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RESEARCH TOPICS

BIOLOGY AND THERAPEUTIC POTENTIAL OF BROWN ADIPOSE TISSUE

Topic Editor
Patrick Seale

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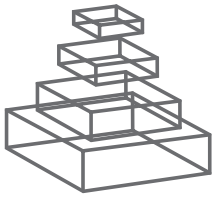

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BIOLOGY AND THERAPEUTIC POTENTIAL OF BROWN ADIPOSE TISSUE

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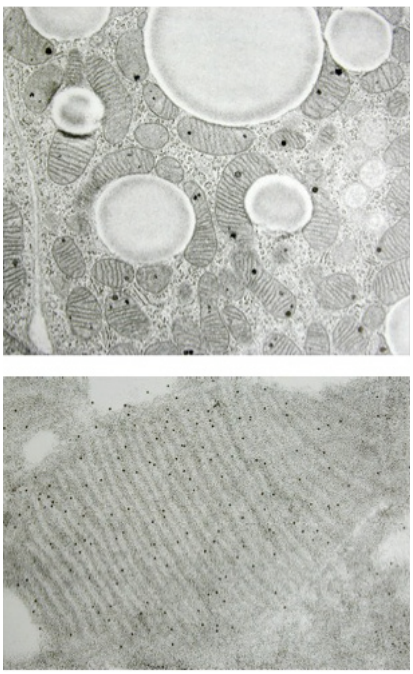

Histology of brown adipocyte and presence of UCP1 in mitochondria. Figure taken from Ricquier D (2011) Uncoupling protein 1 of brown adipocytes, the only uncoupler: a historical perspective. *Front. Endocrin.* 2:85. doi: 10.3389/fendo.2011.00085

Brown adipose tissue (BAT) has long been recognized as a key thermogenic tissue in eutherian mammals. Brown fat cells contain large numbers of highly specialized mitochondria that oxidize fat and carbohydrate to produce heat. This metabolic inefficiency is due to the presence of Ucp1 in the inner mitochondrial membrane of brown fat cells which dissociates the electrochemical gradient from ATP production. BAT presumably evolved to protect animals against hypothermia in response to cold exposure. However, energy expended by BAT has the added benefit of counteracting obesity and associated metabolic disease, at least in rodents. A very large number of studies have consistently shown that mice carrying increased amounts of active brown fat are lean and healthy. Until recently, it was generally believed that the amount of brown fat tissue was negligible in healthy adult humans. However, PET imaging studies have refuted this notion, and revealed the existence of active BAT in most, if not all, adult humans. Notably, there is a very strong inverse correlation between the amount of activated BAT and fatness in humans. Moreover, activated brown fat is lost with ageing, which also correlates with tendency to gain weight. The field must now address whether variation in brown fat activity is a cause or consequence of weight gain. Brown fat cells are localized in discrete depots of BAT and are

also found as clusters interspersed in white fat tissues. The prevalence and function of these so-called "brite" (brown in white) cells in humans remains unknown. The development of strategies to increase the amount and/or activity of brown fat may hold exciting prospects for the treatment of obesity and its associated health consequences. In this Research Topics issue, we would propose to examine the following areas related to brown fat biology:

1. Development of brown and "brite" cells (including: historical/evolutionary perspective, transcriptional pathways, developmental origins)

2. Pathways that influence brown fat cell development (BMP7, TZDs, Prostaglandins, FGF21)

3. Activation of brown fat - focus on sympathetic and sensory innervation (signaling by beta-adrenergic receptors, nerve-fat connections

4. Brown fat thermogenesis in response to cold, diet and hibernation

5. Brown fat in humans including: human brown fat precursors, methods for imaging, factors that influence prevalence, relationship between body mass/obesity and amount of brown fat.

6. Brown fat mitochondria including mechanism of uncoupling (Ucp1 history/structure/function, mitochondrial biogenesis)

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EDITORIAL
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Brown adipose tissue biology and therapeutic potential

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BROWN ADIPOSE TISSUE

Brown adipose tissue (BAT) (**Figure 1**) has been recognized as a key thermogenic tissue in rodents for several decades. However, in the last few years, there has been a resurgent interest in the biology and therapeutic potential of BAT. This has been largely driven by a new understanding that most, if not all, healthy adult humans have significant deposits of BAT which can be activated by cold. Moreover, recent insights into the developmental origins of brown adipose cells and the identification of molecules that regulate brown adipocyte activity have provided a conceptual framework for the design of brown fat-based therapies.

Brown fat cells, when activated, are able to take up and oxidize large amounts of fat and carbohydrate for the purpose of producing heat—a process called adaptive thermogenesis. This transformation of chemical energy into heat at the expense of ATP production is mediated by the presence of Uncoupling Protein-1 (Ucp1) in the inner mitochondrial membrane. Ucp1 catalyzes the leak of protons from the intermembrane space back into the matrix, thus reducing the gradient (and its potential energy) used for ATP production.

BAT presumably evolved to protect animals against hypothermia in response to cold exposure. However, BAT is also known to powerfully counteract obesity and metabolic disease, at least in rodents. Numerous studies over recent years revealed that mice with increased amounts of active brown fat are lean, healthy, and able to resist the harmful metabolic effects of high fat diets. Intriguingly, imaging studies in humans show that brown fat activity is reduced in obesity and aging. The field must now address whether the variation in brown fat activity is a cause or consequence of weight gain. Brown fat cells are localized in discrete depots of BAT and are also found clustered amongst white fat cells in white depots. The prevalence and function of these so-called "beige" or "brite" (brown-in-white) cells in humans remains unknown.

There is a growing consensus in the field that brown fat-targeted therapies hold tremendous promise for the treatment of obesity and associated health consequences. It is also increasingly clear that brown fat can function as an effective sink for disposing of excess glucose and fatty acids. This suggests that brown fat-based therapies could be very effective for treating insulin resistance, type-2 diabetes, and dyslipidemia without necessarily reducing body weight.

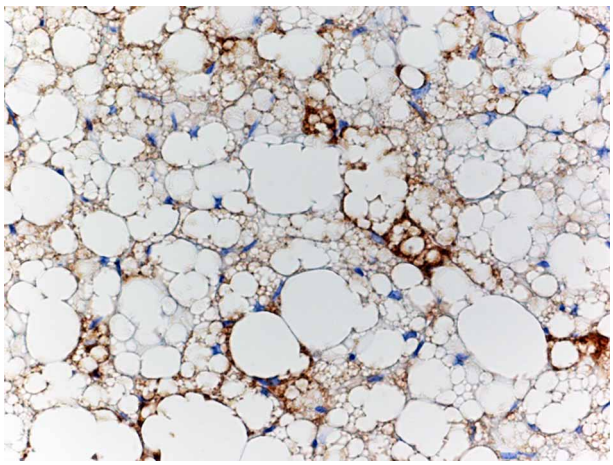

FIGURE 1 | Brown adipose tissue. Immunohistochemical staining of Ucp1 expression in brown adipose tissue showing a mixture of large and small adipocytes.

In this Research Topic, we were able to assemble articles from many of the prominent scientists in the field which focused on many different and important aspects of brown fat biology. The topic begins with a historical perspective (Ricquier, 2012) and also includes reviews and original reports on: test systems to study Ucp1 (Hirschberg et al., 2012); the development of brown adipose cells (Boss and Farmer, 2012; Festuccia et al., 2012; Kozak, 2012; Pisani et al., 2012; Scime, 2012; Yadav and Rane, 2012); the influence of genetics (Kozak, 2012); adrenergic and central control of brown adipocyte activity (Collins, 2012; Morrison et al., 2012); human brown adipose cells and imaging methods (Betz and Enerback, 2012; Hu and Gilsanz, 2012; Muzik et al., 2012; Richard et al., 2012; van Marken Lichtenbelt, 2012); and perspectives for brown adipose-based therapeutics (Betz and Enerback, 2012; Boss and Farmer, 2012). I would like to thank all the contributors and reviewers for their help in putting this interesting and timely collection of articles together.

REFERENCES

Betz, M. J., and Enerback, S. (2012). Therapeutic prospects of metabolically active brown adipose tissue in humans. *Front. Endocrinol.* 2:86. doi: 10.3389/fendo.2011.00086

Boss, O., and Farmer, S. R. (2012). Recruitment of brown adipose tissue as a therapy for obesity-associated diseases. *Front. Endocrinol.* 3:14. doi: 10.3389/fendo.2012.00014

Collins, S. (2012). beta-adrenoceptor signaling networks in adipocytes for recruiting stored fat and energy expenditure. *Front. Endocrinol.* 2:102. doi: 10.3389/fendo.2011.00102

Festuccia, W. T., Blanchard, P. G., and Deshaies, Y. (2012). Control of brown adipose tissue glucose and lipid metabolism by PPARγ. *Front. Endocrinol.* 2:84. doi: 10.3389/fendo.2011.00084

Hirschberg, V., Fromme, T., and Klingenspor, M. (2012). Test systems to study the structure and function of uncoupling protein 1: a critical overview. *Front. Endocrinol.* 2:63. doi: 10.3389/fendo.2011.00063

Hu, H. H., and Gilsanz, V. (2012). Developments in the imaging of brown adipose tissue and its associations with muscle, puberty, and health in children. *Front. Endocrinol.* 2:33. doi: 10.3389/fendo.2011.00033

Kozak, L. P. (2012). The genetics of brown adipocyte induction in white fat depots. *Front. Endocrinol.* 2:64. doi: 10.3389/fendo.2011.00064

Morrison, S. F., Madden, C. J., and Tupone, D. (2012). Central control of brown adipose tissue thermogenesis. *Front. Endocrinol.* 3:5. doi: 10.3389/fendo.2012.00005

Muzik, O., Mangner, T. J., and Granneman, J. G. (2012). Assessment of oxidative metabolism in brown fat using PET imaging. *Front. Endocrinol.* 3:15. doi: 10.3389/fendo.2012.00015

Pisani, D. F., Djedaini, M., Beranger, G. E., Elabd, C., Scheideler, M., Ailhaud, G., et al. (2012). Differentiation of human adipose-derived stem cells into "Brite" (Brown-in-White) adipocytes. *Front. Endocrinol.* 2:87. doi: 10.3389/fendo.2011.00087

Richard, D., Monge-Roffarello, B., Chechi, K., Labbe, S. M., and Turcotte, E. E. (2012). Control and physiological determinants of sympathetically mediated brown adipose tissue thermogenesis. *Front. Endocrinol.* 3:36. doi: 10.3389/fendo.2012.00036

Ricquier, D. (2012). Uncoupling protein 1 of brown adipocytes, the only uncoupler: a historical perspective. *Front. Endocrinol.* 2:85. doi: 10.3389/fendo.2011.00085

Scime, A. (2012). The heat is on: a new avenue to study brown fat formation in humans. *Front. Endocrinol.* 2:118. doi: 10.3389/fendo.2011.00118

van Marken Lichtenbelt, W. (2012). Human brown fat and obesity: methodological aspects. *Front. Endocrinol.* 2:52. doi: 10.3389/fendo.2011.00052

Yadav, H., and Rane, S. G. (2012). TGF-beta/Smad3 signaling regulates brown adipocyte induction in white adipose tissue. *Front. Endocrinol.* 3:35. doi: 10.3389/fendo.2012.00035

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Uncoupling protein 1 of brown adipocytes, the only uncoupler: a historical perspective

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Uncoupling protein 1 (UCP1), is a unique mitochondrial membranous protein devoted to adaptive thermogenesis, a specialized function performed by brown adipocytes. Whereas the family of mitochondrial metabolite carriers comprises ∼40 members, UCP1 is the only memberable to translocate protons through the inner membrane of brown adipocyte mitochondria. By this process, UCP1 uncouples respiration from ATP synthesis and therefore provokes energy dissipation in the form of heat while, also stimulating high levels of fatty acid oxidation. UCP1 homologs were identified but they are biochemically and physiologically different from UCP1. Thirty five years after its identification, UCP1 still appears as a fascinating component. The recent renewal of the interest in human brown adipose tissue makes UCP1 as a potential target for strategies of treatment of metabolic disorders.

Keywords: brown adipocyte, fatty acid, membranous carrier, mitochondria, proton transport, respiration coupling, thermogenesis, uncoupling

INTRODUCTION

FROM PHYSIOLOGICAL MEASUREMENTS OF THERMOGENESIS TO THE IDENTIFICATION OF UCP1

Basal thermogenesis results from the basal activity of many biochemical pathways including ATP-ases and futile cycles. Adaptive thermogenesis is regulated and occurs in particular conditions (cold exposure, arousing from hibernation, food intake. . .). This process involves the recruitment of different cell types and the activation of specific biochemical pathways. Any process that occurs without performing useful work, accumulating intermediates, or concentrating ions has an efficiency of zero from the standpoint of energy conservation or 100% for the purposes of thermogenesis (Nicholls and Locke, 1984). Although ATP-ases contribute, the largest part of heat production by cells probably comes from many metabolic pathways and in particular from oxidation of substrates.

In rodents or in newborns of some species, elegant *in vivo* studies in the 1960s established that brown fat depots were engaged in thermogenesis; this led to an increase in the temperature of the blood in brown fat which was rapidly distributed to heart, brain, kidney, and skeletal muscle (reviews in Nicholls and Locke, 1984; Cannon and Nedergaard, 1985, 2004; Himms-Hagen and Ricquier, 1998). The thermogenic activity of brown adipocytes was confirmed by microcalorimetric determinations of the heat output of excised tissue, isolated brown adipocytes, and isolated brown fat mitochondria (Nedergaard et al., 1977; Seydoux and Girardier, 1977; Ricquier et al., 1979). Physiological or pharmacological experiments established that the ability of animals (mainly rodents) to activate thermogenesis in response to cold exposure correlates with the amount of brown fat and to its activation by the sympathetic nervous system mediates and (Himms-Hagen, 1989).

Thermogenesis is dependent on oxygen consumption and therefore on the ability of cells to oxidize substrates in their mitochondria. Morphologically, brown adipocytes are very unique since they contain an extremely high number of mitochondria; these specialized mitochondria have a highly developed inner membrane, the membrane where the respiratory chain complexes are anchored. In other words, the morphology of brown adipocytes confers on these cells a very high capacity to oxidize substrates (**Figure 1**). Therefore, when heat is required (exposure to the cold, as an example), norepinephrine released by sympathetic nerves rapidly activates brown adipocytes resulting in fatty acid oxidation and heat production. Independently, Smith and Lindberg observed in 1967 that thermogenesis in brown adipose cells resulted from a weak coupling of respiration to ADP phosphorylation, leading to waste of oxidation energy as heat. Some years later, Nicholls and Ricquier showed the presence of a specific 32-kD protein in the inner mitochondrial membrane of brown adipocytes (**Figure 1**) that could uncouple respiration to produce heat rather than ATP (see reviews in Nicholls and Locke, 1984; Cannon and Nedergaard, 2004; Nedergaard et al., 2005). This protein was later termed uncoupling protein UCP and renamed uncoupling protein 1 (UCP1) when UCP2 was identified (Fleury et al., 1997). The obvious thermogenic activity of UCP1 in mitochondria was clearly demonstrated by Kozak et al. (1994) observing the cold sensitive phenotype of the *Ucp*1$^{-/-}$ mouse (Enerbäck et al., 1997). UCP1 activity and regulation are reviewed below.

UCP1: A SPECIFIC PROTON CARRIER UNCOUPLING RESPIRATION FROM ATP SYNTHESIS

UCP1 IS A RESPIRATION UNCOUPLER, SHUNTING THE NORMAL PROTON CIRCUIT AND IS PHYSIOLOGICALLY REGULATED

The physiological measurements of BAT thermogenic activity instigated the search for a mechanism unique to brown adipocyte mitochondria. The search for a respiration uncoupling mechanism, unique to these mitochondria came at a moment when Mitchell had proposed the – rather debated at that time – chemiosmotic theory; a theory explaining that the proton gradient and

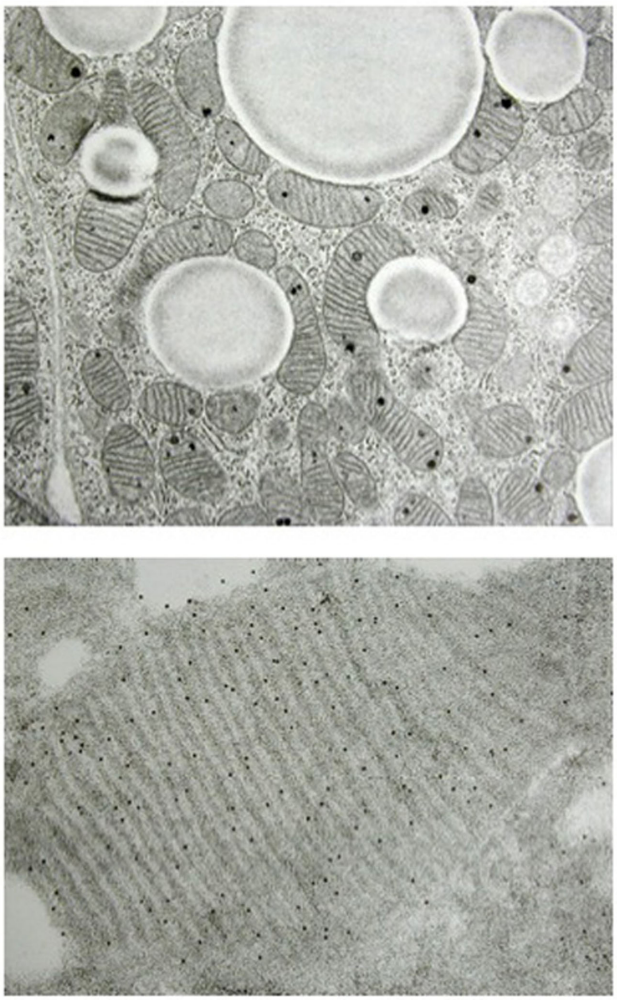

FIGURE 1 | Histology of brown adipocyte and presence of UCP1 in mitochondria. The cytosol of brown adipocytes is characterized by numerous mitochondria and lipid droplets (upper part). Magnification of a brown adipocyte mitochondrion showing parallel cristae and UCP1 detected using antibodies (black dots, lower part). (Figure kindly provided by Dr. Saverio Cinti, University of Ancona).

proton circuit through the inner membrane of mitochondria or chloroplasts, were governing ADP phosphorylation following respiration or exposure to light. According to Mitchell, oxidative phosphorylation is the process by which ADP phosphorylation by the mitochondrial ATP-synthase is coupled to mitochondrial oxygen consumption and to re-oxidation of reduced coenzymes via the proton electrochemical gradient generated by complexes I, III, and IV of respiratory chain. Oxygen then accepts electrons at the level of cytochrome-*c*-oxidase (complex IV) for conversion to water. Therefore, according to Mitchell, energy of the proton gradient ($\Delta\mu_{H+}$) , or the so-called proton-motive force Δp, is used to drive ATP synthesis by ATP-synthase. The proton gradient slows respiratory chain activity and facilitates ATP synthesis. The message here was that a proton circuit linking respiratory chain (protons out) and ATP-synthase (proton re-entry) operated such that oxygen consumption and ATP synthesis were tightly linked (**Figure 2**). Consequently, a proton leak in the inner mitochondrial membrane, distinct from the re-entry of protons via ATP-synthase, would lower the proton gradient and allow energy to be dissipated as heat. The reason for that is that down regulation of the proton gradient lowers the membrane potential which immediately activates the proton pumps and respiratory chain and provokes heat production since oxidation energy is not consumed by the ATP-synthase machinery. Actually, this is what happens when a chemical uncoupler is added to respiring liver or skeletal muscle mitochondria. Taken into consideration this postulate, Nicholls observed that isolated brown adipocyte mitochondria exhibit a very high and unique ion permeability in their inner membrane (review in Nicholls, 2006). This ion permeability, first detected as a chloride permeability, is a proton permeability strongly inhibited in presence of nucleotides, previously shown by Rafael and others to restore the respiratory control of brown adipocyte mitochondria (reviews in Cannon and Nedergaard, 2004; Nicholls, 2006). Photo-affinity labeling of hamster brown fat mitochondria with radioactive nucleotides was used by Heaton et al. (1978) to identify the uncoupling (proton transport) pathway as a 32-kD membranous protein. These data were in agreement with the previous description of this protein, when such a protein was (i) described to be present in rat brown fat mitochondria membrane but absent in liver mitochondria, (ii) the only membrane protein to be significantly increased in brown fat mitochondria following cold-adaptation, and (iii), down regulated when cold-exposed animals returned to the warm (Ricquier and Kader, 1976). Therefore, UCP1 not only plays an important physiological role in Nature, it also occupies a unique position among the family of membranous mitochondrial porters driven by oxidative metabolism: it is the exception that proves the rule of chemi-osmotic theory (Garlid and Jaburek, 1998; **Figure 2**).

UCP1 PROTON TRANSPORT ACTIVITY AND REGULATION

Nicholls developed a methodology to determine the current/voltage relationship of the basal proton leak of mitochondria by titrating down succinate respiration with malonate, allowing to calculate CmH^+, the proton conductance of the inner membrane of liver mitochondria in nanomole $H^+/min^{-1}/mg^{-1}, mv^{-1}$. Doing that with brown fat mitochondria, he found that the freshly prepared mitochondria had an enormous proton conductance and were incapable to maintain more than a few mV of proton-motive force whereas coupled mitochondria exhibit a membrane potential value close to 200 mV. It became clear that UCP1, when active, was acting as a proton translocator (Nicholls, 2006). When thermogenesis is not required, nucleotides bind UCP1 and inhibit its activity. In presence of inhibitory nucleotides, UCP1 has no residual proton conductance and is not leaky (Shabalina et al., 2010). From physiology physiology perspective, the search for a natural activator of UCP1 was logical. When thermogenesis is physiologically required, norepinephrine released by surrounding sympathetic fibers activates lipolysis which increases the level of free fatty acids in brown adipocyte mitochondria. The free fatty acids not only act as substrates for oxidation but also activate UCP1. The demonstration of the major ability of free fatty acids to activate UCP1 came from experiments where albumin

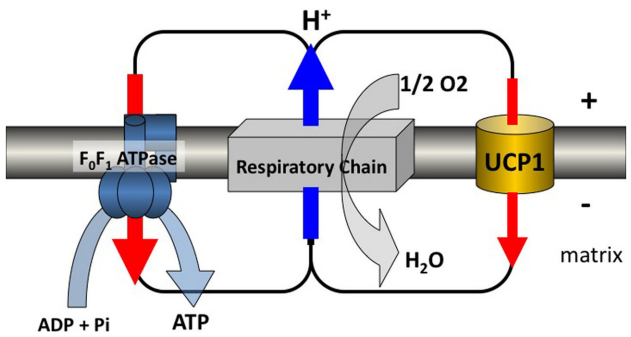

FIGURE 2 | Proton circuit. UCP1 is inserted in the mitochondrial inner membrane where, also present, is a multienzymatic complex called the respiratory chain made of complexes I to IV. The respiratory chain reoxidizes reduced coenzymes and electrons are driven to oxygen. This oxido-reduction step liberates energy which is used to generate an electrochemical gradient of protons across the inner membrane. This gradient is normally consumed by the ATP-synthase which phosphorylates ADP. UCP1 transports protons passively and makes possible a futile cycle of protons across the inner membrane leading to increased energy expenditure. This schema illustrates the situation encountered in brown adipocytes of mammals where a large amount of respiratory chains as well as a large amount of UCP1 are present. Activation of the futile cycling increases considerably energy expenditure and thus heat production by these thermogenic cells. In other cells where homologs of the UCP1 are expressed at much lower level, this pathway would represent a minor contributor to energy expenditure, but might be of importance to avoid oxidative damage (Figure kindly designed by Frédéric Bouillaud).

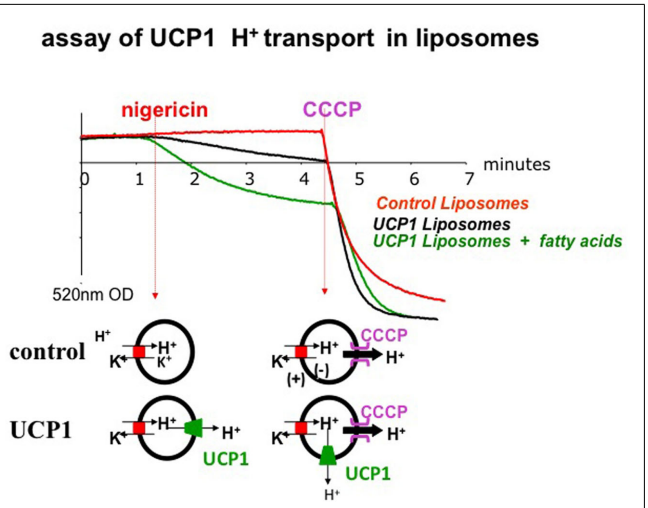

FIGURE 3 | Reconstitution of the proton transport activity of purified UCP1 in liposomes (Mozo et al., 2006). Membrane potential ($\Delta\Psi$) is appreciated using a safranin probe sensitive to membrane potential and allowing a measurement of optical density at 520 nm. In presence of nigericin, H^+ is exchanged against K^+ (H^+ in, K^+ out), there is no charge exchange but a ΔpH is generated. In liposomes containing UCP1, UCP1 dissipates this ΔpH gradient and also increases $\Delta\Psi$ inducing the polarization of the membrane (and a decrease of O.D. signal). The slope of the decrease of the safranin signal is an assay of UCP1 protonophoric activity. Addition of fatty acids to liposome containing UCP1 markedly polarizes the membrane due to activation of the protonophoric activity of UCP1. Upon addition of CCCP, a strong protonophor, H^+ ions go out and positively polarize the liposome membrane on external side.

for free fatty acids were added to isolated brown adipocytes cells and mitochondria. For example, in an elegant experiment, brown adipocyte mitochondria were slowly infused with palmitate to mimic lipolysis. During this procedure, the CmH^+ of UCP1 increased dramatically, the membrane potential decreased, and respiration rose sharply. (Nicholls and Locke, 1984; Ledesma et al., 2002; Cannon and Nedergaard, 2004; Nicholls, 2006). Comparing *wt* and *Ucp*1$^{-/-}$ mice brown fat mitochondria and using a non-metabolizable fatty acid analog, the Stockholm group confirmed the importance of UCP1 in thermogenesis and demonstrated that neither the ability to be metabolized, nor an innate uncoupling activity was a necessary property of UCP1 activators (Shabalina et al., 2008).

Other demonstrations of the proton transport activity of UCP1 were made by researchers who reconstituted its activity in liposomes (see an example in **Figure 3**). In such conditions, free fatty acids activate the proton translocating activity of UCP1 whereas nucleotides (GDP, GTP, ADP, ATP) inhibit it (Strieleman et al., 1985; Winkler and Klingenberg, 1994). According to these data, UCP1 is a purely H^+ translocating protein, the protons being translocated in a carrier-like fashion, instead of by a H^+ channel through the membrane. In other respects, there is still a debate whether fatty acids, (i) only activate proton transport (González-Barroso et al., 1998; Ledesma et al., 2002; Mozo et al., 2006), (ii) participate as a prosthetic group that delivers protons to a site where they are translocated to the matrix, or (iii) are transported as anions by UCP1 as a part of a cycling mechanism completed with the translocation of the protonated form across the lipid bilayer, with a net uptake of a proton (Skulachev, 1988; Garlid and Jaburek, 1998).

UCP1 AA ACID SEQUENCE AND PREDICTED STRUCTURE

The amino-acid sequence of UCP1 was determined following purification by Klingenberg (2010) and Aquila et al. (1985). It was also predicted from the sequencing of cloned cDNA (Bouillaud et al., 1985, 1986). Clearly, UCP1 is partly homologous to members of the anion mitochondrial carriers protein family (also referred to as the metabolite transporters of the mitochondrial inner membrane), including the ADP/ATP carrier and the phosphate carrier. Moreover, like the ADT/ATP carrier and mitochondrial carriers, UCP1 has a tripartite structure comprising three similar sequences of ~100 residues each. Hydropathy plot analysis predicted the existence of six membrane-spanning α-helices (Aquila et al., 1985; Bouillaud et al., 1986). This prediction was supported by an immunological analysis of antigenic sites in UCP1 (Miroux et al., 1993). UCP1 structure has not been resolved yet but is probably close to the structure of the adenine nucleotide translocator (Pebay-Peyroula et al., 2003) and the structure of UCP2 recently identified (Berardi et al., 2011).

UCP1 IS SPECIFIC FOR BROWN ADIPOCYTES AND IS VERY ABUNDANT

The specific activity of UCP1 is unique to brown adipocytes. This was fully demonstrated in mice made null for *Ucp*1, which became hypothermic in a cold ambiance (Enerbäck et al., 1997; Nedergaard et al., 2001). It was also confirmed by recombinant

expression of UCP1 in yeasts or mammalian cells (Casteilla et al., 1990; Bouillaud et al., 1994; González-Barroso et al., 1998). Many experimental approaches including UCP1 mRNA detection or UCP1 immunodetection, as well as the analysis of the activity of a reporter gene drived by *Ucp*1 promotor in transgenic mice, confirmed that *Ucp*1 expression is only observable in brown adipocyte (Cassard-Doulcier et al., 1998; Ricquier and Kozak, 2003).

An additional feature of UCP1 is that it is markedly abundant in brown adipocyte mitochondria where it comprises up to 8% of the total protein. The reason for such an amount is unknown but suggests that UCP1 molecular activity is rather weak.

UCP1 GENE: SPECIFIC EXPRESSION IN BROWN ADIPOCYTES AND REGULATION OF TRANSCRIPTION

CLONING OF CDNAS AND GENE, GENE ORGANIZATION IN RODENTS AND IN HUMANS, TRANSCRIPTIONAL REGULATION

The question of transcriptional regulation of *Ucp*1 has two sides: its unique expression in brown adipocytes, and, the transcriptional activation by norepinephrine and other non-cell-autonomous factors like T_3. In fact, it is not easy to list *cis* elements and *trans*-regulatory factors regulating, either the level of transcription of the gene, or the cell-specific transcription since these two mechanisms are probably controlled by the same factors. UCP1 biosynthesis is largely controlled at the level of transcription which is sharply activated within minutes after exposure of rodents to the cold (Ricquier et al., 1984, 1986; Ricquier and Kozak, 2003). The sympathetic activation of brown adipocytes and the subsequent and immediate rise in cAMP is the primary and main trigger of *Ucp*1 transcription, but other factors such as T_3 (Silva and Rabelo, 1998) and retinoic acid (Alvarez et al., 1995; Larose et al., 1996; Rabelo et al., 1996; Gonzalez-Barroso et al., 2000a) are critical for a full physiological response (Gonzalez-Barroso et al., 2000b). The molecular mechanisms involved in the regulation of rodent and human *UCP*1 transcription have been partially elucidated and a critical 200-bp *cis* region (and more precisely the moiety of this region) having an enhancer activity, located a few kb upstream of the transcriptional start was identified (Cassard-Doulcier et al., 1993; Kozak et al., 1994; Gonzalez-Barroso et al., 2000a). This region is able to bind transcriptional factors such as CREB, CCAAT/enhancer binding proteins α and β, jun, Ets1, thyroid hormone receptors, retinoid X-receptor, and PPARs. Other important regions, in particular cAMP-response elements, were also identified outside of the enhancer region and in the promoter region (Cassard-Doulcier et al., 1994; Kozak et al., 1994; Yubero et al., 1994, 1998; Alvarez et al., 1995; Silva and Rabelo, 1998; Rim and Kozak, 2002; Xue et al., 2005). Xue et al. (2005) proposed that small variations in the levels of several transcriptional components of the *Ucp*1 enhanceosome interact synergistically to achieve large differences in *Ucp*1 expression. In addition to these transcription factors, the co-activator PGC-1α also plays an important role (Puigserver et al., 1998).

GENETIC STUDIES IN HUMAN COHORTS

The human UCP1 gene was mapped to the long arm of chromosome 4 in q31 region (Cassard et al., 1990). A *Bcl*1 polymorphic site was identified at bp-3826 upstream of the TATA box of the *UCP*1 promoter in Bouchard's laboratory (Oppert et al., 1994). Several studies of association of this polymorphism were conducted and revealed that the *UCP*1 A-3826G polymorphism is not a major contributor to obesity development, however, the main observation was a significant association of this polymorphism with fat gain over time (Oppert et al., 1994; Gonzalez-Barroso et al., 2000b).

THE NOVEL UCPS, UCP2, AND UCP3 ARE BIOCHEMICALLY AND PHYSIOLOGICALLY DISTINCT FROM UCP1

UCP2 and UCP3, two Homologs of UCP1, were described in 1997 (Boss et al., 1997; Fleury et al., 1997; Vidal-Puig et al., 1997). Initially, the high level of amino-acid similarity with UCP1 (these new proteins display 57% identity with UCP1) as well as functional assays in yeast were in favor of an uncoupling activity (Fleury et al., 1997; Rial et al., 1999). However, a large number of studies based on physiological measurements of UCP2 or UCP3 expression (such as a marked up-regulation of these two UCPs in skeletal muscles of rodents and humans upon starvation Bevilacqua et al., 2005; Millet et al., 2007) and on analysis of mice null for *Ucp*1 or *Ucp*2, contradicted the first view (Boss et al., 2000; Ricquier and Bouillaud, 2000; Stuart et al., 2001; Rousset et al., 2004). Presently, it is difficult to consider UCP2 or UCP3 as membranous carriers able to uncouple respiration similarly to UCP1 (Nedergaard et al., 1999; Nedergaard and Cannon, 2003). UCP1 is present in a unique tissue the function of which is thermogenesis, whereas UCP2 is widely expressed in tissues and cells (gut, lung, brain, pancreatic islets, immune cells...) and UCP3 is present in skeletal muscles and brown adipose tissue. It appears that these other UCPs are metabolite transporters of the inner mitochondrial inner membrane and can limit the level of reactive oxygen species (Arsenijevic et al., 2000; Harper and Gerrits, 2004). UCP2 is able to inhibit glucose-induced insulin release (Zhang et al., 2001) and it has a transport activity directly or undirectly favoring glucose sparing and fatty acid oxidation (Pecqueur et al., 2008, 2009; Bouillaud, 2009).

CONCLUSION AND PROSPECTIVES: SEARCH FOR ACTIVATORS OR INDUCERS OF UCP1, IDENTIFICATION OF COMPONENTS MIMICKING UCP1, INDUCTION OF THERMOGENIC BROWN ADIPOCYTES

The brown adipocytes remain the cells uniquely able to rapidly burn fatty acids and dissipate oxidation energy as heat. Their activity is strictly dependant on their high content of mitochondria and above all on the presence of UCP1, a very particular membranous carrier, able to disrupt the respiratory-induced $\Delta\mu_H^+$ via a physiologically regulated proton transport activity. Therefore, these cells and UCP1 in particular, offer a chance to find compounds that increase fatty acid oxidation in obese patients and also in patients with metabolic syndrome.

Consequently, understanding the mechanisms which regulate transcription and expression of the human *UCP*1 will facilitate the identification of molecules able to increase the levels of this protein in order to elevate energy expenditure in adult patients. Another approach will be to activate the UCP1 protein itself by searching for specific activators solely interacting with UCP1 (Rial et al., 2011). This is certainly a difficult aim, but since free fatty acids

activate UCP1, modified fatty acids could be engineered. However, such new molecules should be totally specific for UCP1. Another strategy will be to identify chemical compounds able to induce a very slight uncoupling of respiration in tissues such as muscles. However, this later approach is very risky since induction of respiration uncoupling, even moderate, in cells other than brown adipocytes, may lower ATP synthesis and therefore be extremely deleterious. Remember that the use of classical chemical uncoupler, such as 2,4-dinitrophenol, caused serious illness in imprudent individuals and must be strictly banned (Grundlingh et al., 2011). Finally, an interesting approach would be to facilitate the emergence of new brown adipocytes from precursors present in skeletal muscles or in white adipose depots (Seale et al., 2007; Crisan et al., 2008).

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REFERENCES

Alvarez, R., de Andrés, J., Yubero, P., Viñas, O., Mampel, T., Iglesias, R., Giralt, M., and Villarroya, F. (1995). A novel regulatory pathway of brown fat thermogenesis. Retinoic acid is a transcriptional activator of the mitochondrial uncoupling protein gene. *J. Biol. Chem.* 270, 5666–5673.

Aquila, H., Link, T. A., and Klingenberg, M. (1985). The uncoupling protein from brown fat mitochondria is related to the mitochondrial ADP/ATP carrier. Analysis of sequence homologies and of folding of the protein in the membrane. *EMBO J.* 4, 2369–2376.

Arsenijevic, D., Onuma, H., Pecqueur, C., Raimbault, S., Manning, B. S., Miroux, B., Couplan, E., Alves-Guerra, M. C., Goubern, M., Surwit, R., Bouillaud, F., Richard, D., Collins, S., and Ricquier, D. (2000). Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production. *Nat. Genet.* 26, 435–439.

Berardi, M. J., Shih, W. M., Harrison, S. C., and Chou, J. J. (2011). Mitochondrial uncoupling protein 2 structure determined by NMR molecular fragment searching. *Nature* 476, 109–113.

Bevilacqua, L., Ramsey, J. J., Hagopian, K., Weindruch, R., and Harper, M. E. (2005). Long-term caloric restriction increases UCP3 content but decreases proton leak and reactive oxygen species production in rat skeletal muscle mitochondria. *Am. J. Physiol. Endocrinol. Metab.* 289, E429–E438.

Boss, O., Hagen, T., and Lowell, B. B. (2000). Uncoupling proteins 2 and 3: potential regulators of mitochondrial energy metabolism. *Diabetes* 49, 143–156.

Boss, O., Samec, S., Paoloni-Giacobino, A., Rossier, C., Dulloo, A., Seydoux, J., Muzzin, P., and Giacobino, J. P. (1997). Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. *FEBS Lett.* 408, 39–42.

Bouillaud, F. (2009). UCP2, not a physiologically relevant uncoupler but a glucose sparing switch impacting ROS production and glucose sensing. *Biochim. Biophys. Acta* 1787, 377–383.

Bouillaud, F., Arechaga, I., Petit, P. X., Raimbault, S., Levi-Meyrueis, C., Casteilla, L., Laurent, M., Rial, E., and Ricquier, D. (1994). A sequence related to a DNA recognition element is essential for the inhibition by nucleotides of proton transport through the mitochondrial uncoupling protein. *EMBO J.* 13, 1990–1997.

Bouillaud, F., Ricquier, D., Thibault, J., and Weissenbach, J. (1985). Molecular approach to thermogenesis in brown adipose tissue: cDNA cloning of the mitochondrial uncoupling protein. *Proc. Natl. Acad. Sci. U.S.A.* 82, 445–448.

Bouillaud, F., Weissenbach, J., and Ricquier, D. (1986). Complete cDNA-derived aminoacid sequence of rat brown fat uncoupling protein. *J. Biol. Chem.* 261, 1487–1490.

Cannon, B., and Nedergaard, J. (1985). The biochemistry of an inefficient tissue: brown adipose tissue. *Essays Biochem.* 20, 110–164.

Cannon, B., and Nedergaard, J. (2004). Brown adipose tissue: function and physiological significance. *Physiol. Rev.* 84, 277–359.

Cassard, A. M., Bouillaud, F., Mattei, M. G., Hentz, E., Raimbault, S., Thomas, M., and Ricquier, D. (1990). Human uncoupling protein gene: structure, comparison with rat gene and assignment to the long arm of the chromosome 4. *J. Cell. Biochem.* 43, 255–264.

Cassard-Doulcier, A. M., Gelly, C., Bouillaud, F., and Ricquier, D. (1998). The 211-bp enhancer of the rat UCP-1 gene controls specific and regulated expression in the brown adipose tissue. *Biochem. J.* 333, 243–246.

Cassard-Doulcier, A. M., Gelly, C., Fox, N., Schrementi, J., Raimbault, S., Klaus, S., Forest, C., Bouillaud, F., and Ricquier, D. (1993). Tissue-specific and β-adrenergic regulation of the mitochondrial uncoupling protein gene – control by cis-acting elements in the 5′-flanking region. *Mol. Endocrinol.* 7, 497–506.

Cassard-Doulcier, A. M., Larose, M., Matamala, J. C., Champigny, O., Bouillaud, F., and Ricquier, D. (1994). In vitro interactions between nuclear proteins and uncoupling protein gene promoter reveal several putative trans-activating factors including Ets1, retinoid X receptor, thyroid hormone receptor and a CACCC box-binding protein. *J. Biol. Chem.* 269, 24335–24342.

Casteilla, L., Blondel, O., Klaus, S., Raimbault, S., Diolez, P., Moreau, F., Bouillaud, F., and Ricquier, D. (1990). Stable expression of functional mitochondrial uncoupling protein in Chinese hamster ovary cells. *Proc. Natl. Acad. Sci. U.S.A.* 87, 5124–5128.

Crisan, M., Casteilla, L., Lehr, L., Carmona, M., Paoloni-Giacobino, A., Yap, S., Sun, B., Léger, B., Logar, A., Pénicaud, L., Schrauwen, P., Cameron-Smith, D., Russell, A. P., Péault, B., and Giacobino, J. P. (2008). A reservoir of brown adipocyte progenitors in human skeletal muscle. *Stem Cells* 26, 2425–3243.

Enerbäck, S., Jacobsson, A., Simpson, E. M., Guerra, C., Yamashita, H., Harper, M. E., and Kozak, L. P. (1997). Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* 387, 90–94.

Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M. F., Surwit, R. S., Ricquier, D., and Warden, C. H. (1997). Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat. Genet.* 15, 269–272.

Garlid, K. D., and Jaburek, M. (1998). The mechanism of proton transport mediated by mitochondrial uncoupling proteins. *FEBS Lett.* 438, 10–14.

Gonzalez-Barroso, M., Pecqueur, C., Gelly, C., Sanchis, D., Alvez-Guerra, M. C., Bouillaud, F., Ricquier, D., and Cassard-Doulcier, A. M. (2000a). Transcriptional activation of the human UCP1 gene: synergism of retinoids, isoproterenol and thiazolidinedione is mediated by a multipartite response element. *J. Biol. Chem.* 275, 31722–31732.

Gonzalez-Barroso, M., Ricquier, D., and Cassard-Doulcier, A. M. (2000b). The human uncoupling protein-1 gene (UCP1): present status and perspectives in obesity research. *Obes. Rev.* 1, 61–72.

González-Barroso, M. M., Fleury, C., Bouillaud, F., Nicholls, D. G., and Rial, E. (1998). The uncoupling protein UCP1 does not increase the proton conductance of the inner mitochondrial membrane by functioning as a fatty acid anion transporter. *J. Biol. Chem.* 273, 15528–15532.

Grundlingh, J., Dargan, P. I., El-Zanfaly, M., and Wood, D. M. (2011). 2,4-dinitrophenol (DNP): a weight loss agent with significant acute toxicity and risk of death. *J. Med. Toxicol.* 7, 205–212.

Harper, M. E., and Gerrits, M. F. (2004). Mitochondrial uncoupling proteins as potential targets for pharmacological agents. *Curr. Opin. Pharmacol.* 4, 603–607.

Heaton, G. M., Wagenvoord, R. J., Kemp, A., and Nicholls, D. G. (1978). Brown-adipose-tissue mitochondria: photoaffinity labelling of the regulatory site of energy dissipation. *Eur. J. Biochem.* 82, 515–521.

Himms-Hagen, J. (1989). Brown adipose tissue thermogenesis and obesity. *Prog. Lipid Res.* 28, 67–115.

Himms-Hagen, J., and Ricquier, D. (1998). "Brown adipose tissue," in *Handbook of Obesity*, eds G. Bray, C. Bouchard, W. James (New York, Basel, Hong Kong: Marcel Dekker), 415–441.

Klingenberg, M. (2010). Wanderings in bioenergetics and biomembranes. *Biochim. Biophys. Acta* 1797, 579–594.

Kozak, U. C., Kopecky, J., Teisinger, J., Enerbäck, S., Boyer, B., and Kozak, L. P. (1994). An upstream enhancer regulating brown-fat-specific expression of the mitochondrial uncoupling protein gene. *Mol. Cell Biol.* 14, 59–67.

Larose, M., Cassard-Doulcier, A. M., Fleury, C., Serra, F., Champigny, O., Bouillaud, F., and Ricquier, D. (1996). Essential cis-acting elements in rat uncoupling protein gene are in the enhancer. Delineation of a complex element mediating the transcriptional activation by retinoic acid. *J. Biol. Chem.* 271, 31533–31542.

Ledesma, M., Garcià de Lacoba, M., and Rial, E. (2002). The mitochondrial uncoupling proteins. *Genome Biol.* 3. PMID: 12537581.

Millet, L., Vidal, H., Andrealli, F., Larrouy, D., Riou, J. P., Ricquier, D., Laville, M., and Langin, D. (2007). Increased uncoupling protein-2 and uncoupling protein-3 mRNA expression during fasting in obese and lean humans. *J. Clin. Invest.* 100, 2665–2670.

Miroux, B., Frossard, V., Raimbault, S., Ricquier, D., and Bouillaud, F. (1993). The topology of the brown adipose tissue mitochondrial uncoupling protein determined with antibodies against its antigenic sites revealed by a library of fusion proteins. *EMBO J.* 12, 3739–3745.

Mozo, J., Ferry, G., Masscheleyn, S., Miroux, B., Boutin, J. A., and Bouillaud, F. (2006). Assessment of a high-throughput screening methodology for the measurement of purified UCP1 uncoupling activity. *Anal. Biochem.* 15, 201–206.

Nedergaard, J., and Cannon, B. (2003). The "novel" "uncoupling" proteins UCP2 and UCP3: what do they really do? Pros and cons for suggested functions. *Exp. Physiol.* 88, 65–84.

Nedergaard, J., Cannon, B., and Lindberg, O. (1977). Microcalorimetry of isolated mammalian cells. *Nature* 267, 518–520.

Nedergaard, J., Golozoubova, V., Matthias, A., Asadi, A., Jacobsson, A., and Cannon, B. (2001). UCP1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic inefficiency. *Biochim. Biophys. Acta* 1504, 82–106.

Nedergaard, J., Matthias, A., Golozoubova, V., Jacobsson, A., and Cannon, B. (1999). UCP1: the original uncoupling – and perhaps the only one? New perspectives on UCP1, UCP2, and UCP3 in the light of the bioenergetics of the UCP1-ablated mice. *J. Bioenerg. Biomembr.* 31, 475–491.

Nedergaard, J., Ricquier, D., and Kozak, L. P. (2005). Uncoupling proteins: current status andtherapeutic prospects. *EMBO Rep.* 6, 9179–9121.

Nicholls, D. G. (2006). The physiological regulation of uncoupling proteins. *Biochim. Biophys. Acta* 1757, 459–466.

Nicholls, D. G., and Locke, R. M. (1984). Thermogenic mechanisms in brown fat. *Physiol. Rev.* 64, 1–64.

Oppert, J. M., Vohl, M. C., Chagnon, M., Dionne, F. T., Cassard-Doulcier, A. M., Ricquier, D., Pérusse, L., and Bouchard, C. (1994). DNA polymorphism in the uncoupling protein (UCP) gene and human body fat. *Int. J. Obes. Relat. Metab. Disord.* 18, 526–531.

Pebay-Peyroula, E., Dahout-Gonzalez, C., Kahn, R., Trézéguet, V., Lauquin, G. J., and Brandolin, G. (2003). Structure of mitochondrial ADP/ATP carrier in complex with carboxyatractyloside. *Nature* 426, 39–44.

Pecqueur, C., Alves-Guerra, C., Ricquier, D., and Bouillaud, F. (2009). UCP2, a metabolic sensor coupling glucose oxidation to mitochondrial metabolism? *IUBMB Life* 61, 762–767.

Pecqueur, C., Bui, T., Gelly, C., Hauchard, J., Barbot, C., Bouillaud, F., Ricquier, D., Miroux, B., and Thompson, C. B. (2008). Uncoupling protein-2 controls proliferation by promoting fatty acid oxidation and limiting glycolysis-derived pyruvate utilization. *FASEB J.* 22, 9–18.

Puigserver, P., Wu, Z., Park, C. W., Graves, R., Wright, M., and Spiegelman, B. M. (1998). A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 92, 829–839.

Rabelo, R., Reyes, C., Schifman, A., and Silva, J. E. (1996). A complex retinoic acid response element in the uncoupling protein gene defines a novel role for retinoids in thermogenesis. *Endocrinology* 137, 3488–3496.

Rial, E., Gonzalez-Barroso, M., Fleury, C., Iturrizaga, S., Sanchis, D., Jiménez-Jiménez, J., Ricquier, D., Goubern, M., and Bouillaud, F. (1999). Retinoids activate proton transport by the uncoupling proteins UCP1 and UCP2. *EMBO J.* 18, 5827–5833.

Rial, E., Rodríguez-Sánchez, L., Aller, P., Guisado, A., Mar González-Barroso, M., Gallardo-Vara, E., Redondo-Horcajo, M., Castellanos, E., Fernández de la Pradilla, R., and Viso, A. (2011). Development of chromanes as novel inhibitors of the uncoupling proteins. *Chem. Biol.* 25, 264–274.

Ricquier, D., and Bouillaud, F. (2000). The uncoupling protein homologues: UCP1, UCP2, UCP3, StUCP and AtUCP. *Biochem. J.* 345, 161–169.

Ricquier, D., Bouillaud, F., Toumelin, P., Mory, G., Bazin, R., Arch, J., and Pénicaud, L. (1986). Expression of uncoupling protein mRNA in thermogenic or weakly thermogenic brown adipose tissue. Evidence for a rapid β-adrenoceptor-mediated and transcriptionally regulated step during activation of thermogenesis. *J. Biol. Chem.* 261, 13905–13910.

Ricquier, D., Gaillard, J. C., and Turc, J. M. (1979). Microcalorimetry of isolated mitochondria from brown adipose tissue. Effect of guanine-diphosphate. *FEBS Lett.* 99, 203–206.

Ricquier, D., and Kader, J. C. (1976). Mitochondrial protein alteration in active brown fat. A sodium-dodecylsulfate-polyacrylamide gel electrophoretic study. *Biochem. Biophys. Res. Commun.* 73, 577–583.

Ricquier, D., and Kozak, L. K. (2003). "Uncoupling proteins," in *Handbook of Obesity*, eds G. A. Bray and C. Bouchard (New York: Marcel Dekker Inc.), 539–558.

Ricquier, D., Mory, G., Bouillaud, F., Thibault, J., and Weissenbach, J. (1984). Rapid increase of mitochondrial uncoupling protein and its mRNA in stimulated brown adipose tissue. Use of a cDNA probe. *FEBS Lett.* 178, 240–244.

Rim, J. S., and Kozak, L. P. (2002). Regulatory motifs for CREB-binding protein and Nfe2l2 transcription factors in the upstream enhancer of the mitochondrial uncoupling protein 1 gene. *J. Biol. Chem.* 277, 34589–34600.

Rousset, S., Alves-Guerra, M. C., Mozo, J., Miroux, B., Cassard-Doulcier, A. M., Bouillaud, F., and Ricquier, D. (2004). The biology of mitochondrial uncoupling proteins. *Diabetes* 53(Suppl 1), S130–S135.

Seale, P., Kajimura, S., Yang, W., Chin, S., Rohas, L. M., Uldry, M., Tavernier, G., Langin, D., and Spiegelman, B. M. (2007). Transcriptional control of brown fat determination by PRDM16. *Cell Metab.* 6, 38–54.

Seydoux, J., and Girardier, L. (1977). Control of brown fat thermogenesis by the sympathetic nervous system. *Experientia* 33, 1128–1130.

Shabalina, I. G., Backlund, E. C., Bar-Tana, J., Cannon, B., and Nedergaard, J. (2008). Within brown-fat cells, UCP1-mediated fatty acid-induced uncoupling is independent of fatty acid metabolism. *Biochim. Biophys. Acta* 1777, 642–650.

Shabalina, I. G., Ost, M., Petrovic, N., Vrbacky, M., Nedergaard, J., and Cannon, B. (2010). Uncoupling protein-1 is not leaky. *Biochim. Biophys. Acta* 1797, 773–784.

Silva, J. E., and Rabelo, R. (1998). Regulation of the uncoupling protein gene expression. *Eur. J. Endocrinol.* 136, 51–64.

Skulachev, V. P. (1988). Uncoupling: new approaches to an old problem of bioenergetics. *Biochim. Biophys. Acta* 1363, 100–124.

Strieleman, P. J., Schalinske, K., and Shrago, E. (1985). Fatty acid activation of the reconstituted brown adipose tissue mitochondria uncoupling protein. *J. Biol. Chem.* 260, 3402–13405.

Stuart, J. A., Cadenas, S., Jekabsons, M. B., Roussel, D., and Brand, M. D. (2001). Mitochondrial proton leak and the uncoupling protein 1 homologues. *Biochim. Biophys. Acta* 1504, 144–158.

Vidal-Puig, A., Solanes, G., Grujic, D., Flier, J. S., and Lowell, B. B. (1997). UCP3: an uncoupling protein homologue expressed preferentially and abundantly in skeletal muscle and brown adipose tissue. *Biochem. Biophys. Res. Commun.* 1997, 79–82.

Winkler, E., and Klingenberg, M. (1994). Effect of fatty acids on H^+ transport activity of the reconstituted uncoupling protein. *J. Biol. Chem.* 28, 2808–2515.

Xue, B., Coulter, A., Rim, J. S., Koza, R. A., and Kozak, L. P. (2005). Transcriptional synergy and the regulation of Ucp1 during brown adipocyte induction in white fat depots. *Mol. Cell Biol.* 25, 8311–8322.

Yubero, P., Barberá, M. J., Alvarez, R., Viñas, O., Mampel, T., Iglesias, R., Villarroya, F., and Giralt, M. (1998). Dominant negative regulation by c-Jun of transcription of the uncoupling protein-1 gene through a proximal cAMP-regulatory element: a mechanism for repressing basal and norepinephrine-induced expression of the gene before brown adipocyte differentiation. *Mol. Endocrinol.* 12, 1023–1037.

Yubero, P., Manchado, C., Cassard-Doulcier, A. M., Mampel, T., Vinas,

O., Iglesias, R., Giralt, M., and Villarroya, F. (1994). CCAAT/enhancer binding proteins α and β are transcriptional activators of the brown fat uncoupling protein gene promoter. *Biochem. Biophys. Res. Commun.* 198, 653–659.

Zhang, C. Y., Baffy, G., Perret, P., Krauss, S., Peroni, O., Grujic, D., Hagen, T., Vidal-Puig, A. J., Boss, O., Kim, Y. B., Zheng, X. X., Wheeler, M. B., Shulman, G. I., Chan, C. B., and Lowell, B. B. (2001). Uncoupling protein-2 negatively regulates insulin secretion and is a major link between obesity, β cell dysfunction, and type 2 diabetes. *Cell* 105, 745–755.

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Test systems to study the structure and function of uncoupling protein 1: a critical overview

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The discovery of active brown adipose tissue (BAT) in healthy adult humans has renewed interest in the biology of this organ. BAT is capable of distributing nutrient energy in the form of heat allowing small mammals to efficiently defend their body temperature when acutely exposed to the cold. On the other hand BAT might be a target for the treatment of obesity and related diseases, as its pharmacological activation could allow release of excess energy stored in white adipose tissue depots. Energy dissipation in BAT depends on the activity of uncoupling protein 1 (UCP1), therefore a BAT-based obesity therapy requires a detailed understanding of structure and function of UCP1. Although UCP1 has been in the focus of research since its discovery, central questions concerning its mechanistic function and regulation are not yet resolved. They have been addressed in native mitochondria but also in several test systems, which are generally used to lower inter-experimental variability and to simplify analysis conditions. Different test systems have contributed to our current knowledge about UCP1 but of course all of them have certain limitations. We here provide an overview about research on UCP1 structure and function in test systems. So far, these have nearly exclusively been employed to study rodent and not human UCP1. Considering that the amino acid sequence of mouse and human UCP1 is only 79% identical, it will be essential to test whether the human version has a similarly high catalytic activity, allowing a relevant amount of energy dissipation in human BAT. Besides the issue of comparable mechanistic function a sufficiently high expression level of human UCP1 is a further prerequisite for anti-obesity therapeutic potential. Treatments which induce BAT hyperplasia and UCP1 expression in humans might therefore be equally important to discover as mere activators of the thermogenic process.

Keywords: uncoupling protein 1, UCP1, mitochondria, brown adipose tissue

INTRODUCTION

Brown adipose tissue (BAT) is the exclusive site of cold induced non-shivering thermogenesis in mammals and combusts stored nutrient energy as heat (Smith, 1961). In cold exposed rodents, this powerful process can claim more than a quarter of cardiac output and more than half of total oxygen consumption, thereby facilitating heat dissipation up to nearly 500 mW per gram tissue (Foster and Frydman, 1978, 1979; Trayhurn and James, 1978; Puchalski et al., 1987). To achieve this unique capability, brown adipocytes are equipped with a specialized set of cellular features including a large number of mitochondria with dense cristae, multiple small lipid droplets providing high lipolytic rates, high nutrient uptake rates and many more. In the center of all these processes, however, a single essential protein facilitates the crucial mechanism of regulated mitochondrial proton leak across the inner membrane: uncoupling protein 1 (UCP1). Therefore, understanding the structure–function-relationship of UCP1 is of paramount importance to understand brown fat thermogenesis.

The significance of elucidating UCP1 function for biomedical applied science has recently been highlighted by the (re)discovery of active BAT in humans. Radiologists applying fluorodeoxyglucose positron emission tomography (FDG PET) combined with computerized tomography (CT) during tumor diagnosis accidentally visualized the *in vivo* distribution and activity of BAT (Hany et al., 2002). However, the detailed anatomy of human BAT had already been described as early as 1972 and a role of this tissue in diet-induced obesity proposed several years later (Heaton, 1972; Rothwell and Stock, 1979). The allometric comparison of BAT mass and norepinephrine induced thermogenesis capacity across multiple species predicts that mammals larger than 10 kg should have no or insignificant amounts of BAT thermogenesis (Heldmaier, 1971). Accordingly, an *a priori* thermogenic function to defend normothermia in the face of low ambient temperatures is questionable in man. Indeed, estimating the contribution of BAT to cold induced thermogenesis leads to a marginal to negligible fraction (Klingenspor and Fromme, 2012). However, the discovered amounts of BAT are sufficient to burn more than 4 kg of fat during 1 year and may thus very well be a component of organismic energy balance control (Virtanen et al., 2009). Moreover, the targeted pharmacological activation of brown fat energy dissipation is a promising strategy to combat the global obesity epidemic and its associated diseases, but requires understanding how exactly UCP1 structure enables its unique, regulated uncoupling property.

THERMOGENIC FUNCTION OF BAT

While white adipose tissue stores energy in form of triglycerides, BAT is an energy-dissipating organ. Brown adipocytes contain a high number of mitochondria and these contain vast amounts of UCP1 totalling up to 5–8% of mitochondrial protein (Lin and Klingenberg, 1980). Usually the mitochondrial inner membrane is the place where final conversion of nutrient energy into ATP occurs. Combustion of lipids, carbohydrates and proteins results in the production of reduction equivalents, which feed electrons into the respiratory chain. Electrons are passed along a redox gradient until they finally reduce oxygen to water. The energy liberated by the redox reactions drives the export of protons from the matrix into the intermembrane space, creating a proton motive force across the membrane. The ATP synthase utilizes the energy conserved in the proton gradient and phosphorylates ADP to ATP.

In BAT this energy fixation can be interrupted and metabolization of substrates leads to a sustained direct conversion of nutrient energy to heat. Upon adrenergic stimulation lipases mobilize fat from lipid droplets and free fatty acids are rapidly metabolized. Due to the presence of UCP1 in the inner mitochondrial membrane this does not result in ATP production, as UCP1 short-circuits the cycling of protons, which usually couples respiratory chain activity to ATP production (**Figure 1**). In non-stimulated conditions UCP1 is probably inhibited by purine nucleotides, but when activated by free fatty acids, catalyzes a proton flux into the mitochondrial matrix. Thus, the liberation of fatty acids upon cold stimulation fuels heat production dually, by serving as metabolic substrate and by directly activating UCP1.

MOLECULAR ASPECTS OF UCP1 FUNCTION

Despite intense research on UCP1 function several questions concerning its precise mechanism, which might affect its potential as therapeutic target, are unresolved. For example it is still disputed if UCP1 contributes to basal leak of BAT mitochondria, leaving the question if UCP1 would just have to be present or also activated to dissipate excess energy (Parker et al., 2009; Shabalina et al., 2010).

Regulators of UCP1 had been known even before the protein itself was purified and sequenced. Until today the interaction with the inhibitory nucleotides and especially the mechanism of activation by fatty acids is not finally understood. The nucleotide binding of UCP1 has been characterized by measuring GDP binding to wildtype and mutated versions of the protein in heterologous systems. This strategy identified an interacting region in the C-terminal part of the protein as well as individual amino acid residues distributed in the primary sequence providing charges for the interaction with purine nucleotides (Modriansky et al., 1997; Winkler et al., 1997; Echtay et al., 1998). According to the three-dimensional model these residues position in close proximity to each other.

There are different models concerning the activation of UCP1 by fatty acids, being elaborations of two main hypotheses, one claiming that fatty acids are cofactors, needed to form the transport channel for the protons or to overcome nucleotide inhibition (Winkler and Klingenberg, 1994; Klingenberg and Huang, 1999; Shabalina et al., 2004), the other one stating that fatty acids are the substrate of transport. According to this model fatty acid anions would be transported to the intermembrane space by UCP1 and protonated fatty acids would flip–flop back into the matrix to complete proton translocation (Skulachev, 1991; Jezek et al., 1994; Garlid et al., 1998).

So far only one member of the mitochondrial anion transporter family, the ANT, has been crystallized. Three-dimensional models of UCP1 are calculated based on its sequence and the ANT structure, but of course might not reflect the actual situation in the membrane. ANT was crystallized as a monomer whereas almost since the beginning of UCP1 research it was assumed that it functions as a homodimer. This was based on GDP binding stoichiometry, sedimentation analyses, and cross-linking studies (Lin et al., 1980; Lin and Klingenberg, 1982). In a recent review all these findings were carefully reanalyzed, explaining why these results might have been obtained even though UCP1 in BAT mitochondria works as monomer, for example by artificial aggregation in detergents or overestimation of protein content in the presence of detergent (Kunji and Crichton, 2010). Knowing the structure of the wildtype protein or having the ability to compare it to mutant versions of the protein would greatly facilitate to clarify how UCP1 interacts with its known inhibitors and activators or to predict which novel substances might also be able to interact.

Knowledge about protein structure and interacting molecules might also help to learn more about the physiological function of UCP1. It has been shown that UCP1 can be activated by superoxide and reactive alkenals, supporting an involvement in the protection against reactive oxygen species (ROS) damage, according to the mild uncoupling theory (Skulachev, 1996). Functional analysis of isolated BAT mitochondria from wildtype and UCP1 knockout animals supports this theory, as under basal conditions mitochondria from wildtype animals produce less ROS than knockout animals, but reach the same level of ROS production when UCP1 is inhibited by addition of GDP (Oelkrug et al., 2010).

UCP1 ORTHOLOGS AND PARALOGS

Among the UCP1 orthologs rodent UCP1 is the best characterized uncoupling protein but studies of other UCP orthologs and paralogs will be instrumental to elucidate structure–function-relationships. Comparison of the characteristics of uncoupling proteins from different species may reveal potentially crucial conserved residues or domains of which the functional relevance can be tested in targeted mutagenesis studies.

The paralogs UCP2 and UCP3 were discovered due to sequence similarity with UCP1, with 59% for UCP2 and 57% for UCP3 (Boss et al., 1997; Fleury et al., 1997; Gimeno et al., 1997). They differ in their tissue specific expression pattern, as UCP3 is expressed in skeletal muscle, heart and BAT, and UCP2 in multiple tissues, with an important role in immune cells and in beta cells (Pecqueur et al., 2001). For both paralogs no definite physiological function could be assigned as the respective knockout models do not display a clear phenotype (Brand and Esteves, 2005). UCP3 expression is elevated in physiological situations where free fatty acid levels in plasma are elevated. This led to the assumption that UCP3 plays a role in fatty acid metabolism. The fact that the UCP3 knockout mouse is not prone to 3,4-methylenedioxymethamphetamine (MDMA)-induced hyperthermia hinted toward an involvement in thermoregulation. There are several studies showing that UCP2 plays a role in beta cell function and affects glucose stimulated

insulin secretion. Both UCP2 and UCP3, like UCP1, are supposed to protect the cell from excessive production of ROS according to the mild uncoupling theory (Skulachev, 1996). The regulation of the activity of UCP2 and UCP3 seems to differ from UCP1, as they are activated by superoxide and reactive alkenals but not by fatty acids alone. UCP1 orthologs for which uncoupling function has been analyzed, including carp UCP1 (Jastroch et al., 2007), elephant shrew UCP1 (Mzilikazi et al., 2007) and marsupial UCP1 (Polymeropoulos et al., 2011), can be used for comparisons of structure and function. It will be stressed in this review that valid comparisons require the use of suitable expression systems, as otherwise the proteins are characterized on a different mitochondrial background which might contribute to differences in function not related to protein structure. There are also several studies on BAT in larger mammals, like sheep and bovine, which are only exposed to thermal stress after birth and during early juvenile development but not in their adult life history. These studies mainly focus on the aspect of emergence and persistence of the tissue during the postnatal phase and juvenile development (Carstens, 1994; Symonds et al., 2011; Taga et al., 2011), rather than directly testing the function of the uncoupling protein.

HUMAN BAT AND MOLECULAR ASPECTS OF HUMAN UCP1

From a biomedical perspective it is certainly of capital importance to study the human UCP1 protein and its function. This may not be obvious on the first glance but, e.g., mouse and human UCP1 only share less than 80% identity on the amino acid level. The initial three reports of significant amounts of active human BAT in 2009 confirmed the presence of mRNA and protein of human UCP1 in human BAT by immunohistochemistry and quantitative PCR, respectively (Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). These findings have since been reproduced multiple times (Saito et al., 2009; Zingaretti et al., 2009; Svensson et al., 2011). Earlier, human patients with pheochromocytoma have been studied, a neuroendocrine disease leading to excessive release of catecholamines and the appearance of BAT-like tissue depots in the body. Samples of this periadrenal, perirenal, and omental tissue express ∼30 μg UCP1/mg mitochondrial protein, which is a concentration comparable to the expression in the interscapular BAT of mice acclimated to room temperature (**Table 1**; Lean et al., 1986a). Healthy control subjects, however, express only ∼3 μg UCP1/mg mitochondrial protein in perirenal and axillary fat depots (Lean et al., 1986b). It is unclear whether this low concentration is due to a dilution of brown with white adipocyte mitochondria or indeed a far lower level of UCP1 expression in brown adipocytes. Furthermore, other human fat depots not studied so far may display very different UCP1 expression. Even less is known about human UCP1 on the level of functional or mechanistic studies and it remains elusive whether human UCP1 is similarly effective and tightly regulated as its well-studied rodent counterparts. We would like to emphasize the need to remedy this deficit and in the following we systematically present test systems currently available for this challenging task.

TEST SYSTEMS FOR UCP1 FUNCTION

Several test systems have been applied in research on UCP1 function, such as proteoliposomes, yeast, and mammalian cells, including brown adipocytes. Initial studies depended on animal tissue as the sole source for UCP1. They were conducted with mitochondria isolated from animals after exposure to different treatments (cold exposure, high fat diet feeding, drug application) to alter UCP1 expression, or with UCP1 first purified from mitochondria and then reconstituted in proteoliposomes. Cloning and sequencing of UCP1 cDNA and protein (Aquila et al., 1985; Bouillaud et al., 1986) enabled the use of heterologous expression systems either as a source for the purified protein or as a source for mitochondria containing UCP1 at variable concentrations. With these systems it was possible to study the consequences of mutations inserted

Table 1 | Comparison of maximum UCP1-dependent H^+ transport rates in different test systems.

System	UCP1	Mitoprotein (μg/mg)	mV	nmol H^{+*} min^{-1*} μg $UCP1^{-1*}$ mV^{-1}	H^{+*} s^{-1}	Reference
BAT mitochondria	Hamster (cold)	54	180	0.05	5.0	Rial et al. (1983)
	Mouse (RT)	29.8	141	0.02	1.7	Monemdjou et al. (1999)
	Rat (RT)	13	143	0.56	44.0	Esteves et al. (2006)
	Mouse (RT)	29.8	147	0.34	27.5	Shabalina et al. (2006)
	Mouse (RT)	29.8	147	0.31	24.8	Parker et al. (2009)
Liposomes	Rat expressed in yeast				1.2	Murdza-Inglis et al. (1991)
	Hamster expressed in yeast				55.0	Echtay et al. (1998)
	Rat expressed in *E. coli*				11.0	Jaburek and Garlid (2003)
Lipid bilayer	Hamster		180	0.14	14.0	Urbankova et al. (2003)
Yeast mitochondria	Mouse	0.9	138	9.28	704.0	Stuart et al. (2001)
HEK UCP1 mitochondria	Mouse	4.8	155	0.88	74.8	Unpublished data
Brown fat cell mitochondria	Mouse	2.8	141	1.17	90.8	Unpublished data

Values were extracted from tables and figures of publications and UCP1-dependent transport rates were calculated by subtracting respiration of mitochondria from knockout animals or in the presence of GDP. Rates expressed as H^{+*} s^{-1} are calculated from oxygen consumption rates (nmol O^* min^{-1}) and mitochondrial concentrations of UCP1 protein (μg/mg). Considering that maximum UCP1-dependent transport rates are determined at different membrane potentials all systems, except yeast, for which an uncoupling artifact has been published, are in a comparable range.

into the protein and to compare the properties of UCP orthologs and paralogs. Since UCP1 knockout animals have been generated (Enerback et al., 1997), comparison of the bioenergetic properties of normal and UCP1-ablated brown fat mitochondria are another valuable system to study UCP1 function.

In the following we will briefly introduce the principle of different test systems and their most frequent applications and discuss their strengths and weaknesses (**Table 2**). Major insight gained with these test systems concerning the structure–function-relationship of UCP1 will be highlighted. Moreover, we provide a comprehensive summary on which aspects of human UCP1 have been studied so far using these test systems.

PROTEOLIPOSOMES

Liposomes are a standard tool to study the transport function of membrane proteins. The protein(s) of interest are reconstituted into the bilayer phospholipid membrane of vesicles with a diameter of 5–15 nm enclosing an aqueous compartment. This membrane mimics the natural phospholipid environment, provides a lipophilic compartment for protein solubilization and facilitates proper folding. Purified native proteins or recombinantly expressed proteins can be inserted into this membrane by mixing the components in the presence of detergent. Transport characteristics of the inserted membrane protein can be studied by loading the vesicles with specific buffers. Differential buffer composition in- and outside of the vesicle generates the driving force for subsequent experiments where ion flux is measured. The initial study reconstituting UCP1 in this system was published in 1983 (Bouillaud et al., 1983) followed by the demonstration of H^+ transport by UCP1 in proteoliposomes two years later (Klingenberg and Winkler, 1985). The first successful reconstitution of recombinant UCP1 from *E. coli* inclusion bodies was performed by Jaburek and Garlid (2003), who could measure H^+ transport rates comparable to the reconstituted native UCP1 isolated from hamster BAT.

Ion transport is usually detected by dyes, which are quenched by a specific ion or which aggregate when proteoliposomes are polarized. Therefore results are presented as relative transport rates above basal or absolute ion transport rates per time and mg reconstituted protein (**Figure 2**).

The main advantage of the proteoliposome system is that UCP mutants as well as paralogs and orthologs can be directly compared in an identical environment in the absence of secondary influences on UCP function by other mitochondrial or cellular processes. Variation due to different intactness of isolated mitochondria can be excluded and furthermore effects of membrane composition can easily be tested.

A major disadvantage is the inconsistency of results for proteoliposome studies coming from different labs. Variables like protein source (native, recombinant from yeast mitochondria or *E. coli* inclusion bodies), isolation and purification protocol (detergents, purification columns, dialysis steps), and reconstitution conditions (membrane composition, removal of detergent) contribute to highly divergent results concerning various aspects of uncoupling protein function. For example, estimations of correct UCP orientation in vesicles vary between 50 and 85% and transport rates in the range of 1.2–55 $H^{+*}\ s^{-1}$ have been reported (Murdza-Inglis et al., 1991; Echtay et al., 1998) (**Table 1**). Direct comparison with native protein in the same setting seems to be the most feasible approach to evaluate if appropriate experimental conditions have been chosen. Otherwise, incorrect folding or protein aggregation will not be recognized and lead to over- or underestimation of transport activities. A further disadvantage is that transport is analyzed at low driving force. This is a major drawback as the regulation of UCP1 is strongly dependent on membrane potential and shows strong increases in fatty acid induced uncoupling at high driving forces.

Several important findings were achieved by the use of UCP1 proteoliposomes. It was demonstrated that UCP1 is indeed capable of catalyzing a net proton flux and transport rates measured argue for a carrier characteristic (Klingenberg and Winkler, 1985). Furthermore, UCP1 molecules were cross-linked in the isolated state, in mitochondria, and after reconstitution into liposomes and in the liposome system it was shown that forced dimerization does not impair activity, as two cross-linked molecules still bind GDP and still display H^+ transport (Klingenberg and Appel, 1989). Studies with fatty acids and modified analogs or cross-linking of analogs to UCP1 set out to clarify whether fatty acids are essential cofactors or if fatty acid anions are the substrate of transport (Garlid et al., 1996; Jezek et al., 1996; Breen et al., 2006). This question still has not been finally resolved. Liposomes were also suggested to be a suitable test system for large scale screening of molecules to identify novel regulators of UCP1 function (Mozo et al., 2006).

Human UCP1 has been expressed in *E. coli* inclusion bodies and reconstituted for circular dichroism spectroscopy in solution and in liposomes (Ivanova et al., 2010). Remarkably there are no published functional studies in liposomes using the human UCP1, although there are several studies comparing human UCP2 and human UCP3 with rodent UCP1. It seems that the obsolete assumption of BAT disappearance shortly after birth in humans has constrained investigations with the human protein.

PLANAR LIPID BILAYERS

Proteoliposomes spontaneously form a monolayer at the surface of the suspension (Schindler, 1979). Planar lipid bilayers can be formed by the combination of two such monolayers and then have a capacity and dielectrical thickness which is comparable to biological membranes (Montal and Mueller, 1972). The conductance of proteins integrated in these membranes can be analyzed by recording the conductivity of the membrane with a patch clamp amplifier. The first analysis of uncoupling proteins in this system reconstituted purified hamster UCP1 and was performed in 2003 (Urbankova et al., 2003).

The activity of uncoupling proteins in a lipid bilayer membrane is described in current voltage plots which can be used to calculate transported molecules per molecule of the analyzed protein.

Planar lipid bilayer membranes have similar advantages like proteoliposomes, as they allow comparison of different UCPs in a defined background, which can be varied, with respect to membrane phospholipid composition, fatty acids or other protein components. Furthermore, this system allows characterization of the proteins over a broad range of voltages, as the driving force is not applied by buffer loading, but by a voltage source.

As in proteoliposomes the correct folding, insertion, and orientation of purified, native or recombinant proteins into the membrane is critical in this test system. The possibility that cofactors are missing or artifactual transport is detected can hardly be excluded.

With the planar lipid bilayer test system the transport rate of UCP1 isolated from hamster BAT was 14 H^{+*} s^{-1} (Urbankova et al., 2003; **Table 1**). It was shown that fatty acids are obligatory cofactors for UCP1 function and that the activation by fatty acids is membrane potential dependent (Rupprecht et al., 2010). Concerning the debate on the transport mechanism by UCP1, experimental results from this system argue for facilitated flip–flop/transport of deprotonated fatty acids to the intermembrane space. Lipid containing bilayer membranes already display a certain proton conductance, which is dependent on the potential above the membrane. The co-incorporation of uncoupling proteins amplifies this conductance but does not change the membrane potential dependent kinetics (Rupprecht et al., 2010).

Notably the function of human UCP1 has also been analyzed in the planar lipid bilayer system by purification and reconstitution from *E. coli* inclusion bodies. Transport activity of human UCP1 was qualitatively similar to UCP1 isolated from Syrian hamster BAT (Beck et al., 2006). Furthermore it was shown that polyunsaturated fatty acids activate human UCP1 and UCP2 in planar lipid bilayers (Beck et al., 2007).

YEAST

Yeast as a prototypic eukaryotic model organism is easily accessible to genomic modifications. The yeast genome does not contain a UCP1 ortholog, which is a clear advantage for functional studies. Like *E. coli* it can serve for recombinant overexpression of membrane proteins as the starting material for functional studies in proteoliposomes and planar lipid bilayers. Garlid and colleagues, making use of the yeast system to ectopically express UCP1, demonstrated that the protein isolated from yeast and reconstituted into proteoliposomes exhibited characteristics similar to the native UCP1 isolated from BAT (Murdza-Inglis et al., 1991). This opened up new opportunities for mutation studies, giving first insights into structure–function-relationships.

Besides serving for recombinant protein production intact cells or isolated mitochondria from yeast expressing different UCPs can be analyzed to identify consequences of UCP activity for growth, cellular respiration or change of membrane potential as monitored by fluorescent dyes.

Compared to the *E. coli* expression system yeast overcomes the problem of purification and reconstitution of membrane proteins from inclusion bodies because it is a eukaryotic cell, where heterologously expressed uncoupling proteins are inserted into mitochondria, their native environment. Yeast can still be grown easily in large scale, allowing the production of sufficient amounts of UCPs for analyses, with stable conditions. Various yeast strains with deficiencies in possible cofactors are available and can be used to test whether UCP1 function is affected in mitochondria of such a strain. Studies on coenzyme Q deficient strains, for example, demonstrated that coenzyme Q is not a necessary cofactor for uncoupling proteins (Esteves et al., 2004), thus supporting previous findings in proteoliposomes (Jaburek and Garlid, 2003). Yeast has been used for a proteomic study, where the mitochondrial proteome of yeast heterologously expressing UCP1 was compared to that of wildtype yeast (Douette et al., 2006). UCP1 expression leads to a slight increase in mitochondrial mass and upregulation of proteins that help to maintain ATP levels despite uncoupling activity.

Although being a eukaryotic cell, yeast is a single cell organism which precludes analysis of physiological functions of UCP1. Furthermore, Stuart et al. (2001) demonstrated that expression of UCP1 above 1 μg/mg leads to an uncoupling artifact in isolated mitochondria, probably due to improper folding or insertion of the protein in the inner mitochondrial membrane. At a concentration of ∼30 μg/mg UCP1 in brown fat mitochondria of mice kept at room temperature exhibits a transport activity of ∼25 H^{+*} s^{-1}, whereas in yeast even without artifactual uncoupling the activity was ∼700 H^{+*} s^{-1} (**Table 1**).

Most of the structure–function studies on UCP1 have been conducted in yeast. Mutated versions of UCP1 were expressed and either characterized after reconstitution in liposomes or in isolated yeast mitochondria. Of special interest is one study, where protein domains were swapped between UCP1 and UCP3. Hagen and Lowell (2000) demonstrated that the second part of the tripartite structure of UCP1 is necessary and sufficient for activation by fatty acids, which was analyzed further by Jimenez-Jimenez and colleagues. They demonstrated that it is the central matrix loop of UCP1 which is required for fatty acid sensitivity. Exchange of this part of the sequence with the corresponding UCP2 sequence resulted in loss of fatty acid sensitivity (Jimenez-Jimenez et al., 2006).

So far yeast has not been employed as a model organism to study human UCP1 structure and function.

MAMMALIAN CELL LINES

Shortly after the availability of the UCP1 sequence in 1985 the establishment of mammalian test systems was attempted, suitable for the expression of different UCP variants to analyze structure–function-relationships. Chinese hamster ovary (CHO) cells were stably transfected with rat UCP1. The assessment of rat UCP1 function in isolated mitochondria from these cells revealed that they were slightly uncoupled, and respiratory control was regained by addition of 1 mM GDP. This treatment also partially restored membrane potential, but as it did not reach the level of mitochondria from control cells, an uncoupling artifact as published for yeast could not be excluded (Casteilla et al., 1990; Stuart et al., 2001). A further limitation was that the expression level of rat UCP1 in these mitochondria was around 1 μg/mg protein and thus much lower than in BAT mitochondria (**Table 1**). Transient overexpression of UCP1 in HepG2 liver cells led to a decrease in ATP production, but the authors did not try to quantify catalytic activity (Gonzalez-Muniesa et al., 2005). Ectopic overexpression of mouse UCP1 in a fibroblast cell line was applied to characterize in detail the effect on cellular metabolism. The authors found that 3T3L1 cells expressing UCP1 accumulate less lipid due to reduced lipid synthesis, while oxygen consumption is only marginally stimulated. Beta-oxidation and several differentiation markers are not affected, indicating that UCP1 expression in white adipose tissue might provide an approach to reduce lipid accumulation (Si

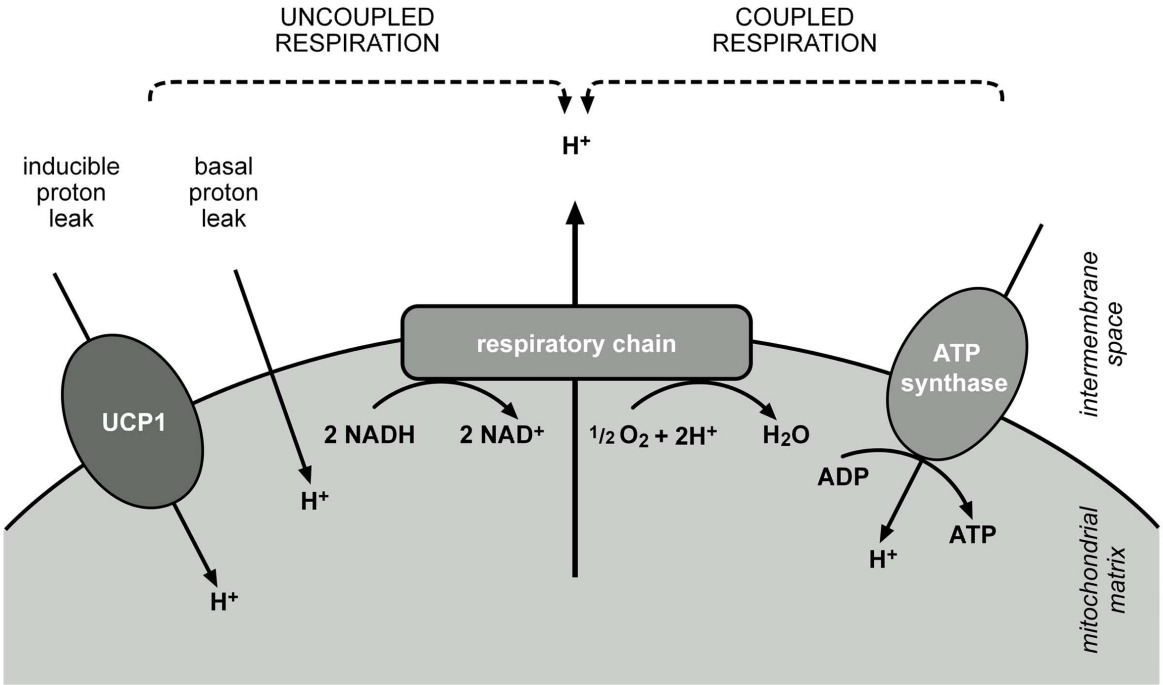

FIGURE 1 | Dissipation of energy from nutrient combustion at the mitochondrial inner membrane. Reduction equivalents feed electrons into the respiratory chain, which are passed along a redox gradient and reduce oxygen to water. Energy released by these reactions is stored as a proton gradient across the membrane, as protons are pumped into the intermembrane space. ATP synthase utilizes this energy and the flux of protons along the enzyme leads to the phosphorylation of ADP to ATP. Activity of the respiratory chain and ATP synthase are coupled by the proton gradient (coupled respiration). Coupling can be reduced by a basal leak of protons across the membrane, present in every cell, or by an inducible proton leak, catalyzed by specific proteins, in case of BAT mitochondria mainly by UCP1 (uncoupled respiration). Proton translocation by leak pathways prevents fixation of energy and releases it as heat.

et al., 2007). In another study, human UCP1 was expressed in INS1E cells, reaching expression levels of 1–1.5 μg/mg mitochondrial protein. UCP1 activity could be induced by addition of fatty acids, leading to an increased respiration and lower membrane potential (Galetti et al., 2009), whereas heterologous expression of human UCP2 in the same system did not affect mitochondrial bioenergetics.

Determination of UCP1 activity in mammalian mitochondria is often performed by the analysis of cellular or mitochondrial respiration but this gives just a rough impression of the coupling state. Only when membrane potential is determined at the same time it is possible to describe proton leak or even proton leak kinetics, as the need for respiratory activity depends largely on the membrane potential which has to be maintained. If two mitochondrial preparations both consume a certain amount of oxygen under non-phosphorylating conditions, those defending a higher membrane potential are more coupled, a fact you would miss by only looking at respiration. Respiration is mostly measured with Clark-type electrodes while membrane potential can be determined with fluorescent dyes or electrodes which detect distribution of lipophilic cations, e.g., $TPMP^+$ (Brand, 1995). Several steady states of membrane potential and oxygen consumption can be titrated and plotted against each other, resulting in a curve describing proton leak kinetics (**Figure 3**). Two curves can be compared by regarding oxygen consumption (respiratory activity) needed to maintain a certain membrane potential.

The obvious advantage of mammalian cell systems is that they represent the natural background on which the probability of proper folding and insertion into the membrane is higher than in other systems, even if UCP1 is expressed in a non-brown fat cell. In our laboratory we have generated a novel mammalian UCP1 expression model by stable transfection of HEK293 cells with an expression vector carrying the mouse UCP1 cDNA under control of the CMV promoter. As exemplified in proton leak measurements UCP1 in isolated mitochondria from these cells is activated by the addition of palmitate (**Figure 3A**). Maximum transport activity of mUCP1 was $\sim 75\,H^+\,s^{-1}$ (**Table 1**). In the presence of GDP this activation is diminished and at 1 mM GDP the proton leak of mitochondria expressing UCP1 at a level of 4.8 μg/mg mitochondrial protein is identical to the proton leak of mitochondria from normal HEK293 cells. This demonstrates that stable expression of UCP1 in these cells at the given expression level does not cause any uncoupling artifact.

Nevertheless, one has to keep in mind that heterologous overexpression may induce stress in a cell which may indirectly impact mitochondrial function. Furthermore, immortalized cell culture models usually develop altered cell physiology during the immortalization process which may for example switch their metabolism

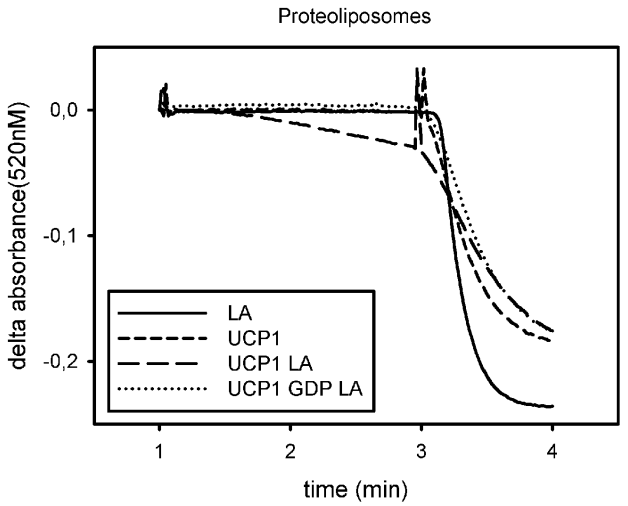

FIGURE 2 | Exemplary trace for analysis of UCP1 function in proteoliposomes. Absorbance of safranin O is plotted against time. Addition of nigericin at minute 1 induces polarization of the liposomes, measured as a decrease in safranin O absorbance, if a proton translocating pathway is present in the vesicles. Addition of CCCP at minute 3 leads to complete polarization and indicates vesicle volume. The initial slope after addition of nigericin is a measure for the proton translocation pathway, if there is one present and active. UCP1 is not active under basal conditions (UCP1) but mediates proton transport in the presence of lauric acid (LA). This can be prevented by addition of GDP (GDP LA). Addition of lauric acid to vesicles not containing UCP1 does not induce proton translocation (LA).

toward glycolysis, as indicated by elevated lactate production, e.g., in the CHO expression system (Casteilla et al., 1990).

So far no one has tested the function of human UCP1 in a heterologous mammalian expression system. Given the many advantages of this easy to manipulate system, future studies will certainly remedy this surprising deficit.

BROWN ADIPOCYTE CELL LINES

Stable brown preadipocyte cell lines have been generated by several different strategies. A first one is isolation, serial passage, and selection of stromal vascular fraction (SVF) cells either from BAT of wildtype mice (Forest et al., 1987) or from BAT hibernoma of transgenic mice with adipose-specific expression of SV40 fragments (Ross et al., 1992). A second strategy has been the cultivation and SV40 immortalization of the SVF from BAT depots of individual mouse embryos or newly born mice (Benito et al., 1993; Klein et al., 1999). These cell lines are maintained as precursor cells, but in response to hormonal stimulation differentiate into brown adipocytes. Therefore they do not only allow analysis of UCP1 function but can also be used to study differentiation of brown adipocytes and regulation of UCP1 expression. The UCP1 expression level in these cells is often low and requires induction by activators of UCP1 transcription for functional analyses.

Studying brown adipocyte cell lines allows analysis in a native background with lower variation than in tissue. In cell culture, molecular biology tools allow overexpression and knockdown of specific factors to test their relevance as cofactors of UCP1 function. A brown adipocyte model can be expected to endogenously feature all interaction partners necessary for optimal UCP1 function, all signaling cascades leading to its activation and comparable metabolic characteristics, substrate specificity, and preference. The possibility to immortalize cells from BAT SVF of individual mice implicates that also transgenic mice can be analyzed, even from models with perinatal mortality. This allows the generation of cell lines carrying knockout/knockin alleles, which had been immensely challenging in the past.

A disadvantage of all cell culture lines is that they change with increasing passage number, somehow limiting the advantage of stability. Low levels of UCP1 expression and the necessity to treat cells with inducing substances further increases variability.

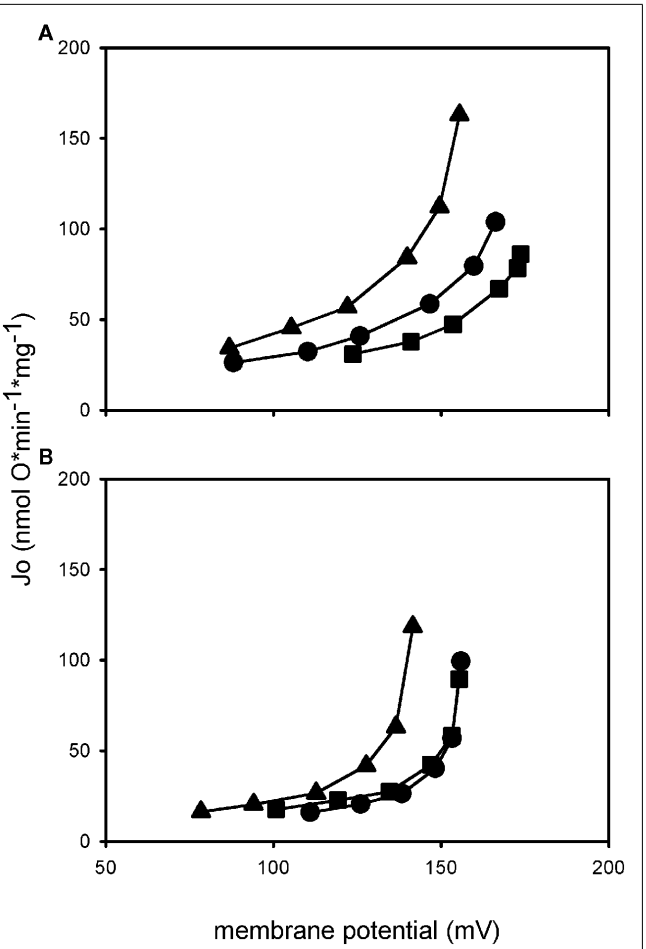

FIGURE 3 | Proton leak kinetics of isolated mitochondria. Mitochondria from HEK UCP1 cells **(A)** and mitochondria from a differentiated brown preadipocyte cell line **(B)**. Addition of palmitate (triangle) in both systems leads to an increase in proton leak compared to basal conditions (circle), indicated by the upwards shift of the curve. Coincubation with GDP (square) prevents the activation by palmitate, demonstrating that it is mediated by UCP1. Proton conductance of HEK UCP1 mitochondria in the presence of GDP is lower than under basal conditions, whereas mitochondria from brown adipocytes are coupled back to basal level.

A recent important finding in cultured brown adipocytes does not concern uncoupling protein function but developmental origin of the cells. It was shown that brown adipocytes and muscle cells originate from the same progenitor, being determined to differentiate into muscle or adipocyte by the absence or presence of PRDM16 (Seale et al., 2007). This finding implies that brown and white fat cells are less related than brown fat and muscle, and underlines the energy-dissipating function of BAT in contrast to the energy conserving function of WAT. Studies in different cell lines have demonstrated the importance of T3, insulin and β-adrenergic signals for brown fat differentiation, but also for retinoids and PPARy (Klaus et al., 1994, 1995; Alvarez et al., 2000; Nedergaard et al., 2005). Even commonly used white adipocyte cell models, mouse embryonic fibroblasts (MEF), can be induced to express UCP1 by treatment with retinoic acid (Mercader et al., 2010).

In our laboratory we have recently started to evaluate UCP1 function in mitochondria isolated from an immortalized brown preadipocyte cell line (kindly provided by B. Spiegelman; Uldry et al., 2006). After differentiation and treatment with isoproterenol and retinal these cells express approximately 2.8 μg UCP1/mg mitochondrial protein. The proton conductance of isolated mitochondria can be increased by the addition of palmitate and this can be prevented by the addition of 1 mM GDP indicating uncoupling protein function (**Figure 3B**). Maximum fatty acid induced UCP1 activity was $\sim 90\, H^{+*}\, s^{-1}$, which is in the same range as maximum UCP1 activity in HEK cells stably expressing UCP1 (**Table 1**).

In 1997, a human brown preadipocyte cell line was generated from infant BAT tissue (Zilberfarb et al., 1997). UCP1 was detected by qPCR and Northern Blot but not further characterized on the functional level, due to low expression level. Very recently a study on precursor cells from human adipose tissue has been published (Lee et al., 2011). With the requirement of long culture times fibroblastic cells can be obtained from the SVF of supraclavicular fat depots, which then can be differentiated and do contain UCP1. No functional studies on human UCP1 have been conducted in these cell lines.

BROWN ADIPOSE TISSUE MITOCHONDRIA FROM WILDTYPE AND MUTANT MICE

One of the first and still widely used approaches to characterize UCP1 is to measure its function and impact on proton leak in isolated BAT mitochondria. Isolation of mitochondria makes them accessible to inhibitory or activating substances which might not pass the cell membrane. Furthermore it is possible to control substrate supply and to monitor membrane potential and respiration without having to correct for cell membrane potential or non-mitochondrial oxygen consumption in the cell. Isolated mitochondria were used for the identification of UCP1 and description of its fundamental characteristics even before it was purified or its sequence was available. Nucleotide binding of UCP1 was characterized, demonstrating pH sensitivity and a higher affinity to GDP and GTP compared to ADP and ATP (Rial et al., 1983; Huang and Klingenberg, 1995). Studies with isolated mitochondria also elucidated structural aspects of UCP1. Tryptic digestion of UCP1 in isolated mitochondria, submitochondrial particles, and proteoliposomes revealed that the C-terminus of the protein is located in the intermembrane space (Eckerskorn and Klingenberg, 1987). Most commonly, mice, rats, hamsters, and guinea pigs have been employed in studies with isolated mitochondria. Comparison of BAT mitochondria from animals exposed to different conditions, e.g., cold adapted and kept at thermoneutrality, allowed to assess the effect of different UCP1 amounts. Analysis of mitochondria from wildtype and knockout mice allows clear assignment of mitochondrial characteristics to the presence and function of UCP1. The creation of transgenic mice even permits analysis of UCP1 function in mitochondria of tissues other than BAT. Ectopically expressed UCP1 in skeletal muscle mitochondria can be fully inhibited with GDP and activated with fatty acids. Furthermore UCP1 seems to be active in the resting state, as superoxide production is lower but reaches control levels when GDP is added (Keipert et al., 2010).

First studies assaying brown fat mitochondria mainly monitored activity of UCP1 by swelling experiments (Nicholls and Lindberg, 1973). Later measurements of respiration and membrane potential were applied to characterize proton leak kinetics, as described above. New technologies now facilitate studies of mitochondrial bioenergetics offering respirometry devices with either high-resolution (OROBOROS Instruments) or multiwell analysis of oxygen consumption and extracellular acidification rates (SEAHORSE BioSciences).

Given that BAT mitochondria are the native environment of UCP1 the protein is correctly folded and assembled in this test system. All necessary cofactors are present and the mitochondrium is equipped to sustain high respiratory activity, enabling maximum UCP1 activity.

In order to make use of these advantages the conditions for mitochondrial isolation must be chosen adequately. Isolation can damage mitochondria, most likely during homogenization of the tissue or by inadequate buffer conditions. It was found, that only in sufficiently low osmotic medium mitochondrial matrix volume expands and allows proper usage of NAD^+ linked substrates (Nicholls and Lindberg, 1972). Effects mediated by other carriers present in the mitochondrial inner membrane have to be excluded by proper controls. Mitochondria come directly from animals which introduces variation due to different life history of different animals. Body weight, exact housing temperature, and age are factors which have impact on mitochondrial characteristics and thus have to be carefully controlled (Porter and Brand, 1993; Harper et al., 1998).

A recent discussion which was based on studies in isolated mitochondria from wildtype and UCP1 knockout mice concerned a possible contribution of UCP1 to basal proton conductance in BAT mitochondria. Parker et al. (2009) found that basal, GDP-insensitive proton leak is higher in BAT mitochondria from wildtype mice compared to UCP1 knockout mice. Shabalina et al. (2010) argued that this is not due to a contribution of UCP1 to basal proton leak, but due to the assay buffer conditions, which selectively harm mitochondria from knockout animals.

Table 2 | Advantages and disadvantages of different UCP1 test systems.

	Advantages	Disadvantages
Proteoliposomes	Completely controlled environment	Susceptible to variation by experimental artifacts Absence of unknown cofactors, regulators, etc.
Planar lipid bilayers	Completely controlled environment High membrane potentials possible	Susceptible to variation by experimental artifacts Absence of unknown cofactors, regulators, etc.
Yeast	Eukaryotic environment: protein modifications, folding, cofactors, etc. Genetically manipulable No endogenous UCP1	High UCP1 levels lead to artificial uncoupling Non-mammalian
Mammalian cell lines	Natural environment for thermogenic UCP1: protein modifications, folding, cofactors, etc. High UCP1 amounts without uncoupling artifacts possible Accessible to molecular biology tools (overexpression, knockdown, etc.)	Altered metabolic phenotypes in immortalized cells, e.g., preferred glycolytic ATP production
Brown adipocyte lines	As above, plus: optimal metabolic environment, natural regulation processes, signaling pathways, and lipid storage	As above, plus: usually low UCP1 abundance Unstable, heterogenous cell systems
Tissue mitochondria	Natural source of UCP1 in its *in vivo* setting optimized for high respiratory activity Availability of manipulated mouse strains	Sophisticated isolation procedures necessary prone to damage mitochondria Time and cost intensive

closer to native background
higher variability, more difficult to manipulate

From artificial to native test systems generally, there is an increase in probability of proper folding and native function with the trade off of increasing variability and reduced possibilities to manipulate the system. Depending on the experimental question there might be a bigger need for simplicity and reproducibility or for a physiological background, and therefore in each case another test system the best choice.

So far there are no studies analyzing human UCP1 in BAT mitochondria, neither in human mitochondria nor after transgenic expression in animal mitochondria.

CONCLUSION AND PERSPECTIVES

The discovery of significant amounts of active BAT in humans has renewed interest in this organ and particularly in UCP1 as a pharmacological target to treat metabolic disease. Unfortunately, very little is known about the physiology of human BAT and most of our supposed knowledge is actually transferred from the study of rodents and other animals. This caveat applies to all aspects of BAT function including neuronal and hormonal regulation, recruitment of tissue hypertrophy and hyperplasia, heat production capacity and the cellular architecture like mitochondrial density and lipid droplet composition. It is equally true for the central question we focus on in this review, the structure and function of UCP1.

We have to keep in mind that the human UCP1 protein only shares less than 80% identity with rodent UCP1. Together with the protein sequences of all eutherian species it forms a group that is markedly different from UCP1 sequences of marsupials or other lower vertebrates (Hughes et al., 2009). It is assumed that this large phylogenetic gap between the eutherian and lower groups represents the acquirement of a regulated, thermogenic function of UCP1, arguing for this very role of human UCP1. On the other hand, as judged by allometric comparison of animals with different masses, a species as large as man should feature only negligible amounts of BAT, relieving selection pressure on efficient uncoupling by UCP1 (Heldmaier, 1971). Such a phenomenon can be observed, for instance, in all species of pigs. Being of the same size as man they have entirely lost an intact UCP1 gene (Berg et al., 2006). Sheep and bovine, however, have retained their UCP1 genes and exhibit functional BAT at least during the postnatal phase.

Activity and regulation of UCP1 proteins of different species have been measured (**Table 1**). Unfortunately, due to the large variation between methods and laboratories it is impossible to dissect the contribution of a species specific difference. It is thus essential to compare UCP1 from different species and including human UCP1 in one test system which does not display artifactual uncoupling due to UCP1 expression and in an experimental setup with low inter-experimental variability (**Table 2**). This will enable us to assess how easily our knowledge on UCP1 function can be transferred to human BAT and provide the groundwork for the search of compounds altering this function.

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REFERENCES

Alvarez, R., Checa, M., Brun, S., Vinas, O., Mampel, T., Iglesias, R., Giralt, M., and Villarroya, F. (2000). Both retinoic-acid-receptor- and retinoid-X-receptor-dependent signalling pathways mediate the induction of the brown-adipose-tissue-uncoupling-protein-1 gene by retinoids. *Biochem. J.* 345(Pt 1), 91–97.

Aquila, H., Link, T. A., and Klingenberg, M. (1985). The uncoupling protein from brown fat mitochondria is related to the mitochondrial ADP/ATP carrier. Analysis of sequence homologies and of folding of the protein in the membrane. *EMBO J.* 4, 2369–2376.

Beck, V., Jaburek, M., Breen, E. P., Porter, R. K., Jezek, P., and Pohl, E. E. (2006). A new automated technique for the reconstitution of hydrophobic proteins into planar bilayer membranes. Studies of human recombinant uncoupling protein 1. *Biochim. Biophys. Acta* 1757, 474–479.

Beck, V., Jaburek, M., Demina, T., Rupprecht, A., Porter, R. K., Jezek, P., and Pohl, E. E. (2007). Polyunsaturated fatty acids activate human uncoupling proteins 1 and 2 in planar lipid bilayers. *FASEB J.* 21, 1137–1144.

Benito, M., Porras, A., and Santos, E. (1993). Establishment of permanent brown adipocyte cell lines achieved by transfection with SV40 large T antigen and ras genes. *Exp. Cell Res.* 209, 248–254.

Berg, F., Gustafson, U., and Andersson, L. (2006). The uncoupling protein 1 gene (UCP1) is disrupted in the pig lineage: a genetic explanation for poor thermoregulation in piglets. *PLoS Genet.* 2, e129. doi:10.1371/journal.pgen.0020129

Boss, O., Samec, S., Paoloni-Giacobino, A., Rossier, C., Dulloo, A., Seydoux, J., Muzzin, P., and Giacobino, J. P. (1997). Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. *FEBS Lett.* 408, 39–42.

Bouillaud, F., Ricquier, D., Gulik-Krzywicki, T., and Gary-Bobo, C. M. (1983). The possible proton translocating activity of the mitochondrial uncoupling protein of brown adipose tissue. Reconstitution studies in liposomes. *FEBS Lett.* 164, 272–276.

Bouillaud, F., Weissenbach, J., and Ricquier, D. (1986). Complete cDNA-derived amino acid sequence of rat brown fat uncoupling protein. *J. Biol. Chem.* 261, 1487–1490.

Brand, M. D. (1995). "Measurement of mitochondrial protonmotive force," in *Bioenergetics. A Practical Approach*, eds G. C. Brown and C. E. Cooper (Oxford: IRL Press), 39–62.

Brand, M. D., and Esteves, T. C. (2005). Physiological functions of the mitochondrial uncoupling proteins UCP2 and UCP3. *Cell Metab.* 2, 85–93.

Breen, E. P., Gouin, S. G., Murphy, A. F., Haines, L. R., Jackson, A. M., Pearson, T. W., Murphy, P. V., and Porter, R. K. (2006). On the mechanism of mitochondrial uncoupling protein 1 function. *J. Biol. Chem.* 281, 2114–2119.

Carstens, G. E. (1994). Cold thermoregulation in the newborn calf. *Vet. Clin. North Am. Food Anim. Pract.* 10, 69–106.

Casteilla, L., Blondel, O., Klaus, S., Raimbault, S., Diolez, P., Moreau, F., Bouillaud, F., and Ricquier, D. (1990). Stable expression of functional mitochondrial uncoupling protein in Chinese hamster ovary cells. *Proc. Natl. Acad. Sci. U.S.A.* 87, 5124–5128.

Cypess, A. M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A. B., Kuo, F. C., Palmer, E. L., Tseng, Y. H., Doria, A., Kolodny, G. M., and Kahn, C. R. (2009). Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* 360, 1509–1517.

Douette, P., Gerkens, P., Navet, R., Leprince, P., De, P. E., and Sluse, F. E. (2006). Uncoupling protein 1 affects the yeast mitoproteome and oxygen free radical production. *Free Radic. Biol. Med.* 40, 303–315.

Echtay, K. S., Bienengraeber, M., Winkler, E., and Klingenberg, M. (1998). In the uncoupling protein (UCP-1) His-214 is involved in the regulation of purine nucleoside triphosphate but not diphosphate binding. *J. Biol. Chem.* 273, 24368–24374.

Eckerskorn, C., and Klingenberg, M. (1987). In the uncoupling protein from brown adipose tissue the C-terminus protrudes to the c-side of the membrane as shown by tryptic cleavage. *FEBS Lett.* 226, 166–170.

Enerback, S., Jacobsson, A., Simpson, E. M., Guerra, C., Yamashita, H., Harper, M. E., and Kozak, L. P. (1997). Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* 387, 90–94.

Esteves, T. C., Echtay, K. S., Jonassen, T., Clarke, C. F., and Brand, M. D. (2004). Ubiquinone is not required for proton conductance by uncoupling protein 1 in yeast mitochondria. *Biochem. J.* 379, 309–315.

Esteves, T. C., Parker, N., and Brand, M. D. (2006). Synergy of fatty acid and reactive alkenal activation of proton conductance through uncoupling protein 1 in mitochondria. *Biochem. J.* 395, 619–628.

Fleury, C., Neverova, M., Collins, S., Raimbault, S., Champigny, O., Levi-Meyrueis, C., Bouillaud, F., Seldin, M. F., Surwit, R. S., Ricquier, D., and Warden, C. H. (1997). Uncoupling protein-2: a novel gene linked to obesity and hyperinsulinemia. *Nat. Genet.* 15, 269–272.

Forest, C., Doglio, A., Ricquier, D., and Ailhaud, G. (1987). A preadipocyte clonal line from mouse brown adipose tissue. Short- and long-term responses to insulin and beta-adrenergics. *Exp. Cell Res.* 168, 218–232.

Foster, D. O., and Frydman, M. L. (1978). Nonshivering thermogenesis in the rat. II. Measurements of blood flow with microspheres point to brown adipose tissue as the dominant site of the calorigenesis induced by noradrenaline. *Can. J. Physiol. Pharmacol.* 56, 110–122.

Foster, D. O., and Frydman, M. L. (1979). Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats reevaluated from changes in tissue blood flow: the dominant role of brown adipose tissue in the replacement of shivering by nonshivering thermogenesis. *Can. J. Physiol. Pharmacol.* 57, 257–270.

Galetti, S., Sarre, A., Perreten, H., Produit-Zengaffinen, N., Muzzin, P., and Assimacopoulos-Jeannet, F. (2009). Fatty acids do not activate UCP2 in pancreatic beta cells: comparison with UCP1. *Pflugers Arch.* 457, 931–940.

Garlid, K. D., Jaburek, M., and Jezek, P. (1998). The mechanism of proton transport mediated by mitochondrial uncoupling proteins. *FEBS Lett.* 438, 10–14.

Garlid, K. D., Orosz, D. E., Modriansky, M., Vassanelli, S., and Jezek, P. (1996). On the mechanism of fatty acid-induced proton transport by mitochondrial uncoupling protein. *J. Biol. Chem.* 271, 2615–2620.

Gimeno, R. E., Dembski, M., Weng, X., Deng, N., Shyjan, A. W., Gimeno, C. J., Iris, F., Ellis, S. J., Woolf, E. A., and Tartaglia, L. A. (1997). Cloning and characterization of an uncoupling protein homolog: a potential molecular mediator of human thermogenesis. *Diabetes* 46, 900–906.

Gonzalez-Muniesa, P., Milagro, F. I., Campion, J., and Martinez, J. A. (2005). Ectopic UCP1 gene expression in HepG2 cells affects ATP production. *J. Physiol. Biochem.* 61, 389–393.

Hagen, T., and Lowell, B. B. (2000). Chimeric proteins between UCP1 and UCP3: the middle third of UCP1 is necessary and sufficient for activation by fatty acids. *Biochem. Biophys. Res. Commun.* 276, 642–648.

Hany, T. F., Gharehpapagh, E., Kamel, E. M., Buck, A., Himms-Hagen, J., and von Schulthess, G. K. (2002). Brown adipose tissue: a factor to consider in symmetrical tracer uptake in the neck and upper chest region. *Eur. J. Nucl. Med. Mol. Imaging* 29, 1393–1398.

Harper, M. E., Monemdjou, S., Ramsey, J. J., and Weindruch, R. (1998). Age-related increase in mitochondrial proton leak and decrease in ATP turnover reactions in mouse hepatocytes. *Am. J. Physiol.* 275, E197–E206.

Heaton, J. M. (1972). The distribution of brown adipose tissue in the human. *J. Anat.* 112, 35–39.

Heldmaier, G. (1971). Zitterfreie Wärmebildung und Körpergröße bei Säugetieren. *Z. Vgl. Physiol.* 73, 222–248.

Huang, S. G., and Klingenberg, M. (1995). Fluorescent nucleotide derivatives as specific probes for the uncoupling protein: thermodynamics and kinetics of binding and the control by pH. *Biochemistry* 34, 349–360.

Hughes, D. A., Jastroch, M., Stoneking, M., and Klingenspor, M. (2009). Molecular evolution of UCP1 and the evolutionary history of mammalian non-shivering thermogenesis. *BMC Evol. Biol.* 9, 4. doi:10.1186/1471-2148-9-4

Ivanova, M. V., Hoang, T., McSorley, F. R., Krnac, G., Smith, M. D., and Jelokhani-Niaraki, M. (2010). A comparative study on conformation and ligand binding of the neuronal uncoupling proteins. *Biochemistry* 49, 512–521.

Jaburek, M., and Garlid, K. D. (2003). Reconstitution of recombinant uncoupling proteins: UCP1, -2, and -3 have similar affinities for ATP and are unaffected by coenzyme Q10. *J. Biol. Chem.* 278, 25825–25831.

Jastroch, M., Buckingham, J. A., Helwig, M., Klingenspor, M., and Brand, M. D. (2007). Functional characterisation of UCP1 in the common carp: uncoupling activity in liver mitochondria and cold-induced expression in the brain. *J. Comp. Physiol. B* 177, 743–752.

Jezek, P., Hanus, J., Semrad, C., and Garlid, K. D. (1996). Photoactivated azido fatty acid irreversibly inhibits anion and proton transport through the mitochondrial uncoupling protein. *J. Biol. Chem.* 271, 6199–6205.

Jezek, P., Orosz, D. E., Modriansky, M., and Garlid, K. D. (1994). Transport of anions and protons by the mitochondrial uncoupling protein and its regulation by nucleotides and fatty acids. A new look at old hypotheses. *J. Biol. Chem.* 269, 26184–26190.

Jimenez-Jimenez, J., Ledesma, A., Zaragoza, P., Gonzalez-Barroso, M. M., and Rial, E. (2006). Fatty acid activation of the uncoupling proteins requires the presence of the central matrix loop from UCP1. *Biochim. Biophys. Acta* 1757, 1292–1296.

Keipert, S., Klaus, S., Heldmaier, G., and Jastroch, M. (2010). UCP1 ectopically expressed in murine muscle displays native function and mitigates mitochondrial superoxide production. *Biochim. Biophys. Acta* 1797, 324–330.

Klaus, S., Choy, L., Champigny, O., Cassard-Doulcier, A. M., Ross, S., Spiegelman, B., and Ricquier, D. (1994). Characterization of the novel brown adipocyte cell line HIB 1B. Adrenergic pathways involved in regulation of uncoupling protein gene expression. *J. Cell Sci.* 107(Pt 1), 313–319.

Klaus, S., Ely, M., Encke, D., and Heldmaier, G. (1995). Functional assessment of white and brown adipocyte development and energy metabolism in cell culture. Dissociation of terminal differentiation and thermogenesis in brown adipocytes. *J. Cell Sci.* 108(Pt 10), 3171–3180.

Klein, J., Fasshauer, M., Ito, M., Lowell, B. B., Benito, M., and Kahn, C. R. (1999). beta(3)-adrenergic stimulation differentially inhibits insulin signaling and decreases insulin-induced glucose uptake in brown adipocytes. *J. Biol. Chem.* 274, 34795–34802.

Klingenberg, M., and Appel, M. (1989). The uncoupling protein dimer can form a disulfide cross-link between the mobile C-terminal SH groups. *Eur. J. Biochem.* 180, 123–131.

Klingenberg, M., and Huang, S. G. (1999). Structure and function of the uncoupling protein from brown adipose tissue. *Biochim. Biophys. Acta* 1415, 271–296.

Klingenberg, M., and Winkler, E. (1985). The reconstituted isolated uncoupling protein is a membrane potential driven H+ translocator. *EMBO J.* 4, 3087–3092.

Klingenspor, M., and Fromme, T. (2012). "Brown adipose tissue," in *Adipose Tissue Biology*, 1st Edn, Vol. 6, ed. M. E. Symonds (Heidelberg: Springer), 413. Available at: http://www.springer.com/biomed/human+physiology/book/978-1-4614-0964-9

Kunji, E. R., and Crichton, P. G. (2010). Mitochondrial carriers function as monomers. *Biochim. Biophys. Acta* 1797, 817–831.

Lean, M. E., James, W. P., Jennings, G., and Trayhurn, P. (1986a). Brown adipose tissue in patients with pheochromocytoma. *Int. J. Obes.* 10, 219–227.

Lean, M. E., James, W. P., Jennings, G., and Trayhurn, P. (1986b). Brown adipose tissue uncoupling protein content in human infants, children and adults. *Clin. Sci.* 71, 291–297.

Lee, P., Swarbrick, M. M., Zhao J. T., and Ho, K. K. (2011). Inducible brown adipogenesis of supraclavicular fat in adult humans. *Endocrinology* 152, 3597–3602.

Lin, C. S., Hackenberg, H., and Klingenberg, E. M. (1980). The uncoupling protein from brown adipose tissue mitochondria is a dimer. A hydrodynamic study. *FEBS Lett.* 113, 304–306.

Lin, C. S., and Klingenberg, M. (1980). Isolation of the uncoupling protein from brown adipose tissue mitochondria. *FEBS Lett.* 113, 299–303.

Lin, C. S., and Klingenberg, M. (1982). Characteristics of the isolated purine nucleotide binding protein from brown fat mitochondria. *Biochemistry* 21, 2950–2956.

Mercader, J., Palou, A., and Bonet, M. L. (2010). Induction of uncoupling protein-1 in mouse embryonic fibroblast-derived adipocytes by retinoic acid. *Obesity (Silver Spring)* 18, 655–662.

Modriansky, M., Murdza-Inglis, D. L., Patel, H. V., Freeman, K. B., and Garlid, K. D. (1997). Identification by site-directed mutagenesis of three arginines in uncoupling protein that are essential for nucleotide binding and inhibition. *J. Biol. Chem.* 272, 24759–24762.

Monemdjou, S., Kozak, L. P., and Harper, M. E. (1999). Mitochondrial proton leak in brown adipose tissue mitochondria of Ucp1-deficient mice is GDP insensitive. *Am. J. Physiol.* 276, E1073–E1082.

Montal, M., and Mueller, P. (1972). Formation of bimolecular membranes from lipid monolayers and a study of their electrical properties. *Proc. Natl. Acad. Sci. U.S.A.* 69, 3561–3566.

Mozo, J., Ferry, G., Masscheleyn, S., Miroux, B., Boutin, J. A., and Bouillaud, F. (2006). Assessment of a high-throughput screening methodology for the measurement of purified UCP1 uncoupling activity. *Anal. Biochem.* 351, 201–206.

Murdza-Inglis, D. L., Patel, H. V., Freeman, K. B., Jezek, P., Orosz, D. E., and Garlid, K. D. (1991). Functional reconstitution of rat uncoupling protein following its high level expression in yeast. *J. Biol. Chem.* 266, 11871–11875.

Mzilikazi, N., Jastroch, M., Meyer, C. W., and Klingenspor, M. (2007). The molecular and biochemical basis of nonshivering thermogenesis in an African endemic mammal, *Elephantulus myurus*. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 293, R2120–R2127.

Nedergaard, J., Petrovic, N., Lindgren, E. M., Jacobsson, A., and Cannon, B. (2005). PPARgamma in the control of brown adipocyte differentiation. *Biochim. Biophys. Acta* 1740, 293–304.

Nicholls, D. G., and Lindberg, O. (1972). Inhibited respiration and ATPase activity of rat liver mitochondria under conditions of matrix condensation. *FEBS Lett.* 25, 61–64.

Nicholls, D. G., and Lindberg, O. (1973). Brown-adipose-tissue mitochondria. The influence of albumin and nucleotides on passive ion permeabilities. *Eur. J. Biochem.* 37, 523–530.

Oelkrug, R., Kutschke, M., Meyer, C. W., Heldmaier, G., and Jastroch, M. (2010). Uncoupling protein 1 decreases superoxide production in brown adipose tissue mitochondria. *J. Biol. Chem.* 285, 21961–21968.

Parker, N., Crichton, P. G., Vidal-Puig, A. J., and Brand, M. D. (2009). Uncoupling protein-1 (UCP1) contributes to the basal proton conductance of brown adipose tissue mitochondria. *J. Bioenerg. Biomembr.* 41, 335–342.

Pecqueur, C., Couplan, E., Bouillaud, F., and Ricquier, D. (2001). Genetic and physiological analysis of the role of uncoupling proteins in human energy homeostasis. *J. Mol. Med.* 79, 48–56.

Polymeropoulos, E. T., Heldmaier, G., Frappell, P. B., McAllan, B. M., Withers, K. W., Klingenspor, M., White, C. R., and Jastroch, M. (2011). Phylogenetic differences of mammalian basal metabolic rate are not explained by mitochondrial basal proton leak. *Proc. Biol. Sci.* doi: 10.1098/rspb.2011.0881. [Epub ahead of print].

Porter, R. K., and Brand, M. D. (1993). Body mass dependence of H+ leak in mitochondria and its relevance to metabolic rate. *Nature* 362, 628–630.

Puchalski, W., Bockler, H., Heldmaier, G., and Langefeld, M. (1987). Organ blood flow and brown adipose tissue oxygen consumption during noradrenaline-induced nonshivering thermogenesis in the Djungarian hamster. *J. Exp. Zool.* 242, 263–271.

Rial, E., Poustie, A., and Nicholls, D. G. (1983). Brown-adipose-tissue mitochondria: the regulation of the 32000-Mr uncoupling protein by fatty acids and purine nucleotides. *Eur. J. Biochem.* 137, 197–203.

Ross, S. R., Choy, L., Graves, R. A., Fox, N., Solevjeva, V., Klaus, S., Ricquier, D., and Spiegelman, B. M. (1992). Hibernoma formation in transgenic mice and isolation of a brown adipocyte cell line expressing the uncoupling protein gene. *Proc. Natl. Acad. Sci. U.S.A.* 89, 7561–7565.

Rothwell, N. J., and Stock, M. J. (1979). A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 281, 31–35.

Rupprecht, A., Sokolenko, E. A., Beck, V., Ninnemann, O., Jaburek, M., Trimbuch, T., Klishin, S. S., Jezek, P., Skulachev, V. P., and Pohl, E. E. (2010). Role of the transmembrane potential in the membrane proton leak. *Biophys. J.* 98, 1503–1511.

Saito, M., Okamatsu-Ogura, Y., Matsushita, M., Watanabe, K., Yoneshiro, T., Nio-Kobayashi, J., Iwanaga, T., Miyagawa, M., Kameya, T., Nakada, K., Kawai, Y., and Tsujisaki, M. (2009). High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* 58, 1526–1531.

Schindler, H. (1979). Exchange and interactions between lipid layers at the surface of a liposome solution. *Biochim. Biophys. Acta* 555, 316–336.

Seale, P., Kajimura, S., Yang, W., Chin, S., Rohas, L. M., Uldry, M., Tavernier, G., Langin, D., and Spiegelman, B. M. (2007). Transcriptional control of brown fat determination by PRDM. *Cell Metab.* 6, 38–54.

Shabalina, I. G., Jacobsson, A., Cannon, B., and Nedergaard, J. (2004). Native UCP1 displays simple competitive kinetics between the regulators purine nucleotides and fatty acids. *J. Biol. Chem.* 279, 38236–38248.

Shabalina, I. G., Ost, M., Petrovic, N., Vrbacky, M., Nedergaard, J., and Cannon, B. (2010). Uncoupling protein-1 is not leaky. *Biochim. Biophys. Acta* 1797, 773–784.

Shabalina, I. G., Petrovic, N., Kramarova, T. V., Hoeks, J., Cannon, B., and Nedergaard, J. (2006). UCP1 and defense against oxidative stress. 4-Hydroxy-2-nonenal effects on brown fat mitochondria are uncoupling protein 1-independent. *J. Biol. Chem.* 281, 13882–13893.

Si, Y., Palani, S., Jayaraman, A., and Lee, K. (2007). Effects of forced uncoupling protein 1 expression in 3T3-L1 cells on mitochondrial function and lipid metabolism. *J. Lipid Res.* 48, 826–836.

Skulachev, V. P. (1991). Fatty acid circuit as a physiological mechanism of uncoupling of oxidative phosphorylation. *FEBS Lett.* 294, 158–162.

Skulachev, V. P. (1996). Role of uncoupled and non-coupled oxidations in maintenance of safely low levels of oxygen and its one-electron reductants. *Q. Rev. Biophys.* 29, 169–202.

Smith, R. E. (1961). Thermogenic activity of the hibernating gland in the cold-acclimated rat. *Physiologist* 4, 113.

Stuart, J. A., Harper, J. A., Brindle, K. M., Jekabsons, M. B., and Brand, M. D. (2001). A mitochondrial uncoupling artifact can be caused by expression of uncoupling protein 1 in yeast. *Biochem. J.* 356, 779–789.

Svensson, P. A., Jernas, M., Sjoholm, K., Hoffmann, J. M., Nilsson, B. E., Hansson, M., and Carlsson, L. M. (2011). Gene expression in human brown adipose tissue. *Int. J. Mol. Med.* 27, 227–232.

Symonds, M. E., Budge, H., Perkins, A. C., and Lomax, M. A. (2011). Adipose tissue development – impact of the early life environment. *Prog. Biophys. Mol. Biol.* 106, 300–306.

Taga, H., Chilliard, Y., Meunier, B., Chambon, C., Picard, B., Zingaretti, M. C., Cinti, S., and Bonnet, M. (2011). Cellular and molecular large-scale features of fetal adipose tissue: is bovine perirenal adipose tissue brown? *J. Cell. Physiol.* doi: 10.1002/jcp.22893. [Epub ahead of print].

Trayhurn, P., and James, W. P. (1978). Thermoregulation and non-shivering thermogenesis in the genetically obese (ob/ob) mouse. *Pflugers Arch.* 373, 189–193.

Uldry, M., Yang, W., St-Pierre, J., Lin, J., Seale, P., and Spiegelman, B. M. (2006). Complementary action of the PGC-1 coactivators in mitochondrial biogenesis and brown fat differentiation. *Cell Metab.* 3, 333–341.

Urbankova, E., Voltchenko, A., Pohl, P., Jezek, P., and Pohl, E. E. (2003). Transport kinetics of uncoupling proteins. Analysis of UCP1 reconstituted in planar lipid bilayers. *J. Biol. Chem.* 278, 32497–32500.

van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, J. M., Kemerink, G. J., Bouvy, N. D., Schrauwen, P., and Teule, G. J. (2009). Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* 360, 1500–1508.

Virtanen, K. A., Lidell, M. E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N. J., Enerback, S., and Nuutila, P. (2009). Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* 360, 1518–1525.

Winkler, E., and Klingenberg, M. (1994). Effect of fatty acids on H+ transport activity of the reconstituted uncoupling protein. *J. Biol. Chem.* 269, 2508–2515.

Winkler, E., Wachter, E., and Klingenberg, M. (1997). Identification of the pH sensor for nucleotide binding in the uncoupling protein from brown adipose tissue. *Biochemistry* 36, 148–155.

Zilberfarb, V., Pietri-Rouxel, F., Jockers, R., Krief, S., Delouis, C., Issad, T., and Strosberg, A. D. (1997). Human immortalized brown adipocytes express functional beta3-adrenoceptor coupled to lipolysis. *J. Cell. Sci.* 110(Pt 7), 801–807.

Zingaretti, M. C., Crosta, F., Vitali, A., Guerrieri, M., Frontini, A., Cannon, B., Nedergaard, J., and Cinti, S. (2009). The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J.* 23, 3113–3120.

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The genetics of brown adipocyte induction in white fat depots

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Evidence that adult humans have functional brown adipose tissue has stirred interest in the possibility that the impressive effectiveness of induction of brown adipocytes to reduce obesity in mice may be translated to the human condition. A major focus recently on the identification of signaling and transcription factor that stimulate the induction of brown adipocytes has come from transgenic and gene KO models. However, these models have created a very complex picture of the regulatory mechanisms for brown fat induction. In this review insights into the critical regulatory pathways involved in brown adipocyte induction in the retroperitoneal fat depot of mice are described from quantitative trait locus (QTL) analysis of allelic variability determining *Ucp*1 levels and brown adipocyte induction in A/J vs. B6 mice. The key observation is that recombinant genotypes, found in recombinant inbred stains and backcross and intercross progeny, show transgressive variation for *Ucp*1 mRNA levels. These genetic crosses also show that the levels of *Ucp*1 mRNA are determined by interactions that control the levels of PPARα, PGC-1α, and type 2 deiodinase (DIO2) and that each factor is controlled by a subset of QTLs that also control *Ucp*1 expression. These results indicate that induction of *Ucp*1 in the retroperitoneal fat depot involves synergy between signaling and transcription factors that vary depending upon the environmental conditions. Inherent in this model is the idea that there is a high level of redundancy that can involve any factor with the potential to influence expression of the core factors, PPARα, PGC-1a, and DIO2.

Keywords: *Ucp*1 mRNA, retroperitoneal fat depot, quantitative trait loci, transgressive variation, recombinant inbred strains of mice, backcross analysis, synergistic interaction of regulatory genes

INTRODUCTION

Approximately 20 transgenic/gene KO models have been described in the literature that increase the number of brown adipocytes in white fat depots and cause the transgenic mice to have increased resistance to diet-induced obesity (DIO;Kozak and Koza, 2010). Although the physiological basis for the resistance to obesity in many of these models is poorly described, the sheer numbers of transgenic models involved provide a compelling case for seriously considering a strategy to combat obesity in humans based upon an increase in brown adipocyte numbers. With the mouse genetic data and the rat pharmaceutical data as backdrops, new evidence that adult humans indeed have brown fat as discrete depots, detectable with PET imaging, has led to renewed efforts to identify mechanisms by which brown adipose tissue (BAT) can be induced in human tissues (Cypess et al., 2009; Van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). For this purpose it behooves us to review the information on BAT induction in the mouse to identify strategies that may be important for its induction in humans. This review will focus on insights that have come from the analysis of quantitative trait loci (QTL) that control variation in *Ucp*1 expression in the retroperitoneal white fat depot of A/J and C57BL/6J mice, in particular on the interaction of natural genetic variation with the environment in the control of *Ucp*1 and its utilization in reducing obesity. These genetic studies, together with what we know about the UCP1 function in the $Ucp1^{-/-}$ mice, indicate that simply reducing ambient temperature can significantly reduce adiposity in obese individuals.

GENETIC VARIATION IN BROWN ADIPOSE TISSUE INDUCTION: AN AVENUE TO REDUCED OBESITY

It has long been known that the expression of BAT was highly variable among different species, reflecting their particular environmental, developmental, and physiological traits and requirements (Smith and Horwitz, 1969). What was not realized, until the past 10–15 years, was the rich genetic variation in BAT expression found within a species (Guerra et al., 1998), a variation that we have only begun to describe in mice and which will certainly be present in other species, including the human. Having described variation in BAT a longterm goal will be to determine the mechanism leading some animals to maximize this genetic trait of increased BAT that can be used to reduce fat stores. Current data indicate that some genetic constitutions make brown adipocyte induction in some individuals more responsive to the environment. Therefore, we need to determine when during development the capacity for modulation of BAT induction is maximal.

INTERSCAPULAR BROWN ADIPOSE TISSUE

In the mouse BAT first appears as a discrete tissue in the interscapular brown adipose tissue (iBAT) region at about 17 days of

gestation (Houstek et al., 1988; Hirning et al., 1989; Giralt et al., 1990). Other discrete BAT depots are found on the top of the kidney in the axial region and in the thoracic cavity; however, when these discrete depots emerge developmentally in the mouse have not been reported to our knowledge (Cannon and Nedergaard, 2004). Following birth, iBAT continues to grow in size until about weaning by a mechanism that involves continued cell proliferation (Staszkiewicz et al., 2009). It has been reported that 65% of the nuclei of iBAT are labeled with BrdU in 10-day-old mice after injection with BrdU on days 3, 4, and 5. The size of iBAT at birth, its rate of growth postnatally and eventual size are identical between C57BL/6J and A/J mice (Xue et al., 2007), two strains which, as we will discuss below, show huge differences in brown adipocyte numbers in selective white fat depots and in their sensitivity to DIO.

BROWN ADIPOCYTES IN WHITE FAT DEPOTS (wBAT)

The induction of brown adipocytes in white fat depots by exposing rats to the cold or treating them with the β3-adrenergic receptor agonist CL 316,243 has been described by several groups (Champigny et al., 1991; Himms-Hagen et al., 1994; Collins et al., 1997). However, the idea that inbred strains of mice vary in their induction of *Ucp*1 in the retroperitoneal fat depot and that this variable inductive response may account for the difference in DIO between A/J and C57BL/6J mice was first advanced by Surwit and coworkers (Collins et al., 1997). This idea suggested the exciting possibility that variant alleles for genes associated with *Ucp*1 expression and the brown adipocyte differentiation program are extant within the common inbred strains, thereby providing a genetic system to identify genes critical for the induction of brown adipocytes in white fat depots (Guerra et al., 1998). As we shall point out below, the genetic variation is restricted to the white fat depots and not observed in iBAT, a phenomenon that is largely recapitulated in the transgenic and gene KO models, that is, induction of brown adipocytes occurs in white fat depots of these transgenic models, but seldom in iBAT.

In the design of a QTL study to identify genes controlling brown fat induction, the selection of the fat depot becomes critical, because each fat depot has its own unique phenotype (Guerra et al., 1998). One is faced with the decision to select the white fat depot that one ascertains, with incomplete data, will be most amenable to analysis and most informative. First of all the iBAT in adult mice is not variable between A/J, B6, and 129/SvJ strains (Almind et al., 2007; Xue et al., 2007). However, large variations in levels of expression are found among tissues and each tissue varies independently among strains (**Figure 1A**). Among the visceral depots, gonadal fat shows large variation between strains, but the expression is low compared to that detected in the inguinal fat depot (Guerra et al., 1998). Mesenteric fat and retroperitoneal fat both have relatively high levels of expression and big differences between strains and they are therefore suitable for a QTL analysis. We selected the retroperitoneal fat for the QTL analysis because it is a fat depot also found in humans (the epididymal fat is not) and it is a well-defined tissue that can be more easily and rapidly excised from the mouse than mesenteric fat. Given these features of *Ucp*1 expression in different white fat depots, we cannot assume that the QTLs found associated with the control of *Ucp*1 mRNA levels in retroperitoneal fat are also controlling *Ucp*1 in other depots, especially the subcutaneous fat.

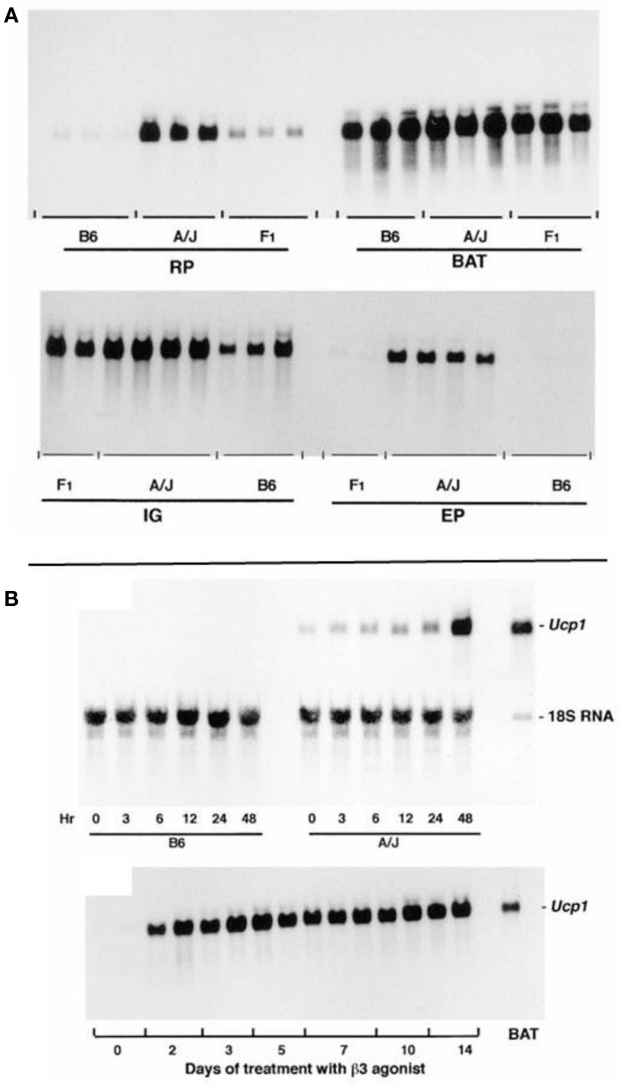

FIGURE 1 | (A) The levels of *Ucp*1 mRNA in the retroperitoneal fat (*RP*), interscapular brown fat (*BAT*), inguinal fat (*IG*), and epididymal fat (*EP*) in total RNA isolated from A/J, B6, and (B6 × A/J) F1 mice are shown. Mice were treated with the β3-adrenergic agonist CL 316,243 for 7 days before isolation of RNA, at a time when induction of *Ucp*1 mRNA had reached a plateau. Ten microgram of RNA from BAT was analyzed and 15 μg from the other fat depots. **(B)** Northern blot analysis of total RNA. (Upper panel) The induction of *Ucp*1 mRNA in the retroperitoneal fat pad of B6 and A/J mice when placed in the cold at 5°C as indicated between 0 and 48 h. Total RNA was isolated from tissues pooled from three mice. Fifteen microgram of RNA was analyzed. (Lower panel) The induction of *Ucp*1 mRNA in the retroperitoneal fat pad after injection of the β3-adrenergic agonist, CL 316,243, once a day in the late afternoon at a dose of 1 mg/kg body wt. RNA (15 μg) from two mice was analyzed individually. A standard of 2.7 μg of interscapular brown fat RNA was included on the gel.

The time course for induction is similar for cold exposure and β3-adrenergic agonist treatment in RP fat with increases in *Ucp*1 mRNA evident by 3 h and stable maximal levels occurring in 48 h

(**Figure 1B**). It is noteworthy that in A/J mice some *Ucp*1 mRNA can be detected at room temperature and induction following exposure to the cold occurs within 3 h.

ALLELIC VARIATION AND RECOMBINANT INBRED STRAINS OF MICE

The phenotypes of obesity and thermogenesis affected by β-AR agonists and ambient temperature in A/J and B6 mice are complex. They involve up-regulation of *Ucp*1 and other molecular components of thermogenesis in existing brown adipocytes in the iBAT depot and *de novo* induction of new differentiated brown adipocyte populations in white adipose tissue; both of which are controlled in part by the sympathetic nervous system (Bartness and Bamshad, 1998). In addition, the phenotypes depend on variation in susceptibility to obesity at 23˚C that exist between A/J and B6 mice and are likely to be independent of BAT thermogenesis (Seldin et al., 1994; Guerra et al., 1998). To unravel the genetic and molecular complexity of brown adipocyte induction in white fat depots and determine its role in energy balance and obesity we utilized two genetic tools; the A × B recombinant inbred (RI) strains and progeny from backcross and intercross matings between A/J and B6 mice and the RI strains (Koza et al., 2000). The A × B RI lines were formed by crossing A/J and B6 mice and intercrossing the resulting F1 progeny to establish an F2 population that were then brother–sister mated for a minimum of 20 generations to establish inbred lines (Bailey, 1971; Taylor, 1981). If a genetic trait is complex, that is, it is controlled by allelic variation at more than one gene, then alleles from each gene associated with the trait in the A/J and B6 parental strain will be fixed in new combinations in the different RI lines. Thus, each RI line with these novel recombinant chromosome patterns will provide potentially unlimited numbers of mice to establish the phenotypes determined by each gene or combinations of genes not present in the original parental lines. As shown in **Figure 2**, the levels of *Ucp*1 mRNA in the retroperitoneal fat depot of the parent A/J mice exposed to a 4˚C ambient temperature for 1 week were 40 times higher than that found in B6 mice (Guerra et al., 1998); however, the novel recombination of alleles in the RI lines generated a continuum of lines of mice with levels of *Ucp*1 that at the lower extreme in A × B10 mice were slightly less than those observed in B6 mice and at the other extreme in A × B8 mice they were 2.5 times higher than that occurring in A/J mice (**Figure 2A**). The number of brown adipocytes in retroperitoneal fat of A × B8 mice approaches that detected in iBAT of B6 mice; however, despite having levels of Ucp1 mRNA similar to A × B8, A × B15 retroperitoneal fat has fewer brown adipocytes that can be detected with UCP1 immunohistology (**Figure 2B**). In addition to providing large numbers of mice with recombinant genotypes to establish phenotypes, comparisons of the strain distribution patterns of the levels of mRNA to the distribution of variant alleles of structural genes with allelic variation provided the first clues that several genes were involved in controlling induction of *Ucp*1 in white fat depots and preliminary assignment of the genes to specific chromosomes (Koza et al., 2000). The chromosomal linkage analysis of genes controlling *Ucp*1 induction will be discussed later with the QTL analysis of brown fat induction in intercross and backcross progeny.

GENETIC VARIABILITY IN THE INDUCTION OF BROWN FAT AND THE OBESE PHENOTYPE

Two questions essential to the relationships between constitutive expression of iBAT and inducible levels of wBAT and susceptibility to DIO can be addressed with the RI lines. The first question is whether the potential for induction of brown adipocytes in white fat determines the development of obesity in the absence of adrenergic stimulation by cold exposure or β3-AR agonist treatment. In other words, does the constitutive level of *Ucp*1 mRNA, which in mice comes from the *Ucp*1 expressed in the discrete iBAT depots and does not show strain variation (Coulter et al., 2003; Almind et al., 2007; Xue et al., 2007), affect the development of obesity. Conceivably this might occur if a cafeteria-like diet directly induced thermogenesis through activation of iBAT *Ucp*1 as proposed by Rothwell and Stock (1979). Accordingly, parental A/J, B6, and six RI lines with variable levels of *Ucp*1 mRNA inducible in white fat were fed a high fat/high sucrose obesogenic diet for 18 weeks at an ambient temperature of 23˚C to establish an obese state for each line (**Figure 3A**). Weight gain averaged from 11 g in A/J mice to 26 g in B6 mice as previously observed (Collins et al., 1997); however, A × B8 mice, which had the highest capacity to induce *Ucp*1 mRNA in white fat depots upon adrenergic stimulation, gained 24 g of fat, which was not significantly different than that observed for B6 mice, which had the lowest level of *Ucp*1 expression (**Figure 3A**; Guerra et al., 1998). This result indicates that in mice fed a high fat diet at 23˚C, the capacity for induction of *Ucp*1/BAT by cold exposure in white fat had no impact on the development of DIO at normal ambient temperatures (23˚C). One could argue that despite similarities in the levels of *Ucp*1 mRNA or protein in the iBAT of A/J and B6 mice, some unknown form of sympathetically mediated activation of iBAT-dependent diet-induced thermogenesis exists among A/J mice at 23˚C to reduce DIO. However, the suppression of increased DIO in B6 *Ucp*1 KO mice, with no capacity for UCP1-dependent thermogenesis by a brown adipocyte, indicates that diet-induced thermogenesis by brown fat is not a factor that increases susceptibility to DIO at 23˚C (Kozak, 2010).

The second question, whether variation in the induction of *Ucp*1 by adrenergic signaling has an impact on the obese state, was addressed in the second part of the experiment. Using the same eight lines of mice with variable levels of obesity, described in **Figure 3A**, were treated with the β3-AR agonist, CL 316,243, for 24 days while continuing to be fed the high fat diet (**Figure 3B**). All mice lost body weight, however, the amount of body weight lost depended upon the levels of *Ucp*1 mRNA that were induced in the white fat depots (**Figures 3B,C**; Guerra et al., 1998), that is, mice with higher levels of adrenergically induced *Ucp*1 mRNA in retroperitoneal fat lost more body weight. Thus, genetically determined variability in the induction of brown adipocytes in white fat depots by adrenergic stimulation is a significant factor in reducing obesity in response to adrenergic stimulation by exposure to cold or drugs. Accordingly, pharmaceuticals that are able to induce the number of brown adipocytes in discrete BAT depots or white fat depots could be an effective anti-obesity strategy.

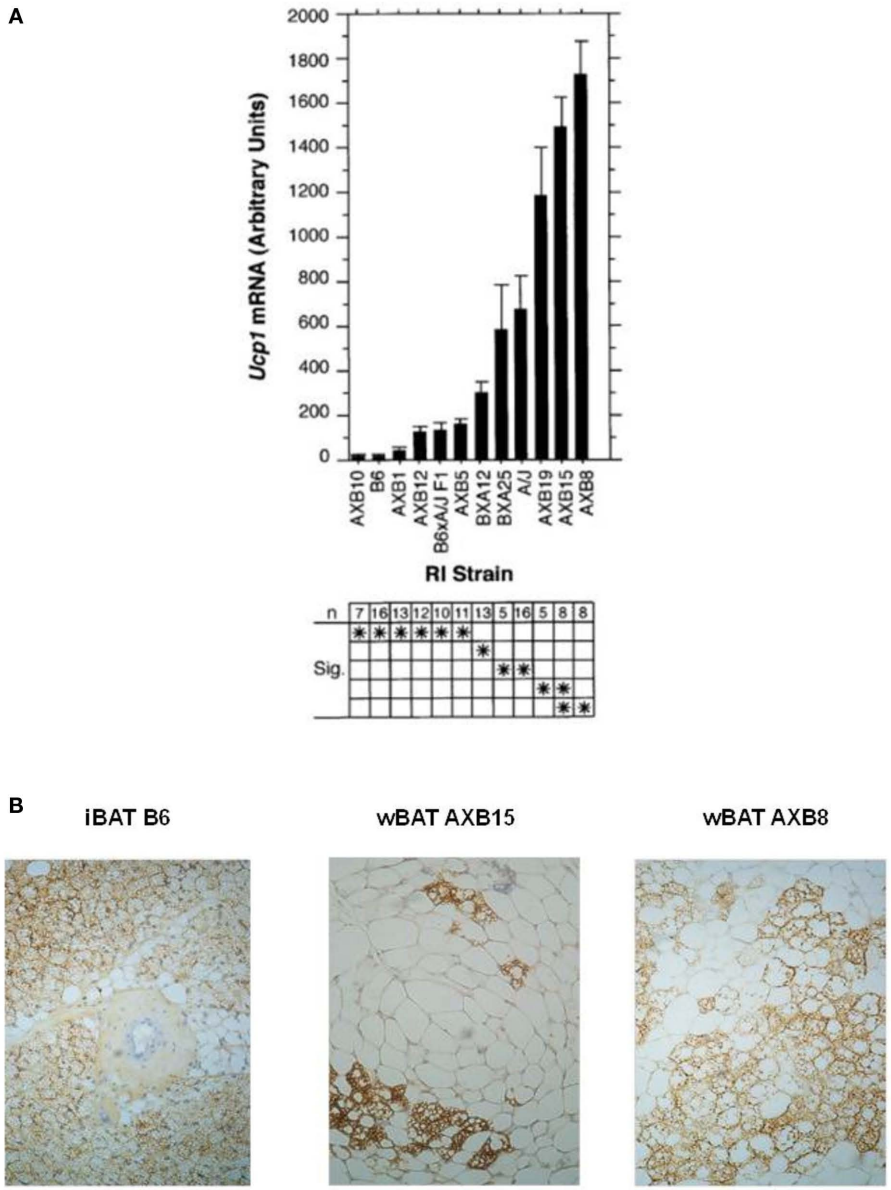

FIGURE 2 | (A) Bar graph shows *Ucp*1 mRNA levels in the retroperitoneal fat pad from parental A/J, B6, F1 hybrid, and RI strains of mice derived from the parental mice. Mice were housed at 5˚C in groups of two to three mice per pen for 7 days before the isolation of RNA. Induction in the cold is similar to that caused by a β3-adrenergic agonist. Data are presented as the mean ± SE. The number of animals (*n*) of each strain and the results of statistical analyses by ANOVA (Fisher's PLSD test in Statview 4.1) are given in the lower chart. Strains sharing asterisks in a row are not significantly different from each other at $P = 0.05$. **(B)** UCP1 immunohistology of paraffin-embedded sections from interscapular brown fat (iBAT) and retroperitoneal fat (wBAT) from A × B8 and A × B15 mice demonstrates the organization of brown adipocytes into clusters that resemble fields of densely packed brown adipocytes characteristic of iBAT. The size of these regions in wBAT may be quite variable, depending upon the strength of the induction.

CHROMOSOMAL MAPPING OF GENES CONTROLLING BAT INDUCTION BY QTL ANALYSIS

Given that there is probably no anti-obesity strategy in mice and rats that is as effective as the induction of brown adipocytes in white fat depots, identifying genes that could enhance the induction of brown adipocytes is an important goal. The analysis of the RI strains provided evidence that the induction of brown adipocytes in white fat upon adrenergic stimulation involved multiple genes; however, neither the number of genes nor their chromosomal location were able to be determined from analysis of strain distribution patterns alone (Koza et al., 2000). To identify the genes controlling brown adipocyte induction, we pursued a QTL analysis in which the primary phenotype was *Ucp*1 mRNA levels in retroperitoneal fat depots after 7 days at 5˚C, initially measured

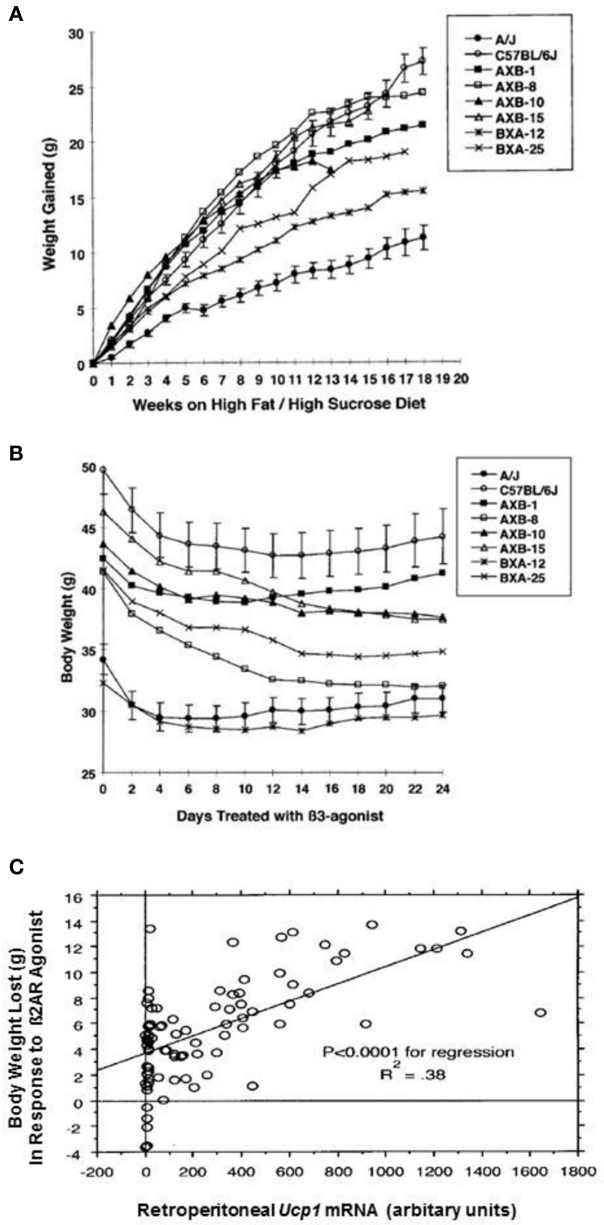

FIGURE 3 | (A) Body weight gained by parental and RI strains fed a high fat/high sucrose diet (Research Diets 12331) starting at 7 week of age and continuing for 18 week. **(B)** After 18 week, mice continued to be fed the high fat/high sucrose diet and were also treated with the β3-adrenergic agonist, CL 316,243, as described (Guerra et al., 1998). Changes in body weight are shown. The mean ± SE for the B6 and A/J parental strains are shown, whereas only the means have been plotted for the RI stains. The number of mice in each group ranges from 9 to 12. **(C)** Regression analysis to evaluate the effects of Ucp1 mRNA in the retroperitoneal fat on body weight loss. *P* is an estimator of the significance of the regression. R^2 is the coefficient of determination; it is the proportion of the dependent variable that is explained by the independent variable. *N* was 86.

by semi-quantitative Northern blots (Guerra et al., 1998) and later by quantitative RT-PCR with TaqMan probes using the ABI sequence detection system (Koza et al., 2000). Since there was a strong correlation between inducible *Ucp*1 mRNA and the content of brown adipocytes determined by immunostaining for UCP1 in the tissues of mice exposed to the cold (Guerra et al., 1998), the *Ucp*1 mRNA sub-phenotype is a reliable surrogate for brown adipocyte induction. The conversion of a white fat depot devoid of brown adipocytes to one rich in brown adipocytes requires the activation of brown fat specific adipogenesis, mitochondrial biogenesis, and increased innervation and vascularization (Cannon and Nedergaard, 2004). However, the most specific molecular marker change is the activation of *Ucp*1. Mice with an inactivated *Ucp*1 gene cannot tolerate an acute exposure to the cold because they have lost an important mechanism for thermogenesis (Enerback et al., 1997). Since the *Ucp*1 expression phenotype is near the end of a complex pathway from cold receptors on the surface of the skin to the processing of signals in the central nervous system that activate signaling and transcription factors in the adipocyte capable of being converted to a brown adipocyte, variable activity of any gene involved at any step of this complex process can be revealed by QTL analysis, if its effects on the levels of *Ucp*1 mRNA are sufficiently robust and precise. Indeed the expression for several genes [PPARα, type 2 deiodinase (DIO2), and PGC-1α] implicated in *Ucp*1 expression are highly variable in white fat of cold exposed mice and show highly significant correlations with *Ucp*1 expression (Coulter et al., 2003; Xue et al., 2005).

Based upon evidence that multilocular brown adipocytes in white fat depots were not labeled with BrdU in rats treated with the β3-AR agonist CL 316,243 (Himms-Hagen et al., 2000), we assumed that cell proliferation of a progenitor brown adipocyte does not occur in white fat depots of adult mice, unlike the cell proliferation that occurs in interscapular BAT during chronic cold exposure (Bukowiecki et al., 1982). Therefore, genes controlling the proliferation of adipocyte progenitors in white fat are assumed not to be involved in the induction of brown adipocytes in white fat during the first 7 days of adrenergic stimulation. Whether cell proliferation occurs following long term cold exposure is another question. This assumption is supported by the evidence on gene expression which showed that maximal levels of *Pgc*-1α mRNA are reached within 1 day of adrenergic stimulation and significant levels of *Ucp*1 mRNA can be detected in A/J mice within 3 h of being exposed to the cold (Coulter et al., 2003). Furthermore, changes in adipocyte morphology showing the emergence of a multilocular adipocyte from a unilocular adipocyte are consistent with the hypothesis that mature unilocular white adipocytes are directly converted to multilocular brown adipocytes (Cinti, 2002). If in fact brown adipocytes arise from mature white adipocytes, then it suggests that the brown adipocyte differentiation program is established during a window of postnatal adipose tissue development between 10 and 21 days of age when brown adipocytes transiently appear and then disappear (Xue et al., 2007). We postulate that since not all adipocytes look like brown adipocytes, this differentiation process has been implemented in selected adipocytes, which resemble mature white adipocytes under certain conditions, at weaning and in adults following adrenergic stimulation by a mechanism that rapidly initiates mitochondrial biogenesis and *Ucp*1 expression. Such a mechanism may not even involve further epigenetic modification of chromatin structure, but merely involve activation of PKA signaling and other mechanisms controlling

cAMP levels. Viewed from this perspective the search for candidate genes controlling the induction of brown adipocytes becomes focused on genetic mechanisms determining the sub-population of white adipocytes in a white fat depot with a chromatin structure unique for brown adipocytes. The brown adipocytes in traditional white fat depots may be masquerading as white adipocytes until they are adrenergically stimulated. A research goal will be to identify the molecular basis of their chromatin structure that enables a rapid transcription of brown adipocyte specific genes. A component of such a mechanism could be RIP140 (Leonardsson et al., 2004; Christian et al., 2005).

THE BACKCROSS MODEL

The molecular analysis of *Ucp*1 regulation has not revealed any brown fat specific signaling or transcription molecules; however, a regulatory model in which brown fat specific expression is determined by a modular molecular mechanism is consistent with existing data (Hansen and Kristiansen, 2006; Kajimura et al., 2008). If an interactive molecular mechanism controls brown adipocyte induction in white fat, perhaps the interactive transgressive mechanism revealed by the analysis of RI lines and backcross and intercross progeny may be the manifestation of such a mechanism at the gene level.

Two cohorts of approximately 400 (A/J × B6) F1 backcross mice were generated to test the idea that the known transcription factors of *Ucp*1 were associated with *Ucp*1 induction phenotypes following 7 days in the cold. Mice in cohort 1 were fed a standard low fat chow (11.9 kcal% fat) diet from weaning to 8 weeks of age when the mice were exposed to cold at 5°C for 7 days. Mice in cohort 2 were fed a high fat diet containing 58 kcal% fat (Research Diets 12331) from weaning until the end of the cold exposure (mice were subjected to cold exposure at 5°C for 7 days at 8 weeks of age). The rationale behind conducting a QTL analysis of mice fed a high fat diet was to assess whether the genes controlling induction of *Ucp*1 were influenced by a cafeteria-like diet, thereby protecting the animal against DIO through increased thermogenesis (Rothwell and Stock, 1979; Cannon and Nedergaard, 2004).

Prior to analysis of gene expression in the backcross mice we assessed the variation in expression of genes implicated in *Ucp*1 transcription between A/J and B6 mice exposed to the cold for 7 days. Differences in expression between the mouse strains were too small for analysis in backcross mice (less than twofold) for the following genes: Pref1, Nfe2l2, SREBP-1c, PPARγ, PPARγ2, CEBP-α, CEBP-β, CEBP-δ, FoxC2, and β3-adrenergic receptor. Robust differences between B6 and A/J were found for PPARα, and *Dio*2 and modest variation for PGC-1α. In addition, the high fat diet showed variable increases in the expression of these latter genes (**Table 1**). The highly significant correlations were found between *Ucp*1 and *Pgc*-1α, *Ppar*α and *Dio*2 (Coulter et al., 2003; Xue et al., 2005). In mice fed a low fat chow diet approximately 55% of the variance in *Ucp*1 levels is associated with the variance in *Pgc*-1α, but less (16%) in mice fed the high fat diet (**Figure 4**). Similar relationships were found between *Ucp*1 and *Ppar*α and *Dio*2 (Xue et al., 2005). In addition, similar to the transgressive variation found for *Ucp*1 in the RI strains, novel allelic recombinations in the backcross progeny generated transgressive phenotypes for each of these genes critical to wBAT induction. The significance of this transgressive variation is that perturbations in the expression levels of these transcription factors through allelic variation underlying the QTLs, together with environmental factors, like diet and ambient temperature, will affect the induction of *Ucp*1 and wBAT. That a large number of transgenes and gene KOs affect wBAT induction through effects on PPARα, PGC-1α, and DIO2 becomes predictable.

Table 1 | Effects of a high fat diet on *Pgc*-1α and *Ucp*1 mRNA levels.

Strain and mRNA	LFD mRNA	HFD mRNA	*P*-value	*n*
AJ *Ucp*1	222 ± 20	186 ± 42	0.43	12
B6 *Ucp*1	9.5 ± 2	33 ± 10	0.02	12
B × AF1 *Ucp*1	38 ± 7	55 ± 12	0.22	12
AJ *Pgc*-1α	13 ± 1	21 ± 2	3.3×10^{-4}	12
B6 *Pgc*-1α	5.7 ± 0.3	9.7 ± 0.9	1.1×10^{-4}	12
B × AF1 *Pgc*-1α	7.4 ± 0.4	9.3 ± 0.6	0.02	12
BC *Ucp*1	67.4 ± 2	77.8 ± 3	7.3×10^{-3}	400
BC *Pgc*-1α	8.2 ± 0.2	11.7 ± 0.3	1.6×10^{-24}	400

A/J, C57BL/6J (B6), (B × A) F1 and backcross mice were exposed to cold at 5°C for 7 days while on chow (4.5 wt% fat, LFD) or high fat diets (36 wt% fat, HFD) from weaning until the end of cold exposure. Pgc-1α mRNA levels increased 43% and Ucp1 levels 15% in backcross mice on a high fat diet.

Using MIT markers chromosomal regions associated with levels of *Ucp*1, *Ppar*α, *Pgc*-1α, and *Dio*2 mRNA in backcross progeny were mapped. The chromosomal linkage studies showed that QTLs on Chromosomes 2, 3, 8, and 19, which are associated with induction of *Ucp*1, were also associated, in part, with induction of *Ppar*α, *Pgc*-1α, and *Dio*2. Furthermore, significant differences were observed for the core QTLs, defined as those controlling *Ucp*1 expression (**Figure 5**). In mice fed a high fat diet, Chromosomes 2, 3, 8, and 19 were associated with levels of *Ucp*1 and *Ppar*α mRNA; however, unlike *Ucp*1 expression, not all QTLs were involved with the *Ppar*α, *Pgc*-1α, and *Dio*2. These associations are shown in **Figure 5**, for backcross mice fed both the low and high fat diets. The complexity of QTLs is greater for mice fed the chow diet than the high fat diet. The identities of the genes in the QTLs which determine the expression phenotypes are not proven, though plausible candidates exist. The fact that the peak of the QTL on Chromosome 8 is very close to *Ucp*1 provides a candidate to account for variation in *Ucp*1 mRNA levels, but it would require a mechanism by which *Ucp*1 also regulates *PPAR*α. Other genes under the peak of the Chromosome 8 QTL appear to be better candidates at this time. The QTL on Chromosome 15 lies close to the location of *Ppar*α. Confirmation that Pparα was a one of the variant alleles could not be confirmed with the *Ppar*α KO mouse (Xue et al., 2005). These linkage associations of *Ucp*1 and these transcription factors are consistent with transgressive variation and the synergy associated with *Ucp*1 induction. A change in the fat and carbohydrate content can change the levels of the transcription factors to select an alternative signaling and transcription factor for wBAT induction. A practical outcome of such a mechanism is that it enhances the range of drug targets for induction of wBAT.

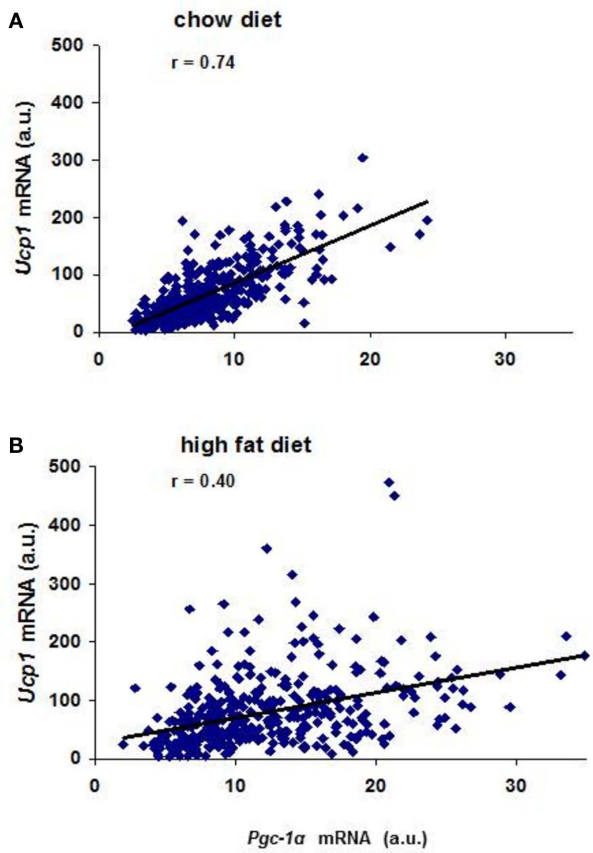

FIGURE 4 | (A) Correlation between *Pgc*-1α and *Ucp*1 mRNA levels in mice fed a 4.5 wt% fat diet. Approximately 400 (B6 × A/J) F1 × A/J backcross males 8 weeks of age were exposed to 5°C for 7 days. **(B)** Correlation between *Pgc*-1α and *Ucp*1 mRNA levels in mice fed a 58-kcal% fat diet. *Pgc*-1α and *Ucp*1 mRNA levels were determined in 400 backcross animals maintained on a high fat diet from weaning to 8 weeks of age and then exposed to the cold for 7 days.

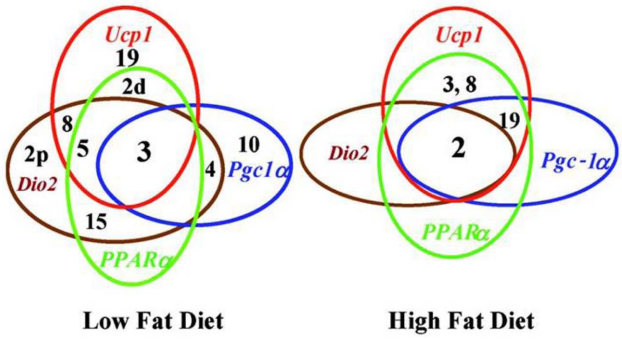

FIGURE 5 | Venn diagrams for chromosomes and gene expression illustrate the overlap of selective chromosomes in regulating genes associated with the regulatory network controlling *Ucp*1 function. Diagram also underscores the profound effects of diet on genes associated with regulatory networks of *Ucp*1 expression. Numbers refer to chromosomes carrying QTLs and those enclosed within a circle indicate those QTL associated with regulation of the indicated gene.

TRANSGRESSIVE VARIATION: A MANIFESTATION OF SYNERGISTIC INTERACTIONS BETWEEN QTLs AND TRANSCRIPTION FACTORS AND SIGNALING MOLECULES THAT CONTROL *UCP*1 EXPRESSION

Synergy in the expression of *Ucp*1 has been observed at two different levels, first at the level of interacting genes and second at the level of transcription and signaling factors implicated in *Ucp*1 expression. The strain distribution of *Ucp*1 mRNA levels in RP fat of cold exposed A × B and B × A RI mice provided clear evidence for the existence of transgressive variation for *Ucp*1 expression, that is, the production of offspring with phenotypes that exceed the parental extremes (**Figure 2**; Guerra et al., 1998; Koza et al., 2000). The novel combinations of alleles at several loci contributing to *Ucp*1 expression in RI strains result in higher levels than that which occurs in the A/J strain. The gene interactions determining transgressive variation have been observed in global studies of gene expression in mice and yeast but rarely analyzed for specific genes (Brem and Kruglyak, 2005; Shockley and Churchill, 2006).The first QTL analysis of the (B6 × A/J) F1 × A/J backcross mice identified Chromosomes 2, 3, 8, and 19 to be associated with *Ucp*1 expression and similar to the RI line data, the levels of *Ucp*1 in the backcross progeny had a range of expression that also showed transgressive variation, that is, they exceeded the levels of *Ucp*1 expression found in the A/J mice, approaching those found in A × B8 mice (Koza et al., 2000; Coulter et al., 2003). Positive effects on *Ucp*1 mRNA levels are associated with an A/J allele at the peak QTL on Chromosomes 2, 3, and 8, whereas the B6 allele on the QTL for Chromosome 19 was associated with elevated *Ucp*1. Accordingly, transgressive variation is based in part upon the interaction of a variant gene encoded by the B6 allele on Chromosome 19 with a variant gene on Chromosomes 2, 3, and/or 8 encoded by an A/J allele. Sixteen sets of mice with allelic assignments possible at Chromosomes 2, 3, 8, and 19 are rank ordered with respect to *Ucp*1 mRNA levels (**Table 2**). As predicted from the strain distribution pattern of the RI lines, A × B8, and seven other strains with levels that exceed A/J mice have A/J alleles at QTLs on Chromosomes 2, 3, and 8, but a B6 allele on Chromosome 19. This interpretation was further tested with an intercross of two RI lines, A × B8 and A × B10 that would assess the association of A/J and B6 alleles at the QTLs on Chromosomes 8 and 19. As shown in **Figure 6A** strong synergistic interactions elevate *Ucp*1 expression in mice homozygous for the A/J allele at *D*8*Mit*45 and homozygous for the B6 allele on Chromosome 19 near *D*19*Mit*86 (∼20 cM). Thus, an A/J allele at *D*19*Mit*86 shows dominance in suppressing the effects of an A/J allele near *D*8*Mit*45 at 40.5 cM (**Figure 6B**). The Chromosome 8 QTL affecting *Ucp*1 mRNA production is near the *Ucp*1 gene itself at 37.7 cM. It is possible that *Ucp*1 itself is structurally variable between A/J and B6 mice and that it directly interacts by a trans-mechanism with a gene located near *D*19*Mit*86. In our efforts to identity the QTLs, congenic lines were generated for QTLs on Chromosomes 8 and 19; however, lines carrying a single QTL failed to show differences in *Ucp*1 expression (L. P. Kozak, unpublished results).

We have shown how trans-acting genes on known chromosomes in a very specific QTL system controlling *Ucp*1 expression

Table 2 | Strain distribution pattern in RI lines in relationship to *Ucp*1 mRNA levels.

Strain	Chromosome 8					RP Ucp1 mRNA	Chromosome 19			
	8M128 31 cM	8M31 34 cM	Ucp1 37.7 cM	8M45 40.5 cM	8M242 47 cM		19M106 18.5 cM	19M86 20 cM	19M99 20.3 cM	19M30 21.4 cM
A/J	A	A	A	A	A	668	A	A	A	A
C57BL/6J	B	B	B	B	B	16	B	B	B	B
(B6 × A/J)F1	AB	AB	AB	AB	AB	129	AB	AB	AB	AB
A × B8	A	A	A	A	A	1719	B	B	B	B
A × B4	A	A	A	A	A	1700	B	B	B	B
A × B15	A	A	A	A	A	1490	B	B	B	B
B × A14	A	A	A	A	B	1439	B	B	B	B
A × B14	A	A	A	A	A	1247	B	B	A	A
A × B19	A	A	A	A	A	1181	B	B	B	B
A × B20	A	A	A	A	A	896	B	B	B	B
A × B18	A	A	A	A	A	630	B	B	B	B
B × A25	A	A	A	A	B	576	B	B	B	B
A × B24	B	B	A	A	A	562	B	B	B	B
B × A11	B	A	A	A	A	387	A	A	A	A
B × A4	A	A	A	A	B	359	A	B	B	B
B × A12	A	A	A	A	A	295	B	B	B	B
A × B6	B	B	B	B	B	226	A	A	A	A
B × A17	B	A	A	A	A	200	B	B	B	B
B × A8	B	A	A	A	A	186	B	B	B	B
A × B5	B	B	B	B	B	153	B	B	B	B
A × B12	B	B	B	B	B	122	A	A	A	B
B × A7	B	B	B	B	B	86	A	A	A	A
A × B2	A	B	B	A	A	77	A	A	A	B
B × A24	B	B	B	B	B	55	A	A	A	A
B × A2	B	B	A	A	A	51	A	A	A	A
B × A13	B	B	B	B	B	51	A	A	A	A
A × B1	B	B	B	B	A	40	B	B	B	B
B × A1	B	B	B	B	B	37	B	B	B	B
A × B10	B	B	B	B	A	13	B	A	A	A

Genotyping data in this table was assembled from the RI strain SDP's in the Mouse Genome Database (The Jackson Laboratory, Bar Harbor, Maine, http://www.informatics.jax.org/riset_form.shtml) and by genotyping of the RI strain DNA's. Microsatellite markers and relative cM positions (MGD) for chromosomes 8 and 19 are noted on the top of the table. Boxed in genotypes indicate RI strains with a recombinations between the markers of each chromosome.

are giving rise to transgressive expression at the molecular level. Previously it was shown that the occurrence of spontaneous ovarian teratomas in RI strains was greater than that observed to occur between progenitor strains (Eppig et al., 1996). A genetic analysis of morphological traits of yeast has revealed a complex QTL system with transgressive variation which has been correlated with gene expression analysis to produced results similar to the regulation of *Ucp*1 and brown fat induction (Nogami et al., 2007). Others have conducted a methodological global gene expression analysis of liver and kidney with chromosome substitution strains between A/J and B6 mice to assess allelic effects on gene expression and found that allelic variation at several chromosomes affect expression of 4209 transcripts (Shockley and Churchill, 2006). Many of the transcripts had levels of expression that exceeded those found in the parental strains, suggestive of transgressive variation (Shockley and Churchill, 2006).

Evidence from backcross and intercross analyses indicated that interactions between a B6 allele on Chromosome 19 with an A/J

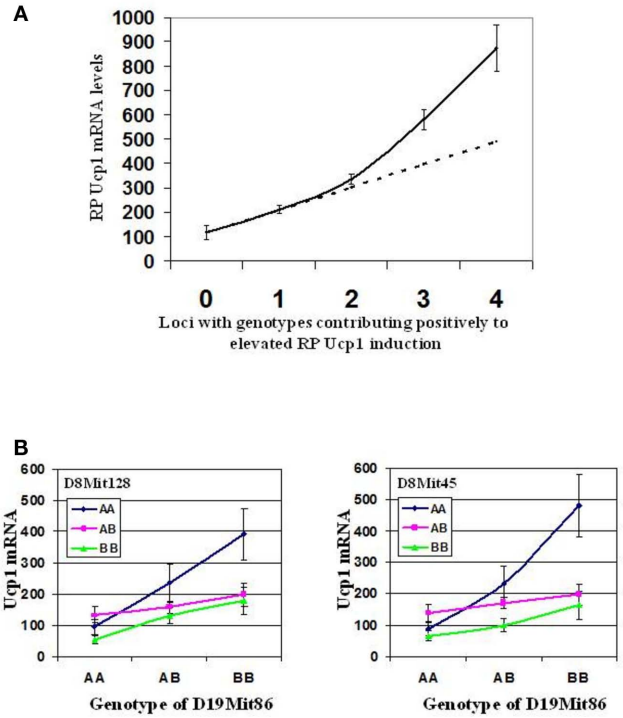

FIGURE 6 | (A) Synergistic effect of *Iba* QTLs on retroperitoneal *Ucp*1 mRNA induction. This is a graphic representation demonstrating the complex epistatic and additive interactions between the four *Iba* loci. The *solid line* represents the experimental retroperitoneal fat (RP) *Ucp*1 mRNA induction levels in relationship to the number of positive contributing loci. The *dashed line* indicates the theoretical value if interactions between *Iba* loci were only additive. **(B)** Effect of interactions between loci near *Ucp*1 (*D8Mit*45, 40.5 cM; *D8Mit*128, 31 cM), and *D19Mit*86 (20 cM) on retroperitoneal fat *Ucp*1 mRNA induction in 286 intercross progeny from A × B10 and A × B8. Line interaction plots demonstrate a strong epistatic interaction between loci on chromosomes 8 and 19. This interaction appears to be stronger for *D8Mit*45, a locus nearer to *Ucp*1 than for *D8Mit*128, the major QTL defined in the (A/J × B6) F1 × A/J backcross. Interaction plot data was generated using Statview v.4.5.

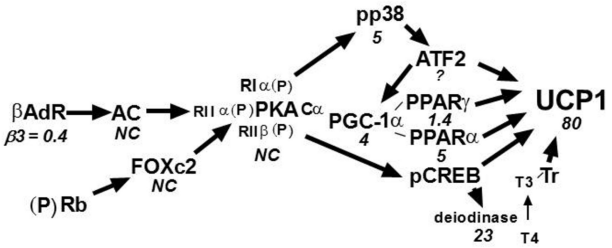

FIGURE 7 | Signaling and transcription components of *Ucp*1 regulation. The number below each regulatory factor refers to the ratio of expression in A/J vs. B6 mice. Except for the β3-adrenergic receptor, expression is higher in B6 than A/J at either the mRNA or protein levels; however, these strain-dependent differences all occur downstream of PKA. Most important is that none of the strain-dependent differences for the regulatory factors approaches the difference observed for *Ucp*1 at either the mRNA or protein levels. This suggests that synergy among the regulatory pathways occurs with a changing cast of factors depending on the genotypes of *Iba* genes and environmental conditions. NC = No difference in expression between A/J and B6.

allele on Chromosome 8 and possibly Chromosome 3 synergize to maximize expression of *Ucp*1. Since we also showed that these QTLs also determined variation in the levels of PPARα, PGC-1α, and *Dio*2 (Xue et al., 2005), we speculate that these transcription factors and signaling molecules, as well as others, interact synergistically to maximize the expression of *Ucp*1. An overview of the signaling and transcription pathways controlling *Ucp*1 expression is presented in **Figure 7**. Below each of the factors is a number that indicates the ratio of expression for that factor between A/J and B6 mice. It is apparent that all increases in expression are found for factors that lie downstream of PKA. There were no differences in expression for PKA or other factors that are upstream of PKA. Therefore, while retinoblastoma (*Rb*) and *Foxc*2 have been implicated in the induction of brown adipocytes in transgenic mice and cell cultures (Cederberg et al., 2001; Hansen et al., 2004), variations in their expression are not associated in determining the differences in the induction of *Ucp*1 in A/J and B6 mice. However, the key feature of this system for the control of *Ucp*1 is that while many regulatory components, known to have essential roles in *Ucp*1 expression, have elevated expression in A/J mice, relative to *Ucp*1 the differences in expression are very modest. For example, there is a four to fivefold difference in expression of the master regulator PGC-1α and other transcription factors between A/J and B6 mice, whereas *Ucp*1 is expressed 80-fold greater in A/J than B6 mice. The regulation of *Ucp*1 is determined by distinct sites for CREB, ATF2, PPARγ/PPARα, LXRα, TR, and interactions by several of the transcription factors with the co-activator PGC-1α (Cassard-Doulcier et al., 1993; Kozak et al., 1994; Sears et al., 1996; Puigserver et al., 1998; Hansen and Kristiansen, 2006; Kajimura et al., 2010). PGC-1α has been shown to be involved in the transcription of *Ucp*1 and additional aspects of mitochondrial biogenesis as co-activator with PPARγ/RXRα and TRβ in cell culture models (Puigserver et al., 1998). Similarly, mice with a targeted mutation for PPARα have no suppression of *Ucp*1 expression in liver or retroperitoneal fat (Kersten et al., 1999; Xue et al., 2005). Therefore, transcription factors with well-documented functions in *Ucp*1 transcription *in vitro* often turn out to be non-essential for its expression when inactivated by gene targeting *in vivo* (Kozak and Koza, 2010). This suggests that many of the sites for transcription of *Ucp*1 are in fact redundant and this redundancy involves not only the DNA regulatory motif; but, extends to the factors themselves. The redundancy facilitates the synergy by which many factors can participate in the determination of the expression of *Ucp*1. It becomes evident how the components of the environment, including diet and ambient temperature, exert their effects on *Ucp*1 through their actions on the factors modulating *Ucp*1 expression.

How does this molecular information on the structure and transcription of the *Ucp*1 gene relate to the read out of the brown fat genetic system as we have studied in A/J and B6 and the associated RI lines derived from these mice? A continuous variability occurs in which mRNA levels are positively correlated with PPARα, PGC-1α, and *Dio*2. We propose that this variability is determined by synergistically interacting genes within the QTLs

on Chromosomes 2, 3, 8, and 19. Thus, the synergy acts at different levels, at one level on the expression of the transcription and signaling mechanism and at another level on the terminal product of the process, that is, on *Ucp*1 expression.

THE DEVELOPMENT OF A BROWN ADIPOCYTE LINEAGE WITHIN WHITE FAT DEPOTS

Our QTL studies to identify genes controlling brown adipocytes in white fat depots revealed additional findings of significant interest. Some of these, such as the variable induction among white fat depots, were important from the perspective of selecting the best tissue to conduct the genetic experiment. For example, the differences between A/J and B6 mice was large for the interperitoneal fat depots, although the rank order of expression was retroperitoneal fat = mesenteric fat ≫ epididymal fat, while the difference between A/J and B6 mice was small in inguinal fat the level of expression was higher than even retroperitoneal fat (Guerra et al., 1998). However, a striking finding was that iBAT, while having the highest level of expression, showed no difference between A/J and B6 (Coulter et al., 2003; Xue et al., 2005). We reasoned that if the induction of brown adipocytes recapitulated early development and was based upon the same molecular mechanisms for all brown adipocytes, then if we analyzed gene and protein expression in brown fat during early development *in utero* and during suckling, *Ucp*1 in interscapular brown fat would be higher in the fetus of A/J compared to B6 mice. However, the analysis showed that at no time during development from the fetus *in utero* until 4 months of age were any strain-dependent differences detected in interscapular brown fat gene expression (Xue et al., 2007; **Figure 8**). In contrast, expression and histological analysis of retroperitoneal fat showed a transient induction of brown adipocytes between 10 and 30 days of age. Importantly, although brown adipocytes initially appeared in the retroperitoneal fat (we have since found that brown adipocytes are induced in the inguinal fat with essentially

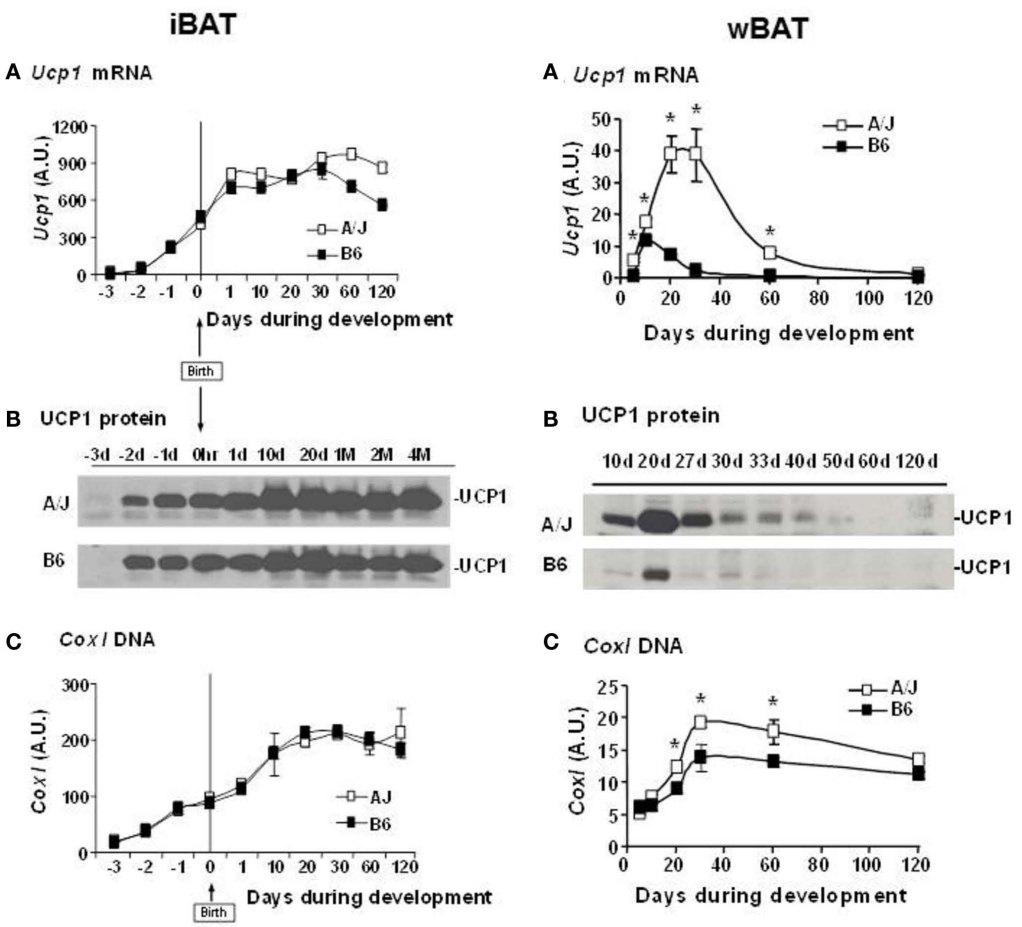

FIGURE 8 | (A) Development of iBAT in A/J and B6. **(A)** *Ucp*1 mRNA, **(B)** UCP1 protein, and **(C)** mitochondrial DNA content in iBAT of A/J and B6 mice during perinatal and postnatal development. For *Ucp*1 mRNA, each time point gives the average value from 4 to 9 individual animals analyzed in duplicate and is expressed relative to cyclophilin. *Cox*1 mitochondrial DNA levels are expressed relative to the nuclear gene *Ucp*2 from the analysis of four mice in duplicate. **(B)**. Induction of UCP1 in RP white fat depot in A/J and B6 during postnatal development. **(A)** *Ucp*1 mRNA, **(B)** UCP1 protein, and **(C)** mitochondrial DNA content in RP of A/J and B6 during postnatal development. $*P < 0.05$ vs. B6. *Ucp*1 mRNA and *Cox*1 DNA is based upon the analysis of three to six animals and Cox1 DNA of four mice per time point. *Ucp*1 mRNA is expressed relative to cyclophilin, while mitochondrial *Cox*1 DNA is reported relative to *Ucp*2.

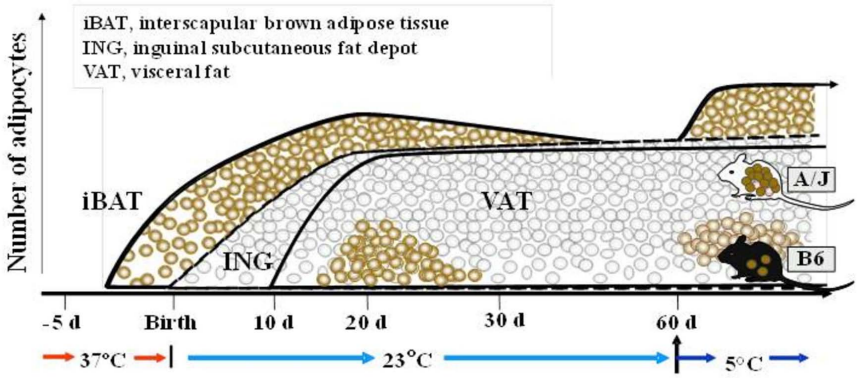

FIGURE 9 | Developmental profiles illustrating the appearance of brown adipocytes in interscapular brown adipose tissue (iBAT) initially at about 4 days before birth. Although the inguinal fat depot (ING) first appears at birth, but the visceral fat (VAT) not until ∼10 days of age, brown adipocytes appear in both depots between 15 and 30 days of age. The induction of brown adipocyte at 20 days of age is higher in A/J than C57Bl/6J (B6) mice, and is induced by in adult animals by adrenergic stimulation in A/J mice, but not B6 mice. This genetic difference in the induction of brown adipocytes among white fat depots is not found in iBAT. This figure has been modified from that found in Xue et al. (2007).

the same time course as retroperitoneal fat) of both A/J and B6 mice, the process was aborted in the B6 mice before the peak of brown adipocyte expression occurred at approximately 24 days of age (Xue et al., 2007). By 2 months of age the brown adipocytes have essentially disappeared from the white fat; however, if mice are exposed to an ambient temperature of 4°C the brown adipocytes are re-induced in A/J mice, but not B6 mice. This genetic variability, characteristic of brown adipocytes found in white fat depots, but not in interscapular brown fat, suggests that the developmental origins of brown adipocytes in interscapular fat are fundamentally different from the brown adipocytes that reside in white fat depots. A conclusion firmly established by the work with PRDM16 and the common progenitor origins for skeletal muscle and iBAT (Seale et al., 2007, 2008).A model of the development of BAT in iBAT and in white fat depots of inducible and non-inducible strain is illustrated in **Figure 9**.

The absence of genetic variability for a trait in a normal animal suggests that the trait is essential for survival of the animal through its function, in this case the presence of iBAT is essential for maintaining a body temperature at birth (Enerback et al., 1997). The newborn mouse, because of its small size and absence of hair, is particularly susceptible to a cold environment. Consequently, brown fat in the discrete depots in the interscapular region and around vital organs is fully functional at birth. On the other hand, brown adipocytes in white fat depots, which appear at ∼20 days of age, are probably not essential for the maintenance of body temperature and therefore more tolerant of genetic variability. Yet whether UCP1 is essential for survival is confounded by the neonatal pig. The pig has poor thermoregulation and no *Ucp*1 in its brown fat due to a deletion of exons 3–5 which occurred about 20 million years ago (Trayhurn et al., 1989; Berg et al., 2006), However, it manages to survive the cold stress of early development, indicating that in the absence of UCP1pigs adapt with alternative mechanisms of thermogenesis. It is interesting that UCP1-deficient mice can also survive the cold if they have a hybrid genetic background, suggesting the importance of novel allelic combinations that can lead to alternative mechanisms of thermogenesis (Hofmann et al., 2001; Anunciado-Koza et al., 2011).

SUMMARY AND PERSPECTIVES

The intent of this review is to provide a perspective on how to apply the discoveries and lessons of the study of the biology of brown fat in rodents to the upcoming investigations on brown fat in humans. The studies in mice indicate that induction of brown adipocytes in white fat depots is highly effective in reducing obesity when adrenergically stimulated. Because iBAT is relatively constant, we do not know how effective it may be in burning off excess calories when adrenergically stimulated. On the other hand the brown adipocytes which are induced in white fat depots by adrenergic stimulation show a rich potential for variable expression; a variation that has been effective in assessing the ability of brown adipocytes to burn off excess calories. Although we do not have the same depth of knowledge of the transcription and signaling pathways, which has been obtained for iBAT, nevertheless, we know that they can be effectively induced from mature white adipocytes by adrenergic signaling. In other words, simply conditioning the wBAT system by implementing a training program consisting of exposure to a reduced ambient temperature, analogous to physical exercise training, could be another useful weapon in the war against obesity.

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REFERENCES

Almind, K., Manieri, M., Sivitz, W. I., Cinti, S., and Kahn, C. R. (2007). Ectopic brown adipose tissue in muscle provides a mechanism for differences in risk of metabolic syndrome in mice. *Proc. Natl. Acad. Sci. U.S.A.* 104, 2366–2371.

Anunciado-Koza, R., Zhang, J., Ukropec, J., Bajpeyi, S., Koza, R. A., Rogers, R. C., Cefalu, W. T., Mynatt, R. L., and Kozak, L. P. (2011). Inactivation of the mitochondrial carrier SLC25A25 (ATP-Mg2+/Pi transporter) reduces physical endurance and metabolic efficiency in mice. *J. Biol. Chem.* 286, 11659–11671.

Bailey, D. W. (1971). Recombinant inbred strains. *Transplantation* 11, 325–327.

Bartness, T. J., and Bamshad, M. (1998). Innervation of mammalian white adipose tissue: implications for the regulation of total body fat. *Am. J. Physiol.* 275, R1399–R1411.

Berg, F., Gustafson, U., and Andersson, L. (2006). The uncoupling protein 1 gene (UCP1) is disrupted in the pig lineage: a genetic explanation for poor thermoregulation in piglets. *PLoS Genet.* 2, e129. doi:10.1371/journal.pgen.0020129

Brem, R. B., and Kruglyak, L. (2005). The lanscape of genetic complexity across 5,700 gene expression traits in yeast. *Proc. Natl. Acad. Sci. U.S.A.* 102, 1572–1577.

Bukowiecki, L., Collet, A. J., Follea, N., Guay, G., and Jahjah, L. (1982). Brown adipose tissue hyperplasia: a fundamental mechanism of adaptation to cold and hyperphagia. *Am. J. Physiol.* 242, E353–E359.

Cannon, B., and Nedergaard, J. (2004). Brown adipose tissue: function and physiological significance. *Physiol. Rev.* 84, 277–359.

Cassard-Doulcier, A. M., Gelly, C., Fox, N., Schrementi, J., Raimbault, S., Klaus, S., Forest, C., Bouillaud, F., and Ricquier, D. (1993). Tissue-specific and beta-adrenergic regulation of the mitochondrial uncoupling protein gene: control by cis-acting elements in the 5′- flanking region. *Mol. Endocrinol.* 7, 497–506.

Cederberg, A., Gronning, L. M., Ahren, B., Tasken, K., Carlsson, P., and Enerback, S. (2001). FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. *Cell* 106, 563–573.

Champigny, O., Ricquier, D., Blondel, O., Mayers, R. M., Briscoe, M. G., and Holloway, B. R. (1991). Beta 3-adrenergic receptor stimulation restores message and expression of brown-fat mitochondrial uncoupling protein in adult dogs. *Proc. Natl. Acad. Sci. U.S.A.* 88, 10774–10777.

Christian, M., Kiskinis, E., Debevec, D., Leonardsson, G., White, R., and Parker, M. G. (2005). RIP140-targeted repression of gene expression in adipocytes. *Mol. Cell. Biol.* 25, 9383–9391.

Cinti, S. (2002). Adipocyte differentiation and transdifferentiation: plasticity of the adipose organ. *J. Endocrinol. Invest.* 25, 823–835.

Collins, S., Daniel, K. W., Petro, A. E., and Surwit, R. S. (1997). Strain-specific response to beta 3-adrenergic receptor agonist treatment of diet-induced obesity in mice. *Endocrinology* 138, 405–413.

Coulter, A. A., Bearden, C. M., Liu, X., Koza, R. A., and Kozak, L. P. (2003). Dietary fat interacts with QTLs controlling induction of Pgc-1 alpha and Ucp1 during conversion of white to brown fat. *Physiol. Genomics* 14, 139–147.

Cypess, A. M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A. B., Kuo, F. C., Palmer, E. L., Tseng, Y. H., Doria, A., Kolodny, G. M., and Kahn, C. R. (2009). Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* 360, 1509–1517.

Enerback, S., Jacobsson, A., Simpson, E. M., Guerra, C., Yamashita, H., Harper, M. E., and Kozak, L. P. (1997). Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* 387, 90–94.

Eppig, J. J., Wigglesworth, K., Varnum, D. S., and Nadeau, J. H. (1996). Genetic regulation of traits essential for spontaneous ovarian teratocarcinogenesis in strain LT/Sv mice: aberrant meiotic cell cycle, oocyte activation, and parthenogenetic development. *Cancer Res.* 56, 5047–5054.

Giralt, M., Martin, I., Iglesias, R., Vinas, O., Villarroya, F., and Mampel, T. (1990). Ontogeny and perinatal modulation of gene expression in rat brown adipose tissue. Unaltered iodothyronine 5′-deiodinase activity is necessary for the response to environmental temperature at birth. *Eur. J. Biochem.* 193, 297–302.

Guerra, C., Koza, R. A., Yamashita, H., Walsh, K., and Kozak, L. P. (1998). Emergence of brown adipocytes in white fat in mice is under genetic control. Effects on body weight and adiposity. *J. Clin. Invest.* 102, 412–420.

Hansen, J. B., Jorgensen, C., Petersen, R. K., Hallenborg, P., De Matteis, R., Boye, H. A., Petrovic, N., Enerback, S., Nedergaard, J., Cinti, S., Te Riele, H., and Kristiansen, K. (2004). Retinoblastoma protein functions as a molecular switch determining white versus brown adipocyte differentiation. *Proc. Natl. Acad. Sci. U.S.A.* 101, 4112–4117.

Hansen, J. B., and Kristiansen, K. (2006). Regulatory circuits controlling white versus brown adipocyte differentiation. *Biochem. J.* 398, 153–168.

Himms-Hagen, J., Cui, J., Danforth, E. Jr., Taatjes, D. J., Lang, S. S., Waters, B. L., and Claus, T. H. (1994). Effect of CL-316,243, a thermogenic beta 3-agonist, on energy balance and brown and white adipose tissues in rats. *Am. J. Physiol.* 266, R1371–R1382.

Himms-Hagen, J., Melnyk, A., Zingaretti, M. C., Ceresi, E., Barbatelli, G., and Cinti, S. (2000). Multilocular fat cells in WAT of CL-316243-treated rats derive directly from white adipocytes. *Am. J. Physiol. Cell Physiol.* 279, C670–C681.

Hirning, U., Schmid, P., Schulz, W. A., Kozak, L. P., and Hameister, H. (1989). In developing brown adipose tissue c-myc protooncogene expression is restricted to early differentiation stages. *Cell Differ. Dev.* 27, 243–248.

Hofmann, W. E., Liu, X., Bearden, C. M., Harper, M.-E., and Kozak, L. P. (2001). Effects of genetic background on thermoregulation and fatty acid-induced uncoupling of mitochondria in UCP1-deficient mice. *J. Biol. Chem.* 276, 12460–12465.

Houstek, J., Kopecky, J., Rychter, Z., and Soukup, T. (1988). Uncoupling protein in embryonic brown adipose tissue–existence of non thermogenic and thermogenic mitochondria. *Biochim. Biophys. Acta* 935, 19–25.

Kajimura, S., Seale, P., and Spiegelman, B. M. (2010). Transcriptional control of brown fat development. *Cell Metab.* 11, 257–262.

Kajimura, S., Seale, P., Tomaru, T., Erdjument-Bromage, H., Cooper, M. P., Ruas, J. L., Chin, S., Tempst, P., Lazar, M. A., and Spiegelman, B. M. (2008). Regulation of the brown and white fat gene programs through a PRDM16/CtBP transcriptional complex. *Genes Dev.* 22, 1397–1409.

Kersten, S., Seydoux, J., Peters, J. M., Gonzalez, F. J., Desvergne, B., and Wahli, W. (1999). Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. *J. Clin. Invest.* 103, 1489–1498.

Koza, R. A., Hohmann, S. M., Guerra, C., Rossmeisl, M., and Kozak, L. P. (2000). Synergistic gene interactions control the induction of the mitochondrial uncoupling protein (Ucp1) gene in white fat tissue. *J. Biol. Chem.* 275, 34486–34492.

Kozak, L. P. (2010). Brown fat and the myth of diet-induced thermogenesis. *Cell Metab.* 11, 263–267.

Kozak, L. P., and Koza, R. A. (2010). The genetics of brown adipose tissue. *Prog. Mol. Biol. Transl. Sci.* 94, 75–123.

Kozak, U. C., Kopecky, J., Teisinger, J., Enerback, S., Boyer, B., and Kozak, L. P. (1994). An upstream enhancer regulating brown-fat-specific expression of the mitochondrial uncoupling protein gene. *Mol. Cell. Biol.* 14, 59–67.

Leonardsson, G., Steel, J. H., Christian, M., Pocock, V., Milligan, S., Bell, J., So, P. W., Medina-Gomez, G., Vidal-Puig, A., White, R., and Parker, M. G. (2004). Nuclear receptor corepressor RIP140 regulates fat accumulation. *Proc. Natl. Acad. Sci. U.S.A.* 101, 8437–8442.

Nogami, S., Ohya, Y., and Yvert, G. (2007). Genetic complexity and quantitative trait loci mapping of yeast morphological traits. *PLoS Genet.* 3, e31. doi:10.1371/journal.pgen.0030031

Puigserver, P., Wu, Z., Park, C. W., Graves, R., Wright, M., and Spiegelman, B. M. (1998). A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 92, 829–839.

Rothwell, N. J., and Stock, M. J. (1979). A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 281, 31–35.

Seale, P., Bjork, B., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scime, A., Devarakonda, S., Conroe, H. M., Erdjument-Bromage, H., Tempst, P., Rudnicki, M. A., Beier, D. R., and Spiegelman, B. M. (2008). PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454, 961–967.

Seale, P., Kajimura, S., Yang, W., Chin, S., Rohas, L. M., Uldry, M., Tavernier, G., Langin, D., and Spiegelman, B. M. (2007). Transcriptional control of brown fat determination by PRDM16. *Cell Metab.* 6, 38–54.

Sears, I. B., Macginnitie, M. A., Kovacs, L. G., and Graves, R. A. (1996). Differentiation-dependent expression of the brown adipocyte uncoupling protein gene: regulation by peroxisome proliferator-activated receptor gamma. *Mol. Cell. Biol.* 16, 3410–3419.

Seldin, M. F., Mott, D., Bhat, D., Petro, A., Kuhn, C. M., Kingsmore, S. F., Bogardus, C., Opara, E., Feinglos, M. N., and Surwit, R. S. (1994). Glycogen synthase: a putative locus for diet-induced hyperglycemia. *J. Clin. Invest.* 94, 269–276.

Shockley, K. R., and Churchill, G. A. (2006). Gene expression analysis of mouse chromosome substitution strains. *Mamm. Genome* 17, 598–614.

Smith, R. E., and Horwitz, B. A. (1969). Brown fat and thermogenesis. *Physiol. Rev.* 49, 330–425.

Staszkiewicz, J., Gimble, J., Cain, C., Dietrich, M., Burk, D., Kirk-Ballard, H., and Gawronska-Kozak, B. (2009). Flow cytometric and immunohistochemical detection of in vivo BrdU-labeled cells in mouse fat depots. *Biochem. Biophys. Res. Commun.* 378, 539–544.

Taylor, B. A. (1981). "Recombinant inbred strains," in *Genetic Variants and Strains of the Laboratory Mouse*, ed. M. C. Green (New York: Gustav Fischer Verlag). 397–407.

Trayhurn, P., Temple, N. J., and Van Aerde, J. (1989). Evidence from immunoblotting studies on uncoupling protein that brown adipose tissue is not present in the domestic pig. *Can. J. Physiol. Pharmacol.* 67, 1480–1485.

Van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, J. M., Kemerink, G. J., Bouvy, N. D., Schrauwen, P., and Teule, G. J. (2009). Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* 360, 1500–1508.

Virtanen, K. A., Lidell, M. E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N. J., Enerback, S., and Nuutila, P. (2009). Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* 360, 1518–1525.

Xue, B., Coulter, A., Rim, J. S., Koza, R. A., and Kozak, L. P. (2005). Transcriptional synergy and the regulation of Ucp1 during brown adipocyte induction in white fat depots. *Mol. Cell. Biol.* 25, 8311–8322.

Xue, B., Rim, J. S., Hogan, J. C., Coulter, A. A., Koza, R. A., and Kozak, L. P. (2007). Genetic variability affects the development of brown adipocytes in white fat but not in interscapular brown fat. *J. Lipid Res.* 48, 41–51.

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TGF-β/Smad3 signaling regulates brown adipocyte induction in white adipose tissue

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Recent identification of active brown fat reserves in adult humans has re-stimulated interest in the role of brown adipocytes in energy homeostasis. In addition, there is accumulating evidence to support the concept of an alteration in energy balance through acquisition of brown fat features in traditional white fat depots. We recently described an important role played by the TGF-β/Smad3 signaling pathway in modulating the appearance of brown adipocytes in traditional white fat, and its implications to thermogenesis, mitochondrial energetics, energy expenditure, and protection from diabetes and obesity. Here we review the data supporting this phenomenon and put into perspective the promise of conversion of white fat to a brown fat state as a potential therapeutic option for obesity and diabetes.

Keywords: TGF-beta, Smad3, obesity, brown fat, white fat

The rise in the worldwide incidence of obesity and, by a tight correlation, diabetes is proof that we are failing as a populace to obey the energy balance equation. Obesity is principally characterized by accumulation of fat in adipose tissue (Gesta et al., 2007; Park et al., 2008). In conditions of energy excess, the white adipose tissue accumulates fat in the form of triglycerides, whilst brown adipose tissue has the potential stimulate energy expenditure by dissipation of fat to produce heat and maintain body temperature. White fat comprises of large unilocular lipid-containing adipocytes with few mitochondria. In contrast, brown fat comprises of small multilocular cells with abundant mitochondria. Brown adipocytes are uniquely characterized by the expression of uncoupling protein-1 (UCP1). Copious amount of brown fat exists in rodents and human infants and it was considered to be non-existent in adult humans. Recent findings that metabolically active brown fat exists in humans (Nedergaard et al., 2007; Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009) have stimulated interest concerning the therapeutic potential of augmenting brown fat to combat metabolic diseases (Enerback, 2010; Nedergaard and Cannon, 2010). Further, it appears that brown fat shares its developmental origin with muscle, and not white fat as it was long presumed (Atit et al., 2006; Timmons et al., 2007). Seale et al. (2008, 2009) provided the formal proof that brown fat is related to skeletal muscle and further showed that the transcription factor PRDM16 determines the fate of $Myf5^{+}$-precursor cells toward brown fat lineage.

BROWN ADIPOCYTE INDUCTION IN WHITE ADIPOSE TISSUE

Brown adipocytes are also found interspersed within the white adipose tissue, in response to chemical or hormonal stimulation, environmental changes, cold exposure, and defined genetic manipulation (Langin, 2009; Lefterova and Lazar, 2009; Frontini and Cinti, 2010). The most well studied models whereby brown adipocytes appear in white fat are upon cold exposure or after stimulation of the beta(3)-adrenoceptor pathways. Cold exposure of mice results in expression of the brown adipocyte marker, UCP1, in inguinal white adipose tissue (Loncar, 1991) and in mesenteric, epididymal, retroperitoneal, inguinal, and periovarian adipose depots upon exposure to cold or to treatment with a beta-adrenoceptor agonist (Cousin et al., 1992). In agreement, beta 3-adrenoceptor knockout mice show suppressed occurrence of brown adipocytes in white fat upon cold exposure (Jimenez et al., 2003). In contrast, transgenic mice overexpressing the beta 1-adrenergic receptor in adipose tissue exhibit abundant appearance of brown fat cells in subcutaneous white adipose tissue and are resistant to obesity (Soloveva et al., 1997). Chronic treatment with the beta3-adrenoceptor agonist, CL 316,243, (Bloom et al., 1992), promotes thermogenesis, and the appearance of multilocular adipocytes in white fat while protecting from high-fat diet-induced obesity (Himms-Hagen et al., 1994). Infusion of CL 316,243 reduced abdominal fat, increased resting metabolic rate, and abundant multilocular brown adipocytes expressing uncoupling protein (UCP) appeared in retroperitoneal white fat (Ghorbani et al., 1997). Similarly, appearance of brown adipocytes in white adipose tissue during CL 316,243-treatment correlated with reversal of obesity and diabetes in Zucker fa/fa rats (Ghorbani and Himms-Hagen, 1997). Also, beta3-adrenergic receptors mediate CL 316,243 agonist-induced effects on energy expenditure, insulin secretion, and food intake (Grujic et al., 1997).

Interestingly, genetic background modulates the relative degree of browning of white adipose tissue. CL 316,243 prevented the development of diet-induced obesity in A/J animals, but not in C57BL/6J animals. In agreement, CL 316,243-treated A/J mice, but not B/6J mice, showed abundant UCP1 expression in white

adipose depots (Collins et al., 1997). Also, significant stain-specific differences in UCP1 transcript levels were seen in various white fat depots derived from A/J and C57BL/6J strains of mice after stimulation of adrenergic signaling (Guerra et al., 1998). Further, cold exposure induced brown adipocytes in retroperitoneal fat of adult A/J mice but not in C57BL/6J mice. In contrast, induction of UCP1 in interscapular brown adipose tissue showed no such strain dependence (Xue et al., 2007).

POSSIBLE MECHANISMS OF BROWNING IN WHITE FAT

TRANSDIFFERENTIATION

Tissue plasticity which allows efficient conversion of white adipocyte to brown adipocyte and vice versa has been proposed as a potential mechanism (Frontini and Cinti, 2010). Thus, cold exposure conditions would promote white-to-brown conversion to fulfill the demand for thermogenesis, whereas, high-fat diet would promote conversion of brown-to-white fat to enable energy storage (Cinti, 2011). It is believed that multilocular fat cells that appear in white fat upon CL 316243-treatment derive from convertible unilocular adipocytes that become multilocular (Himms-Hagen et al., 2000) and that emergence of brown adipocytes in white fat upon cold exposure reflects beta(3)-adrenoceptor-mediated transdifferentiation (Barbatelli et al., 2010). The retinoblastoma (Rb) protein family acts as a molecular switch determining white versus brown adipocyte differentiation. Thus, inactivation of pRB results in the expression of UCP1 in the white adipose tissue and down-regulation of pRB expression is associated with white into brown adipocyte transdifferentiation in response to beta3-adrenergic receptor agonist treatment (Hansen et al., 2004). Further, mice lacking the Rb-family member, p107, possess white adipose tissue with multilocular adipocytes that express elevated levels of UCP1 and PGC-1α, a transcriptional co-activator implicated in mitochondrial biogenesis and adaptive thermogenesis and pRb binds and represses PGC-1α transcription (Scime et al., 2005).

PRECURSOR POOL ORIGIN

Multilocular fat cells that appear in white fat upon CL 316243-treatment may derive from a precursor cell that gives rise to more typical brown adipocytes (Himms-Hagen et al., 2000). To date, such a precursor pool is unidentified. The evidence that the dermis, muscle, and brown fat all originate from the central dermomyotome (Atit et al., 2006) and that immature undifferentiated brown fat cells harbor muscle-specific transcripts and brown preadipocytes exhibit a myogenic signature (Timmons et al., 2007) stimulated a search for a common brown fat/muscle progenitor. Indeed, Seale et al. (2008, 2009) provided evidence that brown fat shares developmental ontogeny with skeletal muscle with the transcription factor PRDM16 promoting the differentiation of skeletal muscle-derived $Myf5^+$ precursor cells toward a classical brown adipocyte lineage. However, they did not detect $Myf5^+$ precursor cell derivatives in the examined white fat. These findings suggest that, either (i) $Myf5^+$ precursor cells do not promote the browning phenomenon in white fat, or (ii) a context dependent role for $Myf5^+$ precursor cells may yet exist with respect to induction of brown adipocytes in white fat, or (iii) other distinct precursor cell pools resident in white fat or other tissue/s may drive the browning phenotype in white fat. Lineage tracing methods with the availability of unique gene expression markers for the three major types of adipose tissue depots – the brown (Zic1), the beige, or brite (Hoxc9), and the white (Tcf21; Walden et al., 2011) – would help identify the potential precursor pool that drives browning of white fat.

AUTOCRINE OR PARACRINE FACTORS

Autocrine or paracrine factors – classic examples being insulin and leptin – play critical roles in regulating not only the cells from which they derive, but also play overarching roles in regulating the biology of other cells that orchestrate glucose homeostasis. This is possible via these factors engaging their specific receptors in the cell type where the action is elicited. Several facts support a notion that a similar such secretagogue might regulate the appearance of brown adipocytes in white fat: (i) the white fat is a reservoir that synthesizes and secretes multiple cytokines and hormones such as leptin, (ii) classical brown fat might produce factors that promote brown adipocyte induction locally as well as at a distance in white fat, and (iii) considering the muscle-specific origin of brown adipocytes, it is logical to assume that a muscle-specific factor might serve as a inducer of brown adipocytes. Indeed, a muscle-derived hormone, irisin, has been identified that promotes induction of brown adipocytes in white fat (Bostrom et al., 2012). Irisin, whose receptor is presently unidentified, is induced in the muscle by PGC-1α expression. It is induced with exercise and a rise in plasma irisin levels correlate with increased energy expenditure, enhanced glucose homeostasis, and protection from obesity. The observation that irisin has paracrine effects on white fat to stimulate induction of a brown fat program has utility in not only furthering the knowledge about browning of white fat but also has obvious therapeutic potential. It is plausible that other factors such as irisin, produced locally in white fat or acting in a paracrine manner, may stimulate the browning of white fat.

Neural circuits

Neural circuits are also recognized as critical modifiers of the white fat to brown fat phenomenon. An enriched environment induced browning of white fat associated with expression of genes involved in brown adipogenesis, thermogenesis, and beta-adrenergic signaling pathways along with improved energy homeostasis and glucose tolerance (Cao et al., 2011). Hypothalamic overexpression of the brain-derived neurotrophic factor (BDNF) promoted a lean phenotype accompanied with acquisition of brown fat features in the white fat, whereas, inhibition of BDNF signaling reversed this phenotype (Cao et al., 2011). Also, hypothalamic neuropeptide Y (NPY) has been implicated in promoting brown fat features in white fat (Chao et al., 2011). Knockdown of NPY expression in the dorsomedial hypothalamus (DMH) reduced white fat depots, increased energy expenditure, and suppressed high-fat diet-induced obesity. Interestingly, these phenomena were accompanied by appearance of brown adipocytes in inguinal white adipose tissue (Chao et al., 2011).

Signaling pathways

Increased FOXC2 expression in adipocytes results in a lean phenotype and protects from hypertriglyceridemia and diet-induced insulin resistance and obesity primarily by increasing the

beta-adrenergic–cAMP–protein kinase A (PKA) signaling pathway (Cederberg et al., 2001). PPARα stimulated the expression of the PRDM16 gene in brown adipocytes and PPARα activation in white adipocytes induced expression of brown fat markers (Hondares et al., 2011). C/EBPα and the corepressors CtBP1 and CtBP2 repress visceral white adipose genes and activate UCP1 transcription during PPARγ agonist-mediated induction of the brown phenotype in white adipocytes (Vernochet et al., 2009). Induction of UCP1 expression in white adipose tissue, but not in classic interscapular brown adipose tissue, is dependent on cyclooxygenase activity (Madsen et al., 2010). Cyclooxygenase (COX)-2, a rate-limiting enzyme in prostaglandin synthesis, is a downstream effector of beta-adrenergic signaling in white adipose tissue and is required for appearance of brown fat features in white fat. Prostaglandin induced differentiation of mesenchymal progenitors toward a brown adipocyte phenotype and overexpression of COX-2 in white fat induced brown adipogenesis in white fat, increased energy expenditure, and protected mice against high-fat diet-induced obesity (Vegiopoulos et al., 2010). Retinoic acid reduces body weight, increases body temperature and adiposity in rodent models and stimulates UCP1 expression in brown adipose tissue and skeletal muscle. Administration of all-trans retinoic acid results in reduced adiposity and adipocyte cell size with a rise in multilocular adipocytes expressing brown fat markers in white fat depots (Mercader et al., 2006). Wnt10b blocks beta3-agonist-induced brown adipose tissue differentiation and suppresses UCP1 expression through repression of PGC-1α, while promoting the appearance of unilocular lipid droplets and expression of white adipocyte genes consistent with conversion of brown fat to white fat (Kang et al., 2005). AMP-kinase (AMPK) signaling is also implicated in the brown fat to white fat conversion. Adipose-specific ablation of an AMP-kinase substrate, desnutrin/ATGL, converts brown fat to a white fat-like tissue where the mice exhibit severely impaired thermogenesis, increased expression of white fat genes and decreased brown fat genes (Ahmadian et al., 2011).

While it is clear that many signals can promote the browning of white fat, the mechanistic details are poorly understood. Further, some of the prior observations utilized cell culture models like primary mouse embryonic fibroblasts differentiated fat cells that harbor both lineages of "brown" fat cells. Also, many of the mouse studies were not performed at thermo-neutral temperature, and instead at an adapted colder temperature of 22–24°C which complicates the role of these molecules in thermogenesis and energy expenditure. Further, whether the repressing of white fat phenotype of these molecules is executed via functional regulation of adaptive thermogenesis or determination of cell fate and it remains unclear how many of these signals regulate the $Myf5^{+}$ lineage brown fat.

TGF-β SIGNALING REGULATES APPEARANCE OF BROWN ADIPOCYTES IN WHITE ADIPOSE TISSUE

We recently elucidated the importance of TGF-β signaling in the appearance of brown adipocytes within the white adipose tissue (Crunkhorn, 2011; Yadav et al., 2011). The TGF-β superfamily member, BMP7, has been implicated in brown adipogenesis (Tseng et al., 2008). Whether, there exists cross-talk between TGF-β and BMP signaling in mediating brown adipogenesis remains to be determined. Similarly, whether BMP signals, or signals emanating from other TGF-β superfamily members, play a role in promoting browning of white fat remains unclear. Moreover, it is also not known if there exists a region-specific importance of TGF-β signaling as it pertains to brown adipocyte development within the different white fat depots, such as the mesenteric, epididymal, retroperitoneal, inguinal, and periovarian adipose depots.

BMP proteins promote differentiation to either white adipocytes or brown adipocytes (Schulz et al., 2011). BMP7 triggers commitment of mesenchymal progenitor cells to a brown adipocyte lineage, and implantation of these cells into nude mice results in development of adipose tissue containing mostly brown adipocytes (Tseng et al., 2008). Subpopulation of adipogenic progenitors (termed ScaPCs) residing in murine brown fat, white fat, and skeletal muscle were isolated and it was shown that muscle and white fat derived Sca-1(+) cells were able to differentiate into brown-like adipocytes upon stimulation with BMP7 (Schulz et al., 2011). Also, Bmp7 knockout embryos show a marked reduction of brown fat, whereas, adenoviral-mediated expression of BMP7 in mice results in a significant increase in brown fat mass and leads to an increase in energy expenditure and a reduction in weight gain (Tseng et al., 2008). However, the role of BMP7 in promoting browning of white fat remains unclear. A more recent study identified Zfp423, a BMP-Smad signaling effector, as a transcriptional regulator of both brown and white preadipocyte differentiation (Gupta et al., 2010), although whether Zfp423 plays a role in promoting brown fat features in white fat is unknown.

The molecular signature that we obtain in white fat derived from Smad3-deficient mice and mice treated with the TGF-β neutralization antibody, 1D11, provides interesting insight into the development of brown adipocytes within the white fat milieu. Thus, white fat from $Smad3^{-/-}$ mice and mice treated with 1D11 antibodies, expresses a preponderance of genes that represent brown fat, mitochondrial, and skeletal muscle biology (Yadav et al., 2011). These findings are in contrast to studies where PPAR γ agonist rosiglitazone promotes norepinephrine augmentable UCP1 gene expression in a subset of white adipocyte cells, without concurrent expression of brown fat/muscle-specific markers (Petrovic et al., 2010). Thus, chronic treatment with rosiglitazone promotes the expression of brown fat genes, mitochondriogenesis, and thermogenesis capacity in a significant subset of white fat cells. However, the cells, referred to as "brite" adipocytes, are devoid of transcripts for classic brown adipocytes markers (including PRDM16) or for myocyte-associated genes. We reconcile these observations as evidence of at least two separate pathways that promote browning in the white fat. Rosiglitazone might promote a pool of brown adipocyte precursors that does not share a muscle origin, while the TGFβ1 effect could act on a pool of cells that might potentially represent a common progenitor for white, brown, and muscle cells. Smad3 cooccupies the genome with cell type specific master transcription factors, including Myod1 in myotubes, PU.1 in pro-B cells, and Oct4 in ESCs (Mullen et al., 2011). It is conceivable that in white fat cells, Smad2/3 interacts with factor/s that specify and maintain cell identity and cellular function. This would also provide plausible explanation for the increased expression of brown fat and skeletal muscle-specific genes in $Smad3^{-/-}$ white fat.

THERAPEUTIC RELEVANCE

Discovering approaches to either prevent fat storage or promote fat dissipation will have a major clinical impact (Tseng et al., 2010). Human white adipocytes have the potential to acquire brown fat cell features (Tiraby et al., 2003). Our findings support the notion that promoting brown adipocyte like features in white fat might favorably alter energy balance and protect from obesity and diabetes (Tiraby and Langin, 2003). Further, our anti-TGF-β antibody studies strongly suggest that this strategy has translational potential in treatment of human obesity and diabetes. Similarly, the role of neuronal signaling regulators, NPY, and BDNF, have been elucidated in the promotion of browning of white fat (Cao et al., 2011; Chao et al., 2011) making these factors amenable for consideration as therapeutic targets. Importantly, the fact that simple changes in the environment promotes hypothalamic BDNF expression and leads to browning of white fat and increased energy dissipation (Cao et al., 2011) is of particular relevance with respect to behavioral modulation as a means to manage and treat obesity and diabetes. Interestingly, nicotine induces browning of white fat, along with associated histological and molecular characteristics, decreases food intake and protects from obesity (Yoshida et al., 1999) thus presenting a potential anti-obesity modality. Furthermore, evidence that prostaglandins, cyclooxygenase 2, and retinoic acid mediated pathways promote the accumulation of brown adipocyte like cells in traditional white fat depots (Madsen et al., 2010; Vegiopoulos et al., 2010; Mercader et al., 2006) argues for utility of these molecules and their downstream effectors as candidate therapeutic targets. Finally, considering its ability to induce browning of white fat and improving glucose homeostasis, the hormone irisin can now be added to list of therapeutic candidates for metabolic disease (Bostrom et al., 2012).

As elevated TGF-β levels are common in many disease conditions, there is a push to develop TGF-β antagonist therapies, including TGF-β neutralization antibodies and small molecule TGF-β receptor antagonists (Yingling et al., 2004). Indeed, such regimens are being clinically evaluated for diseases, such as cancer, fibrosis, scarring, diabetic nephropathy, where elevated TGF-β levels are implicated (Massague et al., 2000; Rane et al., 2006; Massague, 2008). Findings that TGF-β1 levels are elevated with obesity, taken together with our illustration of beneficial effects of the anti-TGFβ neutralization in mouse models of obesity and diabetes, provide a rationale to consider anti-TGF-β modalities for these diseases. The efficacy of 1D11 (α-TGF-β) has been tested in preclinical disease models (Ling et al., 2003; Nam et al., 2008) and a closely related human version of this antibody, designated Fresolimumab, is currently under evaluation in human clinical studies of pulmonary fibrosis, renal disease, and cancer. However, the TGF-β family proteins engage specific receptors in virtually every cell type (Roberts and Sporn, 1985). In addition to the canonical TGF-β/Smad signaling node, cross-talk with other signaling networks is a common feature of TGF-β signals (Derynck and Zhang, 2003) and inhibiting the TGF-β pathway at the ligand–receptor level may damage essential signaling networks that cross-talk with TGF-β.

Although we propose that modulation of TGF-β/Smad3 signaling activates a brown adipocyte like phenotype in rodent white fat, its implication in browning of human white fat is unclear at this time. Further, understanding the molecular pathways of TGF-β/Smad3 signaling as it pertains to appearance of brown adipocytes in white fat depots is essential to decipher mechanistic details. Does TGF-β/Smad3 signaling affect transdifferentiation or precursor cell dynamics as it induces browning of white fat? Which white fat depots are the primary targets, and how do the TGF-β/Smad3 signals harmonize with other molecules, hormones, and pathways that also regulate browning of white fat? Answers to these questions, while yielding greater mechanistic insight, will uncover novel targets for development of rational therapeutics to counter obesity and diabetes.

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REFERENCES

Ahmadian, M., Abbott, M. J., Tang, T., Hudak, C. S., Kim, Y., Bruss, M., Hellerstein, M. K., Lee, H. Y., Samuel, V. T., Shulman, G. I., Wang, Y., Duncan, R. E., Kang, C., and Sul, H. S. (2011). Desnutrin/ATGL is regulated by AMPK and is required for a brown adipose phenotype. *Cell Metab.* 13, 739–748.

Atit, R., Sgaier, S. K., Mohamed, O. A., Taketo, M. M., Dufort, D., Joyner, A. L., Niswander, L., and Conlon, R. A. (2006). Beta-catenin activation is necessary and sufficient to specify the dorsal dermal fate in the mouse. *Dev. Biol.* 296, 164–176.

Barbatelli, G., Murano, I., Madsen, L., Hao, Q., Jimenez, M., Kristiansen, K., Giacobino, J. P., de Matteis, R., and Cinti, S. (2010). The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. *Am. J. Physiol. Endocrinol. Metab.* 298, E1244–E1253.

Bloom, J. D., Dutia, M. D., Johnson, B. D., Wissner, A., Burns, M. G., Largis, E. E., Dolan, J. A., and Claus, T. H. (1992). Disodium (R,R)-5-[2-[[2-(3-chlorophenyl)-2-hydroxyethyl]-amino] propyl]-1,3-benzodioxole-2,2-dicarboxylate (CL 316,243). A potent beta-adrenergic agonist virtually specific for beta 3 receptors. A promising antidiabetic and antiobesity agent. *J. Med. Chem.* 35, 3081–3084.

Bostrom, P., Wu, J., Jedrychowski, M. P., Korde, A., Ye, L., Lo, J. C., Rasbach, K. A., Bostrom, E. A., Choi, J. H., Long, J. Z., Kajimura, S., Zingaretti, M. C., Vind, B. F., Tu, H., Cinti, S., Hojlund, K., Gygi, S. P., and Spiegelman, B. M. (2012). A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* 481, 463–468.

Cao, L., Choi, E. Y., Liu, X., Martin, A., Wang, C., Xu, X., and During, M. J. (2011). White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic-adipocyte axis. *Cell Metab.* 14, 324–338.

Cederberg, A., Gronning, L. M., Ahren, B., Tasken, K., Carlsson, P., and Enerback, S. (2001). FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. *Cell* 106, 563–573.

Chao, P. T., Yang, L., Aja, S., Moran, T. H., and Bi, S. (2011). Knockdown of NPY expression in the promotes development of brown adipocytes and prevents diet-induced obesity. *Cell Metab.* 13, 573–583.

Cinti, S. (2011). Between brown and white: novel aspects of adipocyte differentiation. *Ann. Med.* 43, 104–115.

Collins, S., Daniel, K. W., Petro, A. E., and Surwit, R. S. (1997). Strain-specific response to beta 3-adrenergic receptor agonist treatment of diet-induced obesity in mice. *Endocrinology* 138, 405–413.

Cousin, B., Cinti, S., Morroni, M., Raimbault, S., Ricquier, D., Penicaud, L., and Casteilla, L. (1992). Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization. *J. Cell. Sci.* 103(Pt 4), 931–942.

Crunkhorn, S. (2011). Metabolic disease: turning 'bad' fat into 'good.' *Nat. Rev. Drug Discov.* 10, 659.

Cypess, A. M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A. B., Kuo, F. C., Palmer, E. L., Tseng, Y. H., Doria, A., Kolodny, G. M., and Kahn, C. R. (2009). Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* 360, 1509–1517.

Derynck, R., and Zhang, Y. E. (2003). Smad-dependent and Smad-independent pathways in TGF-beta family signalling. *Nature* 425, 577–584.

Enerback, S. (2010). Human brown adipose tissue. *Cell Metab.* 11, 248–252.

Frontini, A., and Cinti, S. (2010). Distribution and development of brown adipocytes in the murine and human adipose organ. *Cell Metab.* 11, 253–256.

Gesta, S., Tseng, Y. H., and Kahn, C. R. (2007). Developmental origin of fat: tracking obesity to its source. *Cell* 131, 242–256.

Ghorbani, M., Claus, T. H., and Himms-Hagen, J. (1997). Hypertrophy of brown adipocytes in brown and white adipose tissues and reversal of diet-induced obesity in rats treated with a beta3-adrenoceptor agonist. *Biochem. Pharmacol.* 54, 121–131.

Ghorbani, M., and Himms-Hagen, J. (1997). Appearance of brown adipocytes in white adipose tissue during CL 316,243-induced reversal of obesity and diabetes in Zucker fa/fa rats. *Int. J. Obes. Relat. Metab. Disord.* 21, 465–475.

Grujic, D., Susulic, V. S., Harper, M. E., Himms-Hagen, J., Cunningham, B. A., Corkey, B. E., and Lowell, B. B. (1997). Beta3-adrenergic receptors on white and brown adipocytes mediate beta3-selective agonist-induced effects on energy expenditure, insulin secretion, and food intake. A study using transgenic and gene knockout mice. *J. Biol. Chem.* 272, 17686–17693.

Guerra, C., Koza, R. A., Yamashita, H., Walsh, K., and Kozak, L. P. (1998). Emergence of brown adipocytes in white fat in mice is under genetic control. Effects on body weight and adiposity. *J. Clin. Invest.* 102, 412–420.

Gupta, R. K., Arany, Z., Seale, P., Mepani, R. J., Ye, L., Conroe, H. M., Roby, Y. A., Kulaga, H., Reed, R. R., and Spiegelman, B. M. (2010). Transcriptional control of preadipocyte determination by Zfp423. *Nature* 464, 619–623.

Hansen, J. B., Jorgensen, C., Petersen, R. K., Hallenborg, P., de Matteis, R., Boye, H. A., Petrovic, N., Enerback, S., Nedergaard, J., Cinti, S., te Riele, H., and Kristiansen, K. (2004). Retinoblastoma protein functions as a molecular switch determining white versus brown adipocyte differentiation. *Proc. Natl. Acad. Sci. U.S.A.* 101, 4112–4117.

Himms-Hagen, J., Cui, J., Danforth, E. Jr., Taatjes, D. J., Lang, S. S., Waters, B. L., and Claus, T. H. (1994). Effect of CL-316,243, a thermogenic beta 3-agonist, on energy balance and brown and white adipose tissues in rats. *Am. J. Physiol.* 266, R1371–R1382.

Himms-Hagen, J., Melnyk, A., Zingaretti, M. C., Ceresi, E., Barbatelli, G., and Cinti, S. (2000). Multilocular fat cells in WAT of CL-316243-treated rats derive directly from white adipocytes. *Am. J. Physiol. Cell Physiol.* 279, C670–C681.

Hondares, E., Rosell, M., Diaz-Delfin, J., Olmos, Y., Monsalve, M., Iglesias, R., Villarroya, F., and Giralt, M. (2011). PPARalpha induces PGC-1alpha gene expression and contributes to the thermogenic activation of brown fat; involvement of PRDM16. *J. Biol. Chem.* 296, 43112–43122.

Jimenez, M., Barbatelli, G., Allevi, R., Cinti, S., Seydoux, J., Giacobino, J. P., Muzzin, P., and Preitner, F. (2003). Beta 3-adrenoceptor knockout in C57BL/6J mice depresses the occurrence of brown adipocytes in white fat. *Eur. J. Biochem.* 270, 699–705.

Kang, S., Bajnok, L., Longo, K. A., Petersen, R. K., Hansen, J. B., Kristiansen, K., and Macdougald, O. A. (2005). Effects of Wnt signaling on brown adipocyte differentiation and metabolism mediated by PGC-1alpha. *Mol. Cell. Biol.* 25, 1272–1282.

Langin, D. (2009). Recruitment of brown fat and conversion of white into brown adipocytes: strategies to fight the metabolic complications of obesity? *Biochim. Biophys. Acta* 1801, 372–376.

Lefterova, M. I., and Lazar, M. A. (2009). New developments in adipogenesis. *Trends Endocrinol. Metab.* 20, 107–114.

Ling, H., Li, X., Jha, S., Wang, W., Karetskaya, L., Pratt, B., and Ledbetter, S. (2003). Therapeutic role of TGF-beta-neutralizing antibody in mouse cyclosporine A nephropathy: morphologic improvement associated with functional preservation. *J. Am. Soc. Nephrol.* 14, 377–388.

Loncar, D. (1991). Convertible adipose tissue in mice. *Cell Tissue Res.* 266, 149–161.

Madsen, L., Pedersen, L. M., Lillefosse, H. H., Fjaere, E., Bronstad, I., Hao, Q., Petersen, R. K., Hallenborg, P., Ma, T., de Matteis, R., Araujo, P., Mercader, J., Bonet, M. L., Hansen, J. B., Cannon, B., Nedergaard, J., Wang, J., Cinti, S., Voshol, P., Doskeland, S. O., and Kristiansen, K. (2010). UCP1 induction during recruitment of brown adipocytes in white adipose tissue is dependent on cyclooxygenase activity. *PLoS ONE* 5, e11391. doi:10.1371/journal.pone.0011391

Massague, J. (2008). TGFbeta in cancer. *Cell* 134, 215–230.

Massague, J., Blain, S. W., and Lo, R. S. (2000). TGFbeta signaling in growth control, cancer, and heritable disorders. *Cell* 103, 295–309.

Mercader, J., Ribot, J., Murano, I., Felipe, F., Cinti, S., Bonet, M. L., and Palou, A. (2006). Remodeling of white adipose tissue after retinoic acid administration in mice. *Endocrinology* 147, 5325–5332.

Mullen, A. C., Orlando, D. A., Newman, J. J., Loven, J., Kumar, R. M., Bilodeau, S., Reddy, J., Guenther, M. G., Dekoter, R. P., and Young, R. A. (2011). Master transcription factors determine cell-type-specific responses to TGF-beta signaling. *Cell* 147, 565–576.

Nam, J. S., Terabe, M., Mamura, M., Kang, M. J., Chae, H., Stuelten, C., Kohn, E., Tang, B., Sabzevari, H., Anver, M. R., Lawrence, S., Danielpour, D., Lonning, S., Berzofsky, J. A., and Wakefield, L. M. (2008). An anti-transforming growth factor beta antibody suppresses metastasis via cooperative effects on multiple cell compartments. *Cancer Res.* 68, 3835–3843.

Nedergaard, J., Bengtsson, T., and Cannon, B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. *Am. J. Physiol. Endocrinol. Metab.* 293, E444–E452.

Nedergaard, J., and Cannon, B. (2010). The changed metabolic world with human brown adipose tissue: therapeutic visions. *Cell Metab.* 11, 268–272.

Park, K. W., Halperin, D. S., and Tontonoz, P. (2008). Before they were fat: adipocyte progenitors. *Cell Metab.* 8, 454–457.

Petrovic, N., Walden, T. B., Shabalina, I. G., Timmons, J. A., Cannon, B., and Nedergaard, J. (2010). Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. *J. Biol. Chem.* 285, 7153–7164.

Rane, S. G., Lee, J. H., and Lin, H. M. (2006). Transforming growth factor-beta pathway: role in pancreas development and pancreatic disease. *Cytokine Growth Factor Rev.* 17, 107–119.

Roberts, A. B., and Sporn, M. B. (1985). Transforming growth factors. *Cancer Surv.* 4, 683–705.

Schulz, T. J., Huang, T. L., Tran, T. T., Zhang, H., Townsend, K. L., Shadrach, J. L., Cerletti, M., Mcdougall, L. E., Giorgadze, N., Tchkonia, T., Schrier, D., Falb, D., Kirkland, J. L., Wagers, A. J., and Tseng, Y. H. (2011). Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. *Proc. Natl. Acad. Sci. U.S.A.* 108, 143–148.

Scime, A., Grenier, G., Huh, M. S., Gillespie, M. A., Bevilacqua, L., Harper, M. E., and Rudnicki, M. A. (2005). Rb and p107 regulate preadipocyte differentiation into white versus brown fat through repression of PGC-1alpha. *Cell Metab.* 2, 283–295.

Seale, P., Bjork, B., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scime, A., Devarakonda, S., Conroe, H. M., Erdjument-Bromage, H., Tempst, P., Rudnicki, M. A., Beier, D. R., and Spiegelman, B. M. (2008). PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454, 961–967.

Seale, P., Kajimura, S., and Spiegelman, B. M. (2009). Transcriptional control of brown adipocyte development and physiological function – of mice and men. *Genes Dev.* 23, 788–797.

Soloveva, V., Graves, R. A., Rasenick, M. M., Spiegelman, B. M., and Ross, S. R. (1997). Transgenic mice overexpressing the beta 1-adrenergic receptor in adipose tissue are resistant to obesity. *Mol. Endocrinol.* 11, 27–38.

Timmons, J. A., Wennmalm, K., Larsson, O., Walden, T. B., Lassmann, T., Petrovic, N., Hamilton, D. L., Gimeno, R. E., Wahlestedt, C., Baar, K., Nedergaard, J., and Cannon, B. (2007). Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. *Proc. Natl. Acad. Sci. U.S.A.* 104, 4401–4406.

Tiraby, C., and Langin, D. (2003). Conversion from white to brown adipocytes: a strategy for the control of fat mass? *Trends Endocrinol. Metab.* 14, 439–441.

Tiraby, C., Tavernier, G., Lefort, C., Larrouy, D., Bouillaud, F., Ricquier, D., and Langin, D. (2003). Acquirement of brown fat cell features by human

white adipocytes. *J. Biol. Chem.* 278, 33370–33376.

Tseng, Y. H., Cypess, A. M., and Kahn, C. R. (2010). Cellular bioenergetics as a target for obesity therapy. *Nat. Rev. Drug Discov.* 9, 465–482.

Tseng, Y. H., Kokkotou, E., Schulz, T. J., Huang, T. L., Winnay, J. N., Taniguchi, C. M., Tran, T. T., Suzuki, R., Espinoza, D. O., Yamamoto, Y., Ahrens, M. J., Dudley, A. T., Norris, A. W., Kulkarni, R. N., and Kahn, C. R. (2008). New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454, 1000–1004.

van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, J. M., Kemerink, G. J., Bouvy, N. D., Schrauwen, P., and Teule, G. J. (2009). Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* 360, 1500–1508.

Vegiopoulos, A., Muller-Decker, K., Strzoda, D., Schmitt, I., Chichelnitskiy, E., Ostertag, A., Berriel Diaz, M., Rozman, J., Hrabe de Angelis, M., Nusing, R. M., Meyer, C. W., Wahli, W., Klingenspor, M., and Herzig, S. (2010). Cyclooxygenase-2 controls energy homeostasis in mice by de novo recruitment of brown adipocytes. *Science* 328, 1158–1161.

Vernochet, C., Peres, S. B., Davis, K. E., Mcdonald, M. E., Qiang, L., Wang, H., Scherer, P. E., and Farmer, S. R. (2009). C/EBPalpha and the corepressors CtBP1 and CtBP2 regulate repression of select visceral white adipose genes during induction of the brown phenotype in white adipocytes by peroxisome proliferator-activated receptor gamma agonists. *Mol. Cell. Biol.* 29, 4714–4728.

Virtanen, K. A., Lidell, M. E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N. J., Enerback, S., and Nuutila, P. (2009). Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* 360, 1518–1525.

Walden, T. B., Hansen, I. R., Timmons, J. A., Cannon, B., and Nedergaard, J. (2011). Recruited versus non-recruited molecular signatures of brown, "brite" and white adipose tissues. *Am. J. Physiol. Endocrinol. Metab.* 302, E19–E31.

Xue, B., Rim, J. S., Hogan, J. C., Coulter, A. A., Koza, R. A., and Kozak, L. P. (2007). Genetic variability affects the development of brown adipocytes in white fat but not in interscapular brown fat. *J. Lipid Res.* 48, 41–51.

Yadav, H., Quijano, C., Kamaraju, A. K., Gavrilova, O., Malek, R., Chen, W., Zerfas, P., Zhigang, D., Wright, E. C., Stuelten, C., Sun, P., Lonning, S., Skarulis, M., Sumner, A. E., Finkel, T., and Rane, S. G. (2011). Protection from obesity and diabetes by blockade of TGF-beta/Smad3 signaling. *Cell Metab.* 14, 67–79.

Yingling, J. M., Blanchard, K. L., and Sawyer, J. S. (2004). Development of TGF-beta signalling inhibitors for cancer therapy. *Nat. Rev. Drug Discov.* 3, 1011–1022.

Yoshida, T., Sakane, N., Umekawa, T., Kogure, A., Kondo, M., Kumamoto, K., Kawada, T., Nagase, I., and Saito, M. (1999). Nicotine induces uncoupling protein 1 in white adipose tissue of obese mice. *Int. J. Obes. Relat. Metab. Disord.* 23, 570–575.

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Differentiation of human adipose-derived stem cells into "brite" (brown-in-white) adipocytes

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It is well established now that adult humans possess active brown adipose tissue (BAT) which represents a potential pharmacological target to combat obesity and associated diseases. Moreover thermogenic brown-like adipocytes ("brite adipocytes") appear also in mouse white adipose tissue (WAT) upon β3-adrenergic stimulation. We had previously shown that human multipotent adipose-derived stem cells (hMADS) are able to differentiate into cells which exhibit the key properties of human white adipocytes, and then to convert into functional brown adipocytes upon PPARγ activation. In light of a wealth of data indicating that thermogenic adipocytes from BAT and WAT have a distinct cellular origin, we have characterized at the molecular level UCP1 positive hMADS adipocytes from both sexes as brite adipocytes. Conversion of white to brown hMADS adipocytes is dependent on PPARγ activation with rosiglitazone as the most potent agonist and is inhibited by a PPARγ antagonist. In contrast to mouse cellular models, hMADS cells conversion into brown adipocytes is weakly induced by BMP7 treatment and not modulated by activation of the Hedgehog pathway. So far no primary or clonal precursor cells of human brown adipocytes have been obtained that can be used as a tool to develop therapeutic drugs and to gain further insights into the molecular mechanisms of brown adipogenesis in humans. Thus hMADS cells represent a suitable human cell model to delineate the formation and/or the uncoupling capacity of brown/brite adipocytes that could help to dissipate caloric excess intake among individuals.

Keywords: stem cells, rosiglitazone, adipocyte, differentiation, UCP1, brite adipocyte, BAT, WAT

INTRODUCTION

Obesity has reached epidemic proportions globally, with more than one billion adults overweight and at least 300 million of them clinically obese. In addition, at least 155 million children worldwide are overweight or obese, according to the International Obesity Task Force (Hossain et al., 2007). Obesity constitutes a substantial risk factor for hypertension, type 2 diabetes, and cardiovascular diseases implying tremendous burdens for the public health care system. White adipose tissue (WAT) plays a central role in the control of energy homeostasis (Ailhaud et al., 1992; Rosen and Spiegelman, 2006). In contrast to WAT, brown adipose tissue (BAT) is specialized in adaptive thermogenesis in which the uncoupling protein 1 (UCP1) plays a key role (Golozoubova et al., 2006). The involvement of BAT in diet-induced thermogenesis has been recently shown in UCP1-ablated mice raised at thermoneutrality (Feldmann et al., 2009). Of utmost interest, recent data show that active BAT is indeed present as discrete and small depots in healthy adult individuals (Nedergaard et al., 2007; Cypess et al., 2009; Saito et al., 2009; Van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009; Zingaretti et al., 2009) and represents a new target to fight obesity (Fruhbeck et al., 2009; Whittle et al., 2011).

At the cellular level, a myogenic signature of brown adipocytes and cell sorting of muscle and WAT derived progenitors favor a distinct origin from that of white adipocytes (Timmons et al., 2007; Crisan et al., 2008). *In vivo* lineage studies showed that brown adipocytes from brown-fat depots share a common developmental origin with myoblasts (Seale et al., 2008). However, islands of brown adipocytes are also detected in white fat depots of rodents following chronic exposure to cold or pharmacological stimulation of β3-adrenoreceptor (β3-AR). There is indeed evidence that white adipocytes can be converted to brown-like fat cells (Tiraby et al., 2003; Cinti, 2009a,b; Barbatelli et al., 2010). Within traditional WAT depots, a particular type of adipocytes occurs in rodents, corresponding to the "brite" (brown-in-white) adipocytes. These cells express UCP1 and exhibit *in vitro* a thermogenic response to β-AR agonists (Petrovic et al., 2010) that could explain UCP1 expressing cells present as islets surrounded by white adipocytes (Cousin et al., 1992; Xue et al., 2009). Most importantly, *in vivo*, the capacity of obese mice to reduce their fat mass in response to adrenergic stimulation by cold or β3-AR agonist does not depend upon BAT whose amount is genetically invariant among mouse strains but depends upon a genetic variability which affects the development of "brite" adipocytes within

WAT depots (Guerra et al., 1998; Xue et al., 2007). Other external cues are favoring the brown-fat differentiation, e.g., Hedgehog signaling has been characterized as a determinant of brown-fat cell fate (Pospisilik et al., 2010) whereas among bone morphogenetic proteins (BMPs), BMP7 specifically promotes brown adipogenesis of murine multipotent mesenchymal stem cells (Tseng et al., 2008; Schulz et al., 2011).

We have reported the characterization of mesenchymal stem cells from human adipose tissue of young male and female donors [termed human multipotent adipose-derived stem cells (hMADS)] which exhibit at a clonal level a normal karyotype, self-renewal ability, the absence of tumorigenicity (Rodriguez et al., 2004, 2005a; Zaragosi et al., 2006; Elabd et al., 2007; Fontaine et al., 2008), and are able to convert into functional brown-like adipocytes (Elabd et al., 2009). Owing to recent data showing that thermogenic adipocytes from BAT and WAT originate from distinct origin, the question raised whether UCP1-positive hMADS adipocytes from both sexes are classical brown or "brite" adipocytes. Herein we provide evidence at the molecular level that these cells share similarities with mouse brite adipocytes. However, in contrast to mouse cellular models, brown adipogenesis is only slightly induced by BMP7 treatment and not modulated by activation of the Hedgehog pathway. Thus hMADS cells represent a suitable human cell model to gain insights in the formation of brite adipocytes that could help to dissipate caloric excess intake.

MATERIALS AND METHODS

REAGENTS

Cell culture media, serum, buffers, and trypsin were purchased from Lonza Verviers (Verviers, Belgium) and cell culture reagents from Sigma-Aldrich Chimie (Saint-Quentin Fallavier, France). Rosiglitazone was purchased from BertinPharma (Montigny le bretonneux, France).

CELL CULTURE

The establishment and characterization of the multipotency and self-renewal capacity of hMADS cells have been described (Rodriguez et al., 2004, 2005b; Elabd et al., 2007, 2009). In the experiments reported herein hMADS-1, 2, and 3 cells, established respectively, from the umbilical fat pad of a 31-month-old female donor, from the pubic region fat pad of a 5-year-old male donor and the prepubic fat pad of a 4-month-old male, were used between passages 16 and 35 corresponding from 35 to 100 population doublings. Cells were seeded at a density of 4500 cells/cm^2 in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS, 2.5 ng/ml hFGF2, 60 μg/ml penicillin, and 50 μg/ml streptomycin. The medium was changed every other day and hFGF2 was removed when cells reached confluence and were triggered for differentiation at day 2 post-confluence (designated as day 0). Cells were then maintained in DMEM/Ham's F12 media supplemented with 10 μg/ml transferrin, 0.85 μM insulin, 0.2 nM triiodothyronine, 1 μM dexamethasone, 500 μM isobutyl-methylxanthine. Three days later, the medium was changed (dexamethasone and isobutyl-methylxanthine were omitted) and 100 nM rosiglitazone were added for the indicated periods. Media were then changed every other day and cells used at the indicated days. Glycerol-3-phosphate dehydrogenase (GPDH) activity measurements and Oil Red O staining were performed as described previously (Negrel et al., 1978; Bezy et al., 2005).

ISOLATION AND ANALYSIS OF RNA

Total RNA was extracted using TRI-Reagent kit (Euromedex, Souffelweyersheim, France) according to the manufacturer's instructions. Quality control for purity and integrity of RNA were tested by OD (260/280 nM) measurements and ethidium bromide-stained agarose analysis. Reverse transcription-polymerase chain reaction (RT-PCR) analysis was conducted as described previously (Rodriguez et al., 2004; Bezy et al., 2005; Zaragosi et al., 2006; Elabd et al., 2007). Primer sequences, designed using Primer Express software (Applied Biosystems, Courtaboeuf, France), are listed in **Table 1** and were tested for their specificity, efficiency, reproducibility, and dynamic range. For quantitative PCR, final reaction volume was 20 μl using SYBR green master mix (Eurogentec, Angers, France) and assays were run on an ABI Prism 7700 real-time PCR machine (PerkinElmer Life and Analytical Sciences, Boston, MA, USA). An aliquot of PCR products was analyzed on 2% ethidium bromide-stained agarose. The expression of selected genes was normalized to that of TATA-box binding protein (TBP) gene and quantified using the comparative-ΔCt method. TBP expression did not vary along the adipocyte differentiation of hMADS cells; we used also 36B4 and POLR2A genes as housekeeping genes which gave similar data. Human skeletal muscle RNA extracts were obtained from a previous study (Pisani et al., 2010).

MITOCHONDRIA ANALYSIS

Living cells were submitted to 100 nM MitoTracker Red FM (Invitrogen) for 45 min at 37°C. Cells were finally washed with pre-warmed culture medium and visualized with an Axiovert microscope (Carl Zeiss, Le Pecq, France) and pictures were captured and treated with AxioVision software (Carl Zeiss).

CYTOCHROME C OXIDASE ACTIVITY DETERMINATIONS

Cells were disrupted using a polytron in 10 mM Tris pH 8, 1 mM EDTA, and 0.25 M Sucrose containing protease inhibitors. Cell lysate was centrifuged at 750 *g* for 10 min with a pellet corresponding to a nuclear-enriched fraction. The supernatant was then centrifuged at 10 000 *g* for 20 min, the pellet corresponding to a mitochondrial-enriched fraction. The mitochondrial-enriched fraction was used to determine Cytochrome c Oxidase activity according to manufacturer's instructions (Cytochrome c Oxidase Assay kit, Sigma).

STATISTICAL ANALYSIS

Data are expressed as mean values ± SEM and are analyzed using the 2-tailed Student's *t*-test. Differences were considered statistically significant at $P \leq 0.05$.

RESULTS

UCP1, 2, AND 3 EXPRESSION DURING HMADS CELL DIFFERENTIATION

We showed previously that hMADS cells were able to differentiate into white and brown adipocytes depending on the length of activation of PPARγ by rosiglitazone (Elabd et al., 2009). For hMADS-1 cells, originally established from the umbilical fat pad

Table 1 | Sequence of primers used for gene expression analysis.

Gene	Complete name	Forward primer	Reverse primer	Accession N°
UCP1	Uncoupling protein 1	GTGTGCCCAACTGTGCAATG	CCAGGATCCAAGTCGCAAGA	NM021833
UCP2	Uncoupling protein 2	GGCCTCACCGTGAGACCTTAC	TGGCCTTGAACCCAACCAT	NM003355
UCP3	Uncoupling protein 3	TCAGCCCCCTCGACTGTATG	ACTTTCATCAGGGCCCGTTT	NM003356.3
PPARγ	Peroxisome proliferator activated receptor gamma	AGCCTCATGAAGAGCCTTCCA	TCCGGAAGAAACCCTTGCA	NM005037
PGC-1α	PPARg coactivator 1 alpha	CTGTGTCACCACCCAAATCCTTAT	TGTGTCGAGAAAAGGACCTTGA	NM013261
PGC-1β	PPARg coactivator 1 beta	GCGAGAAGTACGGCTTCATCAC	CAGCGCCCTTTGTCAAAGAG	NM13 3263
PRDM16	PR domain containing 16	GAAACTTTATTGCCAATAGTGAGATGA	CCGTCCACGATCTGCATGT	NM022114
CIDEA	Cell death-inducing DFFA-like effector A	GGCAGGTTCACGTGTGGATA	GAAACACAGTGTTTGGCTCAAGA	NM001279
CPT1B	Carnitine palmitoyltransferase 1B	AAACAGTGCCAGGCGGTC	CGTCTGCCAACGCCTTG	NM_152246
ELOVL3	Fatty acid elongase 3	TTGGACCTTGACTTCTGCAA	GGGCTATGGGGAATGAGG	NM152310.1
AGT	Angiotensinogen	ACCTACGTCCACTTCCAAGG	GTTGTCCACCCAGAACTCCT	NM_00 0029.3
MEOX2	Mesenchyme homeobox 2	AGAGGAAAAGCGACAGCTCA	AAGTTCTCTGATTTGCTCTTTGGT	NM005924.4
ZIC1	Zic family member 1	TGGCGCTCACATTCCTCTAT	GCATCTCAGCCCCCTAAAA	NM_00 3412.3
LHX8	LIM homeobox 8	GTCGGACGTCTGGGTTTG	ACGGAAGAAATAGGGGAAGC	NM001001933.1
CtBP1	C-terminal binding protein 1	GCCTCAACGAGCACAACCA	ACCAGGAAGGCCCCTTGTC	NM001012614.1
CtBP2	C-terminal binding protein 2	CTGGTGGACGAGAAAGCCTTA	GGCTGCCCCTCGTATCCT	NM022802.2
NRIP1	Nuclear receptor interacting protein 1	TTGGAGACAGACGAACACTGA	TCTACGCAAGGAGGAGGAGA	NM_00 3489.3
LXR	Liver-X-receptor	CAGGGCTCCAGAAAGAGATG	ACAGCTCCACCGCAGAGT	NM005693.2
GPBAR1	G protein-coupled bile acid receptor 1	CCCAGGCTATCTTCCCAGC	AGCAGGAGCCCATAGACTTCG	NM001077191.1
DiO2	Deiodinase type 2	GTCACTGGTCAGCGTGGTTTT	TTCTTCACATCCCCCAATCCT	NM 000793.5
COX1	Cyclooxygenase 1	AGCAGCTGAGTGGCTATTTCCT	CCAGTTCCAATACCGCAACCG	NM080591.1
COX2	Cyclooxygenase 2	GAATCATTCACCAGGCAAATTG	TCTGTACTGCGGGTGGAACA	NM_00 0963.2
PTGiS	Prostaglandine I2 synthase	GCCACATAGCTCATAAGCTGTAGAAC	AGTTGCTCATCCAGCATTTGC	NM000961.3
PTGiR	Prostaglandine I2 receptor	AGCCTGGGCAAGACTGGAG	TAGGTGAGGTTCCTGCACGAA	NM_00 0960.3
TPK1	Thiamin pyrophosphokinase 1	CAAGGTGTTCGTGGTTGGC	TTGAGTGAGGGCCTCTGGAC	NM_00 1042482.1
FABP4	Fatty acid binding protein 4	TGTGCAGAAATGGGATGGAAA	CAACGTCCCTTGGCTTATGCT	NM_00 1442.2
ADIPOQ	Adiponectin	GCAGTCTGTGGTTCTGATTCCATAC	GCCCTTGAGTCGTGGTTTCC	NM 00 479 7.3
RARRES2	Chemerin	GGAATATTTGTGAGGCTGGAA	CAGGCATTTCCGTTTCCTC	NM002889.3
PANK3	Pantothenate kinase 3	CATTGCAGACGGTGCTATGT	GTTTGTGCAGGTGGAGGTTT	NM024594.3
HOXC9	Homeobox C9	CAGCAAGCACAAAGAGGAGA	CGACGGTCCCTGGTTAAATAC	NM006897.1
DPT	Dermatopontin	CGAGGAGCAACAACCACTTT	CGGCACATTATGAACTTCCA	NM001937.4
LEP	Leptin	AGGGAGACCGAGCGCTTTC	TGCATCTCCACACACCAAACC	NM_00 0230.2
Gli1	Glioma-associated oncogene homolog 1	TGCAGTAAAGCCTTCAGCAATG	TTTTCGCAGCGAGCTAGGAT	NM001167609.1
Myf5	Myogenic factor 5	GATCACCTCCTCAGAGCAACCT	GTGCTGGCAACTGGAGAGAGA	NM005593.2
TBP	TATA-box binding protein	CACGAACCACGGCACTGATT	TTTTCTTGCTGCCAGTCTGGAC	NM003194

of a 31-month-old female donor, a 6-day exposure to rosiglitazone (between days 3 and 9) proved to be optimal for the cells to become white adipocytes. As shown in **Figures 1A,B**, further exposure of the cells to rosiglitazone did not alter the overall adipogenesis level *per se* since treatment between days 3 and 16 brought no change in triglyceride accumulation (Oil Red O staining) and GPDH activity. Under this condition, as shown in **Figure 1C**, a dramatic increase in the expression of UCP1 mRNA was observed. Interestingly, when hMADS-1 cells were first differentiated as above into white adipocytes and rosiglitazone being removed for the next 5 days, a further 2-day rosiglitazone exposure (between days 14 and 16) was sufficient to stimulate the expression of UCP1 (**Figure 1C**). In order to extend these observations, hMADS-2 and hMADS-3 cells, established respectively from the pubic fat pad of a 5-year-old male donor and the prepubic fat pad of a 4-month-old male, were used and gave similar results (**Figure 1C**). Under these conditions, UCP1 protein could be detected by immunoblotting analysis (data not shown).

We then measured for the expression of two other members of the UCP family, UCP2 and UCP3. The expression of UCP2 mRNA was induced during adipogenesis of hMADS-1, 2, and 3 cells, and its expression was partially but not significantly enhanced by the duration of rosiglitazone treatment (**Figure 1D**). Interestingly, UCP3 mRNA was barely detected (100–200 times lower as compared to skeletal muscle cells), was not induced during adipogenesis and remained insensitive to rosiglitazone treatment (**Figure 1E**).

UCP1 INDUCTION IS DEPENDENT UPON PPARγ ACTIVATION

Among members of the thiazolidinedione family, rosiglitazone is known as a high-affinity ligand of PPARγ. We therefore carried out a comparative study with other thiazolidinediones, i.e.,

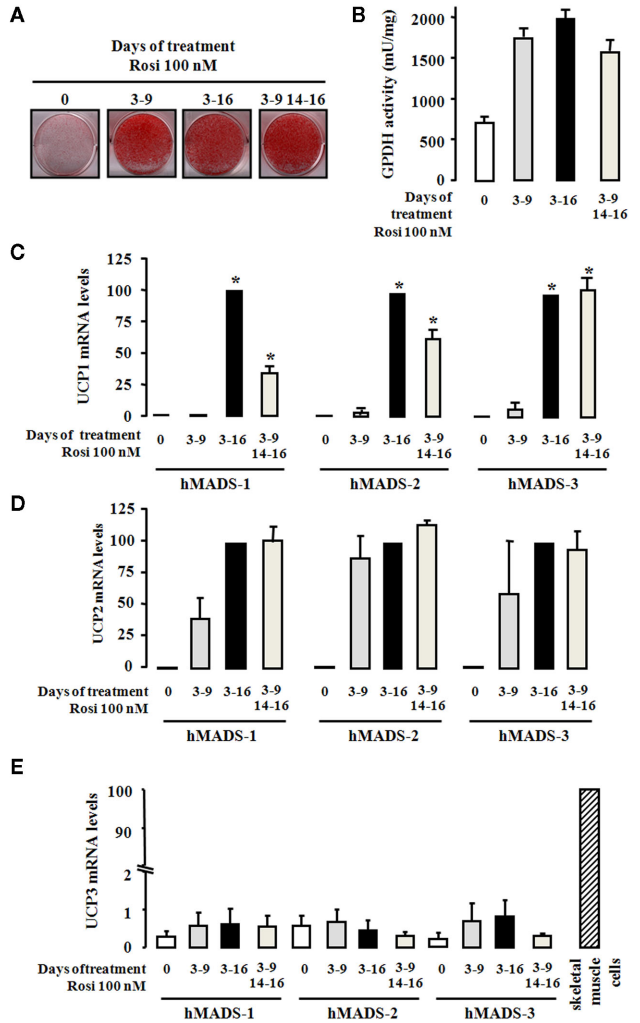

FIGURE 1 | Conversion of hMADS cells into brown- like adipocytes. hMADS-1, 2, and 3 cells were induced to differentiate into adipocytes as described under the Section "Materials and Methods." Hundred nanometer rosiglitazone was added during the indicated periods of treatment. At day 16, **(A)** cells were fixed and stained by Oil Red O, **(B)** GPDH activity was determined, and **(C–E)** UCP1, UCP2, and UCP3 mRNA levels, respectively, were measured by quantitative RT-PCR. Results are mean ± SEM of three **(B,C)** or two **(D,E)** independent experiments. Results in **(C)** and **(D)** are expressed by taking as 100% the value obtained for rosiglitazone treatment between days 3 and 16. In **(E)**, human skeletal muscle cells were used as positive control for UCP3 expression. Results in **(E)** are expressed taking as 100% the value obtained for skeletal muscle cells. $*p < 0.05$ vs. cells treated between days 3 and 9.

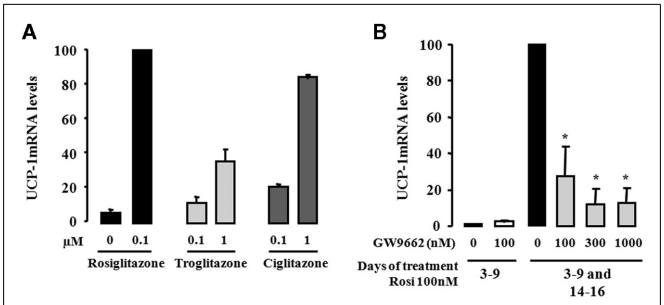

FIGURE 2 | Activation of PPARγ is involved in the conversion of white to brown adipocytes. hMADS-2 cells were induced to differentiate into white adipocytes in the presence of 100 nM rosiglitazone between days 3 and 9, and then treated with various component as indicated between days 14 and 16. **(A)** Effect of various PPARγ agonists (Rosiglitazone, Troglitazone, and Ciglitazone) analyzed by quantitative RT-PCR of UCP1 gene expression. **(B)** Effect of the PPARγ antagonist GW9662 on UCP1 expression stimulated by rosiglitazone. Results are the mean ± SEM of two independent experiments. Results are expressed by taking as 100% the value obtained at 100 nM rosiglitazone. $*p < 0.05$ vs. cells exposed to 100 nM rosiglitazone between days 3–9 and days 14–16.

troglitazone and ciglitazone, with respect to the induction of UCP1 gene expression. For this purpose, hMADS-2 cells were first differentiated into white adipocytes as above, i.e., rosiglitazone treatment between days 3 and 9 followed by its removal for the next 5 days, were then exposed to increasing concentrations of various thiazolidinediones between days 14 and 16. The levels of UCP1 mRNA were analyzed by quantitative RT-PCR at day 16. As shown in **Figure 2A**, troglitazone and ciglitazone were able to induce UCP1 expression in a dose-dependent manner. Clearly, the potency of rosiglitazone was already maximal at a dose where the effects of troglitazone and ciglitazone were weak. In order to gain further insights into the critical role of PPARγ, we tested whether a specific PPARγ antagonist (GW9662) was able to abolish the effect of rosiglitazone on UCP1 expression. As above, hMADS-2 cells were first differentiated into white adipocytes, then treated or not with 100 nM rosiglitazone in the presence or absence of the PPARγ antagonist. As shown in **Figure 2B** the rosiglitazone-induced UCP1 mRNA expression was abolished in a dose-dependent manner by GW9662. Of note, GW9662 showed no effect on untreated hMADS cells. Similar data were observed using hMADS-3 cells (data not shown). Altogether, these observations demonstrated that induction of UCP1 was dependent on PPARγ activation and that rosiglitazone was the most efficient agonist in this process.

CHARACTERIZATION OF MITOCHONDRIOGENESIS IN DIFFERENTIATED hMADS ADIPOCYTES

As abundance of mitochondria is higher in brown as compared to white fat cells, we tested whether mitochondriogenesis was affected in the "browning" process of hMADS cells. Clearly, more mitochondria were observed in brown-like adipocytes as compared to white adipocytes (**Figure 3A**). This observation was further supported by quantitative RT-PCR analysis of CPT1B, a fatty acid transporter of the outer mitochondrial membrane (**Figure 3B**). Under these conditions, CPT1B mRNA levels were 10-fold higher in brown-like adipocytes as compared to white adipocytes. Furthermore, the increase of CPT1B mRNA levels upon rosiglitazone treatment was similar to that observed for UCP1 (**Figure 1C**).

Finally, we measured the Cytochrome c oxidase activity in this fraction as an index of respiratory chain activity. As expected, Cytochrome c oxidase activity was higher in UCP1-containing fractions from hMADS cells chronically treated as compared to cells treated between days 3 and 9 (**Figure 3C**). Thus, hMADS

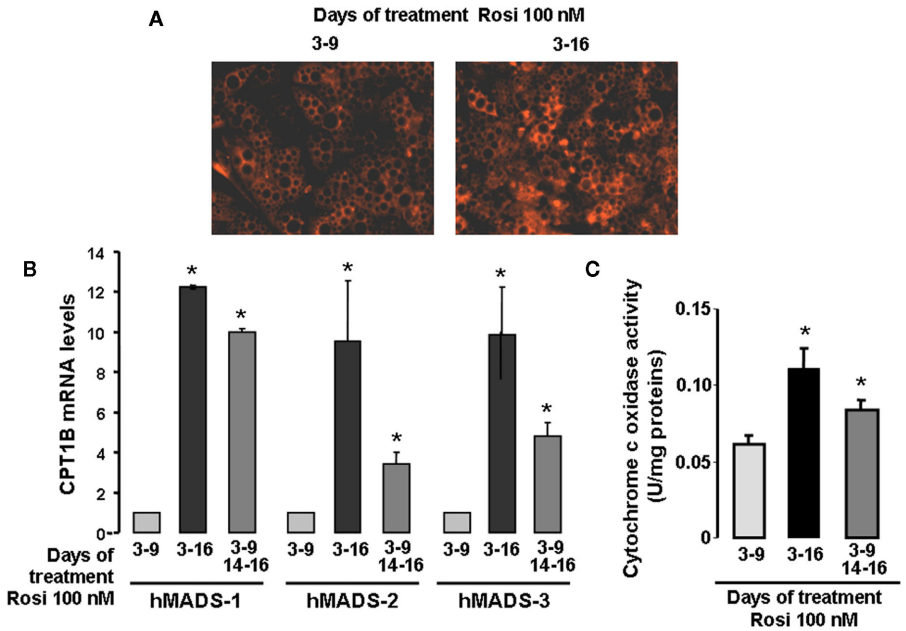

FIGURE 3 | Mitochondriogenesis of hMADS cells after conversion to brown-like adipocytes. hMADS-2 cells were induced to differentiate into adipocytes in the presence of 100 nM rosiglitazone between days 3 and 9 or between days 3 and 16. Alternatively, after removal at day 9, rosiglitazone was re-added or not at day 14 for 2 days. **(A)** Mitochondria visualized by epifluorescence in living cells in the presence of the mitotracker dye, **(B)** CPT1B mRNA levels determined at day 16 by quantitative RT-PCR in hMADS-1, 2, and 3 cells and **(C)** Cytochrome c oxidase activity determined at day 16. Results are mean ± SEM **(B,C)** and are representative experiments **(A)** of three independent experiments performed on different series of cells. $*p < 0.05$ vs. cells treated between days 3 and 9.

adipocytes expressing UCP1 display a significant increase in mitochondriogenesis accompanied by an increase in their respiratory activity; both indicating the acquisition of a brown-like phenotype. As brown-like adipocytes arise from white adipocytes, brown-like adipocytes can be defined at first sight under our conditions as brite adipocytes.

ANALYSIS OF GENE EXPRESSION IN hMADS-DERIVED BRITE ADIPOCYTES

Recently, studies of mouse model have described genes with differential expression in white vs. brown adipocytes (Vernochet et al., 2009; Petrovic et al., 2010). Therefore we aimed at analyzing the expression of a large set of these genes in white vs. brown-like hMADS adipocytes.

First, we analyzed the expression of Myf5 known to be a molecular signature common to brown-fat and skeletal muscle cells (Seale et al., 2008). Myf5 expression was not detectable in proliferating or differentiated hMADS cells originating from three donors (data not shown).

Table 2 summarized the expression values (ΔCt referred to TBP) of different genes in hMADS cells at day 9 of adipocyte differentiation as well as day 16 of white and brite differentiation of hMADS-2 and 3 cells. White and brite hMADS adipocytes expressed classical adipogenic markers (FABP4, ADIPOQ, RARRES2, PANK3, PPARγ, and low level of LXR) at similar levels. In addition, hMADS brite adipocytes expressed UCP1, CPT1B, CIDEA, and ELOVL3, four genes found to be highly expressed in mouse brite adipocytes (Petrovic et al., 2010). Conversely, these cells did not express ZIC1 and LHX8, two genuine brown adipocyte specific genes (Vernochet et al., 2009). Interestingly, hMADS cells expressed PRDM16, MEOX2, and AGT at the same level in both white and brite adipocytes. With respect to white adipocyte specific genes, it is noticeable that leptin (LEP) and DPT but not HOXC9 levels decreased with brite adipocyte formation.

Co-activators (PGC1α, PGC1β) and co-repressors (CtBP1, CtBP2, NRIP1) of PPARγ were also expressed, demonstrating the ability of these cells to modulate PPAR signaling pathways. hMADS cells also expressed other classical components of brown adipocyte signaling such as DiO2 (thyroid hormone pathway), COX1, COX2, PGTiS, and PGTiR (arachidonic acid pathway) and interestingly, an increase in the bile acid receptor, GPBAR1.

Altogether, these observations clearly confirmed the "brite" phenotype of hMADS adipocytes as they display both an origin and a molecular signature distinct from those of genuine brown adipocytes.

EFFECTS OF HEDGEHOG AND BMP SIGNALING EFFECTORS ON BRITE hMADS CELL FORMATION

As conversion of hMADS cells into brite adipocytes is dependent upon PPARγ activation, we became interested in deciphering pathways that are independent of rosiglitazone treatment. It has been shown in mouse models that Hedgehog and BMP pathways modulated the formation of brown adipocytes (Lee et al., 2008; Pospisilik et al., 2010; Schulz et al., 2011). We analyzed UCP1 expression after activation of the Hedgehog pathway, using two known activators of

Table 2 | Analysis of white and brown specific markers.

		hMADS-2				hMADS-3			
		Day 9	Day 16		Day 16	Day 9	Day 16		Day 16
			"White adipocyte"	"Brite adipocyte"	Brite vs. white		"White adipocyte"	"Brite adipocyte"	Brite vs. white
Brown markers	UCP1	0.376	5.231	138.1	**26.393**	0.060	2.400	45.50	**18.958**
	CPT1B	0.322	0.147	1.757	**11.949**	0.020	0.110	1.340	**12.182**
	CIDEA	0.019	0.041	0.076	**1.857**	0.020	0.010	0.028	**2.800**
	ELOVL3	0.449	1.246	2.006	**1.610**	0.600	0.788	1.332	**1.691**
	AGT	0.805	0.140	0.131	0.938	0.275	0.059	0.033	*0.564*
	PRDM16	0.027	0.024	0.017	0.731	0.028	0.036	0.027	0.750
	MEOX 2	0.016	0.019	0.020	1.035	0.030	0.013	0.009	0.705
	ZIC1	nd	nd	nd	nd	0.027	0.018	0.018	1.027
	LHX8	nd	nd	nd	nd	nd	nd	nd	nd
Brown/white markers	FABP4	263.0	94.0	305.0	**3.245**	317.0	206.0	390.0	**1.893**
	ADIPOQ	105.0	31.0	138.0	**4.452**	69.00	27.00	96.00	**3.556**
	RARRES2	0.237	0.513	0.650	1.266	1.002	1.976	1.425	0.721
	PANK 3	2.397	5.035	5.996	1.191	0.979	3.421	2.454	0.717
White markers	HoxC9	0.679	0.514	0.710	1.381	0.591	0.648	0.550	0.849
	DPT	3.264	1.964	1.096	***0.558***	18.306	7.310	6.908	0.945
	LEP	0.707	0.406	0.25	***0.616***	1.392	0.244	0.158	***0.648***
PPAR complex	PGC1α	0.431	0.340	0.494	1.453	0.940	0.320	0.400	1.250
	PGC1β	0.229	0.211	0.220	1.042	0.190	0.190	0.240	1.263
	PPARγ	3.145	13.310	10.598	0.796	10.200	9.500	10.500	1.105
	CtBP1	2.260	7.300	6.200	0.849	1.820	6.100	6.400	1.049
	CtBP2	1.110	1.910	18.200	**9.529**	2.280	2.540	11.700	**4.606**
	NRIP1	1.442	1.066	1.597	1.499	1.140	0.630	0.750	1.190
	LXR	0.110	8.000	12.600	**1.575**	0.150	8.500	20.300	**2.388**
Receptor/enzyme involved in white and brown activation	GPBAR1	0.309	0.120	0.209	**1.743**	0.118	0.056	0.084	1.489
	DiO2	0.060	0.051	0.056	1.103	0.041	0.055	0.057	1.026
	COX2	0.004	0.019	0.010	***0.538***	0.030	0.041	0.039	0.951
	COX1	0.708	0.135	0.125	0.926	0.440	0.140	0.150	1.071
	PTGiS	1.416	nd	nd	nd	0.390	0.910	0.440	***0.484***
	PTGiR	0.008	0.123	0.145	1.178	0.040	0.080	0.118	1.475
	TPK1	1.621	1.551	1.292	0.833	0.633	1.040	0.841	0.809

hMADS-2 and 3 cells were induced to differentiate into adipocytes in the presence of 100 nM rosiglitazone between days 3 and 9 or between days 3 and 16. Expression of various markers described previously was analyzed by quantitative RT-PCR. The expression values (ΔCt referred to TBP) mRNA in hMADS cells at the indicated. Genes indicated in italics and bold are highly expressed in white adipocyte and in brown adipocyte conditions, respectively.

smoothen, i.e., purmorphamine and smoothened agonist (SAG). hMADS cells that were differentiated in the presence of rosiglitazone either between day 3 and 9 or between day 3 and 16 were exposed to the two compounds and analyzed at day 16. As expected, the expression of Gli1 mRNA (**Figure 4A**), a marker of the activation of Hedgehog signaling (Hooper and Scott, 2005), was observed. However, neither purmorphamine nor SAG were able to substitute to rosiglitazone and to modulate the expression of UCP1 or FABP4.

In a similar way we tested whether BMPs could induce the "browning" of hMADS cells. hMADS cells, in the presence or absence of rosiglitazone between days 3 and 9, were treated with or without 10 nM BMP2 or BMP7. As shown in **Figure 4B**, neither BMP2 nor BMP7 affected adipogenesis of hMADS cells, as illustrated by the expression of FABP4 and only induced weakly the formation of brite adipocytes as illustrated by the expression of UCP1 and CIDEA.

DISCUSSION

Recent advances in the developmental analysis of white and brown progenitors demonstrated a distinct origin (Timmons et al., 2007; Crisan et al., 2008). Furthermore, brown-like adipocytes are found in white fat depots upon physiological or pharmacological stimulation and there is evidence that white adipocytes can be converted to brown-like fat cells (Tiraby et al., 2003; Cinti, 2009a,b). A recent study described mouse "brite" adipocytes, obtained upon chronic PPARγ activation of primary cultures of white adipocyte precursors (Petrovic et al., 2010), which may

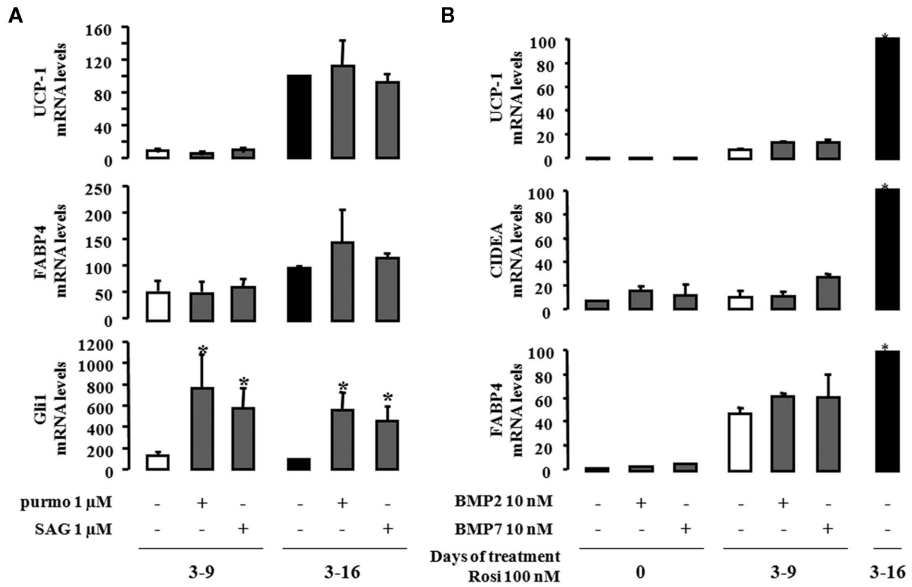

FIGURE 4 | Effect of Hedgehog and BMP signaling in the conversion of white to brown adipocytes. hMADS-2 cells were induced to differentiate into adipocytes in presence of 100 nM rosiglitazone at the indicate times. **(A)**, Purmorphamine (purmo) or smoothened agonist (SAG) as effectors of the Hedgehog signaling pathway were added between days 14 and 18. Their effects were analyzed by measuring UCP1, FABP4, and Gli1 mRNA expression. **(B)** hMADS-2 cells were treated or not with 10 nM BMP2 or BMP7 between days 0 and 16, and analyzed at day 16. Results are the mean ± SEM of three independent experiments and are expressed by taking as 100% the value obtained for 100 nM rosiglitazone treatment between days 14 and 18. $*p < 0.05$ vs. cells treated only by rosiglitazone 100 nM.

explain the UCP1 expression found in islets surrounded by white adipocytes (Cousin et al., 1992; Xue et al., 2009). In adult humans, the increase of white to "brite" adipocyte conversion and activity, taking advantage of the important mass of WAT, could represent a novel strategy to combat overweight/obesity as a result of energy imbalance. Unfortunately, detailed investigations are hampered *ex vivo* by the unavailability of human brite and genuine brown adipocytes.

The observations reported herein emphasize that hMADS cells can differentiate into brite adipocytes. Their differentiation is associated with an increase of mitochondrial markers (**Figure 3**). During proliferation and differentiation, hMADS cells do not express Myf5 indicating that a common signature with myoblasts can be ruled out. Analyzing the expression pattern of genes known to be associated with brown or brite murine adipocytes, clearly demonstrate the brite signature of converted hMADS cells. Indeed, these cells express brown adipocytes markers (PPARα, CPT1B, ELOVL3, CIDEA, PGC1a, and UCP1) and do not (ZIC1, LHX8) or barely express other specific markers (PRDM16, MEOX2) of genuine mouse brown adipocytes. Moreover, hMADS cells express well-known components of brown adipocyte signaling, demonstrating that these cells are able to respond to various signals such as α- and β-adrenergic agonists, bile acids, fatty acids and prostaglandins, thyroid hormone, and the natriuretic peptide (**Table 2**; Rodriguez et al., 2004; Elabd et al., 2009). Therefore, hMADS cells can be used as a human cell model to analyze various brown modulating signals that have been described in rodents. Our results clearly emphasize Hedgehog and BMPs signaling as striking examples of the discrepancies existing between human and rodents (Fontaine et al., 2008; Svensson et al., 2011). Actually, it has been previously shown that activation of Hedgehog signaling *in vivo* and *in vitro* impairs white but not brown adipocyte differentiation (Pospisilik et al., 2010). Herein we showed that treatment of hMADS cells with Hedgehog activators did not affect white or brite adipocyte formation (**Figure 4A**). Furthermore, our data show that BMP7 induces weakly brite adipocyte formation of hMADS cells, in contrast to earlier observations in mouse cell models (Tseng et al., 2008; Schulz et al., 2011) despite the fact that hMADS cells do express functional BMP receptors (Zaragosi et al., 2010; and data not shown).

Taken together, our data demonstrated that differentiated brown-like hMADS adipocytes are representative of the so-called brite adipocytes recently described in mice (Petrovic et al., 2010) though significant differences are observed with regard to signaling cues favoring brown adipogenesis. In conclusion, our human cell model could contribute to shed some light on the mechanisms involved in the browning process of white adipocytes and could help in identifying drugs involved in this phenomenon.

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REFERENCES

Ailhaud, G., Grimaldi, P., and Negrel, R. (1992). Cellular and molecular aspects of adipose tissue development. *Annu. Rev. Nutr.* 12, 207–233.

Barbatelli, G., Murano, I., Madsen, L., Hao, Q., Jimenez, M., Kristiansen, K., Giacobino, J. P., De Matteis, R., and Cinti, S. (2010). The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. *Am. J. Physiol. Endocrinol. Metab.* 298, E1244–E1253.

Bezy, O., Elabd, C., Cochet, O., Petersen, R. K., Kristiansen, K., Dani, C., Ailhaud, G., and Amri, E. Z. (2005). Delta-interacting protein A, a new inhibitory partner of CCAAT/enhancer-binding protein beta, implicated in adipocyte differentiation. *J. Biol. Chem.* 280, 11432–11438.

Cinti, S. (2009a). Reversible physiological transdifferentiation in the adipose organ. *Proc. Nutr. Soc.* 68, 340–349.

Cinti, S. (2009b). Transdifferentiation properties of adipocytes in the adipose organ. *Am. J. Physiol. Endocrinol. Metab.* 297, E977–E986.

Cousin, B., Cinti, S., Morroni, M., Raimbault, S., Ricquier, D., Penicaud, L., and Casteilla, L. (1992). Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization. *J. Cell. Sci.* 103(Pt 4), 931–942.

Crisan, M., Casteilla, L., Lehr, L., Carmona, M., Paoloni-Giacobino, A., Yap, S., Sun, B., Leger, B., Logar, A., Penicaud, L., Schrauwen, P., Cameron-Smith, D., Russell, A. P., Peault, B., and Giacobino, J. P. (2008). A reservoir of brown adipocyte progenitors in human skeletal muscle. *Stem Cells* 26, 2425–2433.

Cypess, A. M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A. B., Kuo, F. C., Palmer, E. L., Tseng, Y. H., Doria, A., Kolodny, G. M., and Kahn, C. R. (2009). Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* 360, 1509–1517.

Elabd, C., Chiellini, C., Carmona, M., Galitzky, J., Cochet, O., Petersen, R., Penicaud, L., Kristiansen, K., Bouloumie, A., Casteilla, L., Dani, C., Ailhaud, G., and Amri, E. Z. (2009). Human multipotent adipose-derived stem cells differentiate into functional brown adipocytes. *Stem Cells* 27, 2753–2760.

Elabd, C., Chiellini, C., Massoudi, A., Cochet, O., Zaragosi, L. E., Trojani, C., Michiels, J. F., Weiss, P., Carle, G., Rochet, N., Dechesne, C. A., Ailhaud, G., Dani, C., and Amri, E. Z. (2007). Human adipose tissue-derived multipotent stem cells differentiate in vitro and in vivo into osteocyte-like cells. *Biochem. Biophys. Res. Commun.* 361, 342–348.

Feldmann, H. M., Golozoubova, V., Cannon, B., and Nedergaard, J. (2009). UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab.* 9, 203–209.

Fontaine, C., Cousin, W., Plaisant, M., Dani, C., and Peraldi, P. (2008). Hedgehog signaling alters adipocyte maturation of human mesenchymal stem cells. *Stem Cells* 26, 1037–1046.

Fruhbeck, G., Becerril, S., Sainz, N., Garrastachu, P., and Garcia-Velloso, M. J. (2009). BAT: a new target for human obesity? *Trends Pharmacol. Sci.* 30, 387–396.

Golozoubova, V., Cannon, B., and Nedergaard, J. (2006). UCP1 is essential for adaptive adrenergic nonshivering thermogenesis. *Am. J. Physiol. Endocrinol. Metab.* 291, E350–E357.

Guerra, C., Koza, R. A., Yamashita, H., Walsh, K., and Kozak, L. P. (1998). Emergence of brown adipocytes in white fat in mice is under genetic control. Effects on body weight and adiposity. *J. Clin. Invest.* 102, 412–420.

Hooper, J. E., and Scott, M. P. (2005). Communicating with Hedgehogs. *Nat. Rev. Mol. Cell Biol.* 6, 306–317.

Hossain, P., Kawar, B., and El Nahas, M. (2007). Obesity and diabetes in the developing world – a growing challenge. *N. Engl. J. Med.* 356, 213–215.

Lee, S. Y., Nakagawa, T., and Reddi, A. H. (2008). Induction of chondrogenesis and expression of superficial zone protein (SZP)/lubricin by mesenchymal progenitors in the infrapatellar fat pad of the knee joint treated with TGF-beta1 and BMP-7. *Biochem. Biophys. Res. Commun.* 376, 148–153.

Nedergaard, J., Bengtsson, T., and Cannon, B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. *Am. J. Physiol. Endocrinol. Metab.* 293, E444–E452.

Negrel, R., Grimaldi, P., and Ailhaud, G. (1978). Establishment of preadipocyte clonal line from epididymal fat pad of ob/ob mouse that responds to insulin and to lipolytic hormones. *Proc. Natl. Acad. Sci. U.S.A.* 75, 6054–6058.

Petrovic, N., Walden, T. B., Shabalina, I. G., Timmons, J. A., Cannon, B., and Nedergaard, J. (2010). Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. *J. Biol. Chem.* 285, 7153–7164.

Pisani, D. F., Dechesne, C. A., Sacconi, S., Delplace, S., Belmonte, N., Cochet, O., Clement, N., Wdziekonski, B., Villageois, A. P., Butori, C., Bagnis, C., Di Santo, J. P., Kurzenne, J. Y., Desnuelle, C., and Dani, C. (2010). Isolation of a highly myogenic CD34-negative subset of human skeletal muscle cells free of adipogenic potential. *Stem Cells* 28, 753–764.

Pospisilik, J. A., Schramek, D., Schnidar, H., Cronin, S. J., Nehme, N. T., Zhang, X., Knauf, C., Cani, P. D., Aumayr, K., Todoric, J., Bayer, M., Haschemi, A., Puviindran, V., Tar, K., Orthofer, M., Neely, G. G., Dietzl, G., Manoukian, A., Funovics, M., Prager, G., Wagner, O., Ferrandon, D., Aberger, F., Hui, C. C., Esterbauer, H., and Penninger, J. M. (2010). Drosophila genome-wide obesity screen reveals hedgehog as a determinant of brown versus white adipose cell fate. *Cell* 140, 148–160.

Rodriguez, A. M., Elabd, C., Amri, E. Z., Ailhaud, G., and Dani, C. (2005a). The human adipose tissue is a source of multipotent stem cells. *Biochimie* 87, 125–128.

Rodriguez, A. M., Pisani, D., Dechesne, C. A., Turc-Carel, C., Kurzenne, J. Y., Wdziekonski, B., Villageois, A., Bagnis, C., Breittmayer, J. P., Groux, H., Ailhaud, G., and Dani, C. (2005b). Transplantation of a multipotent cell population from human adipose tissue induces dystrophin expression in the immunocompetent mdx mouse. *J. Exp. Med.* 201, 1397–1405.

Rodriguez, A. M., Elabd, C., Delteil, F., Astier, J., Vernochet, C., Saint-Marc, P., Guesnet, J., Guezennec, A., Amri, E. Z., Dani, C., and Ailhaud, G. (2004). Adipocyte differentiation of multipotent cells established from human adipose tissue. *Biochem. Biophys. Res. Commun.* 315, 255–263.

Rosen, E. D., and Spiegelman, B. M. (2006). Adipocytes as regulators of energy balance and glucose homeostasis. *Nature* 444, 847–853.

Saito, M., Okamatsu-Ogura, Y., Matsushita, M., Watanabe, K., Yoneshiro, T., Nio-Kobayashi, J., Iwanaga, T., Miyagawa, M., Kameya, T., Nakada, K., Kawai, Y., and Tsujisaki, M. (2009). High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* 58, 1526–1531.

Schulz, T. J., Huang, T. L., Tran, T. T., Zhang, H., Townsend, K. L., Shadrach, J. L., Cerletti, M., Mcdougall, L. E., Giorgadze, N., Tchkonia, T., Schrier, D., Falb, D., Kirkland, J. L., Wagers, A. J., and Tseng, Y. H. (2011). Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. *Proc. Natl. Acad. Sci. U.S.A.* 108, 143–148.

Seale, P., Bjork, B., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scime, A., Devarakonda, S., Conroe, H. M., Erdjument-Bromage, H., Tempst, P., Rudnicki, M. A., Beier, D. R., and Spiegelman, B. M. (2008). PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454, 961–967.

Svensson, P. A., Jernas, M., Sjoholm, K., Hoffmann, J. M., Nilsson, B. E., Hansson, M., and Carlsson, L. M. (2011). Gene expression in human brown adipose tissue. *Int. J. Mol. Med.* 27, 227–232.

Timmons, J. A., Wennmalm, K., Larsson, O., Walden, T. B., Lassmann, T., Petrovic, N., Hamilton, D. L., Gimeno, R. E., Wahlestedt, C., Baar, K., Nedergaard, J., and Cannon, B. (2007). Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. *Proc. Natl. Acad. Sci. U.S.A.* 104, 4401–4406.

Tiraby, C., Tavernier, G., Lefort, C., Larrouy, D., Bouillaud, F., Ricquier, D., and Langin, D. (2003). Acquirement of brown fat cell features by human white adipocytes. *J. Biol. Chem.* 278, 33370–33376.

Tseng, Y. H., Kokkotou, E., Schulz, T. J., Huang, T. L., Winnay, J. N., Taniguchi, C. M., Tran, T. T., Suzuki, R., Espinoza, D. O., Yamamoto, Y., Ahrens, M. J., Dudley, A. T., Norris, A. W., Kulkarni, R. N., and Kahn, C. R. (2008). New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454, 1000–1004.

Van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, J. M., Kemerink, G. J., Bouvy, N. D., Schrauwen, P., and Teule, G. J. (2009). Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* 360, 1500–1508.

Vernochet, C., Peres, S. B., Davis, K. E., Mcdonald, M. E., Qiang, L., Wang, H., Scherer, P. E., and Farmer, S. R. (2009). C/EBPalpha and the corepressors CtBP1 and

CtBP2 regulate repression of select visceral white adipose genes during induction of the brown phenotype in white adipocytes by peroxisome proliferator-activated receptor gamma agonists. *Mol. Cell. Biol.* 29, 4714–4728.

Virtanen, K. A., Lidell, M. E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N. J., Enerback, S., and Nuutila, P. (2009). Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* 360, 1518–1525.

Whittle, A. J., Lopez, M., and Vidal-Puig, A. (2011). Using brown adipose tissue to treat obesity – the central issue. *Trends Mol. Med.* 17, 405–411.

Xue, B., Rim, J. S., Hogan, J. C., Coulter, A. A., Koza, R. A., and Kozak, L. P. (2007). Genetic variability affects the development of brown adipocytes in white fat but not in interscapular brown fat. *J. Lipid Res.* 48, 41–51.

Xue, Y., Petrovic, N., Cao, R., Larsson, O., Lim, S., Chen, S., Feldmann, H. M., Liang, Z., Zhu, Z., Nedergaard, J., Cannon, B., and Cao, Y. (2009). Hypoxia-independent angiogenesis in adipose tissues during cold acclimation. *Cell Metab.* 9, 99–109.

Zaragosi, L. E., Ailhaud, G., and Dani, C. (2006). Autocrine fibroblast growth factor 2 signaling is critical for self-renewal of human multipotent adipose-derived stem cells. *Stem Cells* 24, 2412–2419.

Zaragosi, L. E., Wdziekonski, B., Villageois, P., Keophiphath, M., Maumus, M., Tchkonia, T., Bourlier, V., Mohsen-Kanson, T., Ladoux, A., Elabd, C., Scheideler, M., Trajanoski, Z., Takashima, Y., Amri, E. Z., Lacasa, D., Sengenes, C., Ailhaud, G., Clement, K., Bouloumie, A., Kirkland, J. L., and Dani, C. (2010). Activin a plays a critical role in proliferation and differentiation of human adipose progenitors. *Diabetes* 59, 2513–2521.

Zingaretti, M. C., Crosta, F., Vitali, A., Guerrieri, M., Frontini, A., Cannon, B., Nedergaard, J., and Cinti, S. (2009). The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J.* 23, 3113–3120.

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The heat is on: a new avenue to study brown fat formation in humans

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A commentary on

Differentiation of human adipose-derived stem cells into "brite" (brown-in-white) adipocytes
by Pisani, D., Djedaini, M., Beranger, G. E., Elabd, C., Scheideler, M., Ailhaud, G., and Amri, E.-Z. (2011). Front. Endocrin. 2:87. doi: 10.3389/fendo.2011.00087

The ever increasing rates of obesity and its co-morbidities represent a significant worldwide health problem (Pijl, 2011). An effective and innovative strategy to reduce body weight would be to increase energy expenditure by altering the character of adipose tissue in the body. This approach does not merely aim to eliminate, white adipose tissue (WAT), our reservoirs of energy storage. Rather, its objective is to replace and/or add to WAT depots with highly energetic brown adipocytes. Although brown adipocytes are capable of synthesizing and storing triglyceride, they are functionally different from white adipocytes, playing an important role in heat production, blood triglyceride clearance, and glucose disposal (Cannon and Nedergaard, 2004; Nedergaard et al., 2010; Bartelt et al., 2011).

Both WAT and brown adipose tissue (BAT) are found in specific depots throughout the body (Cinti, 2005). The amount of active BAT found in the supraclavicular region of some human subjects, ~63 g, would be sufficient to metabolize an amount of energy equivalent to about 4 kg of WAT during the course of 1 year (Fruhbeck et al., 2009; Virtanen et al., 2009). Indeed, the weight loss potential of activated BAT is borne out of the estimation that as little as 50 g is sufficient to metabolize 20% of the daily energy needs of an individual (Rothwell and Stock, 1983).

Adipocytes are not restricted to their depot subtype, as brown adipocytes can also be found in WAT and vice versa (Cinti, 2005). Brown adipocytes found interspersed within WAT are known by a variety of names, including adaptive or recruitable fat cells, brown in white (brite) cells, or beige cells (Enerback, 2009; Ishibashi and Seale, 2010; Petrovic et al., 2010). Hence, increasing the numbers of brite cells within WAT depots represents an innovative approach to reduce weight gain.

In this report Pisani et al. (2011) characterize the formation of brite adipocytes *in vitro* from the trans-differentiation of human white adipocytes. For their experiments, they differentiated mesenchymal stem cells, which were isolated from WAT depots of human subjects, first into white adipocytes, and subsequently trans-differentiating them into brown adipocytes. These human multipotent adipose derived stem (hMADS) cells have the potential to differentiate into different mesodermal lineages not restricted to adipocytes, which include myoblasts and osteoblasts (Rodriguez et al., 2005; Elabd et al., 2007, 2009; **Figure 1**). The trans-differentiated white adipocytes formed brown adipocytes with enhanced uncoupling activity with the salient expression of the brown adipocyte marker UCP1. The brown phenotype was further authenticated by the enhanced mitochondriogenesis with increased levels of the fatty acid transporter CPT1B and cytochrome *c* oxidase activity.

Pisani et al. (2011) found the brown adipocytes made from hMADS was dependent on agonist sustained activation of PPARγ, chiefly rosiglitazone. As with brown cell formation in WAT depots of mice, the brown adipocytes that were formed did not appear to have originated from a skeletal muscle, Myf5-expressing progenitor (Seale et al., 2008). However, unlike mouse brown adipocyte differentiation, brown conversion of hMADS cells was not stimulated by BMP7 treatment and was not modulated by activation of the Hedgehog pathway (Tseng et al., 2008; Pospisilik et al., 2010). Moreover, the brown adipocyte determination factor, PRDM16, was expressed at similar levels in both white and brown adipocytes (Seale et al., 2008). This is counter to the recent findings supporting the important involvement of PRDM16 in brite cell formation within the subcutaneous inguinal fat pads of mice (Seale et al., 2011). This discrepancy in hMADS cells may be species specific. However, it does not rule out the possibility that PRDM16 might be controlled at a different level (i.e. protein or activity) in hMADS cells that is not manifested by the differentiation approach used by Pisani et al. (2011).

Although, Pisani et al. (2011) provide compelling evidence for transdifferentiation of white into brown adipocytes *in vitro*, there are other potential avenues to recruit brown adipocytes within WAT depots. Notably, newly formed brown adipocytes in WAT could be derived from: a common white preadipocyte, a WAT specific brown preadipocyte, or by the mobilization of uncommitted MSCs into a brown adipocyte lineage (Scime et al., 2005; **Figure 1**). These alternative avenues to recruit brown adipocytes may depend on a particular physiological state. hMADS cells will now make it possible to study these different potentialities *in vitro*.

Overall, the work of Pisani et al. provides an important approach to understand the *in vitro* mechanisms for human brown adipocyte development in WAT. Unlocking the pathways and cues required for brown cell activation in humans will lead to promising avenues for drug therapies against obesity.

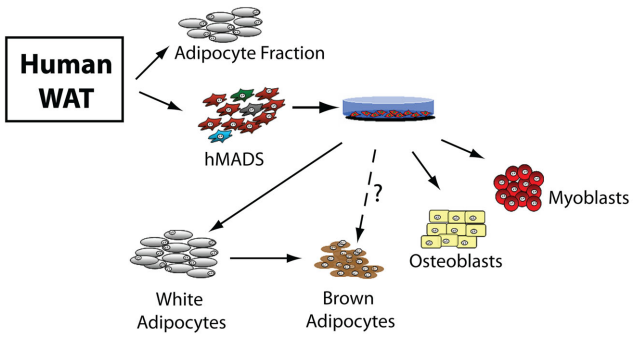

FIGURE 1 | Differentiation potential of hMADS cells. Human multipotent adipose derived stem (hMADS) cells isolated from white adipose tissue (WAT) of human donors can differentiate into osteoblasts, myoblasts, white and brown adipocytes. Although brown adipocytes are formed from the trans-differentiation of white adipocytes, it is not known if there is the potential to differentiate directly from hMADS cells.

REFERENCES

Bartelt, A., Bruns, O. T., Reimer, R., Hohenberg, H., Ittrich, H., Peldschus, K., Kaul, M. G., Tromsdorf, U. I., Weller, H., Waurisch, C., Eychmuller, A., Gordts, P. L., Rinninger, F., Bruegelmann, K., Freund, B., Nielsen, P., Merkel, M., and Heeren, J. (2011). Brown adipose tissue activity controls triglyceride clearance. *Nat. Med.* 17, 200–205.

Cannon, B., and Nedergaard, J. (2004). Brown adipose tissue: function and physiological significance. *Physiol. Rev.* 84, 277–359.

Cinti, S. (2005). The adipose organ. *Prostaglandins Leukot. Essent. Fatty Acids* 73, 9–15.

Elabd, C., Chiellini, C., Carmona, M., Galitzky, J., Cochet, O., Petersen, R., Penicaud, L., Kristiansen, K., Bouloumie, A., Casteilla, L., Dani, C., Ailhaud, G., and Amri, E. Z. (2009). Human multipotent adipose-derived stem cells differentiate into functional brown adipocytes. *Stem Cells* 27, 2753–2760.

Elabd, C., Chiellini, C., Massoudi, A., Cochet, O., Zaragosi, L. E., Trojani, C., Michiels, J. F., Weiss, P., Carle, G., Rochet, N., Dechesne, C. A., Ailhaud, G., Dani, C., and Amri, E. Z. (2007). Human adipose tissue-derived multipotent stem cells differentiate in vitro and in vivo into osteocyte-like cells. *Biochem. Biophys. Res. Commun.* 361, 342–348.

Enerback, S. (2009). The origins of brown adipose tissue. *N. Engl. J. Med.* 360, 2021–2023.

Fruhbeck, G., Becerril, S., Sainz, N., Garrastachu, P., and Garcia-Velloso, M. J. (2009). BAT: a new target for human obesity? *Trends Pharmacol. Sci.* 30, 387–396.

Ishibashi, J., and Seale, P. (2010). Medicine. Beige can be slimming. *Science* 328, 1113–1114.

Nedergaard, J., Bengtsson, T., and Cannon, B. (2010). Three years with adult human brown adipose tissue. *Ann. N. Y. Acad. Sci.* 1212, E20–E36.

Petrovic, N., Walden, T. B., Shabalina, I. G., Timmons, J. A., Cannon, B., and Nedergaard, J. (2010). Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. *J. Biol. Chem.* 285, 7153–7164.

Pijl, H. (2011). Obesity: evolution of a symptom of affluence. *Neth. J. Med.* 69, 159–166.

Pisani, D., Djedaini, M., Beranger, G. E., Elabd, C., Scheideler, M., Ailhaud, G., and Amri, E. -Z. (2011). Differentiation of human adipose-derived stem cells into "brite" (brown-in-white) adipocytes. *Front. Endocrin.* 2:87. doi: 10.3389/fendo.2011.000879

Pospisilik, J. A., Schramek, D., Schnidar, H., Cronin, S. J., Nehme, N. T., Zhang, X., Knauf, C., Cani, P. D., Aumayr, K., Todoric, J., Bayer, M., Haschemi, A., Puviindran, V., Tar, K., Orthofer, M., Neely, G. G., Dietzl, G., Manoukian, A., Funovics, M., Prager, G., Wagner, O., Ferrandon, D., Aberger, F., Hui, C. C., Esterbauer, H., and Penninger, J. M. (2010). *Drosophila* genome-wide obesity screen reveals hedgehog as a determinant of brown versus white adipose cell fate. *Cell* 140, 148–160.

Rodriguez, A. M., Pisani, D., Dechesne, C. A., Turc-Carel, C., Kurzenne, J. Y., Wdziekonski, B., Villageois, A., Bagnis, C., Breittmayer, J. P., Groux, H., Ailhaud, G., and Dani, C. (2005). Transplantation of a multipotent cell population from human adipose tissue induces dystrophin expression in the immunocompetent mdx mouse. *J. Exp. Med.* 201, 1397–1405.

Rothwell, N. J., and Stock, M. J. (1983). Luxuskonsumption, diet-induced thermogenesis and brown fat: the case in favour. *Clin. Sci.* 64, 19–23.

Scime, A., Grenier, G., Huh, M. S., Gillespie, M. A., Bevilacqua, L., Harper, M. E., and Rudnicki, M. A. (2005). Rb and p107 regulate preadipocyte differentiation into white versus brown fat through repression of PGC-1alpha. *Cell Metab.* 2, 283–295.

Seale, P., Bjork, B., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scime, A., Devarakonda, S., Conroe, H. M., Erdjument-Bromage, H., Tempst, P., Rudnicki, M. A., Beier, D. R., and Spiegelman, B. M. (2008). PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454, 961–967.

Seale, P., Conroe, H. M., Estall, J., Kajimura, S., Frontini, A., Ishibashi, J., Cohen, P., Cinti, S., and Spiegelman, B. M. (2011). Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. *J. Clin. Invest.* 121, 96–105.

Tseng, Y. H., Kokkotou, E., Schulz, T. J., Huang, T. L., Winnay, J. N., Taniguchi, C. M., Tran, T. T., Suzuki, R., Espinoza, D. O., Yamamoto, Y., Ahrens, M. J., Dudley, A. T., Norris, A. W., Kulkarni, R. N., and Kahn, C. R. (2008). New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454, 1000–1004.

Virtanen, K. A., Lidell, M. E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N. J., Enerback, S., and Nuutila, P. (2009). Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* 360, 1518–1525.

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Control of brown adipose tissue glucose and lipid metabolism by PPARγ

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Brown adipose tissue (BAT) non-shivering thermogenesis impacts energy homeostasis in rodents and humans. Mitochondrial uncoupling protein 1 in brown fat cells produces heat by dissipating the energy generated by fatty acid and glucose oxidation. In addition to thermogenesis and despite its small relative size, sympathetically activated BAT constitutes an important glucose, fatty acid, and triacylglycerol-clearing organ, and such function could potentially be used to alleviate dyslipidemias, hyperglycemia, and insulin resistance. To date, chronic sympathetic innervation and peroxisome proliferator-activated receptor (PPAR) γ activation are the only recognized inducers of BAT recruitment. Here, we review the major differences between these two BAT inducers in the regulation of lipolysis, fatty acid oxidation, lipid uptake and triacylglycerol synthesis, glucose uptake, and *de novo* lipogenesis. Whereas BAT recruitment through sympathetic drive translates into functional thermogenic activity, PPARγ-mediated recruitment is associated with a reduction in sympathetic activity leading to increased lipid storage in brown adipocytes. The promising therapeutic role of BAT in the treatment of hypertriglyceridemic and hyperglycemic conditions is also discussed.

Keywords: rosiglitazone, PPARγ, brown adipose tissue, sympathetic nervous system, glucose metabolism, lipid metabolism, obesity

INTRODUCTION

Brown adipose tissue (BAT) plays an important role in rodent whole body energy homeostasis due to its extreme ability under demand to dissipate energy as heat (Cannon and Nedergaard, 2004). This process, known as non-shivering thermogenesis, is positively regulated through norepinephrine (NE) release by the sympathetic nerves that densely innervate BAT. Upon sympathetic activation, NE interacts with β_3 adrenergic receptors leading to lipase-mediated triacylglycerol (TAG) hydrolysis. Whereas lipolysis-derived glycerol is recycled back to TAG by glycerokinase (GyK; Festuccia et al., 2003a), lipolysis-derived fatty acids (FA) are directed to the mitochondria where they either undergo oxidation or allosterically activate uncoupling protein (UCP) 1. Active UCP1 dissipates the proton gradient across the inner mitochondrial membrane, thus leading to heat production at the expense of ATP synthesis. FA derived from intracellular TAG hydrolysis are the only activators of UCP1 and mitochondrial uncoupling as evidenced by defective thermogenesis in mice lacking lipolytic adipose triglyceride lipase (ATGL; Zimmermann et al., 2004). Therefore, maintenance of intracellular TAG stores is essential for BAT non-shivering thermogenesis as supported by the marked increase in BAT TAG synthesis in cold-acclimated rats (Cannon and Nedergaard, 2004; Moura et al., 2005).

In addition to thermogenesis, sympathetic innervation is a major regulator of brown adipocyte proliferation, differentiation, and apoptosis implicated in BAT maintenance and function (Cannon and Nedergaard, 2004). Chronic sympathetic activation not only increases BAT mass by enhancing brown adipocyte proliferation and differentiation and by reducing apoptosis (Bronnikov et al., 1999; Lindquist et al., 2000; Cannon and Nedergaard, 2004), but also stimulates mitochondrial biogenesis and the expression of the thermogenic proteins UCP1 and peroxisome proliferator-activated receptor (PPAR) coactivator-1 (PGC-1) α (Puigserver et al., 1998; de Jesus et al., 2001; Silva, 2006) amplifying BAT capacity to produce heat (Cannon and Nedergaard, 2004).

Chronic sympathetic activation was the only recognized inducer of BAT recruitment until the discovery that pharmacological activation of PPARγ also increases BAT mass and UCP1 levels in rodents (Mercer and Trayhurn, 1986; Rothwell et al., 1987; Thurlby et al., 1987; Sell et al., 2004). PPARγ is a nuclear receptor highly expressed in BAT that acts as a master transcriptional regulator of brown adipocyte differentiation required for tissue development, function, and survival (Barak et al., 1999; He et al., 2003; Imai et al., 2004; Gray et al., 2006; Duan et al., 2007; Petrovic et al., 2008). Remarkably, however, PPARγ-mediated BAT recruitment is associated with a reduction in BAT sympathetic activity and thyroid status, which seems to prevent the translation of a high thermogenic capacity (BAT mass and UCP1 content) into increased functional thermogenic activity (Festuccia et al., 2008). Despite being significantly reduced, the residual sympathetic tone remaining under pharmacological PPARγ activation modulates major components of PPARγ-mediated BAT recruitment, including maximal UCP1 expression (Festuccia et al., 2010).

Concomitantly with the upregulation of the thermogenic potential of BAT, PPARγ activation improves its ability to clear and store circulating lipids as TAG, which is associated with important changes in tissue glucose uptake and utilization. In the past few years we have worked toward elucidating the mechanisms whereby PPARγ agonism impacts BAT glucose and lipid metabolism. In the following sections we discuss the major findings of these studies, their integration into current knowledge on the mechanisms of action of PPARγ ligands, and future directions of this research area. Finally, the promising therapeutic role of BAT as an energy expending tissue for obesity treatment and as a glucose and lipid-clearing organ in the treatment of hyperglycemic and hypertriglyceridemic conditions are addressed.

BROWN ADIPOCYTE LIPOLYSIS

Intracellular lipolysis of TAG to FA and glycerol is directly related to BAT thermogenic function such that no thermogenesis can be evoked without activation of lipolysis (Cannon and Nedergaard, 2004). TAG hydrolysis is catalyzed in a stepwise manner by ATGL, hormone-sensitive lipase (HSL), and monoacyglycerol lipase (MGL), which have preferential hydrolytic activity toward TAG, diacylglycerol, and monoacylglycerol, respectively (Zechner et al., 2009). BAT lipolysis is activated by NE released by sympathetic nerves. NE, through activation of β_3 adrenergic receptors and associated adenylyl cyclase, raises cAMP that allosterically interacts and activates protein kinase A, which in turn phosphorylates HSL, thereby inducing its translocation to lipid droplets and increasing its hydrolytic activity.

Peroxisome proliferator-activated receptor γ activation by the agonist rosiglitazone is associated with an upregulation of BAT lipolytic machinery due to increased expression of ATGL and its partner CGI-58 and MGL (Festuccia et al., 2010). Similar to the upregulation of thermogenic genes, such higher lipase levels are not translated into higher functional lipolytic rates due to the inhibition by rosiglitazone of BAT sympathetic activity (Festuccia et al., 2008). In addition, the release of lipolysis-derived FA from BAT is counteracted by their intracellular recycling and re-esterification back to TAG, a process that strongly depends upon the generation of glycerol-3-phosphate (G3P), which is markedly stimulated by rosiglitazone, as discussed below (Festuccia et al., 2009b).

BROWN ADIPOCYTE FATTY ACID OXIDATION AND MITOCHONDRIAL BIOGENESIS

Brown adipose tissue has a marked ability to oxidize FA and glucose as a consequence of high tissue mitochondrial number and oxidative enzymes content. Such high oxidative capacity is translated into high rates of oxygen consumption, making BAT the major body oxygen-consuming organ in situations of active non-shivering thermogenesis (Cannon and Nedergaard, 2004). Lipolysis-derived FA are the preferred substrates oxidized in BAT, providing most of the energy converted to heat by UCP1-mediated mitochondrial uncoupling (Cannon and Nedergaard, 2004). Acutely, sympathetic activation upregulates BAT FA oxidation by increasing lipolysis and intracellular FA availability, and by enhancing carnitine palmitoyl transferase (CPT) 1 activity and FA entry into the mitochondria (Cannon and Nedergaard, 2004).

Chronically, sympathetic activation upregulates BAT oxidative capacity by increasing mitochondrial number, an effect mediated by PGC-1α, an adrenergically modulated coregulator of nuclear receptor function implicated in mitochondrial biogenesis (Puigserver et al., 1998). In contrast to sympathetic activation, PPARγ activation *in vivo* is not associated with changes in BAT mitochondrial number and PGC-1α expression (Festuccia et al., 2010). In brown adipocytes *in vitro*, rosiglitazone does not affect PGC-1α expression but increases mitochondrial number and CPT1 content, such effects being translated into higher oxygen consumption only in the presence of NE (Petrovic et al., 2008). Altogether, these findings indicate a major role of the sympathetic nervous system to enhance brown adipocyte mitochondrial function, such action being potentiated by PPARγ activation.

BROWN ADIPOCYTE LIPID UPTAKE AND TRIACYLGLYCEROL SYNTHESIS

Circulating chylomicron- and very-low-density lipoprotein (VLDL)-bound TAG are the major source of FA incorporated into BAT, a process catalyzed by the endothelium-bound enzyme lipoprotein lipase (LPL), which hydrolyzes circulating TAG to FA and monoacylglycerol for BAT uptake. BAT is a major plasma lipid-clearing organ in rodents and therefore a strong modulator of triglyceridemia (Festuccia et al., 2009b; Laplante et al., 2009; Bartelt et al., 2011). Cold exposure, for example, markedly activates BAT lipid clearance and LPL activity, thus inducing hypotriglyceridemia despite increased liver VLDL–TAG secretion (Mantha and Deshaies, 1998; Moura et al., 2005; Bartelt et al., 2011). In addition, BAT lipid uptake in rodents exposed to cold is favored by an increase in the membrane FA transporter FAT/CD36 (Bartelt et al., 2011).

Similar to cold exposure, pharmacological PPARγ activation in rodents markedly increases BAT TAG clearance and LPL activity, such effects contributing to the hypotriglyceridemic action of PPARγ agonists (Festuccia et al., 2009b; Laplante et al., 2009). Most of the FA taken up by brown adipocytes are directed toward TAG synthesis, a process strongly dependent upon G3P availability (discussed below) and the stepwise enzymatic acylation of its carbon backbone. Initial G3P acylation to lysophosphatidic acid is catalyzed by glycerol-3-phosphate acyltransferase (GPAT; **Figure 1**). Lysophosphatidic acid is then acylated to form phosphatidic acid, which is dephosphorylated to diacylglycerol by phosphatidic acid phosphatase 1 (PAP1, also called lipin) and further acylated to TAG by diacylglycerol acyltransferase (DGAT). In addition to TAG clearance and FA uptake, rosiglitazone activates BAT TAG synthesis by stimulating the activities of GPAT and DGAT. Among the GPAT and DGAT isoforms, rosiglitazone specifically increases mRNA levels of GPAT3 and DGAT1, respectively, both located in the endoplasmic reticulum. GPAT3 and DGAT1 expression is markedly upregulated by PPARγ activation in 3T3-L1 adipocyte (Cao et al., 2006; Yen et al., 2008) and in BAT suggesting direct transcriptional regulation, a hypothesis that remains to be tested. Surprisingly, lipin levels and PAP activity are not affected by rosiglitazone in BAT (Festuccia et al., 2009b), in contrast with the marked stimulation of lipin-1 levels in rat white adipose tissue (WAT; Festuccia and Deshaies, 2009; Festuccia et al., 2009a), and that of lipin-1 expression by pioglitazone in WAT of insulin-resistant humans

(Yao-Borengasser et al., 2006). Noteworthy, PAP specific activity (relative to protein) is 5 to 15-fold higher in BAT than in WAT under basal conditions (Festuccia et al., 2009a); activation of such abundant lipin-1 in BAT may therefore suffice to accommodate the elevated TAG synthetic flux brought by rosiglitazone without the need to increase lipin expression, as is the case in WAT.

BROWN ADIPOCYTE GLUCOSE UPTAKE AND INTRACELLULAR METABOLISM

In BAT, glucose is used not only for G3P synthesis (**Figure 1**), the carbon backbone for FA esterification, and TAG synthesis, and that of FA through *de novo* lipogenesis, but also for energy generation that supports FA esterification to TAG and other adipocyte functions. Significant amounts of glucose in BAT are also stored as glycogen and converted to lactate by anaerobic glycolysis (Cannon and Nedergaard, 2004). BAT displays very high rates of glucose uptake per unit weight, such that even though BAT makes up only a small fraction of rodent body weight, it can constitute a significant glucose-clearing organ especially under sympathetic activation (Cannon and Nedergaard, 2004). Such high rates of BAT glucose uptake in humans as estimated *in vivo* by positron emission tomography are close to those seen in cancer, making BAT a confounding factor for tumor diagnosis. BAT sympathetic activation markedly increases glucose uptake by activating the glucose transporter (GLUT) 1(Cannon and Nedergaard, 2004). In contrast to sympathetic activation, however, BAT rates of glucose uptake and GLUT4 mRNA levels are markedly reduced by rosiglitazone. This contrasts with the well-established PPARγ effects on glucose uptake by subcutaneous WAT, skeletal muscle and heart (Zierath et al., 1998) and excludes a likely contribution of BAT to glucose clearance and the *in vivo* insulin-sensitizing effect of rosiglitazone in rodents.

How rosiglitazone reduces BAT glucose uptake is unknown. It does not seem, however, to involve a direct rosiglitazone effect on BAT as evidenced by the modest increase in glucose uptake and unchanged GLUT4 mRNA levels in isolated brown adipocytes treated with rosiglitazone (Hernandez et al., 2003). Sympathetic innervation, through NE regulation of GLUT1 content and functional activation (Dallner et al., 2006), and insulin, through stimulation of GLUT4 expression and translocation (Teruel et al., 1996), are the major positive regulators of BAT glucose uptake (Cannon and Nedergaard, 2004). It is, therefore, reasonable to suggest that the reduction in both BAT sympathetic drive and plasma insulin levels induced by rosiglitazone (Festuccia et al., 2008) might contribute to reduced BAT glucose uptake.

Likely due to the reduced glucose flux per brown adipocyte, an important consequence of rosiglitazone treatment is a reduction in BAT glycogen content, its synthesis from glucose and the mRNA levels of UDP-linked glucose pyrophosphorylase (UDPG–PPL), which generates UDP-linked glucose for glycogen synthesis. BAT stores significant amounts of glycogen that seem to be an important source of glucose during non-shivering thermogenesis as evidenced by the enhanced glycogen utilization in BAT of rats exposed to cold (Farkas et al., 1999; Jakus et al., 2008). During non-shivering thermogenesis, significant amounts of glucose are converted to lactate by anaerobic glycolysis, producing ATP that might be important in compensating for reduced energy production due to mitochondrial

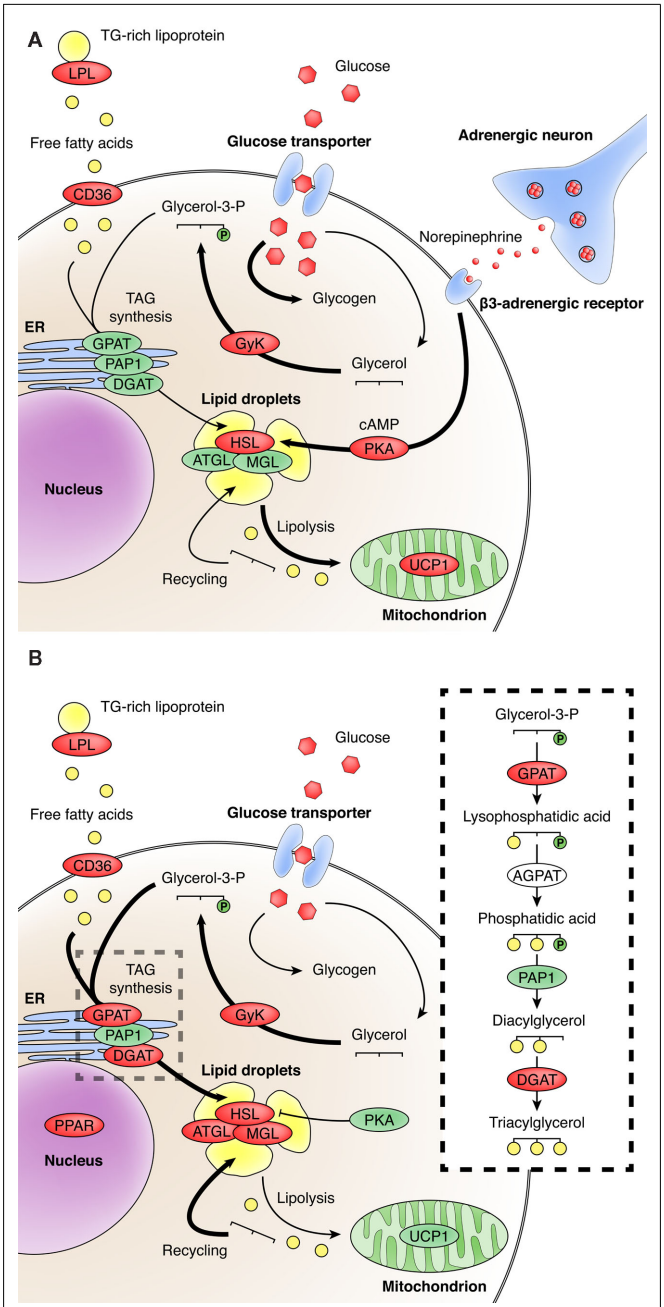

FIGURE 1 | Modulation of brown adipose tissue glucose and lipid metabolism by cold exposure (A) and PPARγ activation (B). Enzymes in red and pathways with thick arrows are activated in response to corresponding stimulus. Abbreviations: AGPAT, acylglycerol-3-phosphate-O-acyltransferase; ATGL, adipose triglyceride lipase; CD36, fatty acid translocase; DGAT, diacylglycerol acyltransferase; GPAT, glycerol 3-phosphate acyltransferase; GyK, glycerokinase; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; MGL, monoacylglycerol lipase; PAP1, phosphatidic acid phosphatase 1; PKA, protein kinase A; UCP1, uncoupling protein 1.

uncoupling (Cannon and Nedergaard, 2004). In addition, glucose seems to play a major role in replenishing citric acid cycle intermediates (anaplerosis; Owen et al., 2002), which is very important for keeping elevated levels of FA oxidation (Cannon and Nedergaard, 2004). Thus the reduction in BAT glucose uptake and glycogen content induced by rosiglitazone might have some deleterious consequences in terms of BAT thermogenic activity.

BROWN ADIPOCYTE G3P GENERATION

TAG synthesis depends on the intracellular levels of G3P. Because adequate levels of TAG for lipolysis and FA production are directly related to BAT thermogenic function, the generation of G3P is maintained under strict control.

The enhanced BAT FA storage induced by PPARγ activation is associated with a marked increase in TAG-glycerol synthesis estimated with the ^3H_2O technique, suggesting an increase in G3P generation by short glycolysis and glyceroneogenesis (Festuccia et al., 2009b). Indeed, glucose incorporation into TAG-glycerol *in vitro* and phosphoenolpyruvate carboxykinase (PEPCK) activity, a key glyceroneogenesis enzyme, are significantly increased by rosiglitazone (Festuccia et al., 2009b). Rosiglitazone also increases BAT GyK activity, which generates G3P by direct phosphorylation of glycerol taken up from the circulation or recycled from TAG hydrolysis. Remarkably, rosiglitazone upregulates GyK even under reduced BAT sympathetic drive (Festuccia et al., 2008), a major positive regulator of BAT GyK (Kawashita et al., 2002; Festuccia et al., 2003a). Acclimation to cold and now PPARγ agonism are to date the sole known situations in which all three possible sources of G3P are concomitantly stimulated in BAT (Festuccia et al., 2003a,b; Moura et al., 2005). Both these situations have in common a marked increase in BAT LPL activity and FA flux, which further illustrates the importance of PPARγ in controlling FA esterification and TAG synthesis.

BAT *DE NOVO* FATTY ACID SYNTHESIS

Along with TAG generation, BAT *de novo* synthesis of FA is activated in thermogenic conditions such as cold exposure and sympathetic stimulation of BAT (Minokoshi et al., 1988; Yu et al., 2002; Moura et al., 2005). Increased *de novo* lipogenesis can be considered as an attempt to maintain TAG levels for subsequent hydrolysis and thermogenesis activation. Rosiglitazone-mediated PPARγ activation, however, is not associated with changes in the rates of BAT *de novo* FA synthesis as estimated *in vivo* by the ^3H_2O technique. This strongly suggests that most of the FA used for TAG synthesis upon PPARγ activation are preformed, likely originating from LPL-mediated hydrolysis of lipoprotein-TAG and albumin-bound non-esterified FA, or recycled from local lipolysis.

PERSPECTIVES

The recent finding of significant amounts of physiologically active BAT in adult humans (Nedergaard et al., 2007) has renewed attention toward the potential implication of this tissue in metabolic homeostasis. Despite its small contribution to whole body weight (∼2% in rodents), BAT has the unique and extraordinary ability on demand to release energy from FA and glucose oxidation as heat by non-shivering thermogenesis. During maximal activation in rodents, BAT is the most oxygen-consuming organ of the body, producing a considerable amount of heat through markedly enhanced FA and glucose oxidation (Cannon and Nedergaard, 2004). Thus BAT can potentially constitute a significant glucose, FA, and TAG-clearing organ especially under sympathetic activation, with possible implications in the treatment of dyslipidemias, hyperglycemia, and insulin resistance. In this regard, pharmacological PPARγ activation emerged as an interesting approach to recruit BAT alternatively to sympathetic activation (Festuccia et al., 2008; Petrovic et al., 2008). PPARγ agonists markedly increase rodent BAT mass and its ability to take up and store FA as TAG. In rodents, BAT significantly contributes to the marked hypolipidemic effects (hypotriglyceridemia and reduced plasma non-esterified FA) of PPARγ agonists (Laplante et al., 2007, 2009). The marked ability of BAT to synthesize TAG under PPARγ activation is related to enhanced generation of G3P and GPAT and DGAT activities. An equivalent activation of BAT TAG synthesis is only seen during cold exposure (Moura et al., 2005), the sole difference being that, under this condition of increased sympathetic activity, newly synthesized TAG is rapidly hydrolyzed to supply FA for thermogenesis, whereas under PPARγ agonism TAG is stored in BAT, probably as a consequence of reduced tissue sympathetic drive. In contrast to lipids, however, BAT glucose uptake does not directly contribute to the improvement in systemic glucose homeostasis induced by rosiglitazone. In fact, glucose uptake per brown adipocyte is reduced by rosiglitazone, in striking contrast with the effects of the agonist in WAT and muscle.

In view of the adverse consequences of pharmacological PPARγ activation such as fluid retention, weight gain, congestive heart failure (Nesto et al., 2003; Lago et al., 2007; Singh et al., 2007), and osteopenia (Kahn et al., 2008) that currently limit the clinical use of PPARγ agonists as insulin sensitizers, progress is being made toward the development of selective PPARγ modulators (SPPARMs). The main goal is to develop SPPARMs that maintain the beneficial effects of PPARγ activation on insulin sensitivity and BAT recruitment without the complications linked with full PPARγ activation.

It is clear, however, that full PPARγ activation is not an efficient strategy to increase BAT thermogenesis and glucose uptake without the concomitant activation of tissue sympathetic activity. An ideal SPPARM would recruit BAT without interfering with basal sympathetic activity, thus enabling functional thermogenic activity. Efficient PPARγ activation could represent an attractive strategy to augment BAT thermogenic capacity and sensitivity to sympathetic stimulation, thus reducing the dosage of β-adrenergic agonists needed to functionally turn on thermogenesis. Further research is clearly needed to elucidate the best strategy to explore the fascinating potential of BAT as an energy dissipating and lipid- and glucose-clearing organ.

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REFERENCES

Barak, Y., Nelson, M. C., Ong, E. S., Jones, Y. Z., Ruiz-Lozano, P., Chien, K. R., Koder, A., and Evans, R. M. (1999). PPAR gamma is required for placental, cardiac, and adipose tissue development. *Mol. Cell* 4, 585–595.

Bartelt, A., Bruns, O. T., Reimer, R., Hohenberg, H., Ittrich, H., Peldschus, K., Kaul, M. G., Tromsdorf, U. I., Weller, H., Waurisch, C., Eychmuller, A., Gordts, P. L., Rinninger, F., Bruegelmann, K., Freund, B., Nielsen, P., Merkel, M., and Heeren, J. (2011). Brown adipose tissue activity controls triglyceride clearance. *Nat. Med.* 17, 200–205.

Bronnikov, G., Bengtsson, T., Kramarova, L., Golozoubova, V., Cannon, B., and Nedergaard, J. (1999). Beta1 to beta3 switch in control of cyclic adenosine monophosphate during brown adipocyte development explains distinct beta-adrenoceptor subtype mediation of proliferation and differentiation. *Endocrinology* 140, 4185–4197.

Cannon, B., and Nedergaard, J. (2004). Brown adipose tissue: function and physiological significance. *Physiol. Rev.* 84, 277–359.

Cao, J., Li, J. L., Li, D., Tobin, J. F., and Gimeno, R. E. (2006). Molecular identification of microsomal acyl-CoA:glycerol-3-phosphate acyltransferase, a key enzyme in de novo triacylglycerol synthesis. *Proc. Natl. Acad. Sci. U.S.A.* 103, 19695–19700.

Dallner, O. S., Chernogubova, E., Brolinson, K. A., and Bengtsson, T. (2006). Beta3-adrenergic receptors stimulate glucose uptake in brown adipocytes by two mechanisms independently of glucose transporter 4 translocation. *Endocrinology* 147, 5730–5739.

de Jesus, L. A., Carvalho, S. D., Ribeiro, M. O., Schneider, M., Kim, S. W., Harney, J. W., Larsen, P. R., and Bianco, A. C. (2001). The type 2 iodothyronine deiodinase is essential for adaptive thermogenesis in brown adipose tissue. *J. Clin. Invest.* 108, 1379–1385.

Duan, S. Z., Ivashchenko, C. Y., Whitesall, S. E., D'alecy, L. G., Duquaine, D. C., Brosius, F. C. III., Gonzalez, F. J., Vinson, C., Pierre, M. A., Milstone, D. S., and Mortensen, R. M. (2007). Hypotension, lipodystrophy, and insulin resistance in generalized PPARgamma-deficient mice rescued from embryonic lethality. *J. Clin. Invest.* 117, 812–822.

Farkas, V., Kelenyi, G., and Sandor, A. (1999). A dramatic accumulation of glycogen in the brown adipose tissue of rats following recovery from cold exposure. *Arch. Biochem. Biophys.* 365, 54–61.

Festuccia, W. T., Blanchard, P. G., Richard, D., and Deshaies, Y. (2010). Basal adrenergic tone is required for maximal stimulation of rat brown adipose tissue UCP1 expression by chronic PPAR-gamma activation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 299, R159–R167.

Festuccia, W. T., Blanchard, P. G., Turcotte, V., Laplante, M., Sariahmetoglu, M., Brindley, D. N., and Deshaies, Y. (2009a). Depot-specific effects of the PPAR{gamma} agonist rosiglitazone on adipose tissue glucose uptake and metabolism. *J. Lipid Res.* 50, 1185–1194.

Festuccia, W. T., Blanchard, P. -G., Turcotte, V., Laplante, M., Sariahmetoglu, M., Brindley, D. N., Richard, D., and Deshaies, Y. (2009b). The PPAR{γ} agonist rosiglitazone enhances rat brown adipose tissue lipogenesis from glucose without altering glucose uptake. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, R1327–R1335.

Festuccia, W. T., and Deshaies, Y. (2009). Depot specificities of PPAR gamma ligand actions on lipid and glucose metabolism and their implication in PPAR gamma-mediated body fat redistribution. *Clin. Lipidol.* 4, 633–642.

Festuccia, W. T., Guerra-Sa, R., Kawashita, N. H., Garofalo, M. A., Evangelista, E. A., Rodrigues, V., Kettelhut, I. C., and Migliorini, R. H. (2003a). Expression of glycerokinase in brown adipose tissue is stimulated by the sympathetic nervous system. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 284, R1536–R1541.

Festuccia, W. T., Kawashita, N. H., Garofalo, M. A., Moura, M. A., Brito, S. R., Kettelhut, I. C., and Migliorini, R. H. (2003b). Control of glyceroneogenic activity in rat brown adipose tissue. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 285, R177–R182.

Festuccia, W. T., Oztezcan, S., Laplante, M., Berthiaume, M., Michel, C., Dohgu, S., Denis, R. G., Brito, M. N., Brito, N. A., Miller, D. S., Banks, W. A., Bartness, T. J., Richard, D., and Deshaies, Y. (2008). Peroxisome proliferator-activated receptor-γ-mediated positive energy balance in the AT is associated with reduced sympathetic drive to adipose tissues and thyroid status. *Endocrinology* 149, 2121–2130.

Gray, S. L., Dalla Nora, E., Backlund, E. C., Manieri, M., Virtue, S., Noland, R. C., O'rahilly, S., Cortright, R. N., Cinti, S., Cannon, B., and Vidal-Puig, A. (2006). Decreased brown adipocyte recruitment and thermogenic capacity in mice with impaired peroxisome proliferator-activated receptor (P465L PPARgamma) function. *Endocrinology* 147, 5708–5714.

He, W., Barak, Y., Hevener, A., Olson, P., Liao, D., Le, J., Nelson, M., Ong, E., Olefsky, J. M., and Evans, R. M. (2003). Adipose-specific peroxisome proliferator-activated receptor γ knockout causes insulin resistance in fat and liver but not in muscle. *Proc. Natl. Acad. Sci. U.S.A.* 100, 15712–15717.

Hernandez, R., Teruel, T., and Lorenzo, M. (2003). Rosiglitazone produces insulin sensitisation by increasing expression of the insulin receptor and its tyrosine kinase activity in brown adipocytes. *Diabetologia* 46, 1618–1628.

Imai, T., Takakuwa, R., Marchand, S., Dentz, E., Bornert, J. M., Messaddeq, N., Wendling, O., Mark, M., Desvergne, B., Wahli, W., Chambon, P., and Metzger, D. (2004). Peroxisome proliferator-activated receptor γ is required in mature white and brown adipocytes for their survival in the mouse. *Proc. Natl. Acad. Sci. U.S.A.* 101, 4543–4547.

Jakus, P. B., Sandor, A., Janaky, T., and Farkas, V. (2008). Cooperation between BAT and WAT of rats in thermogenesis in response to cold, and the mechanism of glycogen accumulation in BAT during reacclimation. *J. Lipid Res.* 49, 332–339.

Kahn, S. E., Zinman, B., Lachin, J. M., Haffner, S. M., Herman, W. H., Holman, R. R., Kravitz, B. G., Yu, D., Heise, M. A., Aftring, R. P., and Viberti, G. (2008). Rosiglitazone-associated fractures in type 2 diabetes: an analysis from a diabetes outcome progression trial (ADOPT). *Diabetes Care* 31, 845–851.

Kawashita, N. H., Festuccia, W. T., Brito, M. N., Moura, M. A., Brito, S. R., Garofalo, M. A., Kettelhut, I. C., and Migliorini, R. H. (2002). Glycerokinase activity in brown adipose tissue: a sympathetic regulation? *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 282, R1185–R1190.

Lago, R. M., Singh, P. P., and Nesto, R. W. (2007). Congestive heart failure and cardiovascular death in patients with prediabetes and type 2 diabetes given thiazolidinediones: a meta-analysis of randomised clinical trials. *Lancet* 370, 1129–1136.

Laplante, M., Festuccia, W. T., Soucy, G., Blanchard, P. G., Renaud, A., Berger, J. P., Olivecrona, G., and Deshaies, Y. (2009). Tissue-specific postprandial clearance is the major determinant of PPARgamma-induced triglyceride lowering in the rat. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, R57–R66.

Laplante, M., Festuccia, W. T., Soucy, G., Gelinas, Y., Lalonde, J., and Deshaies, Y. (2007). Involvement of adipose tissues in the early hypolipidemic action of PPARgamma agonism in the rat. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 292, R1408–R1417.

Lindquist, J. M., Fredriksson, J. M., Rehnmark, S., Cannon, B., and Nedergaard, J. (2000). Beta 3- and alpha1-adrenergic Erk1/2 activation is Src- but not Gi-mediated in Brown adipocytes. *J. Biol. Chem.* 275, 22670–22677.

Mantha, L., and Deshaies, Y. (1998). Beta-adrenergic modulation of triglyceridemia under increased energy expenditure. *Am. J. Physiol.* 274, R1769–R1776.

Mercer, S. W., and Trayhurn, P. (1986). Effects of ciglitazone on insulin resistance and thermogenic responsiveness to acute cold in brown adipose tissue of genetically obese (ob/ob) mice. *FEBS Lett.* 195, 12–16.

Minokoshi, Y., Saito, M., and Shimazu, T. (1988). Sympathetic activation of lipid synthesis in brown adipose tissue in the rat. *J. Physiol. (Lond.)* 398, 361–370.

Moura, M. A., Festuccia, W. T., Kawashita, N. H., Garofalo, M. A., Brito, S. R., Kettelhut, I. C., and Migliorini, R. H. (2005). Brown adipose tissue glyceroneogenesis is activated in rats exposed to cold. *Pflugers Arch.* 449, 463–469.

Nedergaard, J., Bengtsson, T., and Cannon, B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. *Am. J. Physiol. Endocrinol. Metab.* 293, E444–E452.

Nesto, R. W., Bell, D., Bonow, R. O., Fonseca, V., Grundy, S. M., Horton, E. S., Le Winter, M., Porte, D., Semenkovich, C. F., Smith, S., Young, L. H., and Kahn, R. (2003). Thiazolidinedione use, fluid retention, and congestive heart failure: a consensus statement from the American Heart Association and American Diabetes Association. *Circulation* 108, 2941–2948.

Owen, O. E., Kalhan, S. C., and Hanson, R. W. (2002). The key role of anaplerosis and cataplerosis for citric acid cycle function. *J. Biol. Chem.* 277, 30409–30412.

Petrovic, N., Shabalina, I. G., Timmons, J. A., Cannon, B., and Nedergaard, J. (2008). Thermogenically competent nonadrenergic recruitment in brown preadipocytes by a PPARgamma agonist. *Am. J. Physiol. Endocrinol. Metab.* 295, E287–E296.

Puigserver, P., Wu, Z., Park, C. W., Graves, R., Wright, M., and Spiegelman, B. M. (1998). A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* 92, 829–839.

Rothwell, N. J., Stock, M. J., and Tedstone, A. E. (1987). Effects of ciglitazone on energy balance, thermogenesis and brown fat activity in the rat. *Mol. Cell. Endocrinol.* 51, 253–257.

Sell, H., Berger, J. P., Samson, P., Castriota, G., Lalonde, J., Deshaies, Y., and Richard, D. (2004). Peroxisome proliferator-activated receptor γ agonism increases the capacity for sympathetically mediated thermogenesis in lean and ob/ob mice. *Endocrinology* 145, 3925–3934.

Silva, J. E. (2006). Thermogenic mechanisms and their hormonal regulation. *Physiol. Rev.* 86, 435–464.

Singh, S., Loke, Y. K., and Furberg, C. D. (2007). Long-term risk of cardiovascular events with rosiglitazone: a meta-analysis. *JAMA* 298, 1189–1195.

Teruel, T., Valverde, A. M., Benito, M., and Lorenzo, M. (1996). Insulin-like growth factor I and insulin induce adipogenic-related gene expression in fetal brown adipocyte primary cultures. *Biochem. J.* 319(Pt 2), 627–632.

Thurlby, P. L., Wilson, S., and Arch, J. R. (1987). Ciglitazone is not itself thermogenic but increases the potential for thermogenesis in lean mice. *Biosci. Rep.* 7, 573–577.

Yao-Borengasser, A., Rasouli, N., Varma, V., Miles, L. M., Phanavanh, B., Starks, T. N., Phan, J., Spencer, H. J. III., Mcgehee, R. E. Jr., Reue, K., and Kern, P. A. (2006). Lipin expression is attenuated in adipose tissue of insulin-resistant human subjects and increases with peroxisome proliferator-activated receptor γ activation. *Diabetes* 55, 2811–2818.

Yen, C. L., Stone, S. J., Koliwad, S., Harris, C., and Farese, R. V. Jr. (2008). Thematic review series: glycerolipids. DGAT enzymes and triacylglycerol biosynthesis. *J. Lipid Res.* 49, 2283–2301.

Yu, X. X., Lewin, D. A., Forrest, W., and Adams, S. H. (2002). Cold elicits the simultaneous induction of fatty acid synthesis and beta-oxidation in murine brown adipose tissue: prediction from differential gene expression and confirmation in vivo. *FASEB J.* 16, 155–168.

Zechner, R., Kienesberger, P. C., Haemmerle, G., Zimmermann, R., and Lass, A. (2009). Adipose triglyceride lipase and the lipolytic catabolism of cellular fat stores. *J. Lipid Res.* 50, 3–21.

Zierath, J. R., Ryder, J. W., Doebber, T., Woods, J., Wu, M., Ventre, J., Li, Z., Mccrary, C., Berger, J., Zhang, B., and Moller, D. E. (1998). Role of skeletal muscle in thiazolidinedione insulin sensitizer (PPARgamma agonist) action. *Endocrinology* 139, 5034–5041.

Zimmermann, R., Strauss, J. G., Haemmerle, G., Schoiswohl, G., Birner-Gruenberger, R., Riederer, M., Lass, A., Neuberger, G., Eisenhaber, F., Hermetter, A., and Zechner, R. (2004). Fat mobilization in adipose tissue is promoted by adipose triglyceride lipase. *Science* 306, 1383–1386.

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β-Adrenoceptor signaling networks in adipocytes for recruiting stored fat and energy expenditure

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The adipocyte is like a bank: a place to store excess (caloric) cash in times of plenty, and from which one can withdraw savings during "lean times." The β-adrenoceptors (βAR) are the gateways to this mobilization of fat to be consumed in other tissues. This review discusses the βAR signaling pathway(s) in white and brown adipocytes. Studies in rodent models show that brown adipocytes nestled with white fat depots correlate with and are considered a key enabling factor in resistance to diet-induced obesity. Since it is now recognized that adult humans have brown adipocytes, knowing the steps in these signaling pathways may provide the opportunity to manipulate adipocytes to be net consumers of energy.

Keywords: adrenergic, adipocyte, brown, white, signaling, kinase

The catecholamines adrenaline and noradrenaline are well known as the physiological trigger for hydrolyzing stored fat in adipose tissue so that it can be "burned" as fuel in other organs. Adrenaline is commonly referred to as the "fight or flight" hormone. This is because it orchestrates the body's defensive response for rapid recruitment of metabolic fuel from adipose tissue lipolysis, as well as to increase heart rate to enhance tissue perfusion so that organs such as skeletal muscle, and the heart itself, can extract, and metabolize that fuel. These catecholamines control fat cell metabolism in large part through activation of the β-adrenoceptors (βARs). The βARs are members of the large family of G protein-coupled receptors (GPCRs) that are integral membrane proteins of the plasma membrane. The three subtypes of βARs (β_1AR, β_2AR, and β_3AR) are the products of different genes, and adipocytes express all three subtypes. In the mid-late 1980s the discovery of the β_3AR subtype and selective ligands for this receptor led to a period of intense interest in this receptor. This was largely because of the powerful effect of selective β_3AR agonists to promote energy expenditure and weight loss in animal models. However, the relatively poor preliminary performance of human β_3AR agonists in clinical trials (Buemann et al., 2000; Arch, 2002) – and the general belief that adult humans do not possess brown fat – led to the termination of most such research programs.

β-Adrenoceptor activation promotes lipolysis of stored triglyceride in both white and brown adipocytes. In brown adipocytes there is a dense sympathetic nervous system (SNS) innervation and these fatty acids that are released function as both a fuel source and activators of the UCP1 protein, which permits mitochondrial respiration without ATP generation (as discussed elsewhere in this edited volume). Because of this high capacity for uncoupled oxidative respiration, BAT is capable of consuming considerable calories through non-shivering thermogenesis (Lean, 1989; Nedergaard et al., 2001). Its rich vasculature allows the dissemination of this heat to other organs. From detailed studies in rodents we know that prolonged cold acclimation, which increases SNS activation of BAT (and WAT), induces the appearance of brown adipocytes in *all* adipose depots typically considered to be WAT. Brown adipocytes can be found within several typical WAT depots including cervical, axillary, perirenal, periadrenal, and pericardiac depots (Kortelainen et al., 1993; Cinti, 2005). The increase in these cells involves branching and increased activity of the sympathetic nerve fibers within both BAT and WAT (Garofalo et al., 1996; Giordano et al., 1996; De Matteis et al., 1998; Cinti, 1999, 2005). The essential role of the SNS in promoting the increased number and activity of these brown adipocytes is supported by data from mice deficient in the synthesis of noradrenaline or in βARs themselves (Thomas and Palmiter, 1997; Bachman et al., 2002; Jimenez et al., 2002). In these mutants, there is an absence of BAT that is replaced by WAT, and they tend to be obese. A similar loss of functioning BAT is observed in animals that have been maintained at thermoneutrality, a state in which there is little to no SNS activity in fat (Cinti, 1999). All together, these results suggest that β-agonists have the potential to promote postnatal changes in the WAT/BAT ratio. This could have profound implications, for example, in inducing weight loss in humans as is already observed in laboratory animals such as rodents as well as in adult dogs and rhesus monkeys (Arch et al., 1984; Champigny et al., 1991; Cousin et al., 1992; Himms-Hagen et al., 1994; Collins et al., 1997; Fisher et al., 1998). The latter two species, like humans, do not appear to retain large discrete depots of BAT in adulthood.

SIGNAL TRANSDUCTION NETWORKS OF βARs IN ADIPOCYTES

A basic biochemical scheme for the stimulation of adipocyte metabolism by βARs is presented in **Figure 1**. In both brown and white adipocytes activation of βARs sets in motion the classic signaling paradigm in which the receptor interacts with the heterotrimeric GTP-binding protein Gs that, in turn, activates adenylyl cyclase to raise intracellular cAMP levels, the target of which is the cAMP-dependent protein kinase (PKA). In adipocytes PKA phosphorylates several proteins that decorate the lipid droplet (Brasaemle et al., 2004; Brasaemle, 2007; Bickel et al., 2009) as well as lipases that catalyze the hydrolysis of triglycerides (Lass et al., 2011). The interaction of these phosphorylated proteins at the lipid droplet surface culminates in the release of free fatty acids and glycerol and their export into the circulation to be consumed as fuel in other tissues. However, signal transduction through GPCRs, including the βARs, can consist of multi-component complexes capable of activating several kinase pathways either concurrently or sequentially (see Luttrell, 2003 and Reiter and Lefkowitz, 2006 for reviews).

PKA is a well-established downstream agent of the βARs. However, other kinases are also activated by βARs in adipocytes such as ERK, p38 MAP, and AMP kinases (Collins et al., 2004; Gauthier et al., 2008; Omar et al., 2009). The activation of these other kinase classes sometimes depends directly upon PKA, while for others a connection to PKA exists only as a consequence of its lipid mobilizing effects (Gauthier et al., 2008). In addition, PKA in adipocytes is largely Type II, denoting its relative sensitivity to ambient cAMP levels as compared to Type I (Corbin et al., 1975). In adipocytes PKA is also membrane-anchored through interaction with the A-kinase anchoring proteins (AKAPS; McConnachie et al., 2006). Thus there are spatial aspects to PKA activation to consider as well. In that regard an interesting new report identifies optic atrophy-1 (OPA1), a protein best known for its role in mitochondrial "quality control", as an AKAP targeting PKA to the lipid droplet to facilitate perilipin phosphorylation and activation of lipolysis (Pidoux et al., 2011).

G protein-coupled receptors coupled to heterotrimeric Gi antagonize the activation of adenylyl cyclase in response to βARs. With the discovery that GPCRs coupled to Gi can also activate the ERK MAP kinase pathway (Daub et al., 1996; van Biesen et al., 1996), we now know that the picture is a bit more complex. Another dimension to this signaling is that GPCRs can, under certain circumstances, couple to more than one G protein. This is relevant to βAR signaling in adipocytes in that β_2AR was shown to activate ERK following their phosphorylation by PKA. As shown in **Figure 2**, this phosphorylation diminishes β_2AR coupling to Gs and increases coupling to Gi (Zamah et al., 2002; Martin et al., 2004). **Figure 2** also shows that this signaling pathway includes additional phosphorylation events by GPCR kinases (GRKs), which triggers the binding of β-arrestin (Lefkowitz et al., 2006): a scaffolding molecule first shown to be involved in the desensitization of β_2AR to terminate its activation of adenylyl cyclase (Lohse et al., 1990). Subsequently, Luttrell et al. (1999) showed that β-arrestin has broader roles; first showing that it could recruit Src kinase into a complex that leads to ERK activation through proline-rich regions that bind the SH3-domain of Src (see **Figure 2**).

Functionally this aspect of βAR signaling is important for adipocytes because βAR activation of ERK is responsible for about 20–25% of lipolysis (Greenberg et al., 2001; Robidoux et al., 2006). β_3AR in particular is interesting in this regard because it can activate both PKA and ERK by interacting with Gs and Gi interchangeably in the absence of receptor phosphorylation

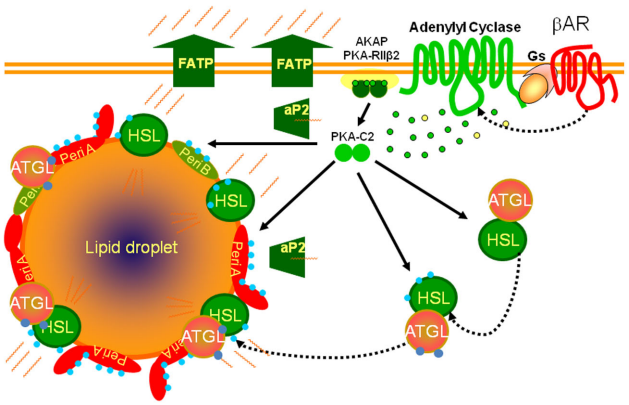

FIGURE 1 | Scheme for βAR signaling cascades controlling lipolysis in adipocytes. βARs activate adenylyl cyclase through their coupling to the heterotrimeric Gs, producing cAMP (green dots) from ATP (yellow dots) to activate the cAMP-dependent protein kinase (PKA), allowing the catalytic subunits (PKA-C2) to be released from the holoenzyme that is anchored to the plasma membrane (AKAP). PKA phosphorylates (blue dots) lipases (HSL: hormone sensitive lipase) and lipid droplet binding proteins such as perilipins (Peri A; Peri B). Adipose triglyceride lipase (ATGL) is phosphorylated but not by PKA. The fatty acids released from triglyceride are chaperoned out of the cell by lipid binding proteins (aP2) and exported through fatty acid transport proteins (FATP).

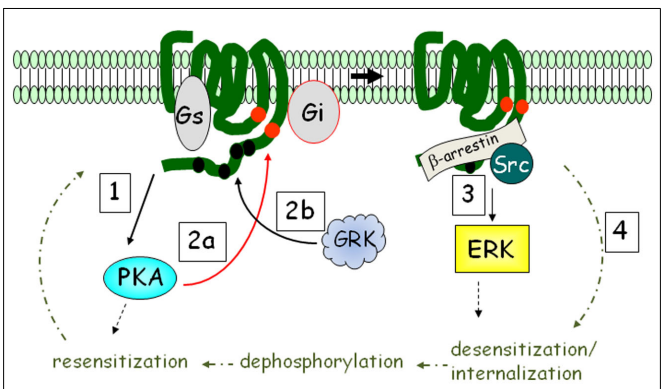

FIGURE 2 | βARs activate ERK MAPK in addition to PKA: the β_2AR mechanism as an example. Upon catecholamine activation, PKA is activated (step 1), and phosphorylates the receptor at intracellular sites (red circles; step 2a). The receptor is also phosphorylated by G protein-coupled receptor kinase (GRK) at multiple sites in the C-terminus (step 2b). PKA phosphorylation interdicts interaction with Gs to favor Gi, while GRK recruits β-arrestin, promoting ERK activation (step 3). β_2AR is subject to rapid desensitization and internalization (step 4), whereupon phosphatases remove the phosphates and the receptor is recycled back to the plasma membrane.

and β-arrestin (Liggett et al., 1993; Soeder et al., 1999). Instead, we identified a novel mechanism by which β_3AR is able to activate ERK. As illustrated in **Figure 3A**, β_3AR contains clusters of proline motifs in the two most important intracellular regions of a GPCR: the third intracellular loop and the carboxyl terminus. These prolines are arranged in the PXXP motif that serves as agonist-dependent docking sites for the SH3-domain of Src. The ability of β_3AR to directly recruit Src kinase in adipocytes is shown in **Figure 3B** (adapted from Cao et al., 2000, and see legend). The β_3AR-selective agonist CL316243 (CL), triggers ERK activation via the endogenous β_3AR in non-transfected (*NT*) cells, and in cells transfected with HA-tagged mouse β_3AR. In the presence of pertussis toxin (PTX), ERK activation was abolished, indicating the requirement for signaling through the heterotrimeric Gi. **Figure 3B** shows that Src kinase co-precipitates with the HA-β_3AR, and this interaction is both β_3-agonist and Gi-dependent. As shown further by Cao et al. there are three to four of these PXXP motifs within all the species homologs of β_3AR, but perturbations of only one or two of these are enough to eliminate ERK activation (Cao et al., 2000; Kumar et al., 2007).

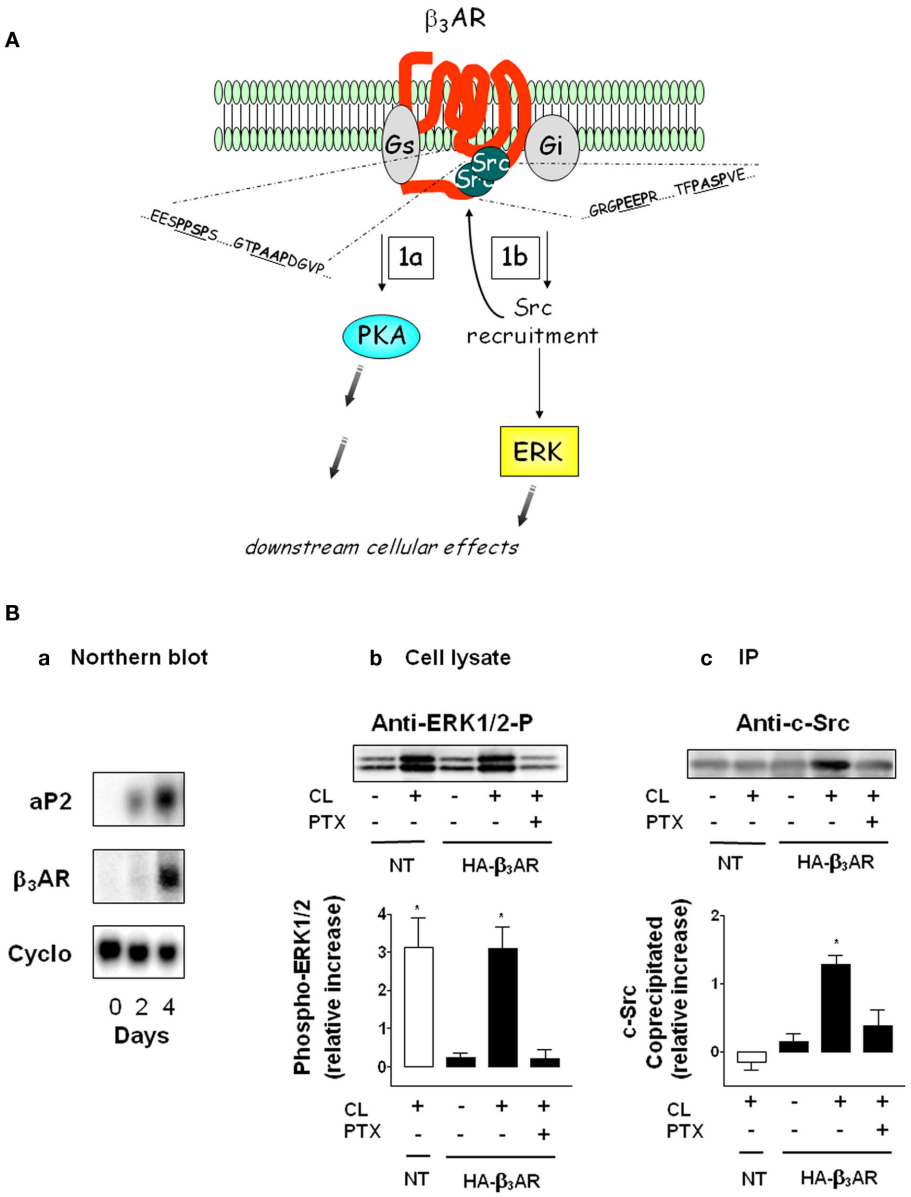

FIGURE 3 | A unique dual signaling mechanism by β_3AR. (A) The receptor can couple to both Gs and Gi to activate PKA (step 1a) and ERK (step 1b). The shaded gray arrows point to subsequent downstream cellular effects. **(B)** Preadipocytes were transfected or not (NT) with hemagglutinin (HA)-tagged β_3AR and differentiated. **(a)** Expression of aP2 and β_3AR mRNA as a function of differentiation on the indicated days. Levels of aP2 are maximal by day 4. Cyclophilin RNA (*Cyclo*) is the internal control. **(b)** Phospho-ERK1/2 in cell lysates in response to the β_3AR agonist CL316243 (CL) in the absence or presence of pertussis toxin (PTX; $n = 3$; mean ± SD). **(c)** Level of c-Src co-precipitated with β_3AR ($n = 3$; mean ± SD). (Adapted from Cao et al., 2000).

These results suggested that there could perhaps be other molecules in addition to Src that might be recruited to the receptor as part of a larger complex. A proteomic search for proteins that could be co-immunoprecipitated with the β_3AR from adipocytes in an agonist-dependent manner identified several proteins that interact with the receptor and the proline motifs, among which was the intermediate filament protein vimentin (Kumar et al., 2007). Vimentin is a component of the lipid droplet in adipocytes (Franke et al., 1987), and was found to be required in adipocytes for both ERK activation and lipolysis (Kumar et al., 2007). Several aspects of this mechanism as well as its significance remain incomplete. These include (i) knowledge of the target(s) of ERK that are responsible for lipolysis; (ii) the mechanism by which vimentin is recruited to the β_3AR, since it does not possess an SH3 binding domain, and therefore perhaps requires a protein intermediate; (iii) the relative importance of ERK in lipolysis in human adipocytes; (iv) what other events might be regulated by this βAR-ERK pathway in adipocytes, such as the control of adipose-secreted factors as one example. Finally, the function of this βAR-activated ERK pathway in brown adipocytes and the role of insulin in these effects has been partly investigated (Klein et al., 2000; Lindquist et al., 2000; Fasshauer et al., 2001) but much work remains to be done both mechanistically and in physiological terms to understand their roles and impact.

In addition to ERK, other MAPK kinases are also activated in response to βARs in adipocytes. p38 MAPK is activated by βARs in white (Moule and Denton, 1998; Mizuno et al., 2002) and brown adipocytes (Cao et al., 2001), including human adipocytes (**Figure 4**). As shown in **Figure 5**, we showed that p38 MAPK activation is indirectly dependent on PKA. An interesting aspect of βAR-dependent activation of MAPKs in adipocytes is that JNK is not activated by βARs or cAMP (Robidoux et al., 2005). Important downstream targets of this pathway in brown adipocytes are the transcription of the *Ucp1* and *PGC-1α* genes (Cao et al., 2001, 2004). Later work proposed p38 MAPK as one of the discriminating factors in the ability of certain mouse strains such as A/J to increase brown adipocyte numbers in WAT during early postnatal growth and development (Xue et al., 2006). p38 MAPK has also been implicated as a downstream factor in the reported permissive property of BMP7 to contribute to brown adipocyte differentiation (Tseng et al., 2008). If we think about how the three βARs activate the same pathways, it becomes clear that although the pathways are the same, the timing of the signaling is not necessarily identical, and signal strength can also be affected. For example, β_3AR sacrifices amplitude of cAMP generation to gain the ability to generate two signaling pathways simultaneously. These concepts are illustrated in **Figure 6**.

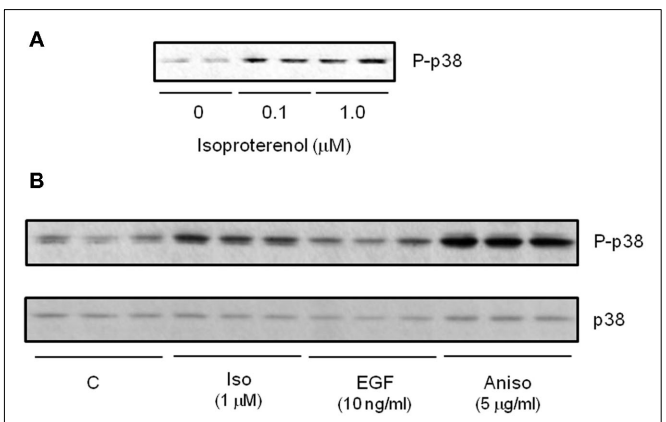

FIGURE 4 | β-agonists increase activation of p38 MAPK in cultured human subcutaneous adipocytes. (A) Western blot measuring isoproterenol (Iso) concentration-response for phospho-p38 MAPK (P-p38). **(B)** Western blot comparing P-p38 response to 15 min treatment with Iso, EGF and the positive control cell stressor Anisomycin. All samples shown are independent biological replicates.

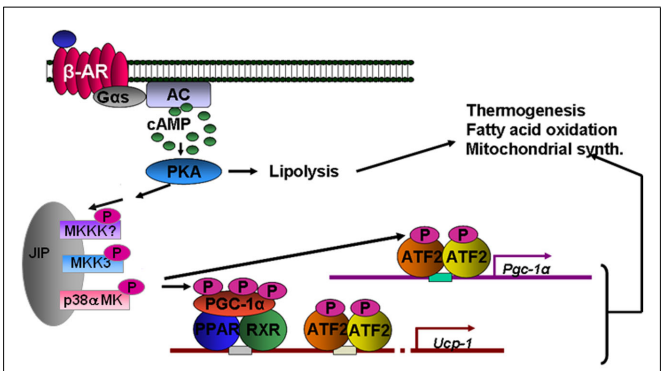

FIGURE 5 | βAR activation of thermogenesis through p38 MAPK. Direct targets of p38 MAPK include peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) and ATF-2. These target the genes for *Pgc-1α* and *Ucp1*. The tandem arrows between PKA and the MAPK module are not yet biochemically defined.

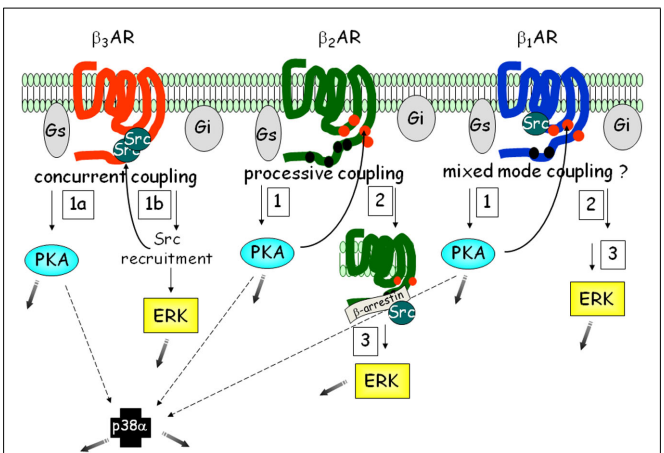

FIGURE 6 | Signaling networks of the βARs in adipocytes. Each of the three βARs is able to activate similar pathways: PKA, ERK, and p38 MAPK. However the order and number of steps involved varies, such that β_3AR represents what we term "concurrent" coupling to Gs vs. Gi, vs. the β_2AR type of "processive" coupling. Less is known about the details of β_1AR. Because β_1AR also contains one of the PXXP motifs as in β_3AR, but it is also phosphorylated, we have provisionally termed in "mixed-mode" coupling. The shaded gray arrows point to subsequent downstream cellular effects.

Mechanistically, the signaling components between PKA and p38 MAPK are not understood, although we have made some progress toward this goal. It appears that p38α MAPK, but not p38β MAPK, is activated in brown adipocytes downstream of βARs and PKA, and that the immediate upstream kinase appears to specifically be MKK3 (Robidoux et al., 2005). In addition, although this βAR → PKA → p38 MAPK pathway also exists in white adipocytes (including human adipocytes) the downstream functional consequences are as yet unknown. We generated mice with adipose-specific deletion of p38α to uncover the role of PKA-dependent p38α MAPK activation in white adipocytes. In the absence of p38α in adipocytes, preliminary evidence suggests that JNK now becomes active, similar to observations made for some other tissue-specific deletions of p38α MAPK (Hui et al., 2007; Perdiguero et al., 2007; Ventura et al., 2007; Wada et al., 2008). Inappropriate JNK activation that occurs in other metabolic tissues such as muscle, liver, and fat has been associated with insulin resistance (Vallerie et al., 2008). Therefore, the complete characterization of these mice will hopefully fill in these gaps in our understanding of the mechanism(s) and physiological interplay between p38 MAPK and JNK in adipocytes and the mechanisms of their control.

Within the last couple of years there has been increasing evidence that AMPK is activated in adipocytes in response to lipolytic stimuli such as βARs (Hutchinson et al., 2005; Koh et al., 2007; Mulligan et al., 2007; Gauthier et al., 2008; Omar et al., 2009). AMPK activation is not necessarily a result of direct involvement of PKA but appears to be more related to lipolysis itself and its effect in reducing the net ATP levels within adipocytes. These findings suggest that lipolysis results in the increase in cellular energy consumption and this is proposed to be due to the stimulation of energy consuming processes such as fatty acid re-esterification and even mitochondrial uncoupling (Gauthier et al., 2008; Yehuda-Shnaidman et al., 2010).

UNCOVERING PATHWAYS THAT SILENCE OR REPRESS ENERGY EXPENDITURE IN BROWN ADIPOCYTES

We spend much effort trying to understand how to "stimulate" or "activate" and "turn on" catabolic pathways such as adaptive thermogenesis in brown adipocytes. However, if there are active mechanisms of repression, we need to identify them in order to release the "brake." Certain targeted gene disruptions in mice that result in a lean phenotype have provided some clues to such repressive pathways. Some of these genes include p70 S6 kinase (Um et al., 2004), estrogen related receptor-α (Luo et al., 2003), co-repressor RIP140 (Leonardsson et al., 2004), the liver X receptors (LXR; Kalaany et al., 2005), and others (Dong et al., 2009; Gao et al., 2009). Although the connections that may exist between these factors are not clear, mechanistically we have uncovered an interesting role at least for the nuclear factors LXRα and RIP140 to function as a regulated repressor complex that blocks βAR-stimulated *Ucp1* gene expression (Wang et al., 2008; Collins et al., 2010). Specifically, LXRα recruits the co-repressor RIP140 to a DR-4 element in the *Ucp1* enhancer adjacent to the PPAR/RXR binding DR-1 motif, leading to suppression of *Ucp1* gene transcription. Given the common target and interaction between LXR and RIP140, we can link the phenotype of these individual mutations to a common mechanism. A necessary element for this repression by LXRα appears to be the availability of an LXR ligand. While the nature of these ligands and the enzymes that control their production are still a matter of much debate (Russell, 2000), at least one suggested candidate, cholesterol-25-hydroxylase, is expressed in white adipocytes at a level 30 times higher than in brown adipocytes (Wang et al., 2008). Therefore, this potential point of control will be an important feature to further explore. Since the ability to expand these reservoirs of "novel" brown adipocytes *in vivo*, to mobilize fuel and to activate thermogenesis all depend on the catecholamine stimulation of βARs, our understanding of the signaling mechanisms of these receptors, and how they themselves are regulated, will be a key factor in harnessing the capacity for increasing these "plastic" brown adipocytes.

CONCLUSION

Although much is known about the signal transduction mechanism of βARs, the future ahead is still full of work to be done. Undoubtedly more surprises will be in store for us as we continue to uncover the signaling networks that βARs control in adipocytes and how we might be able to take advantage of this new knowledge to therapeutically intervene in combating the epidemic of metabolic disease in our midst.

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REFERENCES

Arch, J. R. (2002). beta(3)-Adrenoceptor agonists: potential, pitfalls and progress. *Eur. J. Pharmacol.* 440, 99–107.

Arch, J. R. S., Ainsworth, A. T., Ellis, R. D., Piercy, V., Thody, V. E., Thurlby, P. L., Wilson, C., Wilson, S., and Young, P. (1984). Treatment of obesity with thermogenic β-adrenoceptor agonists: studies on BRL 28630A in rodents. *Int. J. Obes.* 8, 1–11.

Bachman, E. S., Dhillon, H., Zhang, C. Y., Cinti, S., Bianco, A. C., Kobilka, B. K., and Lowell, B. B. (2002). betaAR signaling required for diet-induced thermogenesis and obesity resistance. *Science* 297, 843–845.

Bickel, P. E., Tansey, J. T., and Welte, M. A. (2009). PAT proteins, an ancient family of lipid droplet proteins that regulate cellular lipid stores. *Biochim. Biophys. Acta* 1791, 419–440.

Brasaemle, D. L. (2007). Thematic review series: adipocyte biology. The perilipin family of structural lipid droplet proteins: stabilization of lipid droplets and control of lipolysis. *J. Lipid Res.* 48, 2547–2559.

Brasaemle, D. L., Dolios, G., Shapiro, L., and Wang, R. (2004). Proteomic analysis of proteins associated with lipid droplets of basal and lipolytically stimulated 3T3-L1 adipocytes. *J. Biol. Chem.* 279, 46835–46842.

Buemann, B., Toubro, S., and Astrup, A. (2000). Effects of the two beta3-agonists, ZD7114 and ZD2079 on 24 hour energy expenditure and respiratory quotient in obese subjects. *Int. J. Obes. Relat. Metab. Disord.* 24, 1553–1560.

Cao, W., Daniel, K. W., Robidoux, J., Puigserver, P., Medvedev, A. V., Bai, X., Floering, L. M., Spiegelman, B. M., and Collins, S. (2004). p38 MAP kinase is the central regulator of cAMP-dependent transcription of the brown fat uncoupling protein-1 gene. *Mol. Cell. Biol.* 24, 3057–3067.

Cao, W., Luttrell, L. M., Medvedev, A. V., Pierce, K. L., Daniel, K. W., Dixon, T. M., Lefkowitz, R. J., and Collins, S. (2000). Direct binding of activated c-Src to the β3-adrenergic receptor is required for MAP kinase activation. *J. Biol. Chem.* 275, 38131–38134.

Cao, W., Medvedev, A. V., Daniel, K. W., and Collins, S. (2001). β-Adrenergic activation of p38 MAP kinase in adipocytes: cAMP induction of the uncoupling protein-1 (UCP1) gene requires p38 MAP kinase. *J. Biol. Chem.* 276, 27077–27082.

Champigny, O., Ricquier, D., Blondel, O., Mayers, R. M., Briscoe, M. G., and Holloway, B. R. (1991). Beta 3-adrenergic receptor stimulation restores message and expression of brown-fat mitochondrial uncoupling protein in adult dogs. *Proc. Natl. Acad. Sci. U.S.A.* 88, 10774–10777.

Cinti, S. (1999). *The Adipose Organ.* Milano: Editrice Kurtis.

Cinti, S. (2005). The adipose organ. *Prostaglandins Leukot. Essent. Fatty Acids* 73, 9–15.

Collins, S., Cao, W., and Robidoux, J. (2004). Learning new tricks from old dogs: beta-adrenergic receptors teach new lessons on firing up adipose tissue metabolism. *Mol. Endocrinol.* 18, 2123–2131.

Collins, S., Daniel, K. W., Petro, A. E., and Surwit, R. S. (1997). Strain-specific response to β3-adrenergic receptor agonist treatment of diet-induced obesity in mice. *Endocrinology* 138, 405–413.

Collins, S., Yehuda-Shnaidman, E., and Wang, H. (2010). Positive and negative control of Ucp1 gene transcription and the role of beta-adrenergic signaling networks. *Int. J. Obes. (Lond.)* 34(Suppl. 1), S28–S33.

Corbin, J. D., Keely, S. L., Soderling, T. R., and Park, C. R. (1975). Hormonal regulation of adenosine 3′,5′-monophosphate-dependent protein kinase. *Adv. Cyclic Nucleotide Res.* 5, 265–279.

Cousin, B., Cinti, S., Morroni, M., Raimbault, S., Ricquier, D., Pénicaud, L., and Casteilla, L. (1992). Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization. *J. Cell. Sci.* 103, 931–942.

Daub, H., Weiss, F. U., Wallasch, C., and Ullrich, A. (1996). Role of transactivation of the EGF receptor in signalling by G-protein-coupled receptors. *Nature* 379, 557–560.

De Matteis, R., Ricquier, D., and Cinti, S. (1998). TH-, NPY-, SP-, and CGRP-immunoreactive nerves in interscapular brown adipose tissue of adult rats acclimated at different temperatures: an immunohistochemical study. *J. Neurocytol.* 27, 877–886.

Dong, B., Saha, P. K., Huang, W., Chen, W., Abu-Elheiga, L. A., Wakil, S. J., Stevens, R. D., Ilkayeva, O., Newgard, C. B., Chan, L., and Moore, D. D. (2009). Activation of nuclear receptor CAR ameliorates diabetes and fatty liver disease. *Proc. Natl. Acad. Sci. U.S.A.* 106, 18831–18836.

Fasshauer, M., Klein, J., Kriauciunas, K. M., Ueki, K., Benito, M., and Kahn, C. R. (2001). Essential role of insulin receptor substrate 1 in differentiation of brown adipocytes. *Mol. Cell. Biol.* 21, 319–329.

Fisher, M. H., Amend, A. M., Bach, T. J., Barker, J. M., Brady, E. J., Candelore, M. R., Carroll, D., Cascieri, M. A., Chiu, S. H., Deng, L., Forrest, M. J., Hegarty-Friscino, B., Guan, X. M., Hom, G. J., Hutchins, J. E., Kelly, L. J., Mathvink, R. J., Metzger, J. M., Miller, R. R., Ok, H. O., Parmee, E. R., Saperstein, R., Strader, C. D., Stearns, R. A., and MacIntyre, D. E. (1998). A selective human beta3 adrenergic receptor agonist increases metabolic rate in rhesus monkeys. *J. Clin. Invest.* 101, 2387–2393.

Franke, W. W., Hergt, M., and Grund, C. (1987). Rearrangement of the vimentin cytoskeleton during adipose conversion: formation of an intermediate filament cage around lipid globules. *Cell* 49, 131–141.

Gao, J., He, J., Zhai, Y., Wada, T., and Xie, W. (2009). The constitutive androstane receptor is an anti-obesity nuclear receptor that improves insulin sensitivity. *J. Biol. Chem.* 284, 25984–25992.

Garofalo, M. A., Kettelhut, I. C., Roselino, J. E., and Migliorini, R. H. (1996). Effect of acute cold exposure on norepinephrine turnover rates in rat white adipose tissue. *J. Auton. Nerv. Syst.* 60. 206–208.

Gauthier, M. S., Miyoshi, H., Souza, S. C., Cacicedo, J. M., Saha, A. K., Greenberg, A. S., and Ruderman, N. B. (2008). AMP-activated protein kinase is activated as a consequence of lipolysis in the adipocyte: potential mechanism and physiological relevance. *J. Biol. Chem.* 283, 16514–16524.

Giordano, A., Morroni, M., Santone, G., Marchesi, G. F., and Cinti, S. (1996). Tyrosine hydroxylase, neuropeptide Y, substance P, calcitonin gene-related peptide and vasoactive intestinal peptide in nerves of rat periovarian adipose tissue: an immunohistochemical and ultrastructural investigation. *J. Neurocytol.* 25, 125–136.

Greenberg, A. S., Shen, W. J., Muliro, K., Patel, S., Souza, S. C., Roth, R. A., and Kraemer, F. B. (2001). Stimulation of lipolysis and hormone-sensitive lipase via the extracellular signal-regulated kinase pathway. *J. Biol. Chem.* 276, 45456–45461.

Himms-Hagen, J., Cui, J., Danforth, E. Jr., Taatjes, D. J., Lang, S. S., Waters, B. L., and Claus, T. H. (1994). Effect of CL-316,243, a thermogenic β3-agonist, on energy balance and brown and white adipose tissues in rats. *Am. J. Physiol.* 266, R1371–R1382.

Hui, L., Bakiri, L., Mairhorfer, A., Schweifer, N., Haslinger, C., Kenner, L., Komnenovic, V., Scheuch, H., Beug, H., and Wagner, E. F. (2007). p38alpha suppresses normal and cancer cell proliferation by antagonizing the JNK-c-Jun pathway. *Nat. Genet.* 39, 741–749.

Hutchinson, D. S., Chernogubova, E., Dallner, O. S., Cannon, B., and Bengtsson, T. (2005). Beta-adrenoceptors, but not alpha-adrenoceptors, stimulate AMP-activated protein kinase in brown adipocytes independently of uncoupling protein-1. *Diabetologia* 48, 2386–2395.

Jimenez, M., Léger, B., Canola, K., Lehr, L., Arboit, P., Seydoux, J., Russell, A. P., Giacobino, J. P., Muzzin, P., and Preitner, F. (2002). Beta(1)/beta(2)/beta(3)-adrenoceptor knockout mice are obese and cold-sensitive but have normal lipolytic responses to fasting. *FEBS Lett.* 530, 37–40.

Kalaany, N. Y., Gauthier, K. C., Zavacki, A. M., Mammen, P. P., Kitazume, T., Peterson, J. A., Horton, J. D., Garry, D. J., Bianco, A. C., and Mangelsdorf, D. J. (2005). LXRs regulate the balance between fat storage and oxidation. *Cell Metab.* 1, 231–244.

Klein, J., Fasshauer, M., Benito, M., and Kahn, C. R. (2000). Insulin and the beta3-adrenoceptor differentially regulate uncoupling protein-1 expression. *Mol. Endocrinol.* 14, 764–773.

Koh, H. J., Hirshman, M. F., He, H., Li, Y., Manabe, Y., Balschi, J. A., and Goodyear, L. J. (2007). Adrenaline is a critical mediator of acute exercise-induced AMP-activated protein kinase activation in adipocytes. *Biochem. J.* 403, 473–481.

Kortelainen, M.-L., Pelletier, G., Ricquier, D., and Bukowiecki, L. J. (1993). Immunohistochemical detection of human brown adipose tissue uncoupling protein in an autopsy series. *J. Histochem. Cytochem.* 41, 759–764.

Kumar, N., Robidoux, J., Daniel, K. W., Guzman, G., Floering, L. M., and Collins, S. (2007). Requirement of vimentin filament assembly for beta3-adrenergic receptor activation of ERK MAP kinase and lipolysis. *J. Biol. Chem.* 282, 9244–9250.

Lass, A., Zimmermann, R., Oberer, M., and Zechner, R. (2011). Lipolysis – a highly regulated multi-enzyme complex mediates the catabolism of cellular fat stores. *Prog. Lipid Res.* 50, 14–27.

Lean, M. E. J. (1989). Brown adipose tissue in humans. *Proc. Nutr. Soc.* 48, 243–256.

Lefkowitz, R. J., Rajagopal, K., and Whalen, E. J. (2006). New roles for beta-arrestins in cell signaling: not just for seven-transmembrane receptors. *Mol. Cell* 24, 643–652.

Leonardsson, G., Steel, J. H., Christian, M., Pocock, V., Milligan, S., Bell, J., So, P. W., Medina-Gomez, G., Vidal-Puig, A., White, R., and Parker, M. G. (2004). Nuclear receptor corepressor RIP140 regulates fat accumulation. *Proc. Natl. Acad. Sci. U.S.A.* 101, 8437–8442.

Liggett, S., Freedman, N. J., Schwinn, D. A., and Lefkowitz, R. J. (1993). Structural basis for receptor subtype-specific regulation revealed by a chimeric β3-/β2-adrenergic receptor. *Proc. Natl. Acad. Sci. U.S.A.* 90, 3665–3669.

Lindquist, J. M., Fredriksson, J. M., Rehnmark, S., Cannon, B., and Nedergaard, J. (2000). beta 3- and alpha 1-adrenergic Erk1/2 activation is Src- but not Gi-mediated in brown adipocytes. *J. Biol. Chem.* 275, 22670–22677.

Lohse, M., Benovic, J. L., Codina, J., Caron, M. G., and Lefkowitz, R. J. (1990). β−arrestin: a protein that regulates βadrenergic receptor function. *Science* 248, 1547–1550.

Luo, J., Sladek, R., Carrier, J., Bader, J. A., Richard, D., and Giguère, V. (2003). Reduced fat mass in mice lacking orphan nuclear receptor estrogen-related receptor alpha. *Mol. Cell. Biol.* 23, 7947–7956.

Luttrell, L. (2003). 'Location, location, location': activation and targeting of MAP kinases by G protein-coupled receptors. *J. Mol. Endocrinol.* 30, 117–126.

Luttrell, L. M., Ferguson, S. S., Daaka, Y., Miller, W. E., Maudsley, S., Della Rocca, G. J., Lin, F., Kawakatsu, H., Owada, K., Luttrell, D. K., Caron, M. G., and Lefkowitz, R. J. (1999). Beta-arrestin-dependent formation of beta2 adrenergic receptor-Src protein kinase complexes. *Science* 283, 655–661.

Martin, N. P., Whalen, E. J., Zamah, M. A., Pierce, K. L., and Lefkowitz, R. J. (2004). PKA-mediated phosphorylation of the

beta1-adrenergic receptor promotes Gs/Gi switching. *Cell. Signal.* 16, 1397–1403.

McConnachie, G., Langeberg, L. K., and Scott, J. D. (2006). AKAP signaling complexes: getting to the heart of the matter. *Trends Mol. Med.* 12, 317–323.

Mizuno, K., Kanda, Y., Kuroki, Y., Nishio, M., and Watanabe, Y. (2002). Stimulation of beta(3)-adrenoceptors causes phosphorylation of p38 mitogen-activated protein kinase via a stimulatory G protein-dependent pathway in 3T3-L1 adipocytes. *Br. J. Pharmacol.* 135, 951–960.

Moule, S. K., and Denton, R. M. (1998). The activation of p38 MAPK by the beta-adrenergic agonist isoproterenol in rat epididymal fat cells. *FEBS Lett.* 439, 287–290.

Mulligan, J. D., Gonzalez, A. A., Stewart, A. M., Carey, H. V., and Saupe, K. W. (2007). Upregulation of AMPK during cold exposure occurs via distinct mechanisms in brown and white adipose tissue of the mouse. *J. Physiol. (Lond.)* 580, 677–684.

Nedergaard, J., Golozoubova, V., Matthias, A., Asadi, A., Jacobsson, A., and Cannon, B. (2001). UCP1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic inefficiency. *Biochim. Biophys. Acta* 1504, 82–106.

Omar, B., Zmuda-Trzebiatowska, E., Manganiello, V., Göransson, O., and Degerman, E. (2009). Regulation of AMP-activated protein kinase by cAMP in adipocytes: roles for phosphodiesterases, protein kinase B, protein kinase A, Epac and lipolysis. *Cell. Signal.* 21, 760–766.

Perdiguero, E., Ruiz-Bonilla, V., Gresh, L., Hui, L., Ballestar, E., Sousa-Victor, P., Baeza-Raja, B., Jardí, M., Bosch-Comas, A., Esteller, M., Caelles, C., Serrano, A. L., Wagner, E. F., and Muñoz-Cánoves, P. (2007). Genetic analysis of p38 MAP kinases in myogenesis: fundamental role of p38alpha in abrogating myoblast proliferation. *EMBO J.* 26, 1245–1256.

Pidoux, G., Witczak, O., Jarnæss, E., Myrvold, L., Urlaub, H., Stokka, A. J., Küntziger, T., and Taskén, K. (2011). Optic atrophy 1 is an A-kinase anchoring protein on lipid droplets that mediates adrenergic control of lipolysis. *EMBO J.* 30, 3271–3286.

Reiter, E., and Lefkowitz, R. J. (2006). GRKs and beta-arrestins: roles in receptor silencing, trafficking and signaling. *Trends Endocrinol. Metab.* 17, 159–165.

Robidoux, J., Cao, W., Quan, H., Daniel, K. W., Moukdar, F., Bai, X., Floering, L. M., and Collins, S. (2005). Selective activation of mitogen-activated protein (MAP) kinase kinase 3 and p38{alpha} MAP kinase is essential for cyclic AMP-dependent UCP1 expression in adipocytes. *Mol. Cell. Biol.* 25, 5466–5479.

Robidoux, J., Kumar, N., Daniel, K. W., Moukdar, F., Cyr, M., Medvedev, A. V., and Collins, S. (2006). Maximal beta3-adrenergic regulation of lipolysis involves Src and epidermal growth factor receptor-dependent ERK1/2 activation. *J. Biol. Chem.* 281, 37794–37802.

Russell, D. W. (2000). Oxysterol biosynthetic enzymes. *Biochim. Biophys. Acta* 1529, 126–135.

Soeder, K. S., Snedden, S. K., Cao, W., Della Rocca, G. J., Daniel, K. W., Luttrell, L. M., and Collins, S. (1999). The β3-adrenergic receptor activates mitogen-activated protein kinase in adipocytes through a Gi-dependent mechanism. *J. Biol. Chem.* 274, 12017–12022.

Thomas, S. A., and Palmiter, R. D. (1997). Thermoregulatory and metabolic phenotypes of mice lacking noradrenaline and adrenaline. *Nature* 397, 94–97.

Tseng, Y. H., Kokkotou, E., Schulz, T. J., Huang, T. L., Winnay, J. N., Taniguchi, C. M., Tran, T. T., Suzuki, R., Espinoza, D. O., Yamamoto, Y., Ahrens, M. J., Dudley, A. T., Norris, A. W., Kulkarni, R. N., and Kahn, C. R. (2008). New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454, 1000–1004.

Um, S. H., Frigerio, F., Watanabe, M., Picard, F., Joaquin, M., Sticker, M., Fumagalli, S., Allegrini, P. R., Kozma, S. C., Auwerx, J., and Thomas, G. (2004). Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity. *Nature* 431, 200–205.

Vallerie, S. N., Furuhashi, M., Fucho, R., and Hotamisligil, G. S. (2008). A predominant role for parenchymal c-Jun amino terminal kinase (JNK) in the regulation of systemic insulin sensitivity. *PLoS ONE* 3, e3151. doi:10.1371/journal.pone.0003151

van Biesen, T., Luttrell, L. M., Hawes, B. E., and Lefkowitz, R. J. (1996). Mitogenic signaling via G protein-coupled receptors. *Endocr. Rev.* 17, 698–714.

Ventura, J. J., Tenbaum, S., Perdiguero, E., Huth, M., Guerra, C., Barbacid, M., Pasparakis, M., and Nebreda, A. R. (2007). p38alpha MAP kinase is essential in lung stem and progenitor cell proliferation and differentiation. *Nat. Genet.* 39, 750–758.

Wada, T., Stepniak, E., Hui, L., Leibbrandt, A., Katada, T., Nishina, H., Wagner, E. F., and Penninger, J. M. (2008). Antagonistic control of cell fates by JNK and p38-MAPK signaling. *Cell Death Differ.* 15, 89–93.

Wang, H., Zhang, Y., Yehuda-Shnaidman, E., Medvedev, A. V., Kumar, N., Daniel, K. W., Robidoux, J., Czech, M. P., Mangelsdorf, D. J., and Collins, S. (2008). Liver X receptor is a transcriptional repressor of the uncoupling protein-1 gene and brown adipocyte phenotype. *Mol. Cell. Biol.* 28, 2187–2200.

Xue, B., Rim, J. S., Hogan, J. C., Coulter, A. A., Koza, R. A., and Kozak, L. P. (2006). Genetic variability affects the development of brown adipocytes in white fat, but not in interscapular brown fat. *J. Lipid Res.* 48, 41–51.

Yehuda-Shnaidman, E., Buehrer, B., Pi, J., Kumar, N., and Collins, S. (2010). Acute stimulation of white adipocyte respiration by PKA-induced lipolysis. *Diabetes* 59, 2474–2483.

Zamah, A. M., Delahunty, M., Luttrell, L. M., and Lefkowitz, R. J. (2002). Protein kinase A-mediated phosphorylation of the beta 2-adrenergic receptor regulates its coupling to Gs and Gi. Demonstration in a reconstituted system. *J. Biol. Chem.* 277, 31249–31256.

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Central control of brown adipose tissue thermogenesis

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Thermogenesis, the production of heat energy, is an essential component of the homeostatic repertoire to maintain body temperature during the challenge of low environmental temperature and plays a key role in elevating body temperature during the febrile response to infection. Mitochondrial oxidation in brown adipose tissue (BAT) is a significant source of neurally regulated metabolic heat production in many species from mouse to man. BAT thermogenesis is regulated by neural networks in the central nervous system which responds to feedforward afferent signals from cutaneous and core body thermoreceptors and to feedback signals from brain thermosensitive neurons to activate BAT sympathetic nerve activity. This review summarizes the research leading to a model of the feedforward reflex pathway through which environmental cold stimulates BAT thermogenesis and includes the influence on this thermoregulatory network of the pyrogenic mediator, prostaglandin E_2, to increase body temperature during fever. The cold thermal afferent circuit from cutaneous thermal receptors, through second-order thermosensory neurons in the dorsal horn of the spinal cord ascends to activate neurons in the lateral parabrachial nucleus which drive GABAergic interneurons in the preoptic area (POA) to inhibit warm-sensitive, inhibitory output neurons of the POA. The resulting disinhibition of BAT thermogenesis-promoting neurons in the dorsomedial hypothalamus activates BAT sympathetic premotor neurons in the rostral ventromedial medulla, including the rostral raphe pallidus, which provide excitatory, and possibly disinhibitory, inputs to spinal sympathetic circuits to drive BAT thermogenesis. Other recently recognized central sites influencing BAT thermogenesis and energy expenditure are also described.

Keywords: brown adipose tissue, thermogenesis, thermoregulation, sympathetic nerve activity, preoptic hypothalamus, fever, rostral raphe pallidus, rostral ventromedial medulla

INTRODUCTION

Thermogenesis, the production of heat energy, is an essential component of the homeostatic repertoire to maintain body temperature during the challenge of low environmental temperature. The heat generated during pyrogen-stimulated thermogenesis in brown adipose tissue (BAT) also contributes to fever, a controlled elevation in body temperature that reduces pathogen viability and stimulates immune cell responses. Since energy consumption during thermogenesis can involve oxidation of lipid fuel molecules, regulation of thermogenesis in response to metabolic signals can also contribute to energy balance and regulation of body adipose stores. Indeed, there is increasing interest in devising pharmacological approaches to maintaining the activation of BAT energy consumption as a metabolic furnace to burn the excess calories stored in the white adipose tissue of the obese (Clapham, 2011).

Thermogenesis can occur to a greater or lesser extent in most tissues, since heat generation is a by-product of the inefficiency of mitochondrial ATP production and of ATP utilization. However, CNS thermoregulatory networks can stimulate thermogenesis in response to a cold environment, to a fall in core body temperature or to the presence of pyrogenic cytokines primarily in three tissues: BAT, skeletal muscle, and the heart. In contrast to the indirect nature of shivering thermogenesis in skeletal muscles that are normally used to produce movement and posture, "non-shivering" thermogenesis in BAT is the specific metabolic function of this tissue and is accomplished by the heat generating capacity of a significant facilitated proton leak across the extensive mitochondrial membranes of the brown adipocytes which occurs because of the high expression of uncoupling protein-1 (UCP1) in BAT mitochondria (Cannon and Nedergaard, 2004). The levels of BAT sympathetic nerve activity (SNA), of ganglion cell norepinephrine release and of β3-adrenergic receptor binding to brown adipocytes determine the level of thermogenesis in BAT by regulating both the activity of lipases, such as hormone-sensitive lipase and adipose tissue triacylglycerol lipase, providing the immediate fuel molecules for BAT mitochondria and the level of expression of BAT mitochondrial UCP1 (Cannon and Nedergaard, 2004). Recently, alternatively activated, adipose tissue macrophages have also emerged as a significant source of norepinephrine for the cold-evoked stimulation of BAT thermogenesis (Nguyen et al., 2011).

Brown adipose tissue has developed as an essential thermoregulatory effector in cold defense in rodents and other small mammals (Golozoubova et al., 2006), including infant humans, whose large surface area to body mass ratio suggests that basal metabolism alone would yield insufficient heat to maintain body temperature in cold environments. Recent observations using PET to assess tissue glucose uptake (reviewed in Nedergaard et al., 2007) and

UCP1 analysis of biopsied tissue have confirmed a remarkable amount of metabolically active, BAT in adult humans (Hany et al., 2002; Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). The functional locations of BAT depots in adult humans bear an interesting similarity to those in rodents: a large BAT pad in the vicinity of the upper thorax, individual pads atop each of the paravertebral sympathetic ganglia, and in the vicinity of the adrenal gland and kidney. The stereotyped, but curious localization of BAT pads, exemplified, for instance, by those over each sympathetic ganglion, suggests that, in addition to the overall defense of core temperature in the cold, BAT may also serve an as yet undescribed function to maintain optimal neuronal and synaptic function in specific locations during situations in which the maintenance of core body temperature is challenged. Similar to its function in smaller mammals, adult human BAT activity (assessed by glucose uptake) is highly responsive to beta-adrenergic agonists (Soderlund et al., 2007) and to environmental temperature (Christensen et al., 2006). In addition, the finding of significantly less metabolically active BAT in obese adult human (van Marken Lichtenbelt et al., 2009) has suggested a heretofore unrecognized interaction among BAT energy expenditure, overall energy homeostasis, and body weight.

This review describes our current understanding of the functional organization of the central neural pathways (**Figure 1**), including the core thermoregulatory network, regulating the sympathetic outflow to BAT and, in turn, BAT thermogenesis and energy expenditure.

THERMOREGULATORY CONTROL OF BAT THERMOGENESIS

The core thermoregulatory network in the central nervous system (**Figure 1**) comprises the fundamental pathways through which cutaneous and visceral cold and warm sensation and/or reductions or elevations in brain temperature elicit changes in thermoregulatory effector tissues to counter or protect against changes in the temperature of the brain and other critical organ tissues. As with other thermal effectors (reviewed in Morrison, 2011; Morrison and Blessing, 2011), the thermoregulatory control of BAT thermogenesis occurs through a circuit that includes the pathways transmitting ambient temperature signals from thermal receptors in the skin to the hypothalamic networks that receive and integrate them with brain temperature information to activate efferent pathways to the thermal effectors. Each of the synaptic integration sites in the core thermoregulatory pathway constitutes a potential site for the modulation of BAT thermogenesis by non-thermal signals.

The thermoregulatory control of BAT thermogenesis in response to environmental temperature constitutes a feedforward reflex or "central command" drive, in that the stimulus of a cold environment in contact with skin thermal receptors is not, itself, affected by the evoked thermogenic response, but rather, BAT thermogenesis is initiated to counter a "predicted" fall in body core temperature that would result from the exposure to the cold environment. In contrast, the control of BAT thermogenesis by temperature-sensitive neurons in the brain constitutes a negative feedback reflex in that the warm-sensitive, preoptic area (POA) neurons activated by the increases in brain temperature resulting from stimulated BAT thermogenesis act, in turn, to inhibit sympathetic outflow to BAT.

CUTANEOUS THERMAL RECEPTOR AFFERENT PATHWAY

Cutaneous thermoreception

The thermoregulatory system initiates defensive thermoregulatory responses in response to changes in skin temperature before they affect body core temperature. In this way, exposure to a cold environment can leave core and brain temperatures unaffected or slightly increased (Lomax et al., 1964; Bratincsak and Palkovits, 2005). Such environmental temperature sensation is mediated through cutaneous thermoreceptors which are located in primary sensory nerve endings distributed in the skin. The molecular mechanisms of cutaneous thermoreception appear to reside in the transient receptor potential (TRP) family of cation channels. The strongest data are in support of the TRPM8 as the cutaneous cold receptor TRP channel: TRPM8 is activated by modest cooling ($<27^{\circ}$C; McKemy et al., 2002; Peier et al., 2002), TRPM8-deficient nerve fibers show profound loss of cold sensitivity and TRPM8-deficient mice exhibit a reduced ability to avoid innocuous cold temperatures (Bautista et al., 2007; Colburn et al., 2007; Dhaka et al., 2007). In addition, TRPM8 is activated by menthol (McKemy et al., 2002; Peier et al., 2002) which evokes warm-seeking behavior as well as cold-defensive, physiological responses. TRPV3 and TRPV4 are warm-sensitive TRP channels that are activated by innocuous warm temperatures and are expressed in keratinocytes in skin epidermis. Compared to wild-type mice, mice lacking either TRPV3 or TRPV4 show altered behaviors in discriminating innocuous warm temperatures (Lee et al., 2005; Moqrich et al., 2005).

Intriguing effects on body temperature control and BAT thermogenesis have been reported for agonists and antagonists of TRPV1, a TRP channel that can be activated, *in vitro*, by a noxious range of heat ($>43^{\circ}$C), by protons (pH ≤ 5.9) or by capsaicin (Tominaga et al., 1998). Peripheral or central administration of capsaicin induces hypothermia (Jancso-Gabor et al., 1970; Hori, 1984), an effect beneficial to people living in hot climates, but is ineffective in TRPV1-deficient mice (Caterina et al., 2000). Furthermore, administration of potent TRPV1 antagonists induces hyperthermia (Gavva et al., 2007) by both increasing metabolism and reducing heat loss from the body surface, but not by evoking warm-seeking behavior (Steiner et al., 2007) and this hyperthermic effect is likely exerted by antagonizing TRPV1 located within abdominal viscera (Steiner et al., 2007; Romanovsky et al., 2009). Therefore, tonic activation of peripheral TRPV1, effected by non-thermal stimuli at body temperatures below the threshold for TRPV1 activation, could provide afferent signals to lower body temperature (Romanovsky et al., 2009), however, TRPV1-deficient mice exhibit no obvious deficit in body temperature control (Szelenyi et al., 2004).

In addition to cutaneous thermoreception, thermoreceptive mechanisms exist in body core structures including the brain (e.g., neurons in the POA), spinal cord, and abdomen. The afferent fibers from cold and warm receptors in the abdominal viscera are included among the splanchnic and vagus nerve afferent fibers and their responses to temperature changes are similar to those of cutaneous thermoreceptors (Riedel, 1976; Gupta et al., 1979). Temperature changes in the spinal cord can affect the activity of thermoregulatory neurons in more rostral areas of the brain

(Guieu and Hardy, 1970). TRP channels that are located in the central endings of primary somatosensory fibers in the spinal dorsal horn (Tominaga et al., 1998; Bautista et al., 2007) may sense spinal temperature and could underlie an integration of spinal thermal signals with cutaneous thermal signals at the spinal cord level. Thus, rather than responding directly to changes in environmental temperature, core body thermosensation could play a role (a) in setting the basal tone of thermoregulatory effector efferents including BAT thermogenesis, (b) in enhancing thermoregulatory responses in situations of extreme thermal environments when the feedforward thermoregulatory responses driven by changes in skin temperature have proven inadequate to prevent changes in brain or body core temperature, and (c) in responding to challenges to thermal homeostasis involving shifts in internal body temperature brought about by changes in metabolism (e.g., exercise, hypoglycemia, hypoxia, etc.) or by changes in internal temperature (e.g., intake of cold fluids, hemorrhage, etc.).

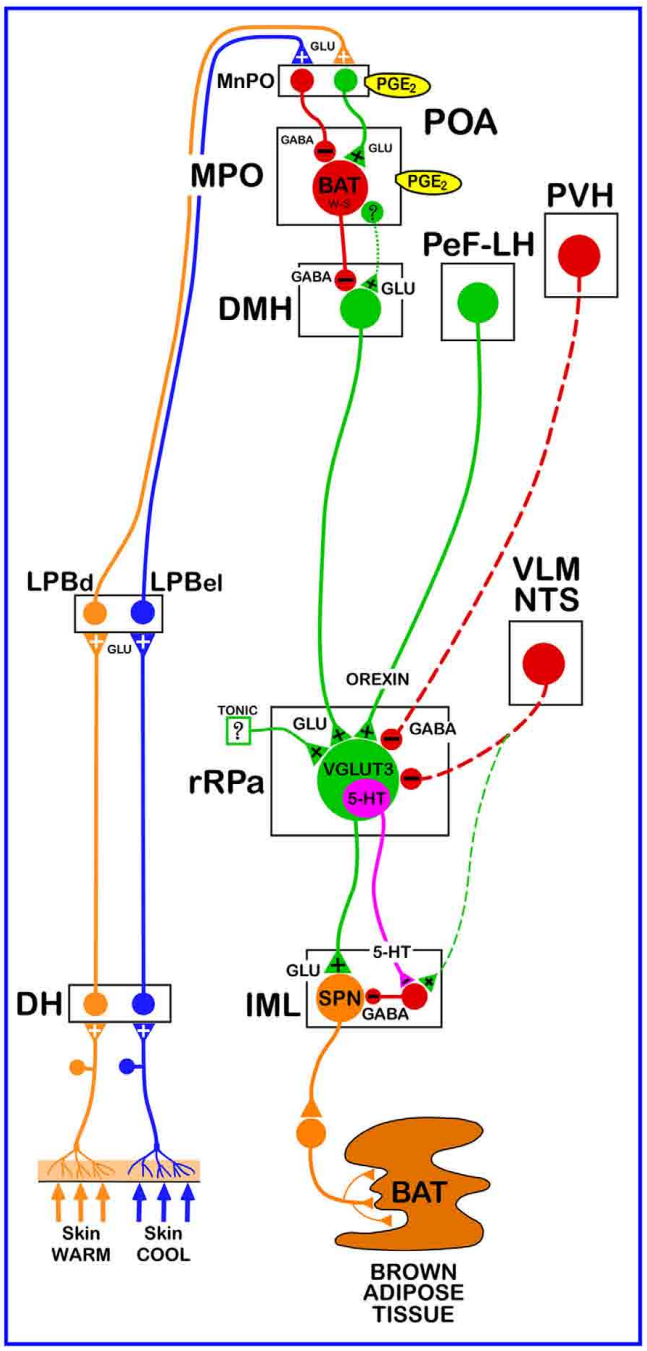

FIGURE 1 | Continued

Dorsal horn

Primary thermal somatosensory fibers deliver thermal information to lamina I neurons in the spinal (or trigeminal) dorsal horn (Craig, 2002; **Figure 1**). Craig and colleagues have described thermoreceptor-specific cells responding linearly to graded, innocuous cooling or warming stimuli, and not being activated further in the noxious temperature range (Andrew and Craig, 2001; Craig et al., 2001). The spinothalamocortical pathway, in which second-order thermosensory neurons in lamina I ascend to synapse on thalamic neurons that, in turn, project to the primary somatosensory cortex, is responsible for conscious

FIGURE 1 | Schematic of the proposed neuroanatomical and neurotransmitter model for the core thermoregulatory network and other CNS sites controlling brown adipose tissue (BAT) thermogenesis. Cool and warm cutaneous thermal sensory receptors transmit signals to respective primary sensory neurons in the dorsal root ganglia which relay this thermal information to second-order thermal sensory neurons in the dorsal horn (DH). Cool sensory DH neurons glutamatergically activate third-order sensory neurons in the external lateral subnucleus of the lateral parabrachial nucleus (LPBel), while warm sensory DH neurons project to third-order sensory neurons in the dorsal subnucleus of the lateral parabrachial nucleus (LPBd). Thermosensory signals for thermoregulatory responses are transmitted from the LPB to the preoptic area (POA) where GABAergic interneurons in the median preoptic (MnPO) subnucleus are activated by glutamatergic inputs from cool-activated neurons in LPBel and inhibit a BAT-regulating population of warm-sensitive (W-S) neurons in the medial preoptic (MPO) subnucleus. In contrast, glutamatergic interneurons in the MnPO, postulated to be excited by glutamatergic inputs from warm-activated neurons in LPBd, excite W-S neurons in MPO. Prostaglandin (PG) E_2 binds to EP3 receptors to inhibit the activity of W-S neurons in the POA. Preoptic W-S neurons providing thermoregulatory control of BAT thermogenesis inhibit BAT sympathoexcitatory neurons in the dorsomedial hypothalamus (DMH) which, when disinhibited during skin cooling, excite BAT sympathetic premotor neurons in the rostral ventromedial medulla, including the rostral raphe pallidus (rRPa), that project to BAT sympathetic preganglionic neurons (SPN) in the spinal intermediolateral nucleus (IML). Some BAT premotor neurons can release glutamate (GLU) to excite BAT SPNs and increase BAT sympathetic nerve activity, while others can release serotonin (5-HT) to interact with 5-HT_{1A} receptors, potentially on inhibitory interneurons in the IML, to increase the BAT sympathetic outflow and thermogenesis. Orexinergic neurons in the perifornical lateral hypothalamus (PeF-LH) project to the rRPa to increase the excitability of BAT sympathetic premotor neurons. Neurons in the paraventricular hypothalamic (PVH) nucleus exert an inhibitory influence on BAT thermogenesis, possibly via a GABAergic input to BAT sympathetic premotor neurons in rRPa. Activation of neurons in the ventrolateral medulla (VLM) or in the nucleus of the solitary tract (NTS) produces an inhibition of BAT thermogenesis, potentially via a non-GABAergic input to rRPa or by activation of spinal inhibitory interneurons in the IML. VGLUT3, vesicular glutamate transporter 3.

perception and discrimination of cutaneous temperature information (Craig et al., 1994; Craig, 2002). However, the spinothalamocortical pathway is not required to initiate or sustain involuntary thermoregulatory responses to environmental cold challenges, since thalamic lesions have no effect on sympathetic thermogenic responses to skin cooling (Nakamura and Morrison, 2008b). However, spinothalamic and trigeminothalamic lamina I neurons do send collateral axons to the lateral parabrachial nucleus (LPB) (Hylden et al., 1989; Li et al., 2006), which, as described below, transmits cutaneous thermosensory information to the core central thermoregulatory network controlling BAT thermogenesis. Another group of afferents likely to influence BAT thermogenesis (Niijima, 1999) arise from both BAT (Bartness et al., 2010) and white adipose tissue (Song et al., 2009). What these afferents sense, the pathways through which this information is relayed centrally and how these adipose afferent signals might influence BAT thermogenesis remain interesting questions.

Lateral parabrachial nucleus

Neurons in the external lateral subnucleus (LPBel) of the LPB and projecting to the median subnucleus (MnPO) of the POA are activated following cold exposure (Bratincsak and Palkovits, 2004; Nakamura and Morrison, 2008b), while those in the dorsal subnucleus (LPBd) are activated in response to skin warming (Bratincsak and Palkovits, 2004; Nakamura and Morrison, 2010). The discharge rate of single, MnPO-projecting LPBel neurons recorded *in vivo* increased markedly in response to skin cooling in a manner paralleling the skin cooling-evoked increases in BAT SNA (Nakamura and Morrison, 2008b). In contrast, single, MnPO-projecting LPBd neurons were excited by skin warming in parallel with the simultaneous inhibition of BAT SNA (Nakamura and Morrison, 2010). The critical role of LPB neurons in transmitting cutaneous, and possibly visceral, thermal sensory information to the hypothalamus to drive BAT thermogenic responses is demonstrated by the elimination of BAT responses to alterations in skin temperature following experimental inactivation of local neurons or blockade of local glutamate receptors in the LPB (Kobayashi and Osaka, 2003; Nakamura and Morrison, 2008b). Similarly, glutamate or other stimulations of LPBel or LPBd neurons can evoke BAT sympathetic and thermogenic responses that parallel those evoked during decreases or increases, respectively, in skin temperature (Nakamura and Morrison, 2008b, 2010). Thus, both cool and warm cutaneous thermosensory signals that are transmitted from spinal dorsal horn or trigeminal neurons to the POA by separate populations of LPB neurons (**Figure 1**) are essential for eliciting rapid responses in BAT thermogenesis to defend body temperature from a variety of thermal challenges. Although nociceptive inputs play only a minor role (Nakamura and Morrison, 2008b), we do not know what other signals are integrated with cutaneous cold afferent inputs to LPBel neurons in the feedforward pathway contributing to drive BAT thermogenesis during environmental cold challenges.

HYPOTHALAMIC MECHANISMS IN THE THERMOREGULATORY CONTROL OF BAT THERMOGENESIS

Within the neural circuits regulating body temperature, the hypothalamus, including the POA, occupies a pivotal position between the cutaneous sensation of ambient temperature and the motor pathways controlling the engagement of thermal effectors (**Figure 1**). Befitting its function as a central integrator of the many dimensions of homeostatic space, the hypothalamus is composed of several interconnected populations of neurons, receives a variety of signals relating to behavioral and emotional state, as well as the condition of the body and the interstitial fluid, and has outputs influencing emotional, behavioral, somatic, and autonomic responses. Control of body temperature is but one of a myriad of interrelated homeostatic functions embedded in the hypothalamic matrix. Despite the anatomical and neurochemical complexity of this brain region, and the many factors that can influence body temperature regulation, considerable progress has been made in understanding the functional organization of the hypothalamic network that controls BAT thermogenesis.

MnPO neurons receive cutaneous thermal signals driving BAT thermoregulatory responses

Within the POA, feedforward, cutaneous cool signaling driving BAT thermogenesis is mediated by glutamatergic inputs from LPBel neurons to neurons in MnPO (**Figure 1**). Stimulation of BAT thermogenesis by activation of LPBel neurons or by skin cooling is blocked by inhibiting neuronal activity in the MnPO (**Figure 2**) or by antagonizing glutamate receptors in the MnPO (Nakamura and Morrison, 2008a,b), and glutamatergic stimulation of MnPO neurons evokes increases in BAT SNA and BAT thermogenesis that are similar to cold-defensive BAT responses (Nakamura and Morrison, 2008a). That the POA subregion receiving thermosensory cold signals is confined to the MnPO is supported by the findings that the projections from LPBel neurons activated by skin cooling terminate mainly in a median part of the POA (Nakamura and Morrison, 2008b) and that glutamatergic stimulation or disinhibition of the MnPO with nanoinjections of NMDA or bicuculline, respectively, evokes physiological responses mimicking cold-defensive responses, while the same stimulation of the MPO or LPO does not (Nakamura and Morrison, 2008a). Thus, activation of MnPO neurons is an essential step in the central mechanism for eliciting cold-defensive BAT thermogenesis to environmental cold challenges (**Figure 1**). MnPO neurons receiving cutaneous thermal signals from LPB neurons also presumably receive other synaptic inputs that could influence the cutaneous thermal afferent regulation of BAT thermogenesis, although the sources of such inputs to these MnPO neurons are unknown. The strong activation of BAT thermogenesis by local nanoinjections of bicuculline into MnPO (Nakamura and Morrison, 2008a) suggests that one such input, at least in anesthetized rats, provides a tonic inhibition of skin cooling-responsive neurons in MnPO.

Stimulation of BAT thermogenesis in response to skin cooling is postulated to occur via a disinhibitory mechanism in which MnPO neurons receiving cutaneous cool signals from LPBel neurons provide a GABA input to the warm-sensitive, inhibitory projection neurons in the MPO (**Figure 1**) to reduce their tonic activity, resulting in disinhibition of neurons in caudal brain regions whose excitation stimulates BAT thermogenesis for cold defense. Consistent with this hypothesis, increases in BAT thermogenesis evoked by skin cooling (**Figure 2**) or by stimulation of MnPO neurons are reversed completely by antagonizing $GABA_A$ receptors in the MPO

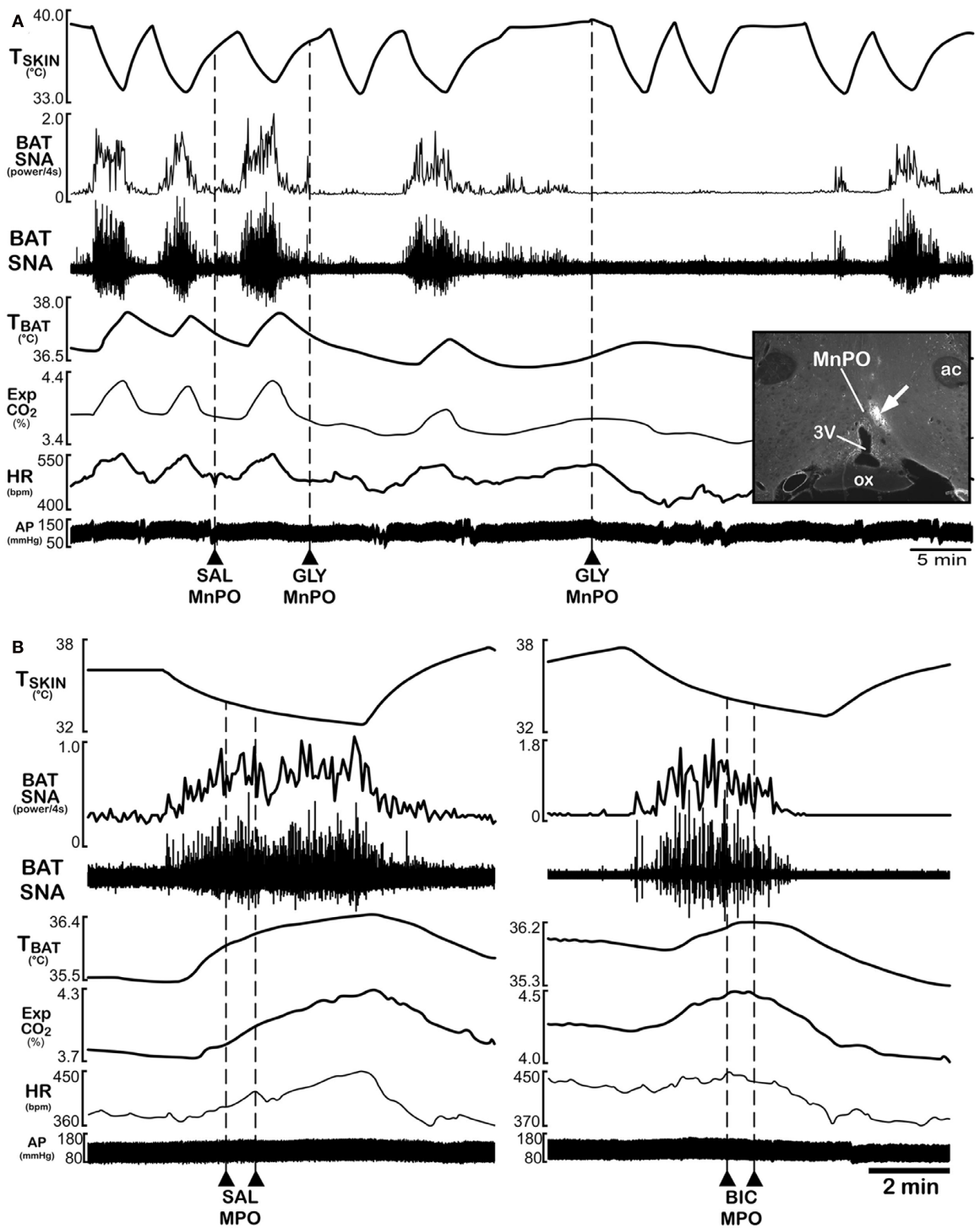

FIGURE 2 | Inhibition of neurons in the median preoptic nucleus (MnPO) or blockade of $GABA_A$ receptors in the medial preoptic nucleus (MPO) prevents skin cooling-evoked BAT thermogenesis. (A) Before and after injection of saline (SAL) vehicle into the MnPO [inset: typical injection site (arrow) in the MnPO; 3v, third ventricle; ox, optic chiasm; ac, anterior commissure], episodes of skin cooling evoke increases in BAT sympathetic nerve activity (SNA), BAT temperature (TBAT), expired CO_2 (Exp CO_2), and heart rate (HR), with no change in arterial pressure (AP). Following nanoinjection of the inhibitory transmitter, glycine (GLY), into the MnPO, skin cooling no longer increases these thermoregulatory parameters. Modified with permission from Nakamura and Morrison (2008a). **(B)** The skin cooling-evoked increases in thermoregulatory parameters, including BAT SNA and TBAT, are unaffected by nanoinjection of saline vehicle into the MPO, but these increases are reversed by blockade of $GABA_A$ receptors in MPO with nanoinjection of bicuculline (BIC). Modified with permission from Nakamura and Morrison (2007).

(Nakamura and Morrison, 2008a). The existence of GABAergic interneurons in the MnPO that innervate the MPO projection neurons is supported by the anatomical observations (a) that some MnPO neurons innervate the MPO (Uschakov et al., 2007), (b) that the MnPO contains many GABAergic neurons (Nakamura et al., 2002), (c) that many neurons in the MnPO, rather than the MPO or LPO, are activated (express Fos protein) in response to reduced environmental temperature (Bratincsak and Palkovits, 2004), and (d) extracellular GABA in the POA is elevated during cold exposure and reduced during heat exposure (Ishiwata et al., 2005).

Warm-sensitive neurons in POA integrate cutaneous and core thermal signals to provide inhibitory regulation of BAT thermogenesis

The conceptual foundation of our current understanding of the role of the hypothalamus in normal body temperature regulation and in the elevated body temperature during fever is the existence of a class of hypothalamic neurons which have intrinsic temperature sensitivity: in the absence of synaptic inputs, their discharge frequency increases as the temperature of their local environment increases. The neurophysiological mechanism underlying the thermosensitivity of warm-sensitive neurons in the POA is thought to reside in a warming-dependent facilitation of the rate of rise of a depolarizing prepotential, due to an heat-induced increase in the inactivation rate of an A-type potassium current, which shortens the intervals between action potentials and thereby increases their firing rates (Boulant, 2006a). Warm-sensitivity could also arise from a heat-induced membrane depolarization that allows warm-sensitive neurons to reach their discharge threshold potential and then determines their discharge frequency (Kobayashi et al., 2006), although the channel mediating such a depolarization has not been identified. Although neurons whose spontaneous discharge frequency is altered by changing the temperature of their local environment exist throughout the CNS, those in the POA and anterior hypothalamus have been most intensely studied because thermoregulatory responses, perhaps with the exception of certain thermoregulatory behaviors (Almeida et al., 2006), are dependent on the integrity of POA neurons. The preeminent importance of central warm-sensitive neurons for the maintenance of normal body temperature can also be appreciated from the relative position of mammalian resting body temperatures well above the freezing point of water, but only a few degrees below the temperature at which proteins begin irreversible denaturation (Romanovsky, 2007).

Initial, *in vivo* recordings in the POA identified neurons with spontaneous discharge at thermoneutral temperatures that increased their discharge during local hypothalamic warming (i.e., putative warm-sensitive neurons; Nakayama et al., 1961, 1963). The POA contains warm-sensitive neurons whose tonic discharge is also reduced by skin cooling and whose thermosensitivity to preoptic temperature is increased when the skin is cooled (Boulant and Hardy, 1974). In subsequent recordings in the POA in hypothalamic slices, the majority of thermosensitive neurons were warm-sensitive (Boulant and Dean, 1986) and the majority of these were GABAergic (Lundius et al., 2010). Further, either skin cooling or direct cooling of the local environment of POA neurons evokes sympathetic thermogenesis in BAT (Imai-Matsumura et al., 1984) and transections immediately caudal to the POA elicit large increases in BAT temperature (Chen et al., 1998). These findings are consistent with a model (**Figure 1**) in which warm-sensitive POA neurons that are tonically active at thermoneutral temperatures, integrate cutaneous and local thermal information, and send inhibitory projections from the MPO to suppress BAT thermogenesis. As ambient temperature is reduced below the thermoneutral zone, such as to a normal "room" temperature of ∼23°C, the discharge rate of warm-sensitive POA neurons is reduced, thereby disinhibiting BAT sympathoexcitatory neurons in more caudal brain sites such as the dorsomedial hypothalamus (DMH) or the rostral raphe pallidus (rRPa; see below) whose increased activity then drives BAT thermogenesis (**Figure 1**). Thus, the firing rates of warm-sensitive projection neurons in the MPO, potentially the principal neurophysiological substrate underlying the thermoregulatory "balance point" (Romanovsky, 2004), are determined by both thermosensory afferent signals from the skin and by the effect of local brain temperature. Other inputs to POA warm-sensitive neurons, such as those postulated from "temperature-insensitive" neurons (Boulant, 2006b), are expected to influence the level of BAT thermogenesis by affecting the basal discharge level of warm-sensitive projection neurons in the MPO or their sensitivity to local and cutaneous thermal signals.

The dorsomedial hypothalamus contains BAT sympathoexcitatory neurons

The observation that transection of the neuraxis immediately caudal to the POA increases BAT SNA and BAT thermogenesis (Chen et al., 1998) suggests that the efferent output of the POA is inhibitory to BAT thermogenesis. However, transections made just caudal to the hypothalamus do not increase basal levels of BAT thermogenesis in normothermic animals (Rothwell et al., 1983) and, in fact, reverse PGE_2-evoked increases in BAT SNA and BAT thermogenesis (Morrison et al., 2004; Rathner and Morrison, 2006). Thus, although a long inhibitory pathway from POA warm-sensitive neurons to medullary BAT sympathetic premotor neurons may contribute to the regulation of BAT thermogenesis, a source of excitatory drive to BAT thermogenesis must exist between the POA and the rostral midbrain.

Convincing data support a role for neurons in the DMH/dorsal hypothalamic area (DMH/DA) in the control of BAT thermogenesis (Dimicco and Zaretsky, 2007), potentially as BAT sympathoexcitatory neurons antecedent to medullary BAT sympathetic premotor neurons in rRPa (**Figure 1**). Administration of endotoxin or cold exposure increases the expression of Fos in neurons of the DMH/DA (Elmquist et al., 1996; Cano et al., 2003; Sarkar et al., 2007). Disinhibition of DMH/DA neurons increases BAT SNA (Cao et al., 2004; **Figure 3A**) and thermogenesis (Zaretskaia et al., 2002), suggesting a tonic GABAergic inhibitory input to BAT thermogenesis-promoting neurons in the DMH/DA (**Figure 1**). This tonic GABAergic input to neurons in the DMH/DA may originate in the POA since POA-derived GABAergic axon swellings make close appositions with DMH neurons, including those that project to the rRPa (Nakamura et al., 2005). In addition, inhibition of neurons in the DMH/DA or blockade of local glutamate receptors in the DMH/DA reverses febrile and cold-evoked excitations

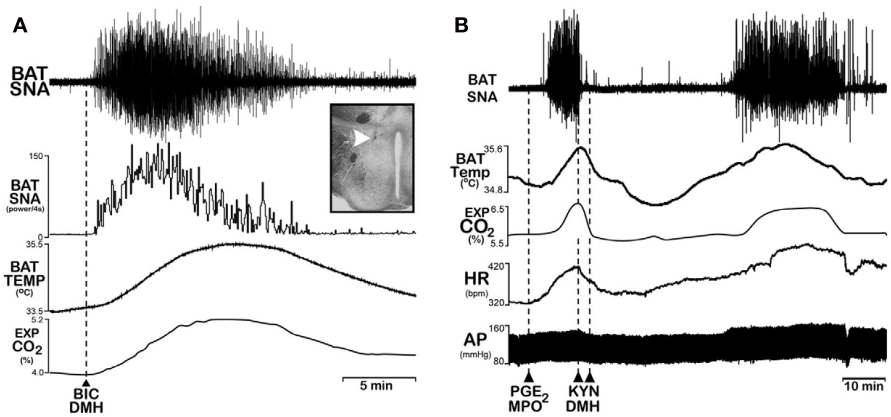

FIGURE 3 | Disinhibition of neurons in the dorsomedial hypothalamus (DMH) increases brown adipose tissue (BAT) thermogenesis and inhibition of neurons in the DMH reverses febrile-evoked BAT sympathetic nerve activity (SNA) and thermogenesis. (A) Nanoinjection of the $GABA_A$ antagonist, bicuculline (BIC), into the DMH, (white arrowhead in inset histological section through the DMH) increased BAT SNA, BAT temperature (BAT Temp), and expired CO_2. Modified with permission from Morrison et al. (2004). **(B)** Nanoinjection of prostaglandin E_2 (PGE_2) into the medial preoptic area (MPO) increased BAT SNA, BAT Temp, Exp CO_2, heart rate (HR), and arterial pressure (AP). Subsequent bilateral nanoinjection of the glutamate receptor antagonist, kynurenate (KYN), into the DMH completely reversed these PGE_2-evoked responses. Modified with permission from Madden and Morrison (2004).

of BAT SNA (**Figure 3B**) and stimulations of BAT thermogenesis (Zaretskaia et al., 2003; Madden and Morrison, 2004; Morrison et al., 2004; Nakamura et al., 2005; Nakamura and Morrison, 2007). Thus, the glutamate-driven activity of neurons in the DMH/DA is required for cold-evoked and febrile activations of BAT thermogenesis, following their disinhibition by a reduction in their GABAergic inhibitory inputs from POA (**Figure 1**). Although the source of the required excitatory input to DMH/DA neurons accounting for their activity following disinhibitory stimuli has yet to be determined, it could arise from cold-sensitive or thermally insensitive neurons in the POA (Boulant, 2006b) or perhaps from an input from the periaqueductal gray (PAG; de Menezes et al., 2009).

Neurons in the DMH/DA do not project directly to BAT sympathetic preganglionic neurons (SPNs), but their monosynaptic projection to the rostral ventromedial medulla (Hermann et al., 1997; Samuels et al., 2002; Nakamura et al., 2005; Yoshida et al., 2009), including the principal site of BAT sympathetic premotor neurons in the rRPa (see below), has been implicated in mediating the effects of DMH/DA neurons on BAT thermogenesis. Glutamate receptor activation in the rRPa is necessary for the increase in BAT SNA and BAT thermogenesis evoked by disinhibition of neurons in the DMH/DA (Cao and Morrison, 2006). Neurons in the DMH/DA that are retrogradely labeled from tracer injections into the rRPa express Fos in response to BAT thermogenic stimuli such as endotoxin, cold exposure or stress (Sarkar et al., 2007; Yoshida et al., 2009), and some DMH/DA neurons that project to the rRPa receive close GABAergic appositions from neurons in the MPO (Nakamura et al., 2005).

In addition to the evidence for a direct monosynaptic pathway from the DMH/DA to the rRPa, both anatomical and physiological evidence suggest a role for neurons in the PAG in determining the level of BAT thermogenesis, potentially by influencing the output from the DMH. Some DMH/DA neurons projecting to the caudal PAG (cPAG) express Fos in response to cold exposure (Yoshida et al., 2005) and some neurons in the cPAG are multisynaptically connected to BAT (Cano et al., 2003), presumably including those that project directly to the raphe (Hermann et al., 1997). Neurons in the cPAG express Fos in response to cold (Cano et al., 2003), although these may not project to the rRPa (Yoshida et al., 2009). Excitation of neurons in cPAG increases BAT temperature, without a concomitant increase in core temperature (Chen et al., 2002), while similar excitation of neurons in the lateral and dorsolateral PAG (dl/lPAG) of conscious rats increases core temperature, the latter being dependent on activity within the DMH (de Menezes et al., 2009). In contrast, in anesthetized and paralyzed rats, skin cooling-evoked stimulation of BAT thermogenesis was unaffected by muscimol injections into the cPAG (Nakamura and Morrison, 2007). The area of the rostral ventromedial PAG (rvmPAG) contains neurons with an inhibitory effect on BAT thermogenesis that are capable of reversing the BAT thermogenesis evoked by PGE_2 injections in to POA or by disinhibition of neurons in DMH/DA (Rathner and Morrison, 2006). Clearly, there is a need for further investigation of the pathways transmitting the sympathetic drive for BAT thermogenesis from the hypothalamus to medullary BAT premotor neurons, and of the roles of various regions of the PAG in regulating BAT thermogenesis.

THE ROSTRAL RAPHE PALLIDUS CONTAINS BAT SYMPATHETIC PREMOTOR NEURONS

Within the hierarchical organization of the central thermoregulatory network, neurons in the rostral ventromedial medulla, centered in the rRPa and extending into nearby raphe magnus nucleus and over the pyramids to the parapyramidal area (PaPy; Bamshad et al., 1999; Oldfield et al., 2002; Cano et al., 2003; Yoshida et al., 2003), play key roles as BAT sympathetic premotor neurons – providing an essential excitatory drive to BAT SPNs in the thoracolumbar spinal cord, which, in turn, excite sympathetic

ganglion cells innervating the BAT pads (**Figure 1**). A comparison of the localization of Fos induced by cold exposure which activates BAT thermogenesis with the locations of retrogradely labeled neurons following virus inoculations of BAT provided function-based evidence that the rRPa (**Figure 4A**) and the ventromedial parvicellular subdivision of the paraventricular hypothalamic (PVH) nucleus are the two potential premotor populations having principal roles in mediating the descending regulation of the spinal sympathetic circuit controlling BAT thermogenesis during cold defense (Cano et al., 2003). Further functional studies have clearly identified the preeminent role of BAT sympathetic premotor neurons in rRPa in the cold-defense activation of BAT thermogenesis; however, the role of the Fos-expressing neurons in PVH remains unknown.

Brown adipose tissue sympathetic premotor neurons in the rRPa receive a potent glutamatergic excitation, as well as GABAergic inhibitory inputs, with the latter predominating under warm conditions to reduce BAT thermogenesis. Relief of this tonically active, GABAergic inhibition as well as an increase in glutamate-mediated excitation, including that from the DMH (Cao and Morrison, 2006), contributes to the cold-evoked and febrile increases in BAT premotor neuronal discharge that drives BAT SNA and BAT heat production. Nanoinjections into rRPa of agonists for either NMDA or non-NMDA glutamate receptors evoke brief, but intense activations of BAT SNA (Madden and Morrison, 2003), indicating that neurons in rRPa capable of increasing the sympathetic drive to BAT express NMDA and non-NMDA subtypes of glutamate receptors. That nanoinjections of bicuculline into the rRPa evoke intense activations of BAT SNA (**Figure 4B**) and BAT energy expenditure (Morrison et al., 1999) that are reduced by glutamate receptor antagonists suggests the existence either of an ongoing or bicuculline-activated excitatory input to rRPa BAT sympathetic premotor neurons.

Conversely, inhibition of neuronal activity or blockade of glutamate receptors in the rRPa reverses the increases in BAT SNA and BAT thermogenesis elicited by a variety of thermogenic stimuli, including skin cooling (**Figure 4C**) and fever (Nakamura et al., 2002; Madden and Morrison, 2003; Morrison, 2003; Nakamura and Morrison, 2007; Ootsuka et al., 2008). Inhibition of rostral ventromedial medullary neurons produces dramatic falls in body temperature in conscious rats (Zaretsky et al., 2003), consistent with an active contribution of BAT sympathetic premotor neurons in the rRPa and BAT thermogenesis to the maintenance of core temperature in a room temperature environment. Other thermogenic stimuli whose activation of BAT thermogenesis is reversed or prevented by inhibition of neural activity in the rRPa include disinhibition of neurons in the DMH (Cao et al., 2004) or in the lateral hypothalamus (Cerri and Morrison, 2005); activation of central mu-opioid receptors (Cao and Morrison, 2005), central melanocortin receptors (Fan et al., 2007) or preoptic CRF receptors (Cerri and Morrison, 2006), and systemic administration of the adipose tissue hormone, leptin (Morrison, 2004). Thus, the rRPa and PaPy regions of the ventromedial medulla contain the principal populations of BAT sympathetic premotor neurons that provide the final common medullospinal pathway (**Figure 1**) for the BAT sympathoexcitatory drive to the spinal network controlling BAT SNA and that are both necessary and sufficient for the

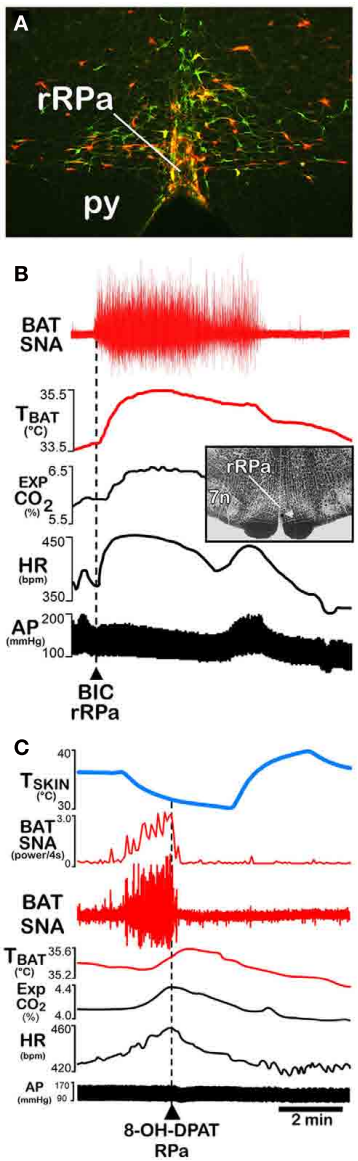

FIGURE 4 | Effects of disinhibition and inhibition of rostral raphe pallidus (rRPa) neurons on brown adipose tissue (BAT) thermogenesis. **(A)** Coronal histological section through the rostral medulla at the level of the facial nucleus and the rRPa, containing immunohistochemically labeled neurons that were trans-synaptically infected following inoculations of BAT with pseudorabies virus (red), that contain the serotonin synthesizing enzyme, tryptophan hydroxylase (green), or that contain both markers (yellow). Py, pyramidal tract. Modified with permission from Cano et al. (2003). **(B)** Disinhibition of neurons in the rRPa [arrowhead in inset histological section through rRPa at the level of the facial nucleus (7n)] with nanoinjections of bicuculline (BIC) elicits dramatic increases in BAT sympathetic nerve activity (SNA), BAT temperature (TBAT), expired CO_2 (Exp CO_2), and heart rate (HR), with little change ion arterial pressure (AP). Modified with permission from Madden and Morrison (2003). **(C)** Inhibition of local neurons in the rRPa with a nanoinjection of the inhibitory serotonin 1A receptor agonist, 8-OH-DPAT, produces a rapid and complete reversal of the skin cooling-evoked increases in BAT SNA and an immediate waning of the accompanying metabolic and cardiac responses, despite the sustained reduction in skin temperature (TSKIN). Modified with permission from Nakamura and Morrison (2007).

BAT thermogenic responses to thermoregulatory (**Figure 1**) and febrile stimuli and to a variety of neurochemical mediators that influence body temperature.

That vesicular glutamate transporter 3 (VGLUT3)-expressing and serotonin-containing neurons in the rostral ventromedial medulla are functionally related to the activation of cold-defensive, BAT thermogenesis is indicated by the findings that a significant percentage of VGLUT3-containing neurons in the rRPa express Fos in response to cold exposure or intracerebroventricular (ICV) PGE_2 (Nakamura et al., 2004a) and that physiologically identified serotonergic neurons in the rRPa increase their firing rate in response to PGE_2 administration or cold exposure (Martin-Cora et al., 2000).

Rats maintained with a brainstem transection just rostral to the superior colliculus (Nautiyal et al., 2008) or with bilateral cuts just caudal to the POA (Blatteis and Banet, 1986) can mount relatively normal cold-defense responses, leading to the conclusion that the POA is not essential for the integration of autonomic thermoregulatory responses in the rat and that circuits caudal to the transection are sufficient to convey cutaneous thermal information to the premotor neurons in the rRPa necessary to evoke cold-defense responses. Why the pathways proposed by these investigators to explain these effects in transected rats are ineffective in intact rats following neuronal inhibition of hypothalamic sites in intact animals (Zaretskaia et al., 2003; Madden and Morrison, 2004; Nakamura and Morrison, 2007, 2008a) remains unexplained. These results may point to an effect of anesthesia or to a response to the transection injury, since they could not be repeated acutely following nearly identical transections rostral to the colliculi in anesthetized rats (Osaka, 2004). Thermally sensitive neurons have been recognized in several sites caudal to the POA and these neurons, rather than cutaneous thermal receptors, may be engaged in eliciting cold-defense responses in rats with transections caudal to the POA. Overall, while these occasional data derived from transected preparations are curious, their relevance to normal thermoregulatory mechanisms in intact animals remains unknown.

SPINAL SYMPATHETIC MECHANISMS INFLUENCING BAT THERMOGENESIS

The discharge of BAT SPNs that determines the level of BAT SNA and BAT thermogenesis, as well as the rhythmic bursting characteristic of BAT SNA, is governed by their supraspinal and segmental inputs as well as those to the network of spinal interneurons that influence BAT SPN excitability. A significant fraction of the BAT sympathetic premotor neurons in rRPa, identified following viral retrograde tracing, are glutamatergic and/or serotonergic neurons. Spinally projecting neurons in the rRPa region can contain phenotypic markers for (a) the VGLUT3, potentially indicative of glutamatergic neurons (Nakamura et al., 2004a; Stornetta et al., 2005); (b) serotonin (5-HT) or tryptophan hydroxylase, a synthetic enzyme for 5-HT (Cano et al., 2003; Nakamura et al., 2004a; Stornetta et al., 2005), and (c) glutamic acid decarboxylase-67 (GAD-67), a marker for GABAergic neurons (Stornetta et al., 2005). Consistent with these findings, 5-HT-containing (Bacon and Smith, 1988; Vera et al., 1990) and VGLUT3-containing terminals synapse on SPNs (Stornetta et al., 2005) or make close appositions with SPN dendrites (Nakamura et al., 2004a,b). In addition, IML-projecting neurons located in the rRPa and the PaPy can contain thyrotropin-releasing hormone (TRH) and substance P (Sasek et al., 1990).

Glutamate and 5-HT play critical roles in the descending excitation of BAT SPNs by their antecedent premotor neurons in the rRPa. The majority of VGLUT3-containing neurons in the rRPa express Fos in response to cold exposure or ICV PGE_2 (Nakamura et al., 2004a), nanoinjection of glutamate or NMDA into the upper thoracic IML activates BAT SNA and BAT thermogenesis (Nakamura et al., 2004a; Madden and Morrison, 2006) and blockade of glutamate receptors in the upper thoracic IML suppresses the increase in BAT thermogenesis evoked by bicuculline injection into rRPa (Nakamura et al., 2004a). Putative serotonergic neurons in the rRPa increase their firing rate in response to cold (Martin-Cora et al., 2000; Nason and Mason, 2006) or PGE_2 administration (Nason and Mason, 2006) and blockade of spinal serotonin receptors reverses the cold-evoked activation of BAT SNA (Madden and Morrison, 2010). Serotonin in the IML can activate BAT SNA and BAT thermogenesis and potentiates the BAT SNA response to NMDA injections into the IML (Madden and Morrison, 2006), such that prior application of serotonin into the IML allows a subsequent subthreshold dose of NMDA to evoke a marked increase in BAT SNA (Madden and Morrison, 2006). The significant role of serotonin-containing neurons in normal cold-defense responses is also supported by the finding that mice that lack almost all central serotonergic neurons show blunted BAT thermogenesis during cold exposure (Hodges et al., 2008). The mechanisms of the interaction between glutamatergic and serotonergic neurotransmission in the IML remain to be elucidated.

Viral inoculations of interscapular BAT (Cano et al., 2003) consistently label a population of spinal interneurons in the vicinity of the IML. Spinal GABAergic interneurons would appear to be among this population since they influence the discharge of SPNs (Deuchars et al., 2005). That such interneurons could receive inputs from the BAT premotor area in the rostral ventromedial medulla is suggested by the demonstration that VGLUT3- and GAD-67-containing terminals synapse on GABAergic neurons in the IML (Stornetta et al., 2005), providing a potential anatomical substrate for a pathway that increases the activity of SPNs through disinhibition. 5-HT-containing terminals form close appositions with GABAergic interneurons in the central autonomic area (Conte et al., 2007) and serotonergic inputs activating 5-HT_{1A} receptors on GABAergic neurons in the IML have been postulated to explain the potentiation of excitatory inputs to BAT SPNs by exogenously applied 5-HT (Madden and Morrison, 2006, 2008a).

Spinal catecholamine release may also modulate the activity of BAT SPNs, considering the excitatory and inhibitory effects of catecholamines on functionally unidentified SPNs (Coote et al., 1981; Miyazaki et al., 1989) and the observation of a dense dopamine beta hydroxylase innervation of SPNs that are synaptically connected to BAT (Cano et al., 2003). Substance P terminals also innervate SPN's (Vera et al., 1990), substance P excites the majority of SPNs (Cammack and Logan, 1996), and intrathecal substance P affects thermoregulation (Dib, 1987), although the latter may be due to an effect on thermal afferent processing in the dorsal horn.

ACTIVATION OF BAT THERMOGENESIS IN FEVER

Fever is a defended elevation in body temperature that plays a significant role in the acute phase reaction stimulated by endogenous pyrogens released during infection or inflammation. PGE_2, which is synthesized in the brain vasculature and in peripheral tissues in response to immune signals (Elmquist et al., 1997; Matsumura et al., 1998; Yamagata et al., 2001), is a powerful endogenous pyrogenic mediator that binds to EP3 receptors in the POA, particularly the MPO and MnPO (Scammell et al., 1996; Nakamura et al., 2000, 2002; Lazarus et al., 2007), to activate BAT thermogenesis in concert with other thermoregulatory effectors to produce a sustained increase in core body temperature. Intravenous PGE_2 is also effective in eliciting the BAT thermogenic component of the febrile response (Ootsuka et al., 2008).

Although we have only a rudimentary understanding of the microcircuitry of the POA thermoregulatory network, we have proposed (Nakamura et al., 2004a, 2005) a model, incorporated in **Figure 1**, for the essential POA mechanisms through which application of PGE_2 into the POA elicits the BAT thermogenic component of the febrile response. As described above for cold-defense responses, under normal conditions, EP3 receptor-expressing POA neurons, potentially including the population of warm-sensitive POA neurons that controls BAT thermogenesis, maintain a tonic GABAergic inhibition of sympathoexcitatory, BAT thermogenesis-promoting neurons in the DMH, and potentially in the rRPa. During infection, PGE_2, produced locally and/or systemically, binds to these EP3 receptors, reducing the activity of BAT-controlling, warm-sensitive neurons in POA, which, in turn, leads to disinhibition of BAT thermogenesis-promoting neurons in DMH/DA and subsequently in rRPa, to drive BAT sympathetic outflow and BAT heat production contributing to an elevated core body temperature. Although the resulting increase in the local temperature of the POA would normally elicit more rapidly rising prepotentials in POA warm-sensitive neurons to inhibit BAT activation, this is offset by the membrane hyperpolarization elicited by EP3 receptor occupancy, thereby allowing a sustained activation of BAT thermogenesis and a maintained fever.

Several experimental findings are consistent with such a model. Binding of PGE_2 to EP3 receptors can inhibit neuronal activity by coupling to inhibitory GTP-binding proteins (Narumiya et al., 1999) and the tonic activity of most warm-sensitive neurons in the POA is inhibited by the E-series of PGs (Schoener and Wang, 1976; Ranels and Griffin, 2003). A population of EP3 receptor-expressing POA neurons multisynaptically innervates BAT (Yoshida et al., 2003) and a subpopulation of EP3 receptor-expressing neurons in the POA projects directly to the DMH (Nakamura et al., 2002, 2005). Interestingly, this subpopulation is separate from the subpopulation of EP3 receptor-expressing POA neurons projecting to the rRPa, although a population of POA neurons that do not express EP3 receptors does send bifurcating axonal projections to both DMH and the rRPa (Nakamura et al., 2009). The majority of EP3 receptor-expressing POA neurons are GABAergic (Nakamura et al., 2002), as are warm-sensitive neurons in POA (Lundius et al., 2010). Antagonizing $GABA_A$ receptors in the DMH evokes a fever-like stimulation of BAT thermogenesis (Morrison, 1999; Morrison et al., 1999; Zaretskaia et al., 2002; Cao et al., 2004) that is also reversed by blockade of glutamate receptors in the rRPa (Cao and Morrison, 2006). Inhibition of POA neurons with a muscimol nanoinjection elicits hyperthermic, cardiovascular, and neuroendocrine responses similar to those evoked by a PGE_2 nanoinjection into the same site (Zaretsky et al., 2006). In addition, ICV PGE_2 application reduces cAMP level in the POA and ICV administration of an inhibitor of phosphodiesterase, a degradation enzyme for cAMP, blunts fever evoked by intra-POA PGE_2 application (Steiner et al., 2002). A marked increase in core temperature and a tachycardia can be elicited by injection of PGE_2 into the paraventricular nucleus of the hypothalamus (PVH) or into the pontine parabrachial nucleus (PBN; Skibicka et al., 2011), although whether neurons in these two sites play a role in fever generation remains to be determined. In this regard, the demonstration that elimination of EP3-R selectively in the POA is sufficient to prevent LPS fever (Lazarus et al., 2007) indicates that EP3-R in PVH or in PBN is not sufficient to support a febrile response or possibly that PGE_2 is not increased in these regions during a febrile stimulus such as LPS.

OREXIN IN rRPa INCREASES BAT THERMOGENESIS

Orexins (hypocretins; de Lecea et al., 1998; Sakurai et al., 1998) are synthesized exclusively in widely projecting (Peyron et al., 1998) neurons in the perifornical area of the lateral hypothalamus (PeF-LH). Orexin neurons influence a variety of functions, including the regulation of food intake (Sakurai et al., 1998). ICV administration of orexin also increases activity and body temperature (Monda et al., 2001). Loss of orexin neurons leads to the disordered sleep patterns of narcolepsy, which is often accompanied by defective energy and metabolic homeostasis, including a high risk for obesity (Kok et al., 2003; Hara et al., 2005) and the potential for altered thermoregulation (Plazzi et al., 2011). Interestingly, systemic orexin, perhaps secreted from the placenta, is required for BAT differentiation and its absence during early development leads to obesity arising from inadequate BAT energy expenditure (Sellayah et al., 2011).

A role for orexin neurons in the PeF-LH in the regulation of BAT thermogenesis has recently been described (Tupone et al., 2011). An anatomical substrate for the influence of orexinergic neurons on BAT thermogenesis is provided by the demonstration that the PeF-LH contains orexinergic neurons that are synaptically coupled to BAT, as shown with viral, trans-synaptic retrograde tracing (Oldfield et al., 2002; Berthoud et al., 2005; Tupone et al., 2011), and that project to the rRPa, as shown with retrograde transport of CTb following injections into the rRPa region containing BAT sympathetic premotor neurons (Tupone et al., 2011). In anesthetized rats, both injections of orexin into rRPa (**Figure 5C**) and glutamatergic activation of orexinergic neurons by nanoinjections of NMDA into the PeF-LH (**Figure 5C**) produced long lasting activations of BAT SNA and BAT thermogenesis, accompanied by marked increases in energy expenditure, indicated by sustained increases in expired CO_2 that paralleled the increases in BAT heat production (**Figure 5A**). Curiously, the strong stimulation of BAT SNA and BAT thermogenesis elicited by injection of orexin into the rRPa or by activation of neurons in the PeF-LH required an ongoing, basal level of BAT SNA, generated in this case by maintaining the rats at a slightly cooled core body temperature. When the rats were warmed to eliminate any basal discharge on the sympathetic

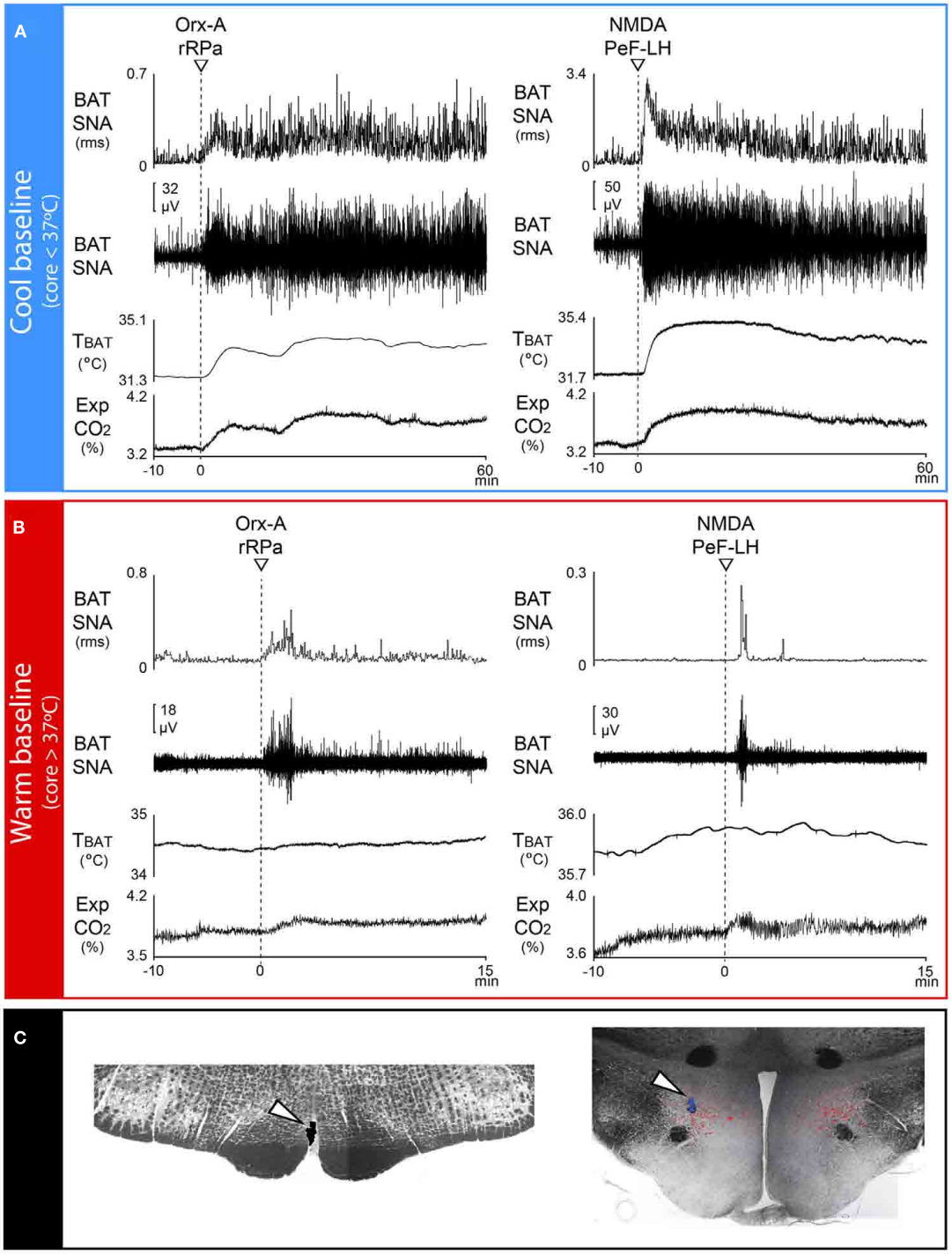

FIGURE 5 | Orexin in the rostral raphe pallidus (rRPa) or activation of neurons in the perifornical lateral hypothalamus (PeF-LH) produces a prolonged increase in BAT sympathetic nerve activity (SNA) and BAT thermogenesis in cool, but not warm rats. (A) Under cool conditions (core temperature <37°C) in which a low level of basal BAT SNA is present, nanoinjections of orexin-A (Orx-A, left panel, dashed line) in the rRPa or of N-methyl-D-aspartate (NMDA, right panel, dashed line) into the PeF-LH elicited prolonged increases in BAT SNA, BAT temperature (TBAT), and expired CO_2 (Exp CO_2). **(B)** Under warm conditions (core temperature >37°C) in which no basal BAT SNA was present, nanoinjections of Orx-A (left panel, dashed line) in rRPa or of NMDA (right panel, dashed line) into the PeF-LH failed to evoke significant changes in BAT SNA, TBAT or Exp CO_2. **(C)** Representative histological sections illustrating nanoinjection sites in the rRPa (left panel, white arrowhead) and in the PeF-LH (right panel, white arrowhead). Note that the NMDA injection sites in the PeF-LH were located in the midst of many neurons immunohistochemically labeled for Orexin-A (red). Modified with permission from Tupone et al. (2011).

nerves to BAT, neither injection of orexin into rRPa nor activation of PeF-LH neurons elicited increases in BAT SNA (Tupone et al., 2011; **Figure 5B**).

These anatomical data demonstrate not only an orexinergic projection from neurons in the PeF-LH to the site of BAT sympathetic premotor neurons in the rRPa, but also a synaptic connection between orexin-containing neurons in PeF-LH and BAT sympathetic premotor neurons. Physiologically, the requirement for an ongoing level of BAT SNA, and thus an activation of BAT sympathetic premotor neurons in rRPa, in order for orexin in rRPa to evoke large and sustained increases in BAT SNA and BAT thermogenesis is consistent with a role for orexin in rRPa to change the gain of the response of BAT sympathetic premotor neurons to their activating, presumably glutamatergic excitatory inputs.

The physiological conditions which activate the orexin neurons in PeF-LH that project to rRPa to modulate the gain of the synaptic drive to BAT sympathetic premotor neurons and facilitate BAT thermogenic responses remains to be determined. Also unknown are the other potential projection targets of the orexin neurons that influence BAT thermogenesis. However, a potential role of orexin neurons in feeding could suggest a simultaneous increase in energy consumption in BAT under conditions of a high level of stored calories. Alternatively, the activation of orexin neurons in awake or aroused states could suggest an accompanying facilitation of BAT thermogenesis to increase brain and core temperatures to optimize performance. In this regard, it would be of interest to determine if the level of activity in orexin neurons parallels the ultradian oscillations in the BAT thermogenesis (Ootsuka et al., 2009). Conversely, a reduction in the activity of the orexinergic input to rRPa may contribute to the reductions in BAT thermogenesis, energy consumption, and body temperature under conditions of sleep, hibernation or starvation.

A ROLE FOR THE VENTROMEDIAL HYPOTHALAMUS IN BAT THERMOGENESIS REMAINS UNCLEAR

The seminal study of Hetherington and Ranson (1940) initiated the interest in the ventromedial hypothalamus (VMH) in energy homeostasis and several succeeding studies have suggested that the VMH affects energy expenditure via sympathetic activation of BAT thermogenesis. Electrical stimulation of the VMH increases BAT thermogenesis (Perkins et al., 1981; Minokoshi et al., 1986) and microinjection of glutamate (Yoshimatsu et al., 1993) or triiodothyronine (Lopez et al., 2010) into VMH increases BAT SNA. Conversely, lesions of the VMH attenuate BAT SNA (Niijima et al., 1984; Sakaguchi et al., 1988). However, the methodologies used in these studies, including electrical stimulation, electrolytic lesions, and large injection volumes (i.e., 100–200 nl for microinjection studies without appropriate anatomical control experiments) significantly limit the conclusions that can be drawn from the resulting data. Furthermore, the close proximity of the VMH to other regions involved in the sympathetic regulation of BAT thermogenesis, including the DMH, the arcuate nucleus and the lateral hypothalamus, and the frequent absence of histological data in these previous studies precludes any conclusions on the role of neurons in the VMH in the control of BAT thermogenesis and BAT energy expenditure. An additional confound is that trans-synaptic retrograde tracing studies have consistently failed to identify any significant population of neurons in the VMH following injection of pseudorabies virus in BAT, even at long post-inoculation times (Bamshad et al., 1999; Oldfield et al., 2002; Cano et al., 2003; Yoshida et al., 2003). Nonetheless, a recent study found impaired diet-induced thermogenesis and lower levels of UCP1 in BAT in mice lacking PI3K specifically in the SF-1 containing neurons of the VMH (Klockener et al., 2011), suggesting a role for VMH neurons in the regulation of BAT. Whether this alteration in BAT UCP1 expression is mediated by a direct effect of altered VMH neuronal discharge on the sympathetic input to BAT, and if so, how this observation might be reconciled with the lack of viral tracer labeling of VMH neurons following inoculation of BAT, remain interesting areas for future study.

INHIBITORY REGULATION OF BAT THERMOGENESIS

HYPOXIA INHIBITS BAT THERMOGENESIS

The brainstem contains the pathways mediating the inhibition of BAT thermogenesis in response to arterial hypoxia, a reflex to restrict oxygen consumption in the face of reduced oxygen availability or compromised oxygen diffusion and transport in the blood. Systemic hypoxia or bolus systemic injections of sodium cyanide produce a prompt and complete reversal of the BAT SNA activations evoked by hypothermia and by PGE_2 in the POA and this response to hypoxia is eliminated by section of the carotid sinus nerves or by inhibition of second-order arterial chemoreceptor sensory neurons in the commissural region of the nucleus of the tractus solitarius (NTS; Madden and Morrison, 2005). Interestingly, hypoxia also eliminates the BAT SNA activation resulting from bicuculline nanoinjection into the rRPa, suggesting that activation of a GABAergic input to BAT sympathetic premotor neurons in rRPa is unlikely to mediate the hypoxic inhibition of BAT thermogenesis. Indirect evidence points to a possible role for a spinal inhibitory mechanism. Similar to arterial hypoxia, disinhibition of neurons in the rostral ventrolateral medulla (RVLM) reduces the BAT SNA activation following bicuculline into the rRPa (Morrison et al., 1999) and both anatomical (Stornetta et al., 2004) and electrophysiological (Deuchars et al., 1997) studies support the existence of a bulbospinal inhibitory pathway to SPNs from the RVLM. The pathway for the hypoxic inhibition of BAT metabolism between the NTS and the BAT SPNs remains to be investigated.

HYPOGLYCEMIA AND GLUCOPRIVATION INHIBIT BAT THERMOGENESIS

Similar to the hypoxia-evoked inhibition of thermogenesis, hypoglycemia and glucoprivation cause hypothermia (Freinkel et al., 1972; Mager et al., 1976), at least in part by inhibiting sympathetically mediated metabolism in BAT (Egawa et al., 1989a; Madden, 2012). This neurally regulated decrease in metabolism reduces cellular oxidative demands during conditions of reduced availability of metabolic fuel. The importance of this adaptive response, which spares scarce glucose resources for use by critical tissues such as the brain, at the expense of thermoregulation, is demonstrated by the observation that prevention of hypothermia during severe hypoglycemia results in increased mortality rates (Buchanan et al., 1991).

Glucoprivation selectively within the lateral hypothalamus reduces BAT SNA by ∼25% (Egawa et al., 1989b), whereas systemic

glucoprivation can completely inhibit BAT SNA (Egawa et al., 1989a; Madden, 2012). More recently we have demonstrated not only that the glucoprivic agent, 2-deoxy-D-glucose (2-DG), reversed the cooling-evoked activation of BAT SNA, but that selective glucoprivation with local nanoinjection of 5-thio-D-glucose (5-TG) within the ventrolateral medulla (VLM) also completely reversed the cooling-evoked activation of BAT SNA (Madden, 2012). Interestingly, intravenous administration of 2-DG did not attenuate the activation of BAT SNA following pontomedullary transection, suggesting that the hypoglycemia-evoked inhibition of BAT SNA is not mediated solely by circuits within the medulla (Madden, 2012). The NPY/catecholaminergic projection from the VLM to the PVH may play an important role in the glucoprivic inhibition of BAT SNA, consistent with the role of VLM neurons in the glucoprivic inhibition of BAT SNA, the ability of neuronal activation in the PVH to inhibit BAT SNA (see below) and the established role of the NPY/catecholaminergic input from the VLM to the PVH in other counterregulatory responses to hypoglycemia (Ritter et al., 2011). Identifying the respective contributions of hypothalamic and medullary regions to the reduced BAT thermogenesis during conditions of reduced availability of metabolic fuel, as well as the characterization of the specific neural pathways by which hypoglycemia influences thermoregulatory neural circuits await further research.

NEURONS IN THE PARAVENTRICULAR HYPOTHALAMIC NUCLEUS INHIBIT BAT THERMOGENESIS

The PVH plays a major role in energy homeostasis, influencing both food intake and energy expenditure. Although the paucisynaptic connection of neurons in the PVH to BAT (Bamshad et al., 1999; Oldfield et al., 2002; Cano et al., 2003; Yoshida et al., 2003) strongly supports a role for these neurons in the sympathetic regulation of BAT thermogenesis, their influence on the regulation of BAT thermogenesis has been controversial. Initially, neurons in the PVH were thought to play a role in the excitation of BAT SNA, since neurons in the dorsal PVH with direct projections to the spinal sympathetic preganglionic cell column are activated during fever (Zhang et al., 2000) and lesions of PVH attenuate fever (Horn et al., 1994; Caldeira et al., 1998; Lu et al., 2001). Curiously, cold-evoked BAT thermogenesis was unaffected by lesions of the PVH (Lu et al., 2001). In contrast, activation of neurons in the PVH has recently been demonstrated to inhibit BAT SNA and BAT thermogenesis (Madden and Morrison, 2009). Unilateral disinhibition of neurons in PVH with nanoinjections of bicuculline or their glutamatergic activation with NMDA injections completely inhibits increases in BAT SNA and BAT thermogenesis elicited by skin and core cooling (**Figure 6A**), by injections of PGE_2 into the MPO that mimic fever, or by disinhibition of neurons in the DMH/DA (Madden and Morrison, 2009). Although activation of PVH neurons could attenuate the increases in BAT SNA and BAT thermogenesis evoked by injections of NMDA into the rRPa, those resulting from bicuculline injections into rRPa were unaffected by disinhibition of PVH neurons (**Figure 6B**), consistent with the PVH-evoked inhibition of BAT SNA being mediated by $GABA_A$ receptors in the rRPa. That neurons in the PVH provide an inhibitory influence on BAT SNA is also supported by the observations that NPY presynaptically inhibits GABA release onto PVH neurons (Cowley et al., 1999) and microinjection of NPY into the PVH decreases BAT SNA (Egawa et al., 1991). These apparent controversies in the relation of PVH neurons to BAT thermogenesis, particularly during fever, might be explained by the presence of subpopulations of PVH neurons mediating contrasting effects or by a role of PVH neurons during fever that involves the stimulation of other fever-supporting effector systems such as the cutaneous vasculature or hormone release, whereas the inhibition of BAT thermogenesis by PVH neurons contributes to a non-febrile homeostatic function.

Controversy also exists concerning the role of melanocortin receptor activation in the PVH in energy expenditure and activation of BAT thermogenesis. Selective rescue of melanocortin-4 receptor (MC4R) expression in neurons of the PVH (and the medial amygdala) in mice lacking expression of MC4R failed to normalize (elevate) their oxygen consumption to wild-type levels (Balthasar et al., 2005). Based on these data it was suggested that PVH MC4Rs do not mediate the energy expenditure effects of melanocortins. In contrast, other groups have demonstrated that microinjection of melanocortin receptor agonists into the PVH increases core and BAT temperatures (Song et al., 2008; Skibicka and Grill, 2009). These effects of melanocortin receptor activation could be mediated by activation of presynaptic MC4Rs, which have been shown to potentiate GABAergic inputs to PVH neurons (Cowley et al., 1999). Indeed, this explanation would reconcile this controversy, since the rescue of MC4R in the study of Balthasar et al. would only rescue the postsynaptic MC4R in PVH neurons and not those located presynaptically and potentially responsible for the effects of exogenously administered melanocortin receptor agonists. This explanation is also consistent with our data indicating that the activity of neurons in the PVH is inhibitory to BAT SNA. The physiological conditions which would stimulate the BAT sympathoinhibitory output from the PVH are unknown, but may include hypoglycemia and chronic intermittent hypoxia. Another interesting possibility is that neurons in the PVH provide a tonic inhibition of BAT thermogenesis and release from this inhibition under specific conditions, such as changes in dietary composition, may activate BAT SNA and BAT energy expenditure.

PONTINE RETRORUBRAL FIELD NEURONS MAINTAIN A TONIC INHIBITION OF BAT THERMOGENESIS

The existence of a tonically active inhibition of BAT thermogenesis from mid-pontine neurons (**Figure 1**) was indicated by the large increases in BAT and/or core temperatures that followed transections of the neuraxis in the vicinity of the pontomedullary junction, but which were absent if transections were made rostral to the pons, but caudal to the DMH (Rothwell et al., 1983; Amini-Sereshki and Zarrindast, 1984). Inactivation of neurons in the vicinity of the pontine retrorubral field produced a similar stimulation of BAT thermogenesis (Shibata et al., 1999). Neither the exact location of the neurons mediating this inhibition nor the physiological basis for its control has been determined.

INHIBITION OF BAT THERMOGENESIS FROM NEURONS IN THE VENTROLATERAL MEDULLA AND THE NUCLEUS OF THE SOLITARY TRACT

The VLM and the NTS contain neurons that comprise fundamental cardiovascular and respiratory regulatory circuits, including

the baroreceptor and arterial chemoreceptor reflex pathways regulating local vasoconstrictor and cardiac sympathetic premotor neurons, as well as the respiratory generating network and its regulation by central chemoreceptors. A role for neurons in these same regions in regulating BAT thermogenesis has been recently described (Cao et al., 2010). Disinhibition of neurons in the VLM with a unilateral nanoinjection of bicuculline elicits a prompt and complete inhibition of the increased BAT SNA and BAT thermogenesis due to skin and core cooling (**Figure 7A**), to injections of PGE_2 into the MPO, to disinhibition of neurons in DMH/DA or

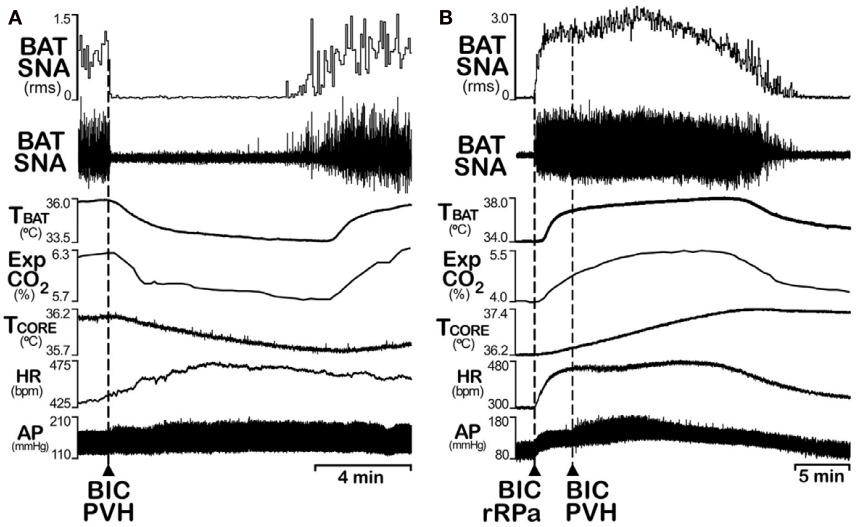

FIGURE 6 | Disinhibition of neurons in the paraventricular hypothalamic (PVH) nucleus inhibits the increases in BAT sympathetic nerve activity (SNA) evoked by cooling, but not those evoked by disinhibition of neurons in the rostral raphe pallidus (rRPa). (A) Nanoinjection of bicuculline (BIC) into the PVH completely reversed the increases in BAT SNA, BAT temperature (TBAT), expired CO_2 (Exp CO_2) produced by whole body cooling. Core body temperature (TCORE) was also reduced; heart rate (HR) and arterial pressure (AP) were increased by BIC in PVH. **(B)** The increases in BAT SNA, TBAT, expired CO_2, TCORE, HR, and AP evoked by nanoinjection of BIC into the rRPa are not affected by nanoinjection of BIC into the PVH. Modified with permission from Madden and Morrison (2009).

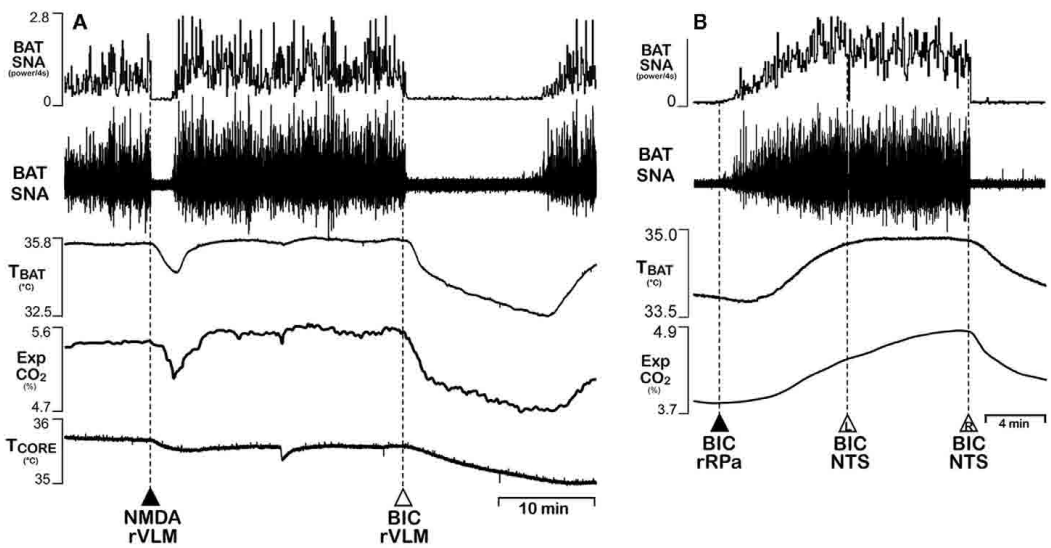

FIGURE 7 | Activation of neurons in the ventrolateral medulla (VLM) or in the nucleus of the solitary tract (NTS) inhibits BAT sympathetic nerve activity (SNA) and BAT thermogenesis. (A) Cooling-evoked increases in BAT SNA, BAT temperature (TBAT), and expired CO_2 (Exp CO_2) were reversed following unilateral nanoinjection of either the glutamate receptor agonist, NMDA, or the GABAA receptor antagonist, bicuculline (BIC), into the rostral VLM (RVLM). Core temperature (TCORE) was also reduced by activation of RVLM neurons. **(B)** Increases in BAT SNA, TBAT, and ExpCO_2 evoked by BIC nanoinjection into the rostral raphe pallidus (rRPa) were reversed by bilateral nanoinjections of BIC into the medial NTS. Modified with permission from Cao et al. (2010).

the rRPa, or to pontomedullary transection (Cao et al., 2010). The VLM region from which inhibitions of BAT SNA could be evoked corresponds to the locations of the A1 and C1 cell groups in the region ventral to the nucleus ambiguous. Similar inhibitions of BAT SNA were also produced by bilateral injections of bicuculline into the medial NTS (**Figure 7B**). However, application of leptin and TRH into the NTS elicits increases in BAT temperature (Hermann et al., 2006). Whether the inhibitions of BAT thermogenesis evoked throughout this rostro-caudal extent of the VLM represent activation of a homogeneous inhibitory mechanism or stimulations of different mechanisms at different rosto-caudal levels remains to be determined, as do the mechanisms through which the VLM and the NTS inhibitions of BAT SNA is effected and the physiological circumstances under which such inhibitions of BAT thermogenesis would normally be elicited. In this regard, both hypoxia and hypoglycemia are strong inhibitory signals for BAT thermogenesis (see above) and the commissural NTS contains second-order chemoreceptor sensory neurons mediating an inhibition of BAT SNA (Madden and Morrison, 2005), and the VLM contains neurons sensitive to glucopenia (Ritter et al., 2001), which also inhibits BAT SNA (Madden and Morrison, 2008b).

SUMMARY

Brown adipose tissue thermogenesis is regulated primarily by a core thermoregulatory neural network which responds to the feedforward afferent signals from cutaneous and core body thermoreceptors and to feedback signals from brain thermosensitive neurons to alter the level of activation of the sympathetic outflow to BAT. We have summarized the research leading to a model (**Figure 1**) of the thermoregulatory reflex pathway through which environmental cold stimulates thermogenesis and includes the influence on this thermoregulatory network of the pyrogenic mediator, PGE_2, to increase body temperature during fever. The cold thermal afferent circuit from cutaneous thermal receptors, through second-order thermosensory neurons in the dorsal horn of the spinal cord ascends to activate neurons in the LPBel which drive GABAergic interneurons in the MnPO to inhibit warm-sensitive, inhibitory output neurons of the MPO. Through the resulting disinhibition of thermogenesis-promoting neurons in the DMH, BAT sympathetic premotor neurons in the rostral ventromedial medulla, including the rRPa, are stimulated to increase the excitation to and responsiveness of the spinal circuits controlling BAT SPN discharge to drive BAT thermogenesis.

Hypoxia and hypoglycemia are strong metabolic regulators of BAT thermogenesis. The activity of neurons in several brain regions can influence the level of BAT thermogenesis and thus could modulate the thermoregulatory control of BAT, although their discharge does not appear to be required to mediate the skin cooling-evoked stimulation of BAT thermogenesis. These include an orexinergic excitation of BAT thermogenesis from neurons in the PeF-LH and inhibitory regulation of BAT thermogenesis by neurons in the PVH, the pontine retrorubral field, the VLM and the NTS.

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REFERENCES

Almeida, M. C., Steiner, A. A., Branco, L. G., and Romanovsky, A. A. (2006). Neural substrate of cold-seeking behavior in endotoxin shock. *PLoS ONE* 1, e1. doi:10.1371/journal.pone.0000001

Amini-Sereshki, L., and Zarrindast, M. R. (1984). Brain stem tonic inhibition of thermoregulation in the rat. *Am. J. Physiol.* 247, R154–R159.

Andrew, D., and Craig, A. D. (2001). Spinothalamic lamina I neurons selectively sensitive to histamine: a central neural pathway for itch. *Nat. Neurosci.* 4, 72–77.

Bacon, S. J., and Smith, A. D. (1988). Preganglionic sympathetic neurones innervating the rat adrenal medulla: immunocytochemical evidence of synaptic input from nerve terminals containing substance P, GABA or 5-hydroxytryptamine. *J. Auton. Nerv. Syst.* 24, 97–122.

Balthasar, N., Dalgaard, L. T., Lee, C. E., Yu, J., Funahashi, H., Williams, T., Ferreira, M., Tang, V., Mcgovern, R. A., Kenny, C. D., Christiansen, L. M., Edelstein, E., Choi, B., Boss, O., Aschkenasi, C., Zhang, C. Y., Mountjoy, K., Kishi, T., Elmquist, J. K., and Lowell, B. B. (2005). Divergence of melanocortin pathways in the control of food intake and energy expenditure. *Cell* 123, 493–505.

Bamshad, M., Song, C. K., and Bartness, T. J. (1999). CNS origins of the sympathetic nervous system outflow to brown adipose tissue. *Am. J. Physiol.* 276, R1569–R1578.

Bartness, T. J., Vaughan, C. H., and Song, C. K. (2010). Sympathetic and sensory innervation of brown adipose tissue. *Int. J. Obes. (Lond.)* 34(Suppl. 1), S36–S42.

Bautista, D. M., Siemens, J., Glazer, J. M., Tsuruda, P. R., Basbaum, A. I., Stucky, C. L., Jordt, S. E., and Julius, D. (2007). The menthol receptor TRPM8 is the principal detector of environmental cold. *Nature* 448, 204–208.

Berthoud, H. R., Patterson, L. M., Sutton, G. M., Morrison, C., and Zheng, H. (2005). Orexin inputs to caudal raphe neurons involved in thermal, cardiovascular, and gastrointestinal regulation. *Histochem. Cell Biol.* 123, 147–156.

Blatteis, C. M., and Banet, M. (1986). Autonomic thermoregulation after separation of the preoptic area from the hypothalamus in rats. *Pflugers Arch.* 406, 480–484.

Boulant, J. A. (2006a). Counterpoint: Heat-induced membrane depolarization of hypothalamic neurons: an unlikely mechanism of central thermosensitivity. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 290, R1481–1484; discussion R1484.

Boulant, J. A. (2006b). Neuronal basis of Hammel's model for set-point thermoregulation. *J. Appl. Physiol.* 100, 1347–1354.

Boulant, J. A., and Dean, J. B. (1986). Temperature receptors in the central nervous system. *Annu. Rev. Physiol.* 48, 639–654.

Boulant, J. A., and Hardy, J. D. (1974). The effect of spinal and skin temperatures on the firing rate and thermosensitivity of preoptic neurones. *J. Physiol. (Lond.)* 240, 639–660.

Bratincsak, A., and Palkovits, M. (2004). Activation of brain areas in rat following warm and cold ambient exposure. *Neuroscience* 127, 385–397.

Bratincsak, A., and Palkovits, M. (2005). Evidence that peripheral rather than intracranial thermal signals induce thermoregulation. *Neuroscience* 135, 525–532.

Buchanan, T. A., Cane, P., Eng, C. C., Sipos, G. F., and Lee, C. (1991). Hypothermia is critical for survival during prolonged insulin-induced hypoglycemia in rats. *Metab. Clin. Exp.* 40, 330–334.

Caldeira, J. C., Franci, C. R., and Pela, I. R. (1998). Bilateral lesion of hypothalamic paraventricular nucleus abolishes fever induced by endotoxin and bradykinin in rats. *Ann. N. Y. Acad. Sci.* 856, 294–297.

Cammack, C., and Logan, S. D. (1996). Excitation of rat sympathetic preganglionic neurones by selective activation of the NK1 receptor. *J. Auton. Nerv. Syst.* 57, 87–92.

Cannon, B., and Nedergaard, J. (2004). Brown adipose tissue: function and physiological significance. *Physiol. Rev.* 84, 277–359.

Cano, G., Passerin, A. M., Schiltz, J. C., Card, J. P., Morrison, S. F., and Sved, A. F. (2003). Anatomical substrates for the central control of sympathetic outflow to interscapular adipose tissue during cold exposure. *J. Comp. Neurol.* 460, 303–326.

Cao, W. H., Fan, W., and Morrison, S. F. (2004). Medullary pathways mediating specific sympathetic responses to activation of dorsomedial hypothalamus. *Neuroscience* 126, 229–240.

Cao, W. H., Madden, C. J., and Morrison, S. F. (2010). Inhibition of brown adipose tissue thermogenesis by neurons in the ventrolateral medulla and in the nucleus tractus solitarius. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 299, R277–R290.

Cao, W. H., and Morrison, S. F. (2005). Brown adipose tissue thermogenesis contributes to fentanyl-evoked hyperthermia. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 288, R723–R732.

Cao, W. H., and Morrison, S. F. (2006). Glutamate receptors in the raphe pallidus mediate brown adipose tissue thermogenesis evoked by activation of dorsomedial hypothalamic neurons. *Neuropharmacology* 51, 426–437.

Caterina, M. J., Leffler, A., Malmberg, A. B., Martin, W. J., Trafton, J., Petersen-Zeitz, K. R., Koltzenburg, M., Basbaum, A. I., and Julius, D. (2000). Impaired nociception and pain sensation in mice lacking the capsaicin receptor. *Science* 288, 306–313.

Cerri, M., and Morrison, S. F. (2005). Activation of lateral hypothalamic neurons stimulates brown adipose tissue thermogenesis. *Neuroscience* 135, 627–638.

Cerri, M., and Morrison, S. F. (2006). Corticotropin releasing factor increases in brown adipose tissue thermogenesis and heart rate through dorsomedial hypothalamus and medullary raphe pallidus. *Neuroscience* 140, 711–721.

Chen, X. M., Hosono, T., Yoda, T., Fukuda, Y., and Kanosue, K. (1998). Efferent projection from the preoptic area for the control of non-shivering thermogenesis in rats. *J. Physiol. (Lond.)* 512(Pt 3), 883–892.

Chen, X. M., Nishi, M., Taniguchi, A., Nagashima, K., Shibata, M., and Kanosue, K. (2002). The caudal periaqueductal gray participates in the activation of brown adipose tissue in rats. *Neurosci. Lett.* 331, 17–20.

Christensen, C. R., Clark, P. B., and Morton, K. A. (2006). Reversal of hypermetabolic brown adipose tissue in F-18 FDG PET imaging. *Clin. Nucl. Med.* 31, 193–196.

Clapham, J. C. (2011). Central control of thermogenesis. *Neuropharmacology* doi: 10.1016/j.neuropharm.2011.10.014. [Epub ahead of print].

Colburn, R. W., Lubin, M. L., Stone, D. J. Jr., Wang, Y., Lawrence, D., D'andrea, M. R., Brandt, M. R., Liu, Y., Flores, C. M., and Qin, N. (2007). Attenuated cold sensitivity in TRPM8 null mice. *Neuron* 54, 379–386.

Conte, D., Deuchars, S. A., and Deuchars, J. (2007). Do serotonin immunoreactive terminals innervate GABAergic neurons in the central autonomic area of the mouse spinal cord. *FASEB J.* 21, A885.

Coote, J. H., Macleod, V. H., Fleetwood-Walker, S., and Gilbey, M. P. (1981). The response of individual sympathetic preganglionic neurones to microelectrophoretically applied endogenous monoamines. *Brain Res.* 215, 135–145.

Cowley, M. A., Pronchuk, N., Fan, W., Dinulescu, D. M., Colmers, W. F., and Cone, R. D. (1999). Integration of NPY, AGRP, and melanocortin signals in the hypothalamic paraventricular nucleus: evidence of a cellular basis for the adipostat. *Neuron* 24, 155–163.

Craig, A. D. (2002). How do you feel? Interoception: the sense of the physiological condition of the body. *Nat. Rev. Neurosci.* 3, 655–666.

Craig, A. D., Bushnell, M. C., Zhang, E. T., and Blomqvist, A. (1994). A thalamic nucleus specific for pain and temperature sensation. *Nature* 372, 770–773.

Craig, A. D., Krout, K., and Andrew, D. (2001). Quantitative response characteristics of thermoreceptive and nociceptive lamina I spinothalamic neurons in the cat. *J. Neurophysiol.* 86, 1459–1480.

Cypess, A. M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A. B., Kuo, F. C., Palmer, E. L., Tseng, Y. H., Doria, A., Kolodny, G. M., and Kahn, C. R. (2009). Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* 360, 1509–1517.

de Lecea, L., Kilduff, T. S., Peyron, C., Gao, X., Foye, P. E., Danielson, P. E., Fukuhara, C., Battenberg, E. L., Gautvik, V. T., Bartlett, F. S. II, Frankel, W. N., Van Den Pol, A. N., Bloom, F. E., Gautvik, K. M., and Sutcliffe, J. G. (1998). The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. *Proc. Natl. Acad. Sci. U.S.A.* 95, 322–327.

de Menezes, R. C., Zaretsky, D. V., Fontes, M. A., and Dimicco, J. A. (2009). Cardiovascular and thermal responses evoked from the periaqueductal grey require neuronal activity in the hypothalamus. *J. Physiol. (Lond.)* 587, 1201–1215.

Deuchars, S. A., Milligan, C. J., Stornetta, R. L., and Deuchars, J. (2005). GABAergic neurons in the central region of the spinal cord: a novel substrate for sympathetic inhibition. *J. Neurosci.* 25, 1063–1070.

Deuchars, S. A., Spyer, K. M., and Gilbey, M. P. (1997). Stimulation within the rostral ventrolateral medulla can evoke monosynaptic GABAergic IPSPs in sympathetic preganglionic neurons in vitro. *J. Neurophysiol.* 77, 229–235.

Dhaka, A., Murray, A. N., Mathur, J., Earley, T. J., Petrus, M. J., and Patapoutian, A. (2007). TRPM8 is required for cold sensation in mice. *Neuron* 54, 371–378.

Dib, B. (1987). Thermoregulatory behaviour induced by intrathecal injection of substance P in the rat. *Eur. J. Pharmacol.* 133, 147–153.

Dimicco, J. A., and Zaretsky, D. V. (2007). The dorsomedial hypothalamus: a new player in thermoregulation. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 292, R47–R63.

Egawa, M., Yoshimatsu, H., and Bray, G. A. (1989a). Effects of 2-deoxy-D-glucose on sympathetic nerve activity to interscapular brown adipose tissue. *Am. J. Physiol.* 257, R1377–R1385.

Egawa, M., Yoshimatsu, H., and Bray, G. A. (1989b). Lateral hypothalamic injection of 2-deoxy-D-glucose suppresses sympathetic activity. *Am. J. Physiol.* 257, R1386–R1392.

Egawa, M., Yoshimatsu, H., and Bray, G. A. (1991). Neuropeptide Y suppresses sympathetic activity to interscapular brown adipose tissue in rats. *Am. J. Physiol.* 260, R328–R334.

Elmquist, J. K., Scammell, T. E., Jacobson, C. D., and Saper, C. B. (1996). Distribution of Fos-like immunoreactivity in the rat brain following intravenous lipopolysaccharide administration. *J. Comp. Neurol.* 371, 85–103.

Elmquist, J. K., Scammell, T. E., and Saper, C. B. (1997). Mechanisms of CNS response to systemic immune challenge: the febrile response. *Trends Neurosci.* 20, 565–570.

Fan, W., Morrison, S. F., Cao, W. H., and Yu, P. (2007). Thermogenesis activated by central melanocortin signaling is dependent on neurons in the rostral raphe pallidus (rRPa) area. *Brain Res.* 1179, 61–69.

Freinkel, N., Metzger, B. E., Harris, E., Robinson, S., and Mager, M. (1972). The hypothermia of hypoglycemia. Studies with 2-deoxy-D-glucose in normal human subjects and mice. *N. Engl. J. Med.* 287, 841–845.

Gavva, N. R., Bannon, A. W., Surapaneni, S., Hovland, D. N. Jr., Lehto, S. G., Gore, A., Juan, T., Deng, H., Han, B., Klionsky, L., Kuang, R., Le, A., Tamir, R., Wang, J., Youngblood, B., Zhu, D., Norman, M. H., Magal, E., Treanor, J. J., and Louis, J. C. (2007). The vanilloid receptor TRPV1 is tonically activated in vivo and involved in body temperature regulation. *J. Neurosci.* 27, 3366–3374.

Golozoubova, V., Cannon, B., and Nedergaard, J. (2006). UCP1 is essential for adaptive adrenergic nonshivering thermogenesis. *Am. J. Physiol. Endocrinol. Metab.* 291, E350–E357.

Guieu, J. D., and Hardy, J. D. (1970). Effects of heating and cooling of the spinal cord on preoptic unit activity. *J. Appl. Physiol.* 29, 675–683.

Gupta, B. N., Nier, K., and Hensel, H. (1979). Cold-sensitive afferents from the abdomen. *Pflugers Arch.* 380, 203–204.

Hany, T. F., Gharehpapagh, E., Kamel, E. M., Buck, A., Himms-Hagen, J., and Von Schulthess, G. K. (2002). Brown adipose tissue: a factor to consider in symmetrical tracer uptake in the neck and upper chest region. *Eur. J. Nucl. Med. Mol. Imaging* 29, 1393–1398.

Hara, J., Yanagisawa, M., and Sakurai, T. (2005). Difference in obesity phenotype between orexin-knockout mice and orexin neuron-deficient mice with same genetic background and environmental conditions. *Neurosci. Lett.* 380, 239–242.

Hermann, D. M., Luppi, P. H., Peyron, C., Hinckel, P., and Jouvet, M. (1997). Afferent projections to the rat nuclei raphe magnus, raphe pallidus and reticularis gigantocellularis pars alpha demonstrated by iontophoretic application of choleratoxin (subunit b). *J. Chem. Neuroanat.* 13, 1–21.

Hermann, G. E., Barnes, M. J., and Rogers, R. C. (2006). Leptin and thyrotropin-releasing hormone: cooperative action in the hindbrain to activate brown adipose thermogenesis. *Brain Res.* 1117, 118–124.

Hetherington, A. W., and Ranson, S. W. (1940). Hypothalamic lesions and adiposity in the rat. *Anat. Rec.* 78, 149–172.

Hodges, M. R., Tattersall, G. J., Harris, M. B., Mcevoy, S. D., Richerson, D. N., Deneris, E. S., Johnson, R. L., Chen, Z. F., and Richerson,

G. B. (2008). Defects in breathing and thermoregulation in mice with near-complete absence of central serotonin neurons. *J. Neurosci.* 28, 2495–2505.

Hori, T. (1984). Capsaicin and central control of thermoregulation. *Pharmacol. Ther.* 26, 389–416.

Horn, T., Wilkinson, M. F., Landgraf, R., and Pittman, Q. J. (1994). Reduced febrile responses to pyrogens after lesions of the hypothalamic paraventricular nucleus. *Am. J. Physiol.* 267, R323–R328.

Hylden, J. L., Anton, F., and Nahin, R. L. (1989). Spinal lamina I projection neurons in the rat: collateral innervation of parabrachial area and thalamus. *Neuroscience* 28, 27–37.

Imai-Matsumura, K., Matsumura, K., and Nakayama, T. (1984). Involvement of ventromedial hypothalamus in brown adipose tissue thermogenesis induced by preoptic cooling in rats. *Jpn. J. Physiol.* 34, 939–943.

Ishiwata, T., Saito, T., Hasegawa, H., Yazawa, T., Kotani, Y., Otokawa, M., and Aihara, Y. (2005). Changes of body temperature and thermoregulatory responses of freely moving rats during GABAergic pharmacological stimulation to the preoptic area and anterior hypothalamus in several ambient temperatures. *Brain Res.* 1048, 32–40.

Jancso-Gabor, A., Szolcsanyi, J., and Jancso, N. (1970). Irreversible impairment of thermoregulation induced by capsaicin and similar pungent substances in rats and guinea-pigs. *J. Physiol. (Lond.)* 206, 495–507.

Klockener, T., Hess, S., Belgardt, B. F., Paeger, L., Verhagen, L. A., Husch, A., Sohn, J. W., Hampel, B., Dhillon, H., Zigman, J. M., Lowell, B. B., Williams, K. W., Elmquist, J. K., Horvath, T. L., Kloppenburg, P., and Bruning, J. C. (2011). High-fat feeding promotes obesity via insulin receptor/PI3K-dependent inhibition of SF-1 VMH neurons. *Nat. Neurosci.* 14, 911–918.

Kobayashi, A., and Osaka, T. (2003). Involvement of the parabrachial nucleus in thermogenesis induced by environmental cooling in the rat. *Pflugers Arch.* 446, 760–765.

Kobayashi, S., Hori, A., Matsumura, K., and Hosokawa, H. (2006). Point: heat-induced membrane depolarization of hypothalamic neurons: a putative mechanism of central thermosensitivity. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 290, R1479–R1480; discussion R1484.

Kok, S. W., Overeem, S., Visscher, T. L., Lammers, G. J., Seidell, J. C., Pijl, H., and Meinders, A. E. (2003). Hypocretin deficiency in narcoleptic humans is associated with abdominal obesity. *Obes. Res.* 11, 1147–1154.

Lazarus, M., Yoshida, K., Coppari, R., Bass, C. E., Mochizuki, T., Lowell, B. B., and Saper, C. B. (2007). EP3 prostaglandin receptors in the median preoptic nucleus are critical for fever responses. *Nat. Neurosci.* 10, 1131–1133.

Lee, H., Iida, T., Mizuno, A., Suzuki, M., and Caterina, M. J. (2005). Altered thermal selection behavior in mice lacking transient receptor potential vanilloid 4. *J. Neurosci.* 25, 1304–1310.

Li, J., Xiong, K., Pang, Y., Dong, Y., Kaneko, T., and Mizuno, N. (2006). Medullary dorsal horn neurons providing axons to both the parabrachial nucleus and thalamus. *J. Comp. Neurol.* 498, 539–551.

Lomax, P., Malveaux, E., and Smith, R. E. (1964). Brain temperatures in the rat during exposure to low environmental temperatures. *Am. J. Physiol.* 207, 736–739.

Lopez, M., Varela, L., Vazquez, M. J., Rodriguez-Cuenca, S., Gonzalez, C. R., Velagapudi, V. R., Morgan, D. A., Schoenmakers, E., Agassandian, K., Lage, R., Martinez De Morentin, P. B., Tovar, S., Nogueiras, R., Carling, D., Lelliott, C., Gallego, R., Oresic, M., Chatterjee, K., Saha, A. K., Rahmouni, K., Dieguez, C., and Vidal-Puig, A. (2010). Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. *Nat. Med.* 16, 1001–1008.

Lu, J., Zhang, Y. H., Chou, T. C., Gaus, S. E., Elmquist, J. K., Shiromani, P., and Saper, C. B. (2001). Contrasting effects of ibotenate lesions of the paraventricular nucleus and subparaventricular zone on sleep-wake cycle and temperature regulation. *J. Neurosci.* 21, 4864–4874.

Lundius, E. G., Sanchez-Alavez, M., Ghochani, Y., Klaus, J., and Tabarean, I. V. (2010). Histamine influences body temperature by acting at H1 and H3 receptors on distinct populations of preoptic neurons. *J. Neurosci.* 30, 4369–4381.

Madden, C. J. (2012). Glucoprivation in the ventrolateral medulla decreases brown adipose tissue sympathetic nerve activity by decreasing the activity of neurons in raphe pallidus. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 302, R224–R232.

Madden, C. J., and Morrison, S. F. (2003). Excitatory amino acid receptor activation in the raphe pallidus area mediates prostaglandin-evoked thermogenesis. *Neuroscience* 122, 5–15.

Madden, C. J., and Morrison, S. F. (2004). Excitatory amino acid receptors in the dorsomedial hypothalamus mediate prostaglandin-evoked thermogenesis in brown adipose tissue. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 286, R320–R325.

Madden, C. J., and Morrison, S. F. (2005). Hypoxic activation of arterial chemoreceptors inhibits sympathetic outflow to brown adipose tissue in rats. *J. Physiol.* 566, 559–573.

Madden, C. J., and Morrison, S. F. (2006). Serotonin potentiates sympathetic responses evoked by spinal NMDA. *J. Physiol. (Lond.)* 577, 525–537.

Madden, C. J., and Morrison, S. F. (2008a). Brown adipose tissue sympathetic nerve activity is potentiated by activation of 5-hydroxytryptamine (5-HT)(1A)/5-HT(7) receptors in the rat spinal cord. *Neuropharmacology* 54, 487–496.

Madden, C. J., and Morrison, S. F. (2008b). Neural circuits involved in glucoprivation-evoked inhibition of metabolism in brown adipose tissue (BAT). *FASEB J.* 22, 950–958.

Madden, C. J., and Morrison, S. F. (2009). Neurons in the paraventricular nucleus of the hypothalamus inhibit sympathetic outflow to brown adipose tissue. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, R831–R843.

Madden, C. J., and Morrison, S. F. (2010). Endogenous activation of spinal 5-hydroxytryptamine (5-HT) receptors contributes to the thermoregulatory activation of brown adipose tissue. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 298, R776–R783.

Mager, M., Robinson, S., and Freinkel, N. (1976). Drug modification of hypothermia induced by CNS glucopenia in the mouse. *J. Appl. Physiol.* 41, 559–564.

Martin-Cora, F. J., Fornal, C. A., Metzler, C. W., and Jacobs, B. L. (2000). Single-unit responses of serotonergic medullary and pontine raphe neurons to environmental cooling in freely moving cats. *Neuroscience* 98, 301–309.

Matsumura, K., Cao, C., Ozaki, M., Morii, H., Nakadate, K., and Watanabe, Y. (1998). Brain endothelial cells express cyclooxygenase-2 during lipopolysaccharide-induced fever: light and electron microscopic immunocytochemical studies. *J. Neurosci.* 18, 6279–6289.

McKemy, D. D., Neuhausser, W. M., and Julius, D. (2002). Identification of a cold receptor reveals a general role for TRP channels in thermosensation. *Nature* 416, 52–58.

Minokoshi, Y., Saito, M., and Shimazu, T. (1986). Sympathetic denervation impairs responses of brown adipose tissue to VMH stimulation. *Am. J. Physiol.* 251, R1005–R1008.

Miyazaki, T., Coote, J. H., and Dun, N. J. (1989). Excitatory and inhibitory effects of epinephrine on neonatal rat sympathetic preganglionic neurons in vitro. *Brain Res.* 497, 108–116.

Monda, M., Viggiano, A., Mondola, P., and De Luca, V. (2001). Inhibition of prostaglandin synthesis reduces hyperthermic reactions induced by hypocretin-1/orexin A. *Brain Res.* 909, 68–74.

Moqrich, A., Hwang, S. W., Earley, T. J., Petrus, M. J., Murray, A. N., Spencer, K. S., Andahazy, M., Story, G. M., and Patapoutian, A. (2005). Impaired thermosensation in mice lacking TRPV3, a heat and camphor sensor in the skin. *Science* 307, 1468–1472.

Morrison, S. F. (1999). RVLM and raphe differentially regulate sympathetic outflows to splanchnic and brown adipose tissue. *Am. J. Physiol.* 276, R962–R973.

Morrison, S. F. (2003). Raphe pallidus neurons mediate prostaglandin E2-evoked increases in brown adipose tissue thermogenesis. *Neuroscience* 121, 17–24.

Morrison, S. F. (2004). Activation of 5-HT1A receptors in raphe pallidus inhibits leptin-evoked increases in brown adipose tissue thermogenesis. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 286, R832–R837.

Morrison, S. F. (2011). 2010 Carl Ludwig distinguished lectureship of the APS neural control and autonomic regulation section: central neural pathways for thermoregulatory cold defense. *J. Appl. Physiol.* 110, 1137–1149.

Morrison, S. F., and Blessing, W. W. (2011). "Central nervous system regulation of body temperature," in *Central Regulation of Autonomic Functions*, 2nd Edn, eds I. J. Llewellyn-Smith and A. J. M. Verberne (New York: Oxford University Press), 324–344.

Morrison, S. F., Cao, W.-H., and Madden, C. J. (2004). Dorsomedial hypothalamic and brainstem pathways controlling thermogenesis in brown adipose tissue. *J. Therm. Biol.* 29, 333–337.

Morrison, S. F., Sved, A. F., and Passerin, A. M. (1999). GABA-mediated inhibition of raphe pallidus neurons regulates sympathetic outflow to brown adipose tissue. *Am. J. Physiol.* 276, R290–R297.

Nakamura, K., Kaneko, T., Yamashita, Y., Hasegawa, H., Katoh, H., and Negishi, M. (2000). Immunohistochemical localization of prostaglandin EP3 receptor in the rat nervous system. *J. Comp. Neurol.* 421, 543–569.

Nakamura, K., Matsumura, K., Hubschle, T., Nakamura, Y., Hioki, H., Fujiyama, F., Boldogkoi, Z., Konig, M., Thiel, H. J., Gerstberger, R., Kobayashi, S., and Kaneko, T. (2004a). Identification of sympathetic premotor neurons in medullary raphe regions mediating fever and other thermoregulatory functions. *J. Neurosci.* 24, 5370–5380.

Nakamura, K., Wu, S. X., Fujiyama, F., Okamoto, K., Hioki, H., and Kaneko, T. (2004b). Independent inputs by VGLUT2- and VGLUT3-positive glutamatergic terminals onto rat sympathetic preganglionic neurons. *Neuroreport* 15, 431–436.

Nakamura, K., Matsumura, K., Kaneko, T., Kobayashi, S., Katoh, H., and Negishi, M. (2002). The rostral raphe pallidus nucleus mediates pyrogenic transmission from the preoptic area. *J. Neurosci.* 22, 4600–4610.

Nakamura, K., and Morrison, S. F. (2007). Central efferent pathways mediating skin cooling-evoked sympathetic thermogenesis in brown adipose tissue. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 292, R127–R136.

Nakamura, K., and Morrison, S. F. (2008a). Preoptic mechanism for cold-defensive responses to skin cooling. *J. Physiol. (Lond.)* 586, 2611–2620.

Nakamura, K., and Morrison, S. F. (2008b). A thermosensory pathway that controls body temperature. *Nat. Neurosci.* 11, 62–71.

Nakamura, K., and Morrison, S. F. (2010). A thermosensory pathway mediating heat-defense responses. *Proc. Natl. Acad. Sci. U.S.A.* 107, 8848–8853.

Nakamura, Y., Nakamura, K., Matsumura, K., Kobayashi, S., Kaneko, T., and Morrison, S. F. (2005). Direct pyrogenic input from prostaglandin EP3 receptor-expressing preoptic neurons to the dorsomedial hypothalamus. *Eur. J. Neurosci.* 22, 3137–3146.

Nakamura, Y., Nakamura, K., and Morrison, S. F. (2009). Different populations of prostaglandin EP3 receptor-expressing preoptic neurons project to two fever-mediating sympathoexcitatory brain regions. *Neuroscience* 161, 614–620.

Nakayama, T., Eisenman, J. S., and Hardy, J. D. (1961). Single unit activity of anterior hypothalamus during local heating. *Science* 134, 560–561.

Nakayama, T., Hammel, H., Hardy, J., and Eisenman, J. (1963). Thermal stimulation of electrical activity of single units of the preoptic region. *Am. J. Physiol.* 204, 1122–1126.

Narumiya, S., Sugimoto, Y., and Ushikubi, F. (1999). Prostanoid receptors: structures, properties, and functions. *Physiol. Rev.* 79, 1193–1226.

Nason, M. W. Jr., and Mason, P. (2006). Medullary raphe neurons facilitate brown adipose tissue activation. *J. Neurosci.* 26, 1190–1198.

Nautiyal, K. M., Dailey, M., Brito, N., Brito, M. N., Harris, R. B., Bartness, T. J., and Grill, H. J. (2008). Energetic responses to cold temperatures in rats lacking forebrain-caudal brain stem connections. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 295, R789–R798.

Nedergaard, J., Bengtsson, T., and Cannon, B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. *Am. J. Physiol. Endocrinol. Metab.* 293, E444–E452.

Nguyen, K. D., Qiu, Y., Cui, X., Goh, Y. P., Mwangi, J., David, T., Mukundan, L., Brombacher, F., Locksley, R. M., and Chawla, A. (2011). Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. *Nature* 480, 104–108.

Niijima, A. (1999). Reflex effects from leptin sensors in the white adipose tissue of the epididymis to the efferent activity of the sympathetic and vagus nerve in the rat. *Neurosci. Lett.* 262, 125–128.

Niijima, A., Rohner-Jeanrenaud, F., and Jeanrenaud, B. (1984). Role of ventromedial hypothalamus on sympathetic efferents of brown adipose tissue. *Am. J. Physiol.* 247, R650–R654.

Oldfield, B. J., Giles, M. E., Watson, A., Anderson, C., Colvill, L. M., and Mckinley, M. J. (2002). The neurochemical characterisation of hypothalamic pathways projecting polysynaptically to brown adipose tissue in the rat. *Neuroscience* 110, 515–526.

Ootsuka, Y., Blessing, W. W., Steiner, A. A., and Romanovsky, A. A. (2008). Fever response to intravenous prostaglandin E2 is mediated by the brain but does not require afferent vagal signaling. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 294, R1294–R303

Ootsuka, Y., De Menezes, R. C., Zaretsky, D. V., Alimoradian, A., Hunt, J., Stefanidis, A., Oldfield, B. J., and Blessing, W. W. (2009). Brown adipose tissue thermogenesis heats brain and body as part of the brain-coordinated ultradian basic rest-activity cycle. *Neuroscience* 164, 849–861.

Osaka, T. (2004). Thermogenesis elicited by skin cooling in anaesthetized rats: lack of contribution of the cerebral cortex. *J. Physiol. (Lond.)* 555, 503–513.

Peier, A. M., Moqrich, A., Hergarden, A. C., Reeve, A. J., Andersson, D. A., Story, G. M., Earley, T. J., Dragoni, I., Mcintyre, P., Bevan, S., and Patapoutian, A. (2002). A TRP channel that senses cold stimuli and menthol. *Cell* 108, 705–715.

Perkins, M. N., Rothwell, N. J., Stock, M. J., and Stone, T. W. (1981). Activation of brown adipose tissue thermogenesis by the ventromedial hypothalamus. *Nature* 289, 401–402.

Peyron, C., Tighe, D. K., Van Den Pol, A. N., De Lecea, L., Heller, H. C., Sutcliffe, J. G., and Kilduff, T. S. (1998). Neurons containing hypocretin (orexin) project to multiple neuronal systems. *J. Neurosci.* 18, 9996–10015.

Plazzi, G., Moghadam, K. K., Maggi, L. S., Donadio, V., Vetrugno, R., Liguori, R., Zoccoli, G., Poli, F., Pizza, F., Pagotto, U., and Ferri, R. (2011). Autonomic disturbances in narcolepsy. *Sleep Med. Rev.* 15, 187–196.

Ranels, H. J., and Griffin, J. D. (2003). The effects of prostaglandin E2 on the firing rate activity of thermosensitive and temperature insensitive neurons in the ventromedial preoptic area of the rat hypothalamus. *Brain Res.* 964, 42–50.

Rathner, J. A., and Morrison, S. F. (2006). Rostral ventromedial periaqueductal gray: a source of inhibition of the sympathetic outflow to brown adipose tissue. *Brain Res.* 1077, 99–107.

Riedel, W. (1976). Warm receptors in the dorsal abdominal wall of the rabbit. *Pflugers Arch.* 361, 205–206.

Ritter, S., Bugarith, K., and Dinh, T. T. (2001). Immunotoxic destruction of distinct catecholamine subgroups produces selective impairment of glucoregulatory responses and neuronal activation. *J. Comp. Neurol.* 432, 197–216.

Ritter, S., Li, A. J., Wang, Q., and Dinh, T. T. (2011). Minireview: The value of looking backward: the essential role of the hindbrain in counterregulatory responses to glucose deficit. *Endocrinology* 152, 4019–4032.

Romanovsky, A. A. (2004). Do fever and anapyrexia exist? Analysis of set point-based definitions. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 287, R992–R995.

Romanovsky, A. A. (2007). Thermoregulation: some concepts have changed. Functional architecture of the thermoregulatory system. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 292, R37–R46.

Romanovsky, A. A., Almeida, M. C., Garami, A., Steiner, A. A., Norman, M. H., Morrison, S. F., Nakamura, K., Burmeister, J. J., and Nucci, T. B. (2009). The transient receptor potential vanilloid-1 channel in thermoregulation: a thermosensor it is not. *Pharmacol. Rev.* 61, 228–261.

Rothwell, N. J., Stock, M. J., and Thexton, A. J. (1983). Decerebration activates thermogenesis in the rat. *J. Physiol. (Lond.)* 342, 15–22.

Sakaguchi, T., Bray, G. A., and Eddlestone, G. (1988). Sympathetic activity following paraventricular or ventromedial hypothalamic lesions in rats. *Brain Res. Bull.* 20, 461–465.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R. M., Tanaka, H., Williams, S. C., Richardson, J. A., Kozlowski, G. P., Wilson, S., Arch, J. R., Buckingham, R. E., Haynes, A. C., Carr, S. A., Annan, R. S., Mcnulty, D. E., Liu, W. S., Terrett, J. A., Elshourbagy, N. A., Bergsma, D. J., and Yanagisawa, M. (1998). Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein-coupled receptors that regulate feeding behavior. *Cell* 92, 573–585.

Samuels, B. C., Zaretsky, D. V., and Dimicco, J. A. (2002). Tachycardia evoked by disinhibition of the dorsomedial hypothalamus in rats is mediated through medullary raphe. *J. Physiol. (Lond.)* 538, 941–946.

Sarkar, S., Zaretskaia, M. V., Zaretsky, D. V., Moreno, M., and Dimicco, J. A. (2007). Stress- and lipopolysaccharide-induced c-fos expression and nNOS in hypothalamic neurons projecting to medullary raphe in rats: a triple immunofluorescent labeling study. *Eur. J. Neurosci.* 26, 2228–2238.

Sasek, C. A., Wessendorf, M. W., and Helke, C. J. (1990). Evidence for coexistence of thyrotropin-releasing hormone, substance P and serotonin in ventral medullary neurons that project to the intermediolateral cell column in the rat. *Neuroscience* 35, 105–119.

Scammell, T. E., Elmquist, J. K., Griffin, J. D., and Saper, C. B. (1996). Ventromedial preoptic prostaglandin E2 activates fever-producing autonomic pathways. *J. Neurosci.* 16, 6246–6254.

Schoener, E. P., and Wang, S. C. (1976). Effects of locally administered prostaglandin E1 on anterior hypothalamic neurons. *Brain Res.* 117, 157–162.

Sellayah, D., Bharaj, P., and Sikder, D. (2011). Orexin is required for brown adipose tissue development, differentiation, and function. *Cell Metab.* 14, 478–490.

Shibata, M., Uno, T., and Hashimoto, M. (1999). Disinhibition of lower midbrain neurons enhances non-shivering thermogenesis in anesthetized rats. *Brain Res.* 833, 242–250.

Skibicka, K. P., Alhadeff, A. L., Leichner, T. M., and Grill, H. J. (2011). Neural controls of prostaglandin 2 pyrogenic, tachycardic, and anorexic actions are anatomically distributed. *Endocrinology* 152, 2400–2408.

Skibicka, K. P., and Grill, H. J. (2009). Hypothalamic and hindbrain melanocortin receptors contribute to the feeding, thermogenic, and cardiovascular action of melanocortins. *Endocrinology* 150, 5351–5361.

Soderlund, V., Larsson, S. A., and Jacobsson, H. (2007). Reduction of FDG uptake in brown adipose tissue in clinical patients by a single dose of propranolol. *Eur. J. Nucl. Med. Mol. Imaging* 34, 1018–1022.

Song, C. K., Schwartz, G. J., and Bartness, T. J. (2009). Anterograde transneuronal viral tract tracing reveals central sensory circuits from white adipose tissue. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 296, R501–R511.

Song, C. K., Vaughan, C. H., Keen-Rhinehart, E., Harris, R. B., Richard, D., and Bartness, T. J. (2008). Melanocortin-4 receptor mRNA expressed in sympathetic outflow neurons to brown adipose tissue: neuroanatomical and functional evidence. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 295, R417–R428.

Steiner, A. A., Antunes-Rodrigues, J., and Branco, L. G. (2002). Role of preoptic second messenger systems (cAMP and cGMP) in the febrile response. *Brain Res.* 944, 135–145.

Steiner, A. A., Turek, V. F., Almeida, M. C., Burmeister, J. J., Oliveira, D. L., Roberts, J. L., Bannon, A. W., Norman, M. H., Louis, J. C., Treanor, J. J., Gavva, N. R., and Romanovsky, A. A. (2007). Nonthermal activation of transient receptor potential vanilloid-1 channels in abdominal viscera tonically inhibits autonomic cold-defense effectors. *J. Neurosci.* 27, 7459–7468.

Stornetta, R. L., Mcquiston, T. J., and Guyenet, P. G. (2004). GABAergic and glycinergic presympathetic neurons of rat medulla oblongata identified by retrograde transport of pseudorabies virus and in situ hybridization. *J. Comp. Neurol.* 479, 257–270.

Stornetta, R. L., Rosin, D. L., Simmons, J. R., Mcquiston, T. J., Vujovic, N., Weston, M. C., and Guyenet, P. G. (2005). Coexpression of vesicular glutamate transporter-3 and gamma-aminobutyric acidergic markers in rat rostral medullary raphe and intermediolateral cell column. *J. Comp. Neurol.* 492, 477–494.

Szelenyi, Z., Hummel, Z., Szolcsanyi, J., and Davis, J. B. (2004). Daily body temperature rhythm and heat tolerance in TRPV1 knockout and capsaicin pretreated mice. *Eur. J. Neurosci.* 19, 1421–1424.

Tominaga, M., Caterina, M. J., Malmberg, A. B., Rosen, T. A., Gilbert, H., Skinner, K., Raumann, B. E., Basbaum, A. I., and Julius, D. (1998). The cloned capsaicin receptor integrates multiple pain-producing stimuli. *Neuron* 21, 531–543.

Tupone, D., Madden, C. J., Cano, G., and Morrison, S. F. (2011). An orexinergic projection from perifornical hypothalamus to raphe pallidus increases rat brown adipose tissue thermogenesis. *J. Neurosci.* 31, 15944–15955.

Uschakov, A., Gong, H., Mcginty, D., and Szymusiak, R. (2007). Efferent projections from the median preoptic nucleus to sleep- and arousal-regulatory nuclei in the rat brain. *Neuroscience* 150, 104–120.

van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, J. M., Kemerink, G. J., Bouvy, N. D., Schrauwen, P., and Teule, G. J. (2009). Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* 360, 1500–1508.

Vera, P. L., Holets, V. R., and Miller, K. E. (1990). Ultrastructural evidence of synaptic contacts between substance P-, enkephalin-, and serotonin-immunoreactive terminals and retrogradely labeled sympathetic preganglionic neurons in the rat: a study using a double-peroxidase procedure. *Synapse* 6, 221–229.

Virtanen, K. A., Lidell, M. E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N. J., Enerback, S., and Nuutila, P. (2009). Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* 360, 1518–1525.

Yamagata, K., Matsumura, K., Inoue, W., Shiraki, T., Suzuki, K., Yasuda, S., Sugiura, H., Cao, C., Watanabe, Y., and Kobayashi, S. (2001). Coexpression of microsomal-type prostaglandin E synthase with cyclooxygenase-2 in brain endothelial cells of rats during endotoxin-induced fever. *J. Neurosci.* 21, 2669–2677.

Yoshida, K., Konishi, M., Nagashima, K., Saper, C. B., and Kanosue, K. (2005). Fos activation in hypothalamic neurons during cold or warm exposure: projections to periaqueductal gray matter. *Neuroscience* 133, 1039–1046.

Yoshida, K., Li, X., Cano, G., Lazarus, M., and Saper, C. B. (2009). Parallel preoptic pathways for thermoregulation. *J. Neurosci.* 29, 11954–11964.

Yoshida, K., Nakamura, K., Matsumura, K., Kanosue, K., Konig, M., Thiel, H. J., Boldogkoi, Z., Toth, I., Roth, J., Gerstberger, R., and Hubschle, T. (2003). Neurons of the rat preoptic area and the raphe pallidus nucleus innervating the brown adipose tissue express the prostaglandin E receptor subtype EP3. *Eur. J. Neurosci.* 18, 1848–1860.

Yoshimatsu, H., Egawa, M., and Bray, G. A. (1993). Sympathetic nerve activity after discrete hypothalamic injections of L-glutamate. *Brain Res.* 601, 121–128.

Zaretskaia, M. V., Zaretsky, D. V., and Dimicco, J. A. (2003). Role of the dorsomedial hypothalamus in thermogenesis and tachycardia caused by microinjection of prostaglandin E2 into the preoptic area in anesthetized rats. *Neurosci. Lett.* 340, 1–4.

Zaretskaia, M. V., Zaretsky, D. V., Shekhar, A., and Dimicco, J. A. (2002). Chemical stimulation of the dorsomedial hypothalamus evokes non-shivering thermogenesis in anesthetized rats. *Brain Res.* 928, 113–125.

Zaretsky, D. V., Hunt, J. L., Zaretskaia, M. V., and Dimicco, J. A. (2006). Microinjection of prostaglandin E2 and muscimol into the preoptic area in conscious rats: comparison of effects on plasma adrenocorticotrophic hormone (ACTH), body temperature, locomotor activity, and cardiovascular function. *Neurosci. Lett.* 397, 291–296.

Zaretsky, D. V., Zaretskaia, M. V., and Dimicco, J. A. (2003). Stimulation and blockade of GABAA receptors in the raphe pallidus: effects on body temperature, heart rate, and blood pressure in conscious rats. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 285, R110–R116.

Zhang, Y. H., Lu, J., Elmquist, J. K., and Saper, C. B. (2000). Lipopolysaccharide activates specific populations of hypothalamic and brainstem neurons that project to the spinal cord. *J. Neurosci.* 20, 6578–6586.

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Human brown fat and obesity: methodological aspects

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Much is known about brown adipose tissue (BAT) in rodents. Its function is to generate heat in response to low environmental temperatures and to diet or overfeeding. The knowledge about BAT in humans is still rather limited despite the recent rediscovery of its functionality in adults. This review highlights the information available on the contribution of BAT in increasing human energy expenditure in relation to obesity. Besides that methodological aspects will be discussed that need special attention in order to unravel the heat producing capacity of human BAT, the recruitment of the tissue, and its functionality.

Keywords: brown adipose tissue, obesity, PET/CT, non-shivering thermogenesis

INTRODUCTION

In the past 3 years more than 40 reviews and commentaries on human brown adipose tissue (BAT) appeared in peer reviewed journals. This revival in the interest in BAT followed the publications on this topic in the NEJM of May 2009 (Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). In the 2-years period that followed only five publications on addition al experimental studies on human BAT appeared (Saito et al., 2009; Zingaretti et al., 2009; Orava et al., 2011; Vijgen et al., 2011; Yoneshiro et al., 2011) and at least eight retrospective patient studies (Au-Yong et al., 2009; Lebron et al., 2010; Pfannenberg et al., 2010; Zukotynski et al., 2010; Gilsanz et al., 2011; Hong et al., 2011; Lee et al., 2011a; Wang et al., 2011). The somewhat misbalanced amount of reviews indicates the strong interest in human BAT by researchers and the interest sparked by the general public. Probably, this attention was already latent, fueled by numerous studies on the topic of non-shivering thermogenesis (NST) and BAT in rodents and humans in the years 1960–1985 and by the promise from animal studies that BAT can dissipate large amounts of energy as body heat at times of obesity pandemic.

The reason for the temporal disinterest in human BAT following 1985 was that some elaborate physiological studies on human BAT in those years concluded that in adults BAT was not of significant importance (Astrup et al., 1985). These studies mainly focused on perirenal BAT, which is abundant in newborns, but relatively scarce in human adults. There was a small revival in the interest in facultative diet induced thermogenesis (DIT) and BAT in humans following the paper by Stock (1999). It was not until the application of the new technique positron emission tomography – computed tomography (PET/CT) combined with a glucose tracer that it became clear that adults do have functional active BAT, but several major depots appeared to exist at other locations than thought before (Nedergaard et al., 2007).

Another important reason for the current interest in BAT physiology comes from series of interesting discoveries in the last 4 years in animal and cell biology studies. Much more is now known about the molecular mechanisms of thermogenesis, the regulation of brown fat development, its cellular lineage specification, and molecular control (for an overview see among others (Seale, 2010).

Therefore, one would expect that after the publications on BAT in 2009, it would take about 1.5 year before an explosion of publications on BAT studies in human adults would appear. However, the relative silence may be explained by the fact that setting up the mix of expertise and the right multidisciplinary laboratory facilities takes more time. Congress abstracts and proceedings indicate that at least several experimental studies are underway to be published. With respect to the reviews published, two of the most elaborate ones are from Fruhbeck et al. (2009) and Tseng et al. (2010).

In the mean time, to what extend can the expectations that were raised to combat obesity be met? In fact, the main composite question is: does human facultative and adaptive thermogenesis (DIT and cold-induced thermogenesis, CIT) have a significant contribution to our energy expenditure, is BAT involved and can these phenomena be used to fight obesity? The recent gathered knowledge with the limited numbers of studies available on the factors that influence prevalence of BAT is reviewed in Nedergaard et al. (2011). The current perspective will therefore be restricted to the (potential) contribution of BAT in increasing energy expenditure to act against obesity and to methodological aspects that need special attention in order to perform sound metabolic studies to enhance our knowledge on the functionality of human BAT.

NON-SHIVERING THERMOGENESIS AND BAT

NON-SHIVERING THERMOGENESIS

Non-shivering thermogenesis is most extensively studied in rodents. The documented level of NST in rodents amounts to more than 150% percent of basal metabolic rate (BMR; Davis et al., 1960). In adult humans the NST under mild cold exposure shows

a large individual variation and varies from 0 to 30% (Dauncey, 1981; Warwick and Busby, 1990; van Ooijen et al., 2001; Claessens-van Ooijen et al., 2006; Celi et al., 2010; Wijers et al., 2010). For a more detailed overview of these studies see van Marken Lichtenbelt and Schrauwen (2011). The maximal level of NST of humans is not known. Rodents, who are fully dependent on BAT for NST, clearly demonstrate that the capacity for NST and BAT activity depends on the level of acclimatization to cold (Golozoubova et al., 2001; Meyer et al., 2011). Heat production in BAT is by mitochondrial uncoupling and is executed by uncoupling protein-1 (UCP1). This is a BAT specific inner-membrane mitochondrial protein. Under "normal" circumstances F (0)F(1)-ATPase uses most of the energy stored in the proton gradient to produce ATP, which is the energy intermediate in the organism. UCP1 causes a reflux of protons into the mitochondrial matrix, bypassing the ATPase. Instead of ATP, heat is dissipated by the uncoupling itself but also by the other parts of the phosphorylation process (Nicholls and Locke, 1984; Klingenberg and Huang, 1999; Cannon and Nedergaard, 2004). In other words the overall cell efficiency to produce ATP drops by mitochondrial uncoupling. UCP1 is a member of a mitochondrial carrier protein family (like UCP2, UCP3). UCP1, however, is the only protein from this family that is shown to mediate NST (Nedergaard et al., 2001) in BAT, at least in rodents.

In humans the classical studies of Davis clearly show that (parallel to rodent studies) during regular cold exposure shivering decreases, while the level of CIT increases (Davis et al., 1960). This indicates that indeed NST increased in adult humans. The anatomical/histological studies by Huttunen et al. (1981) demonstrate high amounts of BAT in Finnish outdoor workers (Huttunen et al., 1981). Combining the results of these studies in humans at least suggest that with increasing NST capacity the amount of BAT increases. Contrary to rodents there are studies that indicate that in humans skeletal muscle NST by cold-induced mitochondrial uncoupling is involved (Wijers et al., 2008, 2011). It is interesting to note that with cold acclimatization, not only the capacity for NST increases, but in parallel the BMR also rises.

In conclusion, human NST can amount up to 30% of BMR, but it is possible that by means of cold acclimatization higher levels are possible. There is a large individual variation, which can in part be explained by different levels of cold acclimatization. These levels of cold acclimatization may in turn be linked to body composition (see below).

BAT OR BRITE

Adult human brown fat was anatomically described (Cramer, 1920; Heaton, 1972; Tanuma et al., 1975, 1976) many years ago, and significant amounts of BAT have for long been demonstrated in adults living in cold environments from samples obtained by necropsy (Huttunen et al., 1981). More recently, unrelated pursuits within nuclear medicine, using FDG-PET/CT scans, indicated that BAT is present and active in adult patients (Hany et al., 2002; Cohade et al., 2003; Yeung et al., 2003; Nedergaard et al., 2007), followed by experimental studies on cold activated BAT (Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). With a PET scan performed 45–60 min after intravenous injection of ^{18}F-2-fluoro-2-deoxyglucose (FDG), glucose uptake can be visualized. FDG is taken up via members of the sodium-independent glucose transporter family (such as GLUT1, GLUT3, and GLUT4). Therefore it provides a measure of the volume of brown fat and a measure of glucose uptake in BAT, especially so with the dynamic PET/CT scans. From these scans, however, it cannot be determined how pure (brown adipocyte density relative to white adipocytes) the BAT tissue is. Alternatively BAT perfusion has recently been measured using PET/CT in humans using labeled water ($[^{15}O]H_2O$; Orava et al., 2011).

Animal and cell line studies have revealed much information on the embryonic development of BAT and skeletal muscle (Seale et al., 2008). Brown adipocytes from distinct depots and skeletal muscle cells share common precursors. Subscapular and perirenal BAT in rodents consists of almost 100% brown adipocytes. In contrast, brown-like adipocytes emerging in white adipose tissue ("BRITE-brown-in-white adipocytes") originate from other precursors, that are shared with white adipocytes (Petrovic et al., 2010). This tissue thus consists of a mixture of brown and white adipocytes. It is also possible that BRITE cells come from transdifferentiation of white adipocytes (Cinti, 2009). A very recent cell culture study provided the first evidence of inducible brown adipogenesis in humans (Lee et al., 2011b).

To address the specific origin of human brown fat cells definite anatomical, histological, and molecular measurements are needed. Some information is already available. To some extend there is a shift in the BAT depots from newborns to adults (**Figure 1**). Metabolic studies related to brown fat or NST in newborns are very scarce (but see for instance; Karlberg et al., 1965). However, older histological studies show that there is a large interscapular depot in newborns, resembling that of rodents, but probably more mixed with white adipocytes (Merklin, 1974). The most pure BAT depots in newborns are located posterior cervical, perirenal, and suprailiac. Most of the interscapular and suprailiac depots disappear during growth. Also the perirenal BAT is much more abundant in newborns than in adults. In older children at least from the age of 9 year and in adult humans the most important sites are supraclavicular, axillary, and paravertebral (Hong et al., 2011; van Marken Lichtenbelt and Schrauwen, 2011). Although these sites are also reported in newborns it is possible that in adults much of these tissues consist of later recruited brown(like) adipocytes. It therefore is possible that these sites in fact are BRITE cell depots. Indeed, all histological observations of BAT in humans show that brown adipocytes are mixed with white adipocytes (Heaton, 1972; Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Zingaretti et al., 2009). Even in the major supraclavicular depots the tissue consists of a mixture of brown and white cells (Virtanen et al., 2009). The cell composition of human BAT and whether the distinction between BAT and BRITE has functional consequences remains to be investigated. Nevertheless, it is likely that the density of the brown fat (like) adipocytes determines the mass-specific metabolic capacity of the tissue.

BAT AND NST

Rodents fully depend on BAT with respect to NST (Cannon and Nedergaard, 2004). The quantitative contribution of BAT to NST in humans still needs to be elucidated. Up to this day, dedicated studies on human cold activated BAT combined with measurements on energy expenditure are restricted to five studies (Saito

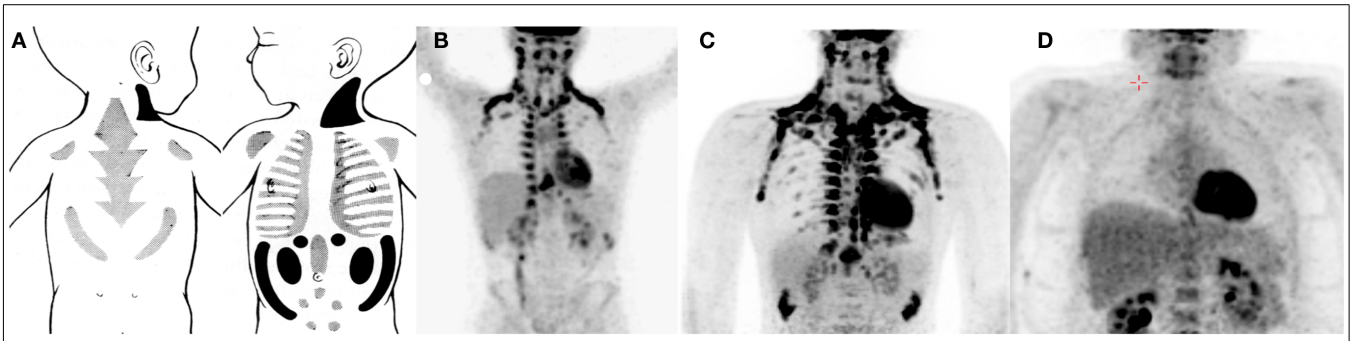

FIGURE 1 | Examples of brown adipose tissue in the newborn [(A) Merklin, 1974], 13-year-old boy [(B) Hong et al., 2011], adult male [(C) van Marken Lichtenbelt et al., 2009], and a morbid obese male [(D) Vijgen et al., 2011]. Note that the drawings of BAT in newborns are based on anatomical studies and that the scan 13-year-old boy was carried out without a specific cooling protocol.

et al., 2009; van Marken Lichtenbelt et al., 2009; Orava et al., 2011; Vijgen et al., 2011; Yoneshiro et al., 2011). Two of these studies show that subjects with BAT have higher CIT (Vijgen et al., 2011; Yoneshiro et al., 2011). Only one study shows that there is a correlation between BAT activity (i.e., FDG uptake) and NST (Yoneshiro et al., 2011). One study shows an association between BAT perfusion (by [^{15}O]H_2O PET/CT) and energy expenditure in the cold (Orava et al., 2011). However in this study no distinction has been made between CIT and RMR. Another study shows that there is a relation between BAT activity and RMR (van Marken Lichtenbelt et al., 2009). It is disputed whether the RMR measurements (at 22˚C) in this study were really under thermoneutral conditions, because 28˚C is reported to be thermoneutral (Nedergaard et al., 2011). However, the thermoneutral conditions were established in naked or very sparsely clothed humans (Erikson et al., 1956; Dauncey, 1981), while during the BAT tests the subjects wore more clothes (0.49 clo). Therefore, indeed RMR was measured. Higher levels of BAT could indicate higher levels of cold acclimatization, which in turn are related to elevated levels of RMR. It should be noted, however, that the issue of measurements conditions during thermoneutral conditions and during mild cold does deserve special attention (see below).

In summary, the available studies at best provide a relation between BAT FDG-activity and NST, but from these data the contribution of BAT cannot be quantified. Prudent calculations based on maximal BAT oxygen consumption in rodents combined with allometric considerations, and based on PET/CT scans reveal a contribution of activated BAT of 3–5% of BMR (Virtanen et al., 2009; van Marken Lichtenbelt and Schrauwen, 2011). These numbers are based on 50 g of BAT (based on FDG-PET/CT). From a physiological and practical point of view a 5% contribution is significant. Moreover, the individual variation is high, meaning that higher contributions are likely. Based on PET/CT the amount of BAT ranges from 0 to 130 g (van Marken Lichtenbelt et al., 2009; Orava et al., 2011). For the calculations several assumptions were made that must be verified. For actual quantification of human BAT energy expenditure more information is needed about the actual amount of BAT, the brown fat cell density of the tissue, and the mass-specific (or cell specific) BAT energy expenditure. Moreover it is possible that BRITE cells from less distinct depots have an additional contribution to NST. They have not yet been mapped in humans, because their FDG uptake is below the detection limit of PET/CT. However these cells may be abundant in many white adipose tissue, and therefore can have a significant contribution.

Another important aspect not yet studied is to what extent BAT needs to be activated to have a significant effect on energy expenditure. May be maximal activation of BAT needs uncomfortable low temperatures. It is likely that temperatures just below the thermoneutral zone do not activate BAT maximally, but are acceptable from a comfort point of view. Studies are needed to find out to what extend sub maximally activated BAT increases thermogenesis. It is likely that those conditions will be more readily accepted in our daily living environment such as offices and dwellings.

DIET INDUCED THERMOGENESIS AN BAT

DIET INDUCED THERMOGENESIS

Diet induced thermogenesis can be subdivided in an obligatory part and a facultative part. Obligatory DIT encompasses the heat generated by digestion, absorption, and processing of the food. The facultative DIT is the regulated heat production used to dissipate food energy. This part is affected by for instance glucose tolerance, insulin sensitivity, body fat (distribution), and the sympathetic nervous system (SNS) activity (Valensi et al., 1998; Camastra et al., 1999). The SNS activity in its turn is elevated due to feeding (Young and Landsberg, 1977). Especially carbohydrate intake (Schwartz et al., 1999) and a very low protein diets increase the facultative thermogenesis (Stock, 1999). On the other hand the activity of the SNS is reduced in response to starvation (Young and Landsberg, 1977). The SNS-response to feeding significantly contributes to DIT (Ravussin and Swinburn, 1992).

The SNS is concerned in both the facultative part of DIT and NST. Where NST is a means of protecting the body against cold, the facultative part of DIT is a mechanism for enriching nutrient poor diets by disposing of the excess non-essential energy (Stock, 1999). Thus, facultative DIT increases as the diet becomes unbalanced (e.g., low protein content). On the other hand the increase in DIT after a high protein diet results from the high metabolic costs of metabolizing protein and is a form of obligatory thermogenesis not involving the SNS. One of the first studies on facultative DIT was on pigs showing that animals with a rather extreme low

protein diet had a much higher DIT than those with a high protein weaning diet (Miller and Payne, 1962). Several studies followed that indicted that the SNS was involved. Stirling and Stock (1968) showed that both CIT and DIT involve SNS activation of heat production.

In humans such experiments are very rare. One study compared the variations in thermogenesis in subjects exposed to both mild cold (3 days) and overfeeding (3 days, 160% of normal energy intake; Wijers et al., 2007). The changes in energy expenditure during mild cold exposure and overfeeding were significantly correlated. These results thus reveal that both overfeeding-induced and mild CIT are individual specific and may share common regulating mechanisms, confirming results from animal studies (Feldmann et al., 2009). Indeed, there was a significant correlation between fasting norepinephrine plasma concentrations and energy expenditure in both situations. Although fasting catecholamine levels may not be the best indicator, this result is in line with SNS involvement.

DIT AND BAT

It was not until the publication of Rothwel and Stock (1979) that the link between DIT and BAT was established in rodents, although this link has recently been disputed (Kozak, 2010).

Are there any indications for a relation between the food intake or diet composition and BAT activity in humans? Currently there are no dedicated studies available. From studies from Nuclear Medicine carried out to reduce BAT FDG uptake for cancer research, it is evident that the diet composition may affect FDG uptake in BAT. A high fat low carbohydrate diet reduced BAT uptake compared to a fasted condition (Williams and Kolodny, 2008). This was explained by the fact that fatty acids are used as main fuel and secondly by the Randle effect (fatty acids loading inhibits glucose metabolism). This study however, does not provide evidence for nutritional *activation* of BAT in humans. It does show that nutritional manipulation can affect BAT activity, in this case reduced glucose (or FDG) uptake. However, there are no elaborate studies that show that food intake can increase BAT activity.

Indirectly, there is some evidence of involvement of BAT in DIT in humans. That is, insulin, which normally is increased after eating, increases human BAT metabolism (Orava et al., 2011). Glucose uptake was measured with FDG-PET/CT, while plasma insulin concentration was elevated by using euglycemic hyperinsulinemic clamp technique (1 mU/kg/min). It turned out that Insulin-stimulated glucose uptake rate in BAT was higher than in white adipose tissue, similar to skeletal muscle, but less than cold stimulated glucose uptake in BAT. This indicates that food intake, which elevates insulin levels, may activate BAT as well. Interestingly, while during cold exposure BAT both glucose uptake and blood perfusion are elevated, insulin induced glucose uptake was not accompanied with an increase in blood perfusion. Probably, the glucose is transported into the brown adipocyte without an increase in thermogenesis. If certain diets increase both glucose uptake and/or perfusion in BAT awaits investigation.

In conclusion, there is no dietary intervention study in humans available that shows a relation between food intake, dietary composition, or overfeeding and BAT activity. Such studies simply still need to be performed.

BAT AND OBESITY

Obese subjects tend to show a blunted NST under the same mild cold situation compared to lean subjects (Claessens-van Ooijen et al., 2006; Wijers et al., 2010). The reason for this can be twofold: NST is impaired in obese subjects or obese people just need a cooler environment to trigger NST. The latter can be attributed to the insulation properties of subcutaneous fat (Bar-or et al., 1969; Claessens-van Ooijen et al., 2006; Savastano et al., 2009), surface-to-volume ratio (Kurz et al., 1995), or skin vasoconstrictive reaction to cold (Valensi et al., 2000). These properties can reduce body heat loss from the skin. This in turn affects the trigger for NST. Indeed, it has been shown that the individual variation in 24 h NST was negatively related to changes in distal skin temperatures (van Marken Lichtenbelt et al., 2002). On the other hand, a blunted NST in the obese can also be caused by a different cold-induced autonomic responsiveness (Matsumoto et al., 1999, 2001) or to a reduced TSH and thyroid hormone response (Lean et al., 1988). From these observed differences in the mechanisms involved in heat loss and heat production, it cannot be deduced whether reduced hormonal or neural responses are a consequence of obesity or a causal factor for the risk of weight gain. Finally, there are indications of a genetic component involved in thermogenesis and BAT that come from UCP1 and beta-adrenergic receptor polymorphism studies (see van Marken Lichtenbelt and Schrauwen, 2011).

Several retrospective studies show that BAT prevalence and the amount of active BAT is lower in obese subjects (Cypess et al., 2009; Pfannenberg et al., 2010; Wang et al., 2011). However, retrospective studies generally show a lower prevalence, because these patient studies were not designed for activating BAT (Nedergaard et al., 2011; van Marken Lichtenbelt and Schrauwen, 2011). Using a standardized cold exposure protocol we showed that BAT activity was significantly negatively related to body mass index (BMI) and to percent body fat (van Marken Lichtenbelt et al., 2009). In a follow-up study on morbid obese subjects we extended the range of BMI and body fat percentage (Vijgen et al., 2011). In this study we used an individualized protocol and cooled the subjects to maximal non-shivering conditions. The combined results clearly confirm the negative relationship between active BAT and BMI or body fat (**Figure 2**). The subjects were in the age range of 18–32 years (lean to obese) and 25–51 years (morbid obese subjects). In both studies most subjects were young adults. This means that the relation with body fat is independent of age. Based on the combined data of two other studies (Saito et al., 2009; Zingaretti et al., 2009) it was postulated that the interrelationship between age and obesity may obscure the relationship between BAT and obesity (Nedergaard et al., 2011). Our results however, clearly show the relation between BAT and obesity is independent of age. This does not mean that the interrelationship does not exist. A reduced active BAT in elderly can at least partly be explained by increased adiposity with age. In other words the relation between BAT and age may be obscured by differences in body composition. Future studies should try to measure BAT in elderly with varying body compositions.

BODY COMPOSITION MEASUREMENT

Most human BAT studies use body fat (by dual X-ray absorptiometry, DXA) or BMI as an indication of obesity. Because BAT

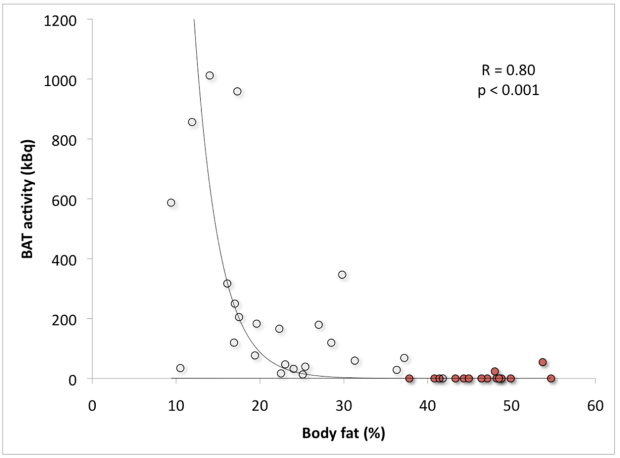

FIGURE 2 | Brown adipose tissue activity in relation to body fat percentage. The open dots indicate the study group ranging from lean to morbidly obese obese (age range: 18–32 years; van Marken Lichtenbelt et al., 2009) and the red dots indicate a second group of morbidly obese subjects (age range: 25–51 years; Vijgen et al., 2011).

activation by cold depends on the insulation properties of the body, preferably more information about body fat distribution then DXA gives should be obtained. Especially the amount of subcutaneous body fat and skeletal muscle in the limbs is needed. DXA provides to some extend a possibility to map body fat, but with scanning techniques such as magneto resonance imaging (MRI) or CT more details on subcutaneous body fat can be determined. DXA is the most widely used technique for determining whole body fat percentage, but it is not very accurate on an individual level. It is used because of convenience, not because of accuracy. In fact DXA has a substantial error up to 6.7% compared to the gold standard (four-compartment model; Wong et al., 2002; van Marken Lichtenbelt et al., 2004; Minderico et al., 2008). This means that the DXA results are comparable to methods such as skinfold thickness and bioimpedance analyses. A serious problem is that the DXA companies do not provide details about their assumptions and calculations and comparisons of different instruments indicate a large inter-instrument variability. More attention should be given to the errors involved and that a significant part of the variation in metabolic studies potentially can be ascribed to the variation in body composition (DXA) results. Most accurate method is the three-compartment model combining underwater weighing with deuterium dilution (or the four-compartment model including a DXA bone scan; useful in subjects with low bone mineral densities). Unfortunately these techniques do not provide information about the body fat distribution. Alternatively imaging techniques such as CT and MRI are very useful because they do provide details on body fat distribution and subcutaneous fat. CT has better resolution than DXA but provides greater ionizing radiation exposure. MRI provides detailed information but needs a longer imaging time and specialized equipment for acquisition and analysis (Silver et al., 2010).

METABOLIC STUDIES AND ENVIRONMENTAL TEMPERATURE

Metabolic studies on BAT activity using (mild) cold exposure need to be carried out correctly, preferably in a standardized fashion. Both the cold and the thermal neutral conditions have to be selected carefully. Wrong environmental temperatures can result in wrong conclusion as has been shown recently with rodents that are in general raised and housed at temperatures below their thermoneutral zone. These conditions resulted in the wrong conclusions that UCP1 knock out mice fail to become obese (Feldmann et al., 2009). The mice shivered in order to keep warm. Feldmann et al. (2009) elegantly demonstrated that when these mice were kept at thermoneutrality, UCP1 ablation in itself did induce obesity.

Cold exposure experiments in humans should be carried out with the temperature conditions adjusted to the individual. This holds for both the cold situation (e.g., maximal non-shivering conditions without shivering; Vijgen et al., 2011) and for the thermoneutral condition (Kingma et al., 2011). Both conditions are important because the level of NST is calculated by the difference in energy expenditure of each condition. The importance of individual attuned environmental conditions in human metabolic studies has very often been neglected.

Many factors influence the thermoneutral zone, such as body composition (degree of obesity), fat distribution, clothing, energy expenditure, age, and gender (Kingma et al., 2011). These factors have the potential to introduce bias in study results and therefore need to be taken into consideration in studies on energy metabolism, obesity, medical conditions, thermal comfort, or vigilance. It is needed to go back to the basics of human thermoregulation. For instance, when baseline conditions are carried out at too low temperatures some NST may already occur. The NST calculated after cold exposure would then result in too low values. For more examples and the determination of the thermoneutral zone see Kingma et al. (2011).

The same applies for the experiments under mild cold conditions. The goal is to establish maximal BAT activity and NST under non-shivering conditions. During shivering much of the FDG label will be taken up by the skeletal muscle and the relation between CIT and BAT activity will be obscured by shivering thermogenesis due to competition in FDG uptake in the tissues. The few dedicated human BAT studies use a standard, more, or less fixed protocol under mild cold conditions (around 16°C) sometimes with additional ice cooling of the feet. It is disputed whether ice (or cold pressor test) is an effective way of stimulating BAT, because it works systemically (Nedergaard et al., 2011). BAT, however, is normally selectively stimulated via sympathetic nerve fibers that are activated by the coldsensitive centers in the brain.

In our recent studies we cool subjects until the start of shivering, which is monitored by EMG and questionnaires (Vijgen et al., 2011). Then we raise the temperature slightly, so that the shivering just stops. In this way we want to achieve maximal NST. It is assumed that the NST level will not increase when shivering starts, although this is hard to prove. Our current trials also indicate that the rate of cooling may affect the level of NST with a lower level of NST during fast cooling due to an earlier onset of shivering. Finally, we also obtained indications that for activating

BAT it matters which parts of the body skin are exposed to the cooling medium.

In conclusion, several studies indicate differences in BAT activity and thermogenesis between individuals and between groups (lean and obese), but for more subtle and physiological relevant differences more care should be taken in the study design and the attuning of environmental conditions.

OTHER TISSUES INVOLVED – SKELETAL MUSCLE

Theoretically other tissues and other processes than mitochondrial uncoupling (e.g., Ca-cycling, transmembrane ion leak) may be responsible for the increase in energy expenditure upon cold exposure. Skeletal muscle is potentially a significant contributor to NST as indicated in older studies using adrenalin, ephedrine, or carbohydrate induced thermogenesis (Astrup et al., 1985, 1986; Simonsen et al., 1992). An estimated contribution amounts to 35–67% of the CIT or DIT (Simonsen et al., 1992; van Baak, 2008). More recently, it is shown that human NST in response to cold exposure is accompanied by and significantly related to mitochondrial uncoupling in skeletal muscle (Wijers et al., 2008, 2011). The last study also indicates that both BAT and skeletal muscle play a role in NST with their relative contribution depending on which beta receptors are involved (beta 2 for skeletal muscle; beta 3 for BAT). More details on the involvement of skeletal muscle are given in van Marken Lichtenbelt and Schrauwen (2011).

The actual *in vivo* increased energy expenditure of skeletal muscle and of BAT during cold have not been studied. Moreover for a comparison about the contribution of the different tissues one relies on various techniques of which the results are not quantitatively comparable. For skeletal muscle biopsies and cell respiration measurements are used, while for BAT volume and activity the glucose tracer FDG provides the information. For a sound comparison between the contributions of each tissue, comparable techniques are needed. New techniques such as MRI, functional MRI (fMRI), or magneto resonance spectrometry (MRS) may in future be applied in both skeletal muscle and BAT (Barker and Armstrong, 2010; Hu et al., 2010). Alternatively, the implementation and development of (dynamic) PET techniques combining FDG and fatty acid tracers, tracers measuring blood perfusion (Orava et al., 2011), sympathetic innervation, thermogenesis, or oxygen consumption also are promising in this respect (Baba et al., 2007; Madar et al., 2011).

BAT RECRUITMENT

Finally, in relation to obesity it is important to know whether brown fat (both BAT and BRITE) can be recruited and then used to increase energy expenditure. In the case of obesity, the question is whether obesity develops because obese people are prone to have less BAT or whether during the development of obesity BAT gradually disappears. The latter can be explained by the larger insulation of the subcutaneous fat layer and the smaller surface-to-volume ratio. Therefore obese people are less often in a situation where NST is needed compared to their lean counterparts.

In this respect it is important to study whether BAT can be recruited by cold acclimatization or dietary intervention. No intervention studies are available yet, except for the earlier mentioned study by Davis (1961) on cold acclimatization. Recent data on morbidly obese subjects show that indeed BAT can be recruited by weight loss (Vijgen et al., in review), indicating that low levels or absence of BAT is a result of obesity.

CONCLUSION

Adult humans do show NST up to 30% of BMR and BAT seems to contribute significantly. Contrary to rodent studies skeletal muscle thermogenesis may also be involved. There is a clear (negative) relation between BAT and the degree of obesity and there is an indication that weight loss can recruit BAT. However, more basic studies are needed on cold acclimatization and dietary interventions in relation to BAT activity.

More knowledge is needed about the relative presence in BAT and BRITE in humans and the actual BAT metabolic activity in comparison with other tissues. There exist several new and promising techniques that deserve more attention, such as dynamic PET/CT, the use of new PET tracers, and techniques such as fMRI and MRS should be explored. Cold exposure studies should be carried out in a more standardized fashion, taken into account the individual variation in thermoneutral zone and in ambient temperature to induce NST. For the metabolic studies on BAT related to obesity body composition methods should include fat distribution, especially the measurement of subcutaneous fat.

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REFERENCES

Astrup, A., Bulow, J., Christensen, N. J., Madsen, J., and Quaade, F. (1986). Facultative thermogenesis induced by carbohydrate: a skeletal muscle component mediated by epinephrine. *Am. J. Physiol.* 250, E226–E229.

Astrup, A., Bulow, J., Madsen, J., and Christensen, N. J. (1985). Contribution of BAT and skeletal muscle to thermogenesis induced by ephedrine in man. *Am. J. Physiol.* 248, E507–E515.

Au-Yong, I. T., Thorn, N., Ganatra, R., Perkins, A. C., and Symonds, M. E. (2009). Brown adipose tissue and seasonal variation in humans. *Diabetes* 58, 2583–2587.

Baba, S., Engles, J. M., Huso, D. L., Ishimori, T., and Wahl, R. L. (2007). Comparison of uptake of multiple clinical radiotracers into brown adipose tissue under cold-stimulated and nonstimulated conditions. *J. Nucl. Med.* 48, 1715–1723.

Barker, A. R., and Armstrong, N. (2010). Insights into developmental muscle metabolism through the use of 31P-magnetic resonance spectroscopy: a review. *Pediatr. Exerc. Sci.* 22, 350–368.

Bar-or, O., Lundegren, H. M., and Buskirk, E. R. (1969). Heat tolerance of exercising obese and lean women. *J. Appl. Physiol.* 26, 403–409.

Camastra, S., Bonora, E., Del, S. Prato, Rett, K., Weck, M., and Ferrannini, E. (1999). Effect of obesity and insulin resistance on resting and glucose-induced thermogenesis in man. EGIR (European Group for the Study of Insulin Resistance). *Int. J. Obes. Relat. Metab. Disord.* 23, 1307–1313.

Cannon, B., and Nedergaard, J. (2004). Brown adipose tissue: function and physiological significance. *Physiol. Rev.* 84, 277–359.

Celi, F., Butler, P. W., Brychta, R. J., Linderman, J. D., Alberobello, A. T., Smith, S., Courville, A. B., Lai, E. W., Costello, R., Skarulis, M., Csako, G., Remaley, A., Pacak, K., and Chen, K. Y. (2010). Minimal changes in environmental temperature result in a significant increase in energy expenditure and changes in the hormonal homeostasis in healthy adults. *Eur. J. Endocrinol.* 163, 863–872.

Cinti, S. (2009). Transdifferentiation properties of adipocytes in the adipose organ. *Am. J. Physiol. Endocrinol. Metab.* 297, E977–E986.

Claessens-van Ooijen, A. M., Westerterp, K. R., Wouters, L., Schoffelen, P. F., van Steenhoven, A. A., and van Marken Lichtenbelt, W. D. (2006). Heat production and body temperature during cooling and rewarming in overweight and lean men. *Obesity (Silver Spring)* 14, 1914–1920.

Cohade, C., Osman, M., Pannu, H. K., and Wahl, R. L. (2003). Uptake in supraclavicular area fat ("USA-Fat"): description on 18F-FDG PET/CT. *J. Nucl. Med.* 44, 170–176.

Cramer, W. (1920). On glandular adipose tissue, and its relation to the endocrine organs and the vitamin problem. *Br. J. Exp. Pathol.* 1, 184–196.

Cypess, A. M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A. B., Kuo, F. C., Palmer, E. L., Tseng, Y. H., Doria, A., Kolodny, G. M., and Kahn, C. R. (2009). Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* 360, 1509–1517.

Dauncey, M. J. (1981). Influence of mild cold on 24 h energy expenditure, resting metabolism and diet-induced thermogenesis. *Br. J. Nutr.* 45, 257–267.

Davis, T. R., Johnston, D. R., Bell, F. C., and Cremer, B. J. (1960). Regulation of shivering and non-shivering heat production during acclimation of rats. *Am. J. Physiol.* 198, 471–475.

Davis, T. R. A. (1961). Chamber cold acclimatization in man. *J. Appl. Physiol.* 16, 1011–1015.

Erikson, H., Krog, J., Andersen, K. L., and Scholander, P. F. (1956). The critical temperature in naked man. *Acta Physiol. Scand.* 37, 35–39.

Feldmann, H. M., Golozoubova, V., Cannon, B., and Nedergaard, J. (2009). UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab.* 9, 203–209.

Fruhbeck, G., Becerril, S., Sainz, N., Garrastachu, P., and Garcia-Velloso, M. J. (2009). BAT: a new target for human obesity? *Trends Pharmacol. Sci.* 30, 387–396.

Gilsanz, V., Chung, S. A., Jackson, H., Dorey, F. J., and Hu, H. H. (2011). Functional brown adipose tissue is related to muscle volume in children and adolescents. *J. Pediatr.* 158, 722–726.

Golozoubova, V., Hohtola, E., Matthias, A., Jacobsson, A., Cannon, B., and Nedergaard, J. (2001). Only UCP1 can mediate adaptive nonshivering thermogenesis in the cold. *FASEB J.* 15, 2048–2050.

Hany, T. F., Gharehpapagh, E., Kamel, E. M., Buck, A., Himms-Hagen, J., and von Schulthess, G. K. (2002). Brown adipose tissue: a factor to consider in symmetrical tracer uptake in the neck and upper chest region. *Eur. J. Nucl. Med. Mol. Imaging* 29, 1393–1398.

Heaton, J. M. (1972). The distribution of brown adipose tissue in the human. *J. Anat.* 112, 35–39.

Hong, T. S., Shammas, A., Charron, M., Zukotynski, K. A., Drubach, L. A., and Lim, R. (2011). Brown adipose tissue 18F-FDG uptake in pediatric PET/CT imaging. *Pediatr. Radiol.* 41, 759–768.

Hu, H. H., Smith, D. L. Jr., Nayak, K. S., Goran, M. I., and Nagy, T. R. (2010). Identification of brown adipose tissue in mice with fat-water IDEAL-MRI. *J. Magn. Reson. Imaging* 31, 1195–1202.

Huttunen, P., Hirvonen, J., and Kinnula, V. (1981). The occurrence of brown adipose tissue in outdoor workers. *Eur. J. Appl. Physiol. Occup. Physiol.* 46, 339–345.

Karlberg, P., Moore, R. E., and Oliver, T. K. Jr. (1965). Thermogenic and cardiovascular responses of the newborn baby to noradrenaline. *Acta Paediatr. Scand.* 54, 225–238.

Kingma, B. R. M., Frijns, A. J. H., and van Marken Lichtenbelt, W. D. (2011). The thermoneutral zone: implications for metabolic studies. *Front. Biosci. (Schol. Ed.)* [Epub ahead of print].

Klingenberg, M., and Huang, S. G. (1999). Structure and function of the uncoupling protein from brown adipose tissue. *Biochim. Biophys. Acta* 1415, 271–296.

Kozak, L. P. (2010). Brown fat and the myth of diet-induced thermogenesis. *Cell Metab.* 11, 263–267.

Kurz, A., Sessler, D. I., Christensen, R., and Dechert, M. (1995). Heat balance and distribution during the core-temperature plateau in anesthetized humans. *Anesthesiology* 83, 491–499.

Lean, M. E., Murgatroyd, P. R., Rothnie, I., Reid, I. W., and Harvey, R. (1988). Metabolic and thyroidal responses to mild cold are abnormal in obese diabetic women. *Clin. Endocrinol. (Oxf.)* 28, 665–673.

Lebron, L., Chou, A. J., and Carrasquillo, J. A. (2010). Interesting image. Unilateral F-18 FDG uptake in the neck, in patients with sympathetic denervation. *Clin. Nucl. Med.* 35, 899–901.

Lee, P., Zhao, J. T., Swarbrick, M. M., Gracie, G., Bova, R., Greenfield, J. R., Freund, J., and Ho, K. K. (2011a). High prevalence of brown adipose tissue in adult humans. *J. Clin. Endocrinol. Metab.* 96, 2450–2455.

Lee, P., Swarbrick, M. M., Ting, J. Zhao, and Ho, K. K. (2011b). Inducible brown adipogenesis of supraclavicular fat in adult humans. *Endocrinology* 152, 3597–3602.

Madar, I., Isoda, T., Finley, P., Angle, J., and Wahl, R. (2011). 18F-fluorobenzyl triphenyl phosphonium: a noninvasive sensor of brown adipose tissue thermogenesis. *J. Nucl. Med.* 52, 808–814.

Matsumoto, T., Miyawaki, C., Ue, H., Kanda, T., Yoshitake, Y., and Moritani, T. (2001). Comparison of thermogenic sympathetic response to food intake between obese and non-obese young women. *Obes. Res.* 9, 78–85.

Matsumoto, T., Miyawaki, T., Ue, H., Kanda, T., Zenji, C., and Moritani, T. (1999). Autonomic responsiveness to acute cold exposure in obese and non-obese young women. *Int. J. Obes. Relat. Metab. Disord.* 23, 793–800.

Merklin, R. J. (1974). Growth and distribution of human fetal brown fat. *Anat. Rec.* 178, 637–645.

Meyer, C. W., Willershauser, M., Jastroch, M., Rourke, B. C., Fromme, T., Oelkrug, R., Heldmaier, G., and Klingenspor, M. (2011). Adaptive thermogenesis and thermal conductance in wild-type and UCP1-KO mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 299, R1396–R1406.

Miller, D. S., and Payne, P. R. (1962). Weight-maintenance and food intake. *J. Nutr.* 78, 225–262.

Minderico, C. S., Silva, A. M., Keller, K., Branco, T. L., Martins, S. S., Palmeira, A. L., Barata, J. T., Carnero, E. A., Rocha, P. M., Teixeira, P. J., and Sardinha, L. B. (2008). Usefulness of different techniques for measuring body composition changes during weight loss in overweight and obese women. *Br. J. Nutr.* 99, 432–441.

Nedergaard, J., Bengtsson, T., and Cannon, B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. *Am. J. Physiol. Endocrinol. Metab.* 293, E444–E452.

Nedergaard, J., Bengtsson, T., and Cannon, B. (2011). Three years with adult human brown adipose tissue. *Ann. N. Y. Acad. Sci.* 1212, E20–E36.

Nedergaard, J., Golozoubova, V., Matthias, A., Asadi, A., Jacobsson, A., and Cannon, B. (2001). UCP1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic inefficiency. *Biochim. Biophys. Acta* 1504, 82–106.

Nicholls, D. G., and Locke, R. M. (1984). Thermogenic mechanisms in brown fat. *Physiol. Rev.* 64, 1–64.

Orava, J., Nuutila, P., Lidell, M. E., Oikonen, V., Noponen, T., Viljanen, T., Scheinin, M., Taittonen, M., Niemi, T., Enerback, S., and Virtanen, K. A. (2011). Different metabolic responses of human brown adipose tissue to activation by cold and insulin. *Cell Metab.* 14, 272–279.

Petrovic, N., Walden, T. B., Shabalina, I. G., Timmons, J. A., Cannon, B., and Nedergaard, J. (2010). Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. *J. Biol. Chem.* 285, 7153–7164.

Pfannenberg, C., Werner, M. K., Ripkens, S., Stef, I., Deckert, A., Schmadl, M., Reimold, M., Haring, H. U., Claussen, C. D., and Stefan, N. (2010). Impact of age on the relationships of brown adipose tissue with sex and adiposity in humans. *Diabetes* 59, 1789–1793.

Ravussin, E., and Swinburn, B. A. (1992). Pathophysiology of obesity. *Lancet* 340, 404–408.

Rothwell, N. J., and Stock, M. J. (1979). A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 281, 31–35.

Saito, M., Okamatsu-Ogura, Y., Matsushita, M., Watanabe, K., Yoneshiro, T., Nio-Kobayashi, J., Iwanaga, T., Miyagawa, M., Kameya, T., Nakada, K., Kawai, Y., and Tsujisaki, M. (2009). High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* 58, 1526–1531.

Savastano, D. M., Gorbach, A. M., Eden, H. S., Brady, S. M., Reynolds, J. C., and Yanovski, J. A. (2009). Adiposity and human regional body temperature. *Am. J. Clin. Nutr.* 90, 1124–1131.

Schwartz, M. W., Baskin, D. G., Kaiyala, K. J., and Woods, S. C. (1999). Model for the regulation of energy balance and adiposity by the central nervous system. *Am. J. Clin. Nutr.* 69, 584–596.

Seale, P. (2010). Transcriptional control of brown adipocyte development and thermogenesis. *Int. J. Obes. (Lond.)* 34(Suppl. 1), S17–S22.

Seale, P., Bjork, B., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scime, A., Devarakonda, S., Conroe, H. M., Erdjument-Bromage, H., Tempst, P., Rudnicki, M. A., Beier, D. R., and Spiegelman, B. M. (2008). PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454, 961–967.

Silver, H. J., Welch, E. B., Avison, M. J., and Niswender, K. D. (2010). Imaging body composition in obesity and weight loss: challenges and opportunities. *Diabetes Metab. Syndr. Obes.* 3, 337–347.

Simonsen, L., Bulow, J., Madsen, J., and Christensen, N. J. (1992). Thermogenic response to epinephrine in the forearm and abdominal subcutaneous adipose tissue. *Am. J. Physiol.* 263, E850–E855.

Stirling, J. L., and Stock, M. J. (1968). Metabolic origins of thermogenesis induced by diet. *Nature* 220, 801–802.

Stock, M. J. (1999). Gluttony and thermogenesis revisited. *Int. J. Obes.* 23, 1105–1117.

Tanuma, Y., Ohata, M., Ito, T., and Yokochi, C. (1976). Possible function of human brown adipose tissue as suggested by observation on perirenal brown fats from necropsy cases of variable age groups. *Arch. Histol. Jpn.* 39, 117–145.

Tanuma, Y., Tamamoto, M., Ito, T., and Yokochi, C. (1975). The occurrence of brown adipose tissue in perirenal fat in Japanese. *Arch. Histol. Jpn.* 38, 43–70.

Tseng, Y. H., Cypess, A. M., and Kahn, C. R. (2010). Cellular bioenergetics as a target for obesity therapy. *Nat. Rev. Drug Discov.* 9, 465–482.

Valensi, P., Lormeau, B., Dabbech, M., Miossec, P., Paries, J., Dauchy, F., and Attali, J. R. (1998). Glucose-induced thermogenesis, inhibition of lipid oxidation rate and autonomic dysfunction in non-diabetic obese women. *Int. J. Obes. Relat. Metab. Disord.* 22, 494–499.

Valensi, P., Smagghue, O., Pariès, J., Velayoudon, P., Lormeau, B., and Attali, J. R. (2000). Impairment of skin vasoconstrictive response to sympathetic activation in obese patients: influence of rheological disorders. *Metab. Clin. Exp.* 49, 600–606.

van Baak, M. A. (2008). Meal-induced activation of the sympathetic nervous system and its cardiovascular and thermogenic effects in man. *Physiol. Behav.* 94, 178–186.

van Marken Lichtenbelt, W. D., Hartgens, F., Vollaard, N. B. J., Ebbing, S., and Kuipers, H. (2004). Body composition changes in bodybuilders: a method comparison. *Med. Sci. Sports Exerc.* 36, 490–497.

van Marken Lichtenbelt, W. D., and Schrauwen, P. (2011). Implications of non-shivering thermogenesis for energy balance regulation in humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 301, R285–R296.

van Marken Lichtenbelt, W. D., Schrauwen, P., van De Kerckhove, S., and Westerterp-Plantenga, M. S. (2002). Individual variation in body temperature and energy expenditure in response to mild cold. *Am. J. Physiol.* 282, E1077–E1083.

van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, M. A. F. L., Kemerink, G. J., Bouvy, N. D., Schrauwen, P., and Teule, G. J. J. (2009). Cold-activated brown adipose tissue in healthy adult men. *N. Engl. J. Med.* 360, 1500–1508.

van Ooijen, A. M. J., van Marken Lichtenbelt, W. D., and Westerterp, K. R. (2001). Individual differences in body temperature and the relation to energy expenditure: the influence of mild cold. *J. Therm. Biol.* 26, 455–459.

Vijgen, G. H. E. J., Bouvy, N. D., Teule, G. J. J., Brans, B., Schrauwen, P., and van Marken Lichtenbelt, W. D. (2011). Brown adipose tissue in morbidly obese subjects. *PLoS ONE* 6, e17247. doi:10.1371/journal.pone.0017247

Virtanen, K. A., Lidell, M. E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N. J., Enerback, S., and Nuutila, P. (2009). Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* 360, 1518–1525.

Wang, Q., Zhang, M., Ning, G., Gu, W., Su, T., Xu, M., Li, B., and Wang, W. (2011). Brown adipose tissue in humans is activated by elevated plasma catecholamines levels and is inversely related to central obesity. *PLoS ONE* 6, e21006. doi:10.1371/journal.pone.0021006

Warwick, P. M., and Busby, R. (1990). Influence of mild cold on 24 h energy expenditure in "normally" clothed adults. *Br. J. Nutr.* 63, 481–488.

Wijers, S. L., Saris, W., and van Marken Lichtenbelt, W. D. (2010). Cold induced adaptive thermogenesis in lean and obese. *Obesity (Silver Spring)* 18, 1092–1099.

Wijers, S. L. J., Saris, W. H. M., and van Marken Lichtenbelt, W. D. (2007). Individual thermogenic responses to mild cold and overfeeding are closely related. *J. Clin. Endocrinol. Metab.* 92, 4299–4305.

Wijers, S. L. J., Schrauwen, P., Saris, W. H. M., and van Marken Lichtenbelt, W. D. (2008). Human skeletal muscle mitochondrial uncoupling is associated with cold induced adaptive thermogenesis. *PLoS ONE* 3, e1777. doi:10.1371/journal.pone.0001777

Wijers, S. L. J., Schrauwen, P., van Baak, M. A., Saris, W. H. M., and van Marken Lichtenbelt, W. D. (2011). Beta-adrenergic receptor blockade does not inhibit cold induced thermogenesis in humans: possible involvement of brown adipose tissue. *J. Clin. Endocrinol. Metab.* 96, E598–E605.

Williams, G., and Kolodny, G. M. (2008). Method for decreasing uptake of 18F-FDG by hypermetabolic brown adipose tissue on PET. *AJR Am. J. Roentgenol.* 190, 1406–1409.

Wong, W. W., Hergenroeder, A. C., Stuff, J. E., Butte, J. E., Smith, E. O., and Ellis, K. J. (2002). Evaluating body fat in girls and female adolescents: advantages and disadvantages of dual-energy X-ray absorptiometry. *Am. J. Clin. Nutr.* 76, 384–389.

Yeung, H. W., Grewal, R. K., Gonen, M., Schoder, H., and Larson, S. M. (2003). Patterns of (18)F-FDG uptake in adipose tissue and muscle: a potential source of false-positives for PET. *J. Nucl. Med.* 44, 1789–1796.

Yoneshiro, T., Aita, S., Matsushita, M., Kameya, T., Nakada, K., Kawai, Y., and Saito, M. (2011). Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. *Obesity (Silver Spring)* 19, 13–16.

Young, J. B., and Landsberg, L. (1977). Stimulation of the sympathetic nervous system during sucrose feeding. *Nature* 269, 615–617.

Zingaretti, M. C., Crosta, F., Vitali, A., Guerrieri, M., Frontini, A., Cannon, B., Nedergaard, J., and Cinti, S. (2009). The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J.* 23, 3113–3120.

Zukotynski, K. A., Fahey, F. H., Laffin, S., Davis, R., Treves, S. T., Grant, F. D., and Drubach, L. A. (2010). Seasonal variation in the effect of constant ambient temperature of 24 degrees C in reducing FDG uptake by brown adipose tissue in children. *Eur. J. Nucl. Med. Mol. Imaging* 37, 1854–1860.

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Control and physiological determinants of sympathetically mediated brown adipose tissue thermogenesis

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Brown adipose tissue (BAT) represents a remarkable heat-producing tissue. The thermogenic potential of BAT is conferred by uncoupling protein 1, a protein found uniquely in brown adipocytes. BAT activity and capacity is controlled by the sympathetic nervous system (SNS), which densely innervates brown fat depots. SNS-mediated BAT thermogenesis is essentially governed by hypothalamic and brainstem neurons. BAT activity is also modulated by brain energy balance pathways including the very significant brain melanocortin system, suggesting a genuine involvement of SNS-mediated BAT thermogenesis in energy homeostasis. The use of positron emission tomography/computed tomography scanning has revealed the presence of well-defined BAT depots in the cervical, clavicular, and paraspinal areas in adult humans. The prevalence of these depots is higher in subjects exposed to low temperature and is also higher in women compared to men. Moreover, the prevalence of BAT decreases with age and body fat mass, suggesting that BAT could be involved in energy balance regulation and obesity in humans. This short review summarizes recent progress made in our understanding of the control of SNS-mediated BAT thermogenesis and of the determinants of BAT prevalence or detection in humans.

Keywords: energy balance, energy expenditure, melanocortin system, cold exposure, age, sex, environmental temperature, positron emission tomography

INTRODUCTION

Brown adipose tissue (BAT) is a specialized tissue whose main function is to produce heat. In small mammals, it is located in discrete depots and has largely been investigated for its role in thermogenesis (Cannon and Nedergaard, 2004; Sell et al., 2004a; Richard et al., 2010; Richard and Picard, 2011). The heat-producing capacity of BAT is such that it allows small mammals such as rats and mice to live at temperatures as low as 4°C without shivering.

The activation of BAT thermogenesis is under the strict control of the sympathetic nervous system (SNS). BAT is richly innervated by the postganglionic neurons of the efferent branches of the SNS (Bartness et al., 2010). These neurons release noradrenaline (NA), whose action on β-adrenergic receptors triggers the breakdown of triglycerides into fatty acids, which not only serve as energy source for thermogenesis but also act as the stimulators of BAT thermogenic activity (Nicholls and Locke, 1984). The SNS activity in BAT is governed by the brain autonomic centers including hypothalamus and brainstem, which are regions involved in the cold-induced thermogenesis (Morrison, 2011) and energy balance regulation (Richard, 2007; Richard and Picard, 2011).

We now have evidence that BAT is not only found in small mammals but also in adult humans (Nedergaard et al., 2007; Cannon and Nedergaard, 2010; Enerback, 2010a,b; Richard et al., 2010). A number of studies involving imaging procedures such as positron emission tomography/computed tomography (PET/CT) scanning have revealed symmetrical distribution of metabolically active brown fat depots around cervical, clavicular, and paraspinal regions in humans (Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009; Zingaretti et al., 2009). Recent progress has also been made in our understanding of various factors that affect the prevalence and activity of BAT in humans (Cypess et al., 2009; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Ouellet et al., 2011; Wang et al., 2011; Yoneshiro et al., 2011a).

This short article is aimed at reviewing (i) the brain circuits, essentially those pertaining to the metabolic brain melanocortin system, that control SNS-mediated BAT thermogenesis and (ii) our current understanding of the factors determining the prevalence or detection of BAT depots in humans.

BAT THERMOGENESIS

Brown adipose tissue was first observed in 1551 by the Swiss naturalist Konrad Gessner, who described it as being "neither fat nor flesh (*nec pinguitudo nec caro*)" (Gesner, 1551; Cannon and Nedergaard, 2008). The brownish appearance of BAT indeed contrasts with that of the white adipose tissue (WAT) whose role is to store lipids predominantly. Furthermore, brown adipocytes have a developmental origin that largely differs from that of the white adipocytes (Seale et al., 2008; Tseng et al., 2008). In that respect, brown but not white adipocytes develop from myogenic factor 5 (Myf5)-expressing myoblasts under the action of the transcription factor PRD1-BF1-RIZ1 homologous domain-containing 16 (PRDM16; Cannon and Nedergaard, 2008; Kajimura et al., 2010; Seale, 2010). The extraordinary thermogenic capacity of BAT is conferred by the presence of uncoupling protein 1 (UCP1), a protein uniquely found in the brown adipocytes.

UCP1 is a mitochondrial inner membrane protein that is capable of uncoupling mitochondrial oxidation from ATP synthesis, in a process that triggers heat production through enhanced cellular respiration (Nicholls and Locke, 1984).

Owing to its tremendous thermogenic potential, BAT is viewed as a potential target to increase energy expenditure and combat obesity. Animal data support a role for BAT thermogenesis in energy homeostasis. Chronic reduction in SNS-mediated BAT thermogenesis parallels the reduction in energy expenditure exhibited by various animal models of obesity (also exhibiting enhanced energy intake) such as the leptin deficient *ob*/*ob* mouse (Trayhurn et al., 1982), leptin-receptor deficient *db*/*db* mouse (Goodbody and Trayhurn, 1981), leptin-resistant *fa*/*fa* rat (Marchington et al., 1983), and melanocortin-4 receptor (MC4R)-ablated obese mice (Ste et al., 2000; Balthasar et al., 2005). A decrease in SNS-mediated BAT thermogenesis associated with reduced energy expenditure has also been reported in animals seeking to spare energy such as rats subjected to energy restriction (Rothwell and Stock, 1982) as well as in lactating (Trayhurn and Richard, 1985) and pregnant rodents (Andrews et al., 1986).

Further support for the role of BAT in energy balance was the recent finding that *Ucp1*-ablated mice displayed a reduction in adaptive thermogenesis accompanied by an increase in fat gain, which was exacerbated by high-fat feeding (Feldmann et al., 2009). It is noteworthy that *Ucp1*-ablated mice gained excess fat only when they were housed at a thermoneutral temperature (29°C). Interestingly, lack of UCP1 *per se* would apparently not increase metabolic efficiency in the *Ucp1*-deficient mice housed below 29°C (Kozak and Anunciado-Koza, 2008), except in old mice (Kontani et al., 2005), or in mice chronically fed an energy-dense palatable diet (Nedergaard et al., 2001). The absence of changes in the energy balance following *Ucp1* loss/gain-of-function under certain circumstances, should not be considered to invalidate the importance of BAT in energy balance. It is important to consider that the complex brain circuitries controlling SNS-mediated BAT thermogenesis can and do activate alternative thermogenic pathways in animals with defective BAT. A well-developed/organized BAT thermogenic machinery can certainly be an asset upon which the brain can rely to efficiently control energy expenditure and hence regulate energy balance.

BRAIN CIRCUITS INVOLVED IN SNS-MEDIATED CONTROL OF BAT THERMOGENESIS AND ENERGY HOMEOSTASIS

Brown adipose tissue depots are richly innervated by SNS postganglionic neurons, and it is through the SNS, and hence via adrenergic receptors, that BAT thermogenesis is physiologically controlled (Cannon and Nedergaard, 2004; Bartness et al., 2010). Although thermogenic capacity of BAT can be significantly enhanced by the use of agents such as peroxisome proliferator-activated receptor γ (PPARγ) agonists, the actual thermogenic activity of the tissue is critically dependent upon the adrenergic tone to BAT (Sell et al., 2004b; Festuccia et al., 2008). Cold exposure and overfeeding increases noradrenaline (NA) turnover rate in BAT (Landsberg et al., 1984) and, remarkably, neither cold nor overfeeding induce thermogenic activity in mice lacking β-adrenoreceptors (β-less mice; Bachman et al., 2002; Jimenez et al., 2002; Lowell and Bachman, 2003).

Sympathetic nervous system-mediated BAT thermogenesis is controlled by the hypothalamic and brainstem nuclei such as the preoptic area (POA), arcuate hypothalamic nucleus (ARC), paraventricular hypothalamic nucleus (PVH), dorsomedial hypothalamic nucleus (DMH), periaqueductal gray (PAG), and raphé pallidus (RPA; Grill, 2006; Berthoud and Morrison, 2008; Richard et al., 2010; Morrison, 2011; Richard and Picard, 2011). All of these nuclei contain neurons expressing chemical mediators and receptors involved in the control of SNS-mediated BAT thermogenesis. The brain circuits involved in energy balance regulation differ slightly from those involved in thermoregulatory thermogenesis. The latter have been the subject of excellent reviews elsewhere (Morrison, 2011; Nakamura, 2011) and will not be discussed here. This section will review the recent advances made in our understanding of the melanocortin system, which represents the most significant regulator of energy homeostasis.

THE MELANOCORTIN SYSTEM IS A KEY PLAYER IN THE CONTROL OF SNS-MEDIATED BAT THERMOGENESIS IN RELATION TO ENERGY HOMEOSTASIS

The brain "metabolic" melanocortin system consists of (i) ARC neurons producing the proopiomelanocortin (POMC) fragment, α-melanocyte-stimulating hormone (αMSH), (ii) neurons harboring the melanocortin receptor 4 (MC4R), (iii) and ARC neurons synthesizing agouti-related protein (AgRP), an endogenous antagonist of the MC4R. The ARC neurons that synthesize AgRP also express NPY, which is a well-recognized anabolic neuropeptide (Herzog, 2003; Nguyen et al., 2011). The "catabolic" role played by the brain melanocortin system has been acknowledged previously (Garfield et al., 2009; Mountjoy, 2010; Corander and Coll, 2011; De Jonghe et al., 2011; Pandit et al., 2011). Loss-of-function mutations of the key players of this system (for instance POMC or MC4R) have been reported to cause massive obesity in laboratory animals (De Jonghe et al., 2011) and humans (Farooqi and O'Rahilly, 2006; Tao, 2010).

It is noteworthy that ARC POMC and AgRP/NPY neurons are known to be leptin-sensitive, which further corroborates the role of the melanocortin system in energy homeostasis. Upon activation by leptin, POMC neurons release the catabolic fragment α-MSH from nerve terminals found in several brain regions that express MC4R. Moreover, leptin inhibits AgRP/NPY neurons and MC4R deficiency has been shown to blunt the ability of leptin (be it injected centrally or peripherally) to increase Ucp1 expression in BAT and WAT (Zhang et al., 2005).

HYPOTHETICAL MELANOCORTINERGIC CIRCUITS CONTROLLING SNS-MEDIATED BAT THERMOGENESIS

In an attempt to delineate the circuits pertaining to the melanocortin system controlling SNS-mediated BAT thermogenesis, we have identified numerous brain populations of MC4R mRNA-harboring neurons that are polysynaptically (through several synapses) connected to BAT, using transneuronal viral retrograde tract-tracing technique (Song et al., 2008). It was estimated that 84, 83, and 77% of the neurons expressing MC4R found in the PVH, PAG, and POA brain regions, respectively, were connected to interscapular BAT in rats (Song et al., 2008). Since the absolute total number of neurons from each of these three region

that connect to BAT is very high in, one can confidently infer that MC4R neurons found in the PVH, POA, and PAG brain regions are all unquestionably part of the circuits that control BAT thermogenesis (Morrison, 2011; Richard and Picard, 2011).

An increase in BAT thermogenesis was reported when MC4R agonist melanotan 2 (MT2) was injected in the PVH (Song et al., 2008), further demonstrating that the MC4R neurons of the PVH promote BAT-mediated thermogenesis. The phenotype of the MC4R neurons of PVH that are connected to BAT remains to be ascertained. However, it is likely that neurons expressing oxytocin (OT) and the cannabinoid receptor 1 (CB1; Richard et al., 2009; Gelez et al., 2010; Ghamari-Langroudi et al., 2011) could directly project to the intermediolateral column (IML) of the spinal cord in order to control the SNS neurons linked to BAT (Song et al., 2008). Recent data from our laboratory has further demonstrated that CB1 agonism can blunt MT2-induced oxygen consumption and UCP1 expression in BAT (Roffarello and Richard, unpublished results), pointing toward an interaction between the melanocortin and cannabinoid pathways in the regulation of BAT-mediated thermogenesis.

With 83% of its 1337 MC4R positive neurons (some 1165 neurons) synaptically linked to the iBAT (Song et al., 2008), the PAG would represent the next brain region (after PVH) containing the largest population of MC4R neurons connected to BAT (Song et al., 2008). This neuro-anatomical finding leaves little doubt about the potential involvement of PAG in the MC4R-agonism-mediated regulation of BAT thermogenesis. The ventrolateral division of the PAG has been reported to receive direct projections from the ARC (Guo and Longhurst, 2010; Li et al., 2010a) and then to project to the RPA (Li et al., 2010b), wherefrom 5-hydroxytryptamine neurons in turn were found to project to the IML, likely to control the SNS outflow to BAT (Morrison, 1999, 2011). In addition, other studies have demonstrated a direct involvement of the PAG in the regulation of BAT-mediated thermogenesis (de Menezes et al., 2006; Rathner and Morrison, 2006).

After the PVH, the POA comprises the second largest population of hypothalamic MC4R neurons that are connected to iBAT (Song et al., 2008). The role of the POA in regulating BAT thermogenesis has been acknowledged previously (Nakamura et al., 2005; Nakamura, 2011). However the neuronal circuits through which MC4R neurons of POA control BAT-mediated thermogenesis have yet to be delineated. The POA contains gamma-aminobutyric acid (GABA) neurons that project to the DMH, where they inhibit excitatory neurons that send projections to the brainstem in order to connect with the premotor neurons governing the activity of the SNS efferent neurons (Nakamura et al., 2005; Morrison, 2011; Nakamura, 2011). Interestingly, similar to prostaglandin E2 (PGE2; Nakamura et al., 2005; Morrison, 2011; Nakamura, 2011), MC4R POA neurons could inhibit the POA GABA neurons projecting to the DMH to ultimately release their inhibition on neurons promoting SNS activity to BAT.

Overall, it is apparent that the melanocortin system exerts its control on the SNS-mediated BAT thermogenesis via various neuronal populations of hypothalamus and brainstem regions. Future studies would be required to better characterize these neurons and extend our current understanding on this subject.

BAT IN ADULT HUMANS

In the last decade, various studies using PET/CT scanning with ^{18}fluorodeoxyglucose (^{18}FDG; often aimed at screening tumors) have demonstrated the presence of metabolically active fat depots within the cervical, supraclavicular, and paraspinal regions in adults humans (Nedergaard et al., 2007; Cannon and Nedergaard, 2010; Enerback, 2010a,b; Richard et al., 2010). These depots were characterized as being BAT with cells having multilocular cytoplasm and abundant mitochondria (Zingaretti et al., 2009). Moreover, these fat depots were reported to have rich SNS-innervations and to express Ucp1, the ultimate marker of the brown adipocytes (Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009; Zingaretti et al., 2009). Additionally, ^{18}FDG-detected BAT was found to express mRNAs encoding other proteins such as type II iodothyronine deiodinase (DIO2), PPARγ-coactivator-1α (PGC1α), PRDM16, and β3-adrenergic receptor (Virtanen et al., 2009), all of which are known to be the key players in mediating BAT thermogenesis.

The prevalence of ^{18}FDG-detectable BAT is determined by a series of factors including age, sex, body mass index (BMI), outdoor temperature preceding PET/CT scanning and the diabetes status of the individuals. The detection of metabolically active BAT has been shown to (i) decrease with age (Cypess et al., 2009; Saito et al., 2009; Zingaretti et al., 2009), (ii) inversely correlate with BMI and fat content (Cypess et al., 2009; Saito et al., 2009; Zingaretti et al., 2009), (iii) increase with exposure to low temperatures (Cohade et al., 2003a; Garcia et al., 2006; Kim et al., 2008; Cypess et al., 2009; Saito et al., 2009), (iv) be higher in women than men (Cypess et al., 2009), and (v) to be lower in diabetic than non-diabetic patients (Cypess et al., 2009). In large cohorts of adult humans screened for cancer, the detection/prevalence of BAT was estimated to be relatively low (5–10% and below; Cypess et al., 2009; Ouellet et al., 2011). This obviously represents an underestimation of the true prevalence of BAT. In fact, the presence of BAT is not ineluctably detectable during PET scans in cancer screening investigations, as BAT needs to be stimulated to exhibit ^{18}FDG uptake. The true prevalence of BAT is much higher than 5–10% and we (Ouellet et al., 2012) and others (van Marken Lichtenbelt et al., 2009) have reported a near 100% BAT prevalence in cold-exposed young human subjects. PET/CT investigations with large cohorts have nonetheless proved invaluable for meaningfully scrutinizing various determinants of the BAT prevalence.

BAT DISTRIBUTION IN CHILDREN AND ADULT HUMANS DIFFERS FROM THAT OF HUMAN NEONATES AND SMALL MAMMALS

The pattern of BAT distribution in adult humans differs from that seen in human neonates (Enerback, 2010a) and small laboratory rodents such as rats and mice (Cannon and Nedergaard, 2004). In contrast to rodents, humans do not possess a prominent interscapular BAT depot. In adult humans (Ouellet et al., 2011) as well as in children and adolescents (Drubach et al., 2011), the most easily detectable and the most prominent BAT depot is located in the supraclavicular area. This depot exhibited the highest ^{18}FDG uptake activity following cold exposure (van Marken Lichtenbelt et al., 2009) and was also described as

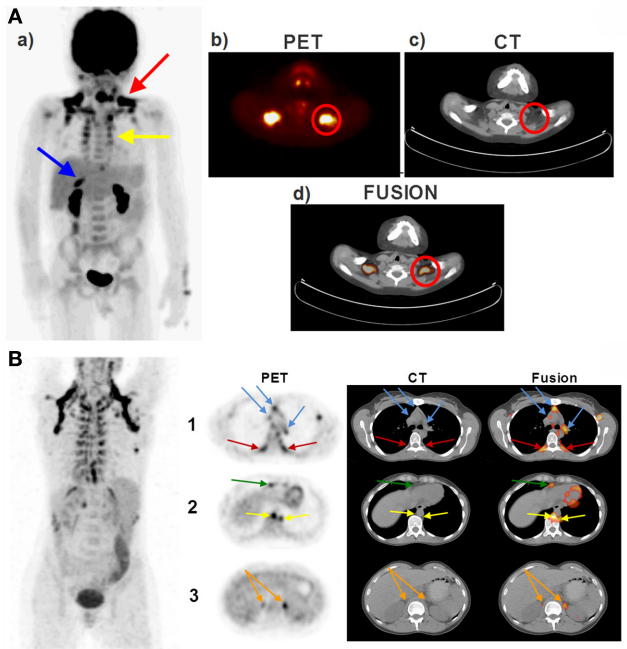

FIGURE 1 | Brown adipose tissue (BAT) distribution in a pediatric (A) and adult (B) human. (A) The PET/CT scans of a 5-year-old boy taken 60 min after an i.v. injection of 5 mCi ^{18}FDG. Red arrow shows the supraclavicular BAT, yellow arrow shows the peri-spinal BAT depots and blue arrow shows the peri-adrenal BAT depot in **(a)** coronal view (postero-anterior projection). The Supraclavicular BAT is also illustrated in a transversal series of **(b)** PET, **(c)** CT, and **(d)** fusion views of the cervical–supraclavicular region. Red circles denote the supraclavicular BAT in **(b–d)**. **(B)** The PET/CT scans of a 35-year-old woman taken 60 min after an i.v. injection of 5 mCi ^{18}FDG. First row shows the transversal section of the aortic arch. Red arrows denote the peri-spinal BAT depots and the blue arrows point to the mediastinal BAT depots on PET (left panel), CT (middle panel), and fusion scans (right panel). Second row shows the transversal section of the hepatocardiac section, where yellow arrows point to the peri-aortic BAT depots and the green arrow points to the pericardial BAT depots. Third row shows the transversal section of the adrenal gland, where orange arrows point to the peri-adrenal BAT depots. PET/CT, positron emission tomography/computed tomography; ^{18}FDG, ^{18}fluorodeoxyglucose; i.v., intravenous; BAT, brown adipose tissue.

the USA-fat (Uptake in Supraclavicular Area Fat) in one of the decisive studies identifying BAT using PET/CT scanning (Cohade et al., 2003b). In addition, BAT is also noticeably seen in the cervical and paraspinal areas in both children and in adults (Enerback, 2010a; Richard et al., 2010; Richard and Picard, 2011). **Figure 1A** demonstrates the PET/CT images of a 5-year-old boy showing the presence of ^{18}FDG uptake activity in various fat depots, whereas **Figure 1B** represents the PET/CT images of a 35-year-old women showing the presence of ^{18}FDG-detectable BAT in the supraclavicular, peri-spinal, mediastinal-, pericardial, peri-aortic, and peri-adrenal sites. We (Chechi et al., unpublished observations) and others (Sacks et al., 2009; Sacks and Fain, 2011) have recently observed that human epicardial fat depot expresses significant levels of Ucp1, indicating that BAT is present around the heart.

HUMAN BAT ACTIVITY IS UNDER THE CONTROL OF THE SNS

The detection of ^{18}FDG-detectable BAT in humans is also modulated by the use of β-blockers (Tatsumi et al., 2004; Jacobsson et al., 2005; Parysow et al., 2007; Soderlund et al., 2007; Cypess et al., 2009). Soderlund et al. (2007) demonstrated that a single dose of 80 mg of propranolol (taken 2 h before FDG administration) led to the complete attenuation of ^{18}FDG uptake in BAT. On the other hand, patients with pheochromocytoma were reported to exhibit intense ^{18}FDG uptake in BAT (Hadi et al., 2007; Kuji et al., 2008; Yamaga et al., 2008), which disappeared following the resection of the catecholamine-secreting tumors (Hadi et al., 2007; Yamaga et al., 2008).

BAT PREVALENCE/DETECTION DECREASES WITH AGE

Age represents a significant determinant of ^{18}FDG-detectable BAT (Truong et al., 2004; Gelfand et al., 2005; Cypess et al., 2009; Saito et al., 2009). In cohorts of patients under cancer surveillance, people exhibiting ^{18}FDG uptake sites were reported to be on average younger than those showing no sign of ^{18}FDG-detectable BAT (Cypess et al., 2009; Zingaretti et al., 2009; Ouellet et al., 2011). In a recent investigation aimed at assessing the prevalence and determinants of BAT ^{18}FDG uptake in children and adolescents screened for tumors, Drubach et al. (2011) reported prevalence of detectable BAT of above 40%, with the highest BAT activity seen in the 13- to 15-years-old age group. A prevalence of above 40% in children/adolescents tested in conditions that do not necessarily activate BAT has to be considered as being high and much higher than that of 5–10% seen in cohorts of adult patients tested in similar conditions. Consistent with this, in our cohort of adult patients tested for cancer, we observed a decrease in the detection/prevalence of BAT with age in both men and women (Ouellet et al., 2011). In addition, Saito et al. (2009) reported a prevalence of cold-induced BAT ^{18}FDG uptake in 52% (16/31) of subjects aged between 23 and 35 years, compared to 8% (2/24) of subjects aged between 38 and 65 years. Altogether, the literature suggests that age reduces the thermogenic capacity of BAT, which renders BAT detection by PET/CT less probable (Richard et al., 2010).

BAT PREVALENCE/DETECTION DECREASES WITH INCREASED FATNESS

The detection of BAT appears strongly influenced by BMI and body fat in humans of all age groups (Cypess et al., 2009; Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Drubach et al., 2011; Ouellet et al., 2011; Wang et al., 2011; Yoneshiro et al., 2011a). Specifically, BAT activity was reported to be inversely correlated to BMI (Cypess et al., 2009; Ouellet et al., 2011), body fat (Saito et al., 2009; van Marken Lichtenbelt et al., 2009; Yoneshiro et al., 2011a), and visceral obesity (Wang et al., 2011). Noteworthily, Saito et al. (2009) reported that the only two subjects with active BAT (out of 24) in an age group of 38–65 years were the two leanest subjects. The same investigators also provided evidence to support a negative association between BAT activity and the accumulation of body fat with age (Yoneshiro et al., 2011a). van Marken Lichtenbelt et al. (2009), who reported an inverse relationship between cold-induced ^{18}FDG uptake activity in BAT with both BMI and percentage of body fat, emphasized that the sole subject (out of 24), who resisted cold-induced BAT ^{18}FDG uptake in their study,

was the one displaying the largest BMI (38.7) and percentage of body fat (41.8%).

BAT DETECTION/PREVALENCE INCREASES WITH LOW TEMPERATURE

In addition to age and body fat mass, the outdoor temperature on the day of ^{18}FDG PET/CT scanning may be a major determinant of BAT detection. Expectedly, the colder the temperature at the time of scanning, the higher is the prevalence of ^{18}FDG-detectable BAT (Cohade et al., 2003a; Kim et al., 2008; Ouellet et al., 2011). Not surprisingly, this BAT prevalence varies with seasons, being higher in winter than in summer (Cohade et al., 2003a; Au-Yong et al., 2009; Cypess et al., 2009; Saito et al., 2009; Ouellet et al., 2011). Winter could enhance the stimulating effect of acute cold exposure on the ^{18}FDG uptake in BAT (Saito et al., 2009), likely by increasing BAT capacity (Richard et al., 2010; Ouellet et al., 2011). The seasonal effects on BAT detection has also been linked to changes in the photoperiod (Au-Yong et al., 2009). However, we found that the outdoor temperature on the day of testing was a stronger determinant of BAT detection than the photoperiod (Ouellet et al., 2011). The notion that environmental temperature is a major determinant of ^{18}FDG-detectable BAT prevalence is also consonant with the early data reported by Huttunen et al. (1981), who demonstrated a higher prevalence of BAT-related enzymes in the adipose tissue biopsies of outdoor workers compared to indoor workers.

BAT DETECTION/PREVALENCE IS HIGHER IN WOMEN THAN MEN

Sex represents another factor that influences BAT detection in humans, since women exhibit higher prevalence than men (Hany et al., 2002; Cohade et al., 2003b; Truong et al., 2004; Rousseau et al., 2006; Cypess et al., 2009). Cypess et al. (2009) reported positive scans for ^{18}FDG uptake sites in 76 of 1013 women (7.5%) and 30 of 959 men (3.1%), corresponding to a female/male ratio of more than 2:1. We also observed a similar pattern of BAT prevalence in women compared to men in our cohort (Ouellet et al., 2011). In addition, women were reported to exhibit greater BAT mass and higher ^{18}FDG uptake activity (Cypess et al., 2009). Interestingly, we observed that the sex effect on the detection/prevalence of BAT tended to disappear in aging humans (Ouellet et al., 2011), indicating the potential role of female sex hormones in BAT thermogenesis. In that respect, it is worth mentioning that the prevalence of detectable BAT did not differ between boys and girls prior to puberty (Drubach et al., 2011).

The reason as to why women exhibit a higher detection of ^{18}FDG-BAT (after puberty and prior to menopause) is not known. The possibility however exists that women experience more cold sensation at a given temperature than men, which is supported by human and animal studies (Quevedo et al., 1998; Rodriguez et al., 2001; Rodriguez-Cuenca et al., 2002; Valle et al., 2007). This hypothesis needs to be further substantiated in future human cohorts.

DIABETES LOWERS DETECTION OF BAT

We recently demonstrated that prevalence of ^{18}FDG-detectable BAT was lower in the diabetic subjects (1.8%) than the non-diabetic subjects (11.5%; Ouellet et al., 2011), which is in line with the observations made by Cypess et al. (2009). However, the mechanisms linking diabetes to a reduced detection of BAT remain to be delineated.

BAT-MEDIATED THERMOGENESIS MAY REPRESENT A SIGNIFICANT ENERGY-CONSUMING PROCESS IN HUMANS

Our recent series of experiments with ^{11}C-acetate (Ouellet et al., 2012), a PET tracer used to assess tissue oxidative metabolism (Labbe et al., 2011), convincingly demonstrated the metabolic importance of BAT in non-shivering thermogenesis in young adults. Our subjects were studied under well-controlled cold exposure conditions designed to minimize shivering (Ouellet et al., 2012). We observed a significant 1.8-fold increase in the total energy expenditure (excess expenditure of some 250 kcal over 3 h of cold exposure), which was suggested to be due, in large part, to an increase in BAT-mediated non-shivering thermogenesis associated with an increase in intracellular fat depletion. We indeed observed a significant increase in the radio-density of BAT after cold exposure, indicating that BAT triglyceride content was reduced by cold exposure (Ouellet et al., 2012). By demonstrating the involvement of BAT in the non-shivering thermogenesis of young adults, our results (Ouellet et al., 2012) add to the previous findings that BAT detection is reduced by aging and fatness, and lend further support for the participation of BAT in energy homeostasis. Incidentally, the relative difficulty in detecting adaptive thermogenesis in humans (Schoeller, 2001) does not necessarily argue against the role of BAT thermogenesis in energy homeostasis, as it parallels the difficulty in detecting BAT. Future metabolic studies conducted in the cold-adapted young individuals would possibly help to delineate the role of BAT thermogenesis in controlling energy expenditure in humans. Based on reasonable assumptions, Virtanen et al. (2009) estimated that annual energy expenditure associated with the submaximal stimulation of about 60 g of human BAT depot could burn more than 4 kg of body fat. Such an estimate is in line with that of Rothwell and Stock (1983), who pioneered the view that active brown fat can have profound influence on human energy metabolism.

CONCLUSION

Recent advances in our understanding of numerous brain pathways that drive the SNS outflow to BAT (Bartness and Song, 2007; Song et al., 2008; Bartness et al., 2010; Morrison, 2011; Richard and Picard, 2011) using sophisticated techniques like transneuronal viral retrograde tract-tracing have fueled the current interest in studying the brain systems involved in energy balance regulation (Bartness et al., 2010; Richard et al., 2010; Richard and Picard, 2011). One such system includes the brain metabolic melanocortin system, which acts via the activation of MC4R, thereby controlling energy expenditure while enhancing BAT thermogenesis (Ste et al., 2000; Butler, 2006). Numerous brain populations of MC4R mRNA-harboring neurons that are synaptically connected to BAT (Song et al., 2008) and WAT (Song et al., 2005) have been identified. Although the functional circuits involving these neurons in the control of BAT thermogenesis remain to be delineated, the available data supports a role of the PVH, PAG, POA. Future studies are warranted to further understand the role of melanocortin system and other neuronal pathways contributing to the regulation of SNS-mediated BAT thermogenesis.

The revitalized interest in understanding the role of BAT thermogenesis in obesity is largely due to the recent demonstration that BAT exists in substantial amount in adult humans (Nedergaard et al., 2007; Enerback, 2010b; Nedergaard and Cannon, 2010; Ouellet et al., 2011; Richard and Picard, 2011; Yoneshiro et al., 2011b). In addition, new sites (such as epicardial adipose tissue) exhibiting the presence of brown adipocytes are being recognized in humans. Our recent observations demonstrating that BAT is involved in non-shivering thermogenesis in humans (Ouellet et al., 2012), coupled with the observations that ^{18}FDG uptake in BAT was blunted by aging and fatness tend to support the view that BAT-mediated thermogenesis is a critical player in human energy balance. Indeed, a better understanding of factors affecting the prevalence, distribution, and regulation of BAT thermogenesis would open up new avenues for intervening with human obesity.

REFERENCES

Andrews, J. F., Richard, D., Jennings, G., and Trayhurn, P. (1986). Brown adipose tissue thermogenesis during pregnancy in mice. *Ann. Nutr. Metab.* 30, 87–93.

Au-Yong, I. T., Thorn, N., Ganatra, R., Perkins, A. C., and Symonds, M. E. (2009). Brown adipose tissue and seasonal variation in people. *Diabetes* 58, 2583–2587.

Bachman, E. S., Dhillon, H., Zhang, C. Y., Cinti, S., Bianco, A. C., Kobilka, B. K., and Lowell, B. B. (2002). Beta AR signaling required for diet-induced thermogenesis and obesity resistance. *Science* 297, 843–845.

Balthasar, N., Dalgaard, L. T., Lee, C. E., Yu, J., Funahashi, H., Williams, T., Ferreira, M., Tang, V., R. A. McGovern, Kenny, C. D., Christiansen, L. M., Edelstein, E., Choi, B., Boss, O., Aschkenasi, C., Zhang, C. Y., Mountjoy, K., Kishi, T., Elmquist, J. K., and Lowell, B. B. (2005). Divergence of melanocortin pathways in the control of food intake and energy expenditure. *Cell* 123, 493–505.

Bartness, T. J., and Song, C. K. (2007). Brain-adipose tissue neural crosstalk. *Physiol. Behav.* 91, 343–351.

Bartness, T. J., Vaughan, C. H., and Song, C. K. (2010). Sympathetic and sensory innervation of brown adipose tissue. *Int. J. Obes. (Lond.)* 34(Suppl. 1), S36–S42.

Berthoud, H. R., and Morrison, C. (2008). The brain, appetite, and obesity. *Annu. Rev. Psychol.* 59, 55–92.

Butler, A. A. (2006). The melanocortin system and energy balance. *Peptides* 27, 281–290.

Cannon, B., and Nedergaard, J. (2004). Brown adipose tissue: function and physiological significance. *Physiol. Rev.* 84, 277–359.

Cannon, B., and Nedergaard, J. (2008). Developmental biology: neither fat nor flesh. *Nature* 454, 947–948.

Cannon, B., and Nedergaard, J. (2010). Metabolic consequences of the presence or absence of the thermogenic capacity of brown adipose tissue in mice (and probably in humans). *Int. J. Obes. (Lond.)* 34(Suppl. 1), S7–S16.

Cohade, C., Mourtzikos, K. A., and Wahl, R. L. (2003a). "USA-fat": prevalence is related to ambient outdoor temperature-evaluation with 18F-FDG PET/CT. *J. Nucl. Med.* 44, 1267–1270.

Cohade, C., Osman, M., Pannu, H. K., and Wahl, R. L. (2003b). Uptake in supraclavicular area fat ("USA-fat"): description on 18F-FDG PET/CT. *J. Nucl. Med.* 44, 170–176.

Corander, M. P., and Coll, A. P. (2011). Melanocortins and body weight regulation: glucocorticoids, agouti-related protein and beyond. *Eur. J. Pharmacol.* 660, 111–118.

Cypess, A. M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A. B., Kuo, F. C., Palmer, E. L., Tseng, Y. H., Doria, A., Kolodny, G. M., and Kahn, C. R. (2009). Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* 360, 1509–1517.

De Jonghe, B. C., Hayes, M. R., and Bence, K. K. (2011). Melanocortin control of energy balance: evidence from rodent models. *Cell. Mol. Life Sci.* 68, 2569–2588.

de Menezes, R. C., Zaretsky, D. V., Fontes, M. A., and DiMicco, J. A. (2006). Microinjection of muscimol into caudal periaqueductal gray lowers body temperature and attenuates increases in temperature and activity evoked from the dorsomedial hypothalamus. *Brain Res.* 1092, 129–137.

Drubach, L. A., Palmer, E. L. III, Connolly, L. P., Baker, A., Zurakowski, D., and Cypess, A. M. (2011). Pediatric brown adipose tissue: detection, epidemiology, and differences from adults. *J. Pediatr.* 159, 939–944.

Enerback, S. (2010a). Human brown adipose tissue. *Cell Metab.* 11, 248–252.

Enerback, S. (2010b). Brown adipose tissue in humans. *Int. J. Obes.* 34, S43–S46.

Farooqi, S., and O'Rahilly, S. (2006). Genetics of obesity in humans. *Endocr. Rev.* 27, 710–718.

Feldmann, H. M., Golozoubova, V., Cannon, B., and Nedergaard, J. (2009). UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab.* 9, 203–209.

Festuccia, W. T., Oztezcan, S., Laplante, M., Berthiaume, M., Michel, C., Dohgu, S., Denis, R. G., Brito, M. N., Brito, N. A., Miller, D. S., Banks, W. A., Bartness, T. J., Richard, D., and Deshaies, Y. (2008). Peroxisome proliferator-activated receptor-gamma-mediated positive energy balance in the rat is associated with reduced sympathetic drive to adipose tissues and thyroid status. *Endocrinology* 149, 2121–2130.

Garcia, C. A., Van, D. Nostrand, Atkins, F., Acio, E., Butler, C., Esposito, G., Kulkarni, K., and Majd, M. (2006). Reduction of brown fat 2-deoxy-2-[F-18]fluoro-D-glucose uptake by controlling environmental temperature prior to positron emission tomography scan. *Mol. Imaging Biol.* 8, 24–29.

Garfield, A. S., Lam, D. D., Marston, O. J., Przydzial, M. J., and Heisler, L. K. (2009). Role of central melanocortin pathways in energy homeostasis. *Trends Endocrinol. Metab.* 20, 203–215.

Gelez, H., Poirier, S., Facchinetti, P., Allers, K. A., Wayman, C., Alexandre, L., and Giuliano, F. (2010). Neuroanatomical evidence for a role of central melanocortin-4 receptors and oxytocin in the efferent control of the rodent clitoris and vagina. *J. Sex. Med.* 7, 2056–2067.

Gelfand, M. J., O'Hara M. S, Curtwright, L. A., and Maclean, J. R. (2005). Premedication to block [(18)F]FDG uptake in the brown adipose tissue of pediatric and adolescent patients. *Pediatr. Radiol.* 35, 984–990.

Gessner, K. (1551). Conradi Gesneri medici Tigurine Historiae Animalium Lib. I de Quadripedibus uiuiparis, 842.

Ghamari-Langroudi, M., Srisai, D., and Cone, R. D. (2011). Multinodal regulation of the arcuate/paraventricular nucleus circuit by leptin. *Proc. Natl. Acad. Sci. U.S.A.* 108, 355–360.

Goodbody, A. E., and Trayhurn, P. (1981). GDP binding to brown-adipose-tissue mitochondria of diabetic – obese (db/db) mice. Decreased binding in both the obese and pre-obese states. *Biochem. J.* 194, 1019–1022.

Grill, H. J. (2006). Distributed neural control of energy balance: contributions from hindbrain and hypothalamus. *Obesity (Silver Spring)* 14(Suppl. 5), 216S–221S.

Guo, Z. L., and Longhurst, J. C. (2010). Activation of reciprocal pathways between arcuate nucleus and ventrolateral periaqueductal gray during electroacupuncture: involvement of VGLUT3. *Brain Res.* 1360, 77–88.

Hadi, M., Chen, C. C., Whatley, M., Pacak, K., and Carrasquillo, J. A. (2007). Brown fat imaging with (18)F-6-fluorodopamine PET/CT, (18)F-FDG PET/CT, and (123)I-MIBG SPECT: a study of patients being evaluated for pheochromocytoma. *J. Nucl. Med.* 48, 1077–1083.

Hany, T. F., Gharehpapagh, E., Kamel, E. M., Buck, A., Himms-Hagen, J., and von Schulthess, G. K. (2002). Brown adipose tissue: a factor to consider in symmetrical tracer uptake in the neck and upper chest region. *Eur. J. Nucl. Med. Mol. Imaging* 29, 1393–1398.

Herzog, H. (2003). Neuropeptide Y and energy homeostasis: insights from Y receptor knockout models. *Eur. J. Pharmacol.* 480, 21–29.

Huttunen, P., Hirvonen, J., and Kinnula, V. (1981). The occurrence of brown adipose tissue in outdoor workers. *Eur. J. Appl. Physiol. Occup. Physiol.* 46, 339–345.

Jacobsson, H., Bruzelius, M., and Larsson, S. A. (2005). Reduction of FDG uptake in brown adipose tissue by propranolol. *Eur. J. Nucl. Med. Mol. Imaging* 32, 1130.

Jimenez, M., Leger, B., Canola, K., Lehr, L., Arboit, P., Seydoux, J., Russell, A. P., Giacobino, J. P., Muzzin, P., and Preitner, F. (2002). Beta(1)/beta(2)/beta(3)-adrenoceptor knockout mice are obese and cold-sensitive but have normal lipolytic responses to fasting. *FEBS Lett.* 530, 37–40.

Kajimura, S., Seale, P., and Spiegelman, B. M. (2010). Transcriptional control of brown fat development. *Cell Metab.* 11, 257–262.

Kim, S., Krynyckyi, B. R., Machac, J., and Kim, C. K. (2008). Temporal relation between temperature change and FDG uptake in brown adipose tissue. *Eur. J. Nucl. Med. Mol. Imaging* 35, 984–989.

Kontani, Y., Wang, Y., Kimura, K., Inokuma, K. I., Saito, M., Suzuki-Miura, T., Wang, Z., Sato, Y., Mori, N., and Yamashita, H. (2005). UCP1 deficiency increases susceptibility to diet-induced obesity with age. *Aging Cell* 4, 147–155.

Kozak, L. P., and Anunciado-Koza, R. (2008). UCP1: its involvement and utility in obesity. *Int. J. Obes. (Lond.)* 32(Suppl. 7), S32–S38.

Kuji, I., Imabayashi, E., Minagawa, A., Matsuda, H., and Miyauchi, T. (2008). Brown adipose tissue demonstrating intense FDG uptake in a patient with mediastinal pheochromocytoma. *Ann. Nucl. Med.* 22, 231–235.

Labbe, S. M., Croteau, E., Grenier-Larouche, T., Frisch, F., Ouellet, R., Langlois, R., Guerin, B., Turcotte, E. E., and Carpentier, A. C. (2011). Normal postprandial nonesterified fatty acid uptake in muscles despite increased circulating Fatty acids in type 2 diabetes. *Diabetes* 60, 408–415.

Landsberg, L., Saville, M. E., and Young, J. B. (1984). Sympathoadrenal system and regulation of thermogenesis. *Am. J. Physiol.* 247(Pt 1), E181–E189.

Li, P., Tjen-A-Looi, S. C., Guo, Z. L., and Longhurst, J. C. (2010a). An arcuate-ventrolateral periaqueductal gray reciprocal circuit participates in electroacupuncture cardiovascular inhibition. *Auton. Neurosci.* 158, 13–23.

Li, P., Tjen-A-Looi, S. C., and Longhurst, J. C. (2010b). Nucleus raphe pallidus participates in midbrain-medullary cardiovascular sympathoinhibition during electroacupuncture. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 299, R1369–R1376.

Lowell, B. B., and Bachman, E. S. (2003). Beta-Adrenergic receptors, diet-induced thermogenesis, and obesity. *J. Biol. Chem.* 278, 29385–29388.

Marchington, D., Rothwell, N. J., Stock, M. J., and York, D. A. (1983). Energy balance, diet-induced thermogenesis and brown adipose tissue in lean and obese (fa/fa) Zucker rats after adrenalectomy. *J. Nutr.* 113, 1395–1402.

Morrison, S. F. (1999). RVLM and raphe differentially regulate sympathetic outflows to splanchnic and brown adipose tissue. *Am. J. Physiol.* 276(Pt 2), R962–R973.

Morrison, S. F. (2011). Carl Ludwig distinguished lectureship of the APS neural control and autonomic regulation section: central neural pathways for thermoregulatory cold defense. *J. Appl. Physiol.* 110, 1137–1149.

Mountjoy, K. G. (2010). Functions for pro-opiomelanocortin-derived peptides in obesity and diabetes. *Biochem. J.* 428, 305–324.

Nakamura, K. (2011). Invited review: central circuitries for body temperature regulation and fever. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 301, R1207–R1208.

Nakamura, Y., Nakamura, K., Matsumura, K., Kobayashi, S., Kaneko, T., and Morrison, S. F. (2005). Direct pyrogenic input from prostaglandin EP3 receptor-expressing preoptic neurons to the dorsomedial hypothalamus. *Eur. J. Neurosci.* 22, 3137–3146.

Nedergaard, J., Bengtsson, T., and Cannon, B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. *Am. J. Physiol. Endocrinol. Metab.* 293, E444–E452.

Nedergaard, J., and Cannon, B. (2010). The changed metabolic world with human brown adipose tissue: therapeutic visions. *Cell Metab.* 11, 268–272.

Nedergaard, J., Golozoubova, V., Matthias, A., Shabalina, I., Ohba, K., Ohlson, K., Jacobsson, A., and Cannon, B. (2001). Life without UCP1: mitochondrial, cellular and organismal characteristics of the UCP1-ablated mice. *Biochem. Soc. Trans.* 29(Pt 6), 756–763.

Nguyen, A. D., Herzog, H., and Sainsbury, A. (2011). Neuropeptide Y and peptide YY: important regulators of energy metabolism. *Curr. Opin. Endocrinol. Diabetes Obes.* 18, 56–60.

Nicholls, D. G., and Locke, R. M. (1984). Thermogenic mechanisms in brown fat. *Physiol. Rev.* 64, 1–64.

Ouellet, V., Labbe, S. M., Blondin, D. P., Phoenix, S., Guerin, B., Haman, F., Turcotte, E. E., Richard, D., and Carpentier, A. C. (2012). Brown adipose tissue oxidative metabolism contributes to energy expenditure during acute cold exposure in humans. *J. Clin. Invest.* 122, 545–552.

Ouellet, V., Routhier-Labadie, A., Bellemare, W., Lakhal-Chaieb, L., Turcotte, E., Carpentier, A. C., and Richard, D. (2011). Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. *J. Clin. Endocrinol. Metab.* 96, 192–199.

Pandit, R., de Jong, J. W., Vanderschuren, L. J., and Adan, R. A. (2011). Neurobiology of overeating and obesity: the role of melanocortins and beyond. *Eur. J. Pharmacol.* 660, 28–42.

Parysow, O., Mollerach, A. M., Jager, V., Racioppi, S., San, J. Roman, and Gerbaudo, V. H. (2007). Low-dose oral propranolol could reduce brown adipose tissue F-18 FDG uptake in patients undergoing PET scans. *Clin. Nucl. Med.* 32, 351–357.

Quevedo, S., Roca, P., Pico, C., and Palou, A. (1998). Sex-associated differences in cold-induced UCP1 synthesis in rodent brown adipose tissue. *Pflugers Arch.* 436, 689–695.

Rathner, J. A., and Morrison, S. F. (2006). Rostral ventromedial periaqueductal gray: a source of inhibition of the sympathetic outflow to brown adipose tissue. *Brain Res.* 1077, 99–107.

Richard, D. (2007). Energy expenditure: a critical determinant of energy balance with key hypothalamic controls. *Minerva Endocrinol.* 32, 173–183.

Richard, D., Carpentier, A. C., Dore, G., Ouellet, V., and Picard, F. (2010). Determinants of brown adipocyte development and thermