-glutamine neurotransmitter cycle and *de novo* glutamate synthesis (22–24). Abundant evidence shows that neurons lack the anaplerotic enzyme pyruvate carboxylase required for *de novo* synthesis of TCA cycle intermediates (19, 21, 25). Through pyruvate carboxylase-mediated anaplerosis in astrocytes pyruvate and bicarbonate enter the TCA cycle to replenish carbon skeletons lost via glutamine efflux. The subsequently formed TCA cycle intermediate  $\alpha$ -ketoglutarate is converted to glutamate by transamination via aspartate aminotransferase or reductive amination via glutamate dehydrogenase. Glutamate can be subsequently converted to glutamine by glutamine synthetase (15), an enzyme exclusively expressed in astrocytes (26). Numerous *in vivo*  $^{13}\text{C}$  MRS studies have established that the glutamate-glutamine neurotransmitter cycle between astrocytes and neurons is a major metabolic flux in brain (12, 13, 16, 20, 23). In the meanwhile, glutamine efflux from the brain is highly significant (27). Many studies have demonstrated that *de novo* synthesis of glutamate is a significant metabolic pathway essential for maintaining glutamate/glutamine homeostasis in the central nervous system (16, 18).

### Glutamate and Glutamatergic Hyperactivity in Bipolar Disorder

Glutamate is the major excitatory neurotransmitter in the central nervous system. Although the pathophysiology of bipolar disorder is still poorly understood, growing evidence suggests that glutamatergic abnormalities play a key role in the pathogenesis and treatment of bipolar disorder. For example, many rodent studies have demonstrated that mood stabilizers modulate glutamatergic receptors while manipulation

of glutamatergic receptors causes significant changes in mood-associated behaviors (28, 29). Post-mortem studies of bipolar disorder have also produced evidence of excitotoxicity in the frontal cortex (30), altered glutamatergic function on both presynaptic and post-synaptic sides, and abnormal excitatory synaptic connections (31, 32). In keeping with the preclinical and post-mortem findings of glutamatergic hyperactivity in bipolar disorder an *in vivo* transcranial magnetic stimulation study has reported impaired cortical inhibition in bipolar disorder (33).

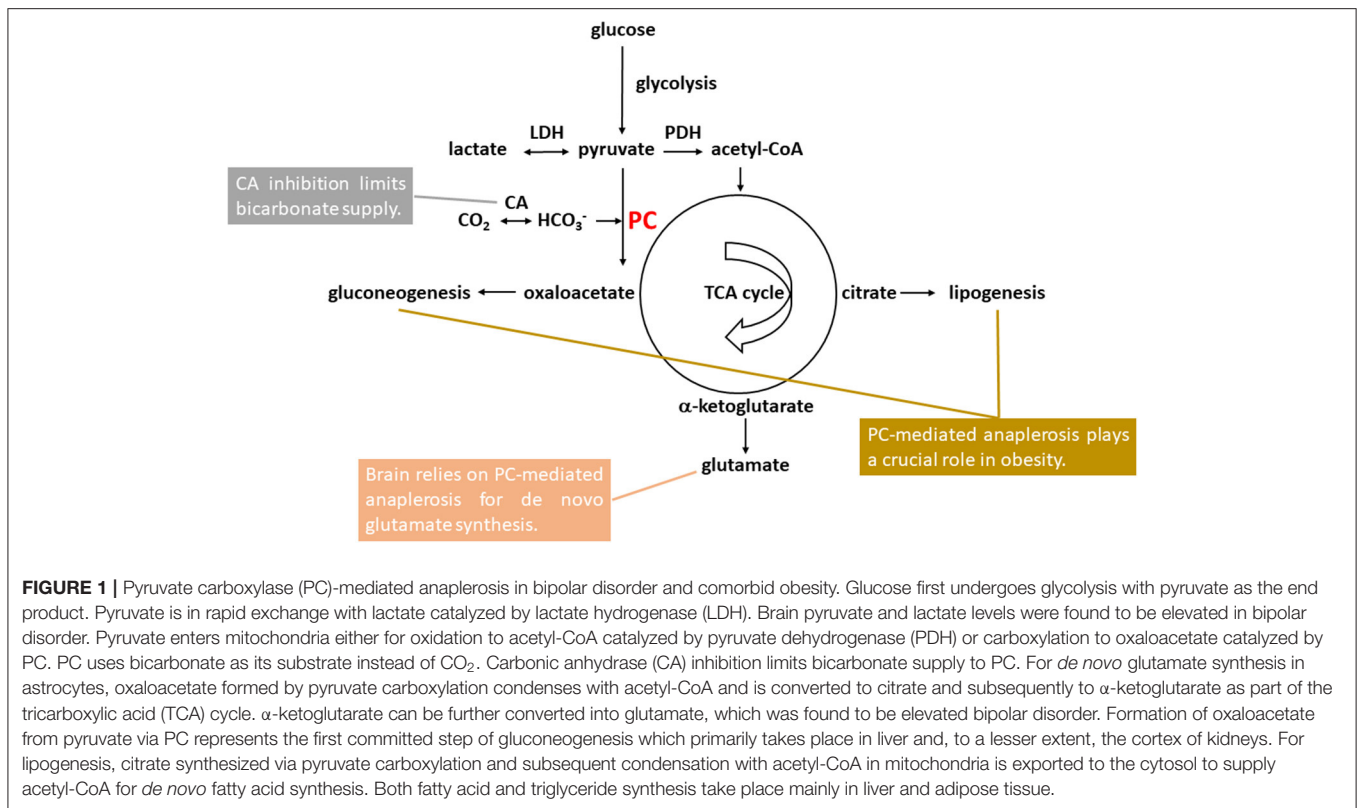
High glutamate + glutamine levels were shown to correlate with cognitive impairment in many brain disorders associated with glutamatergic abnormalities (34). The increased glutamate availability suggests activity-dependent vesicular glutamate release of larger quantal size because vesicle glutamate filling levels are dependent on the concentration of cytoplasmic glutamate to be packaged into synaptic vesicles (35). As excessive glutamate activates ionotropic receptors in extra-synaptic sites and causes neurotoxicity by calcium influx and generation of free radicals including nitric oxide, the sustained elevation of glutamate levels therefore may be a significant part of the pathogenesis of the widespread glutamatergic abnormalities in bipolar disorder (36).

### Elevated Glutamate Levels in Bipolar Disorder Can Be Explained by Increased Pyruvate Carboxylase-Mediated Anaplerosis in Brain

Despite the highly reproducible evidence of elevated brain glutamate levels in bipolar disorder from numerous *in vivo* MRS studies, to the best of our knowledge, a connection between the MRS results and pyruvate carboxylase-mediated anaplerosis has not been made in the literature. However, several drugs used in the treatment of bipolar disorder have important links to pyruvate carboxylase. For example, carbamazepine has long been a therapeutic option for bipolar disorder. It has been used in the treatment of bipolar disorder in both acute mania and maintenance therapy. In rats chronically administered dietary carbamazepine the abundance and activity of biotinylated pyruvate carboxylase were significantly reduced in both liver and brain (37, 38). The potential connection between the efficacy of carbamazepine in bipolar disorder treatment and its effect on pyruvate carboxylase has yet to be investigated.

The important role of the mitochondrial enzyme pyruvate carboxylase in brain function is also well-recognized clinically. Pyruvate carboxylase deficiency, a rare autosomal recessive inborn error of metabolism, is characterized by impairment of lactate metabolism and gluconeogenesis, producing severe lactic acidosis accompanied by compromised psychomotor development and intellectual disability (39). Certain drugs used in the treatment of bipolar disorder improve cerebral metabolism. For example, lithium was demonstrated to enhance oxidative phosphorylation in post-mortem human brain tissue (40) and quetiapine reduced lactate in rapid cycling manic bipolar patients (41).

Despite numerous variations across the studies (e.g., patient selection, disease state, medication history, and  $^1\text{H}$  MRS



**FIGURE 1 |** Pyruvate carboxylase (PC)-mediated anaplerosis in bipolar disorder and comorbid obesity. Glucose first undergoes glycolysis with pyruvate as the end product. Pyruvate is in rapid exchange with lactate catalyzed by lactate hydrogenase (LDH). Brain pyruvate and lactate levels were found to be elevated in bipolar disorder. Pyruvate enters mitochondria either for oxidation to acetyl-CoA catalyzed by pyruvate dehydrogenase (PDH) or carboxylation to oxaloacetate catalyzed by PC. PC uses bicarbonate as its substrate instead of  $\text{CO}_2$ . Carbonic anhydrase (CA) inhibition limits bicarbonate supply to PC. For *de novo* glutamate synthesis in astrocytes, oxaloacetate formed by pyruvate carboxylation condenses with acetyl-CoA and is converted to citrate and subsequently to  $\alpha$ -ketoglutarate as part of the tricarboxylic acid (TCA) cycle.  $\alpha$ -ketoglutarate can be further converted into glutamate, which was found to be elevated bipolar disorder. Formation of oxaloacetate from pyruvate via PC represents the first committed step of gluconeogenesis which primarily takes place in liver and, to a lesser extent, the cortex of kidneys. For lipogenesis, citrate synthesized via pyruvate carboxylation and subsequent condensation with acetyl-CoA in mitochondria is exported to the cytosol to supply acetyl-CoA for *de novo* fatty acid synthesis. Both fatty acid and triglyceride synthesis take place mainly in liver and adipose tissue.

methodologies) *in vivo*  $^1\text{H}$  MRS studies of bipolar disorder have found elevated glutamate or glutamate + glutamine with surprisingly high consistency (8, 11, 36, 42–45). Consistent, mood phase-independent elevation in glutamate levels in the frontal brain areas was measured in adult bipolar disorder patients by many  $^1\text{H}$  MRS studies (43) while treatment of bipolar disorder patients by lithium and valproate resulted in glutamate + glutamine reduction (45). A meta-analysis (11) of the  $^1\text{H}$  MRS measurement of glutamate + glutamine found elevated glutamate + glutamine levels in bipolar patients when compared with healthy controls with an effect size of 0.72 and a 95% confidence interval of 0.17–1.27 ( $p = 0.01$ ) for the pooled studies that reported glutamate + glutamine in all area of the brain (nine studies with 162 bipolar disorder patients and 165 healthy controls). Analyses of medicated and non-medicated bipolar disorder patients found that the effect size for glutamate level increase in non-medicated patients was much higher (1.91;  $p = 0.03$ ) than in medicated patients (0.31;  $p = 0.03$ ), consistent with that medications decreased brain glutamate. Increased serum  $\alpha$ -ketoglutarate and glutamate and increased glutamate in post-mortem brain tissue samples obtained from bipolar disorder individuals have also been reported (46–49). For instance, plasma glutamate levels in patients with bipolar mania ( $n = 20$ ) were significantly higher in both mania phase ( $46 \pm 19 \mu\text{M}$ ,  $p = 0.03$ ) and remission ( $57 \pm 27 \mu\text{M}$ ,  $p = 0.04$ ) than matched controls ( $36 \pm 9 \mu\text{M}$ ,  $n = 20$ ) (46). After correcting for post-mortem changes the level of glutamate at the time of death measured from post-mortem frontal cortex samples (Brodmann area 6) of bipolar disorder patients ( $15.33 \pm 5.72 \text{ nmol/mg tissue}$ ,  $n = 15$ ) was found to be significantly higher than in the normal control

samples ( $10.68 \pm 2.59 \text{ nmol/mg tissue}$ ,  $n = 15$ ,  $p = 0.013$ ) (47). In contrast, only a few studies have reported no change in glutamate + glutamine or reduced glutamate + glutamine in brain areas studied (36).

It is well-known in the neurochemical literature that brain relies on pyruvate carboxylase-mediated anaplerosis for *de novo* glutamate synthesis (15–21). Because of the unique role of pyruvate carboxylase in brain glutamate formation the highly consistent findings of elevated glutamate or glutamate + glutamine levels in bipolar disorder observed by *in vivo*  $^1\text{H}$  MRS, serum and post-mortem studies can be readily explained by increased pyruvate carboxylase-mediated anaplerosis in brain of patients with bipolar disorders. This explanation is also supported by the significant comorbidity between bipolar disorder and obesity as described in section Glutamate, Bipolar Disorder, and Comorbid Obesity.

### Elevated Glutamate Levels in Bipolar Disorder Is Consistent With a Chronic Mismatch Between Glucose Utilization and Oxidative Metabolism

A large body of evidence has consistently demonstrated that there is a significant mismatch or uncoupling between glucose utilization and oxidative metabolism in stimulated brain accompanying increased glutamatergic activities (50). Similar mismatches have also been observed in brain after vigorous physical exercise (51). Many functional  $^1\text{H}$  MRS studies have reported transient elevation of glutamate or glutamate + glutamine levels in activated brain tissue in

response to stimuli or tasks (52). In preclinical studies, increased glutamatergic activities were found to cause an increase in pyruvate carboxylation, resulting in enlarged glutamate and glutamine pools (17). These results suggest that enhanced glutamatergic activity increases *de novo* synthesis of glutamate from glucose (17). It should be noted that literature evidence for a transient increase in glutamate levels following a functional task or stimulus is not conclusive (52). Recent *in vivo*  $^{13}\text{C}$  MRS studies of anesthetized rodents found that acute stimulation did not increase pyruvate carboxylase-mediated anaplerotic flux rate in brain (53, 54).

The molar ratio of the arterio-venous difference of oxygen to glucose +  $\frac{1}{2}$  lactate is commonly referred to as the oxygen-to-carbohydrate index. The oxygen-to-carbohydrate index is reduced when more glucose and lactate are taken up into the brain than are oxidized to  $\text{CO}_2$ . As lactate accumulation can only account for a portion of the large decrease in oxygen-to-carbohydrate index accompanying the mismatch between stimulation of glucose utilization and oxidative metabolism, it has been proposed that increased *de novo* glutamate synthesis via pyruvate carboxylase-mediated anaplerosis contributes to the large decrease in oxygen-to-carbohydrate index when glucose utilization outpaces oxidative metabolism during stimulation of brain activity (17, 51).

$^{31}\text{P}$  and  $^1\text{H}$  MRS studies have found reduced oxidative phosphorylation and elevated lactate and glutamate + glutamine levels in brain in bipolar disorder, indicating impaired oxidative metabolism (7–10, 36, 42, 43). In contrast, positron emission tomography (PET) studies using [ $^{18}\text{F}$ ]fluorodeoxyglucose have reported small or no differences between healthy controls and bipolar disorder patients in glucose utilization rate in the prefrontal cortex or brain as a whole (55–58). There is no consensus in the directionality of the reported differences by the PET studies (55–58). Considering the variations across the PET studies, the lack of consensus in the direction of the changes suggests that the overall abnormalities in cerebral glucose utilization in bipolar disorder are likely very small. The  $^{31}\text{P}$  and  $^1\text{H}$  MRS and PET results, taken together, indicate that there is a considerable mismatch between oxidative metabolism and glucose utilization in brain in bipolar disorder. Therefore, the elevated glutamate + glutamine levels are consistent with mitochondrial dysfunction and a chronic mismatch between glucose utilization and oxidative metabolism in bipolar disorder accompanied by incomplete carbohydrate oxidation and increased pyruvate carboxylase-mediated anaplerosis.

## GLUTAMATE, BIPOLAR DISORDER, AND COMORBID OBESITY

### Glutamate Levels and Body Mass Index in Bipolar Disorder

Bipolar disorder and obesity are positively associated (59–62) with cardiovascular disease as the most common cause of death in bipolar disorder patients (63). Bipolar disorder patients are two-thirds more likely to be obese than the age-, race-, and sex-adjusted general population (60). A study of the association between body weight and bipolar illness in drug-naïve patients

reported that ~41% of untreated patients with bipolar disorder were overweight or obese (59). Obese bipolar disorder patients also have a more severe mood illness than normal weight patients (61). The underlying causes of the effects of obesity on bipolar disorder are still being investigated (61, 64). Recent neuroimaging studies reported that structural and neurochemical abnormalities in brain characteristic of bipolar disorder were more prominent with higher body mass index (65, 66). In particular, the increase in bilateral hippocampal glutamate + glutamine in patients with first-episode mania measured by  $^1\text{H}$  MRS was found to be more pronounced with higher body mass index (67). In comparison, the correlation between glutamate + glutamine and body mass index in healthy individuals was insignificant (67).

### Obesity Is Associated With Increased Pyruvate Carboxylase-Mediated Anaplerosis

Pyruvate carboxylase plays a crucial role in lipogenesis and gluconeogenesis in mammals. It converts pyruvate and bicarbonate into oxaloacetate for further conversion into citrate which is then exported from mitochondria and cleaved in cytosol to supply precursors for *de novo* fatty acid synthesis [(68); Figure 1]. The activity of pyruvate carboxylase is dramatically increased during adipocyte differentiation. Over expression of pyruvate carboxylase is associated with obesity and type 2 diabetes (69). Of the four gluconeogenic enzymes (phosphoenolpyruvate carboxykinase, fructose-1,6-bisphosphatase, glucose-6-phosphatase, and pyruvate carboxylase) pyruvate carboxylase reaction is the first committed step and likely rate-limiting in gluconeogenesis (70). The pyruvate carboxylase reaction provides oxaloacetate for subsequent conversion into phosphoenolpyruvate by phosphoenolpyruvate carboxykinase and regulates hepatic glucose production. In humans, increased hepatic pyruvate carboxylase expression was closely correlated with plasma glycemia, indicating that hepatic pyruvate carboxylase is a key determinant of gluconeogenesis in liver (71). Animal studies have demonstrated that increased pyruvate carboxylase flux is an important pathway responsible for increased hepatic glucose production in diabetes development (72). Furthermore, selective inhibition of pyruvate carboxylase expression in liver and adipose tissue significantly reduced adiposity, plasma lipid levels and hepatic steatosis (71). A recent *in vivo*  $^1\text{H}$  and  $^{13}\text{C}$  MRS study of a mouse model of high-fat diet consumption has also found significantly elevated glutamate and glutamate + glutamine levels as well as increased pyruvate carboxylase-mediated anaplerotic flux rate in the hypothalamus of treated animals (73). Taken together, the above evidence demonstrates that increased pyruvate carboxylase-mediated anaplerosis is a metabolic hallmark of obesity.

### Carbonic Anhydrase Inhibition in Bipolar Disorder and Obesity

Catalysis by carbonic anhydrase is necessary to speed up the reversible hydration of  $\text{CO}_2$  for a variety of biological processes. In the central nervous system carbonic anhydrase

inhibition enhances inhibitory neurotransmission. Many anticonvulsants are strong carbonic anhydrase inhibitors. Adjunctive acetazolamide, a sulfonamide carbonic anhydrase inhibitor, improved prophylactic efficacy in 44% of the treatment-resistant bipolar disorder patients (74). One of the common adverse effects of acetazolamide is weight loss. Adjunctive topiramate and zonisamide have been used in the treatment of bipolar disorder. They are also strong carbonic anhydrase inhibitors and caused persistent weight loss in obese patients (75–79). Of the three anticonvulsants, the efficacy of topiramate in the treatment of bipolar disorder has been demonstrated by many studies (77). Topiramate also caused substantial weight loss in patients with bipolar disorders in those studies (77).

Inhibition of carbonic anhydrase limits the access of CO<sub>2</sub>-fixing enzymes pyruvate carboxylase and acetyl-CoA carboxylase to bicarbonate and decreases pyruvate carboxylase-mediated anaplerosis in peripheral tissues (Figure 1). It has been demonstrated that carbonic anhydrase activity is required for optimal activity of hepatic pyruvate carboxylase in *de novo* synthesis of both fatty acids and non-saponifiable lipids (80). Carbonic anhydrase inhibitors are known to inhibit *de novo* lipogenesis and gluconeogenesis in liver (81, 82). In cultured adipocytes inhibition of carbonic anhydrase by sulfonamides also significantly decreased lipogenesis (83).

Carbonic anhydrase in brain is predominantly expressed in glial and choroid cells (84–88). The much lesser carbonic anhydrase expression in neurons facilitates rapid removal of CO<sub>2</sub>, which is generated by the highly active neuronal oxidative metabolism, from neurons by free diffusion. This distinct distribution of intracellular carbonic anhydrase in brain leads to the conversion of CO<sub>2</sub> into bicarbonate primarily in astrocytes, rendering astrocytes as sinks of CO<sub>2</sub> (89). In cultured astrocytes inhibition of carbonic anhydrase caused a large reduction in pyruvate carboxylase-mediated CO<sub>2</sub> fixation by limiting the supply of bicarbonate to pyruvate carboxylase, resulting in reduced TCA cycle intermediate levels and reduced glutamate production (90). Since in the central nervous system astrocytes are the predominant site for both CO<sub>2</sub> hydration catalyzed by carbonic anhydrase and pyruvate carboxylation catalyzed by pyruvate carboxylase (Figure 1), limitation of *de novo* synthesis of glutamate by carbonic anhydrase inhibition may play a significant role in the antiepileptic properties and mood stabilization effects of anticonvulsants that are also carbonic anhydrase inhibitors. Therefore, both mechanistic and clinical studies of carbonic anhydrase inhibition support the proposed connections among bipolar disorder, obesity and pyruvate carboxylase-mediated anaplerosis.

## Pyruvate Carboxylase-Mediated Anaplerosis Is a Potential Therapeutic Target for Bipolar Disorder and Comorbid Obesity

A single pyruvate carboxylase isoform is expressed in humans and found in mitochondria only (91). Pyruvate carboxylase

expression is regulated by complex mechanisms and many exogenous and endogenous modulators (80, 91). Many modulators of pyruvate carboxylase pass the blood brain barrier (80) therefore may affect pyruvate carboxylase activities in both peripheral tissues and the brain. Obesity and diabetes are associated with increased pyruvate carboxylase expression in liver and adipose tissue (69). In contrast, insulin inhibits pyruvate carboxylase expression in liver (80). In the central nervous system increased pyruvate supply was found to augment pyruvate carboxylase-mediated anaplerotic flux and glutamate production in astrocytes (15).

Previous studies have shown that pharmacological inhibition of pyruvate carboxylase by phenylacetic acid markedly reduced hepatic gluconeogenesis in rats (92). The effects of pyruvate carboxylase on glucose and lipid metabolism in several rodent models were measured using a specific antisense oligonucleotide to selectively decrease pyruvate carboxylase expression in liver and adipose tissue (71). The specific antisense oligonucleotide approach significantly reduced plasma glucose concentrations and endogenous glucose production. In a high-fat-diet rat model, pyruvate carboxylase antisense oligonucleotide reduced adiposity, plasma lipid levels, and hepatic steatosis (71). It has been suggested that pyruvate carboxylase is a potential therapeutic target for several diseases associated with obesity (71, 92). As the experimental findings discussed here indicate that elevated pyruvate carboxylation may be a significant part of the pathogenesis of glutamatergic hyperactivity and comorbid obesity in bipolar disorder, designing inhibitors of pyruvate carboxylase to pharmacologically modulate pyruvate carboxylase-mediated anaplerosis may be a useful new treatment strategy for bipolar disorder and comorbid obesity.

## CONCLUSIONS

Increased pyruvate carboxylase-mediated anaplerosis can readily explain the elevated glutamate or glutamate + glutamine levels in brain in bipolar disorder observed by *in vivo* <sup>1</sup>H MRS. Multiple independent lines of evidence suggest that increased pyruvate carboxylase-mediated anaplerosis is a common mechanism underlying glutamatergic hyperactivity and the significant positive association between bipolar disorder and obesity. As the increased prevalence of obesity in bipolar disorder is associated with illness severity and poor treatment outcomes development of preventive and treatment strategies targeting pyruvate carboxylase-mediated anaplerosis may be warranted.

## AUTHOR CONTRIBUTIONS

JS performed literature analysis and proposed the hypothesis. JT performed literature search. JS and JT wrote the paper. Both authors reviewed the manuscript and agreed on its final version.

## FUNDING

This work was supported by the Intramural Research Program of National Institute of Mental Health, NIH (ZIA MH002803).

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**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.

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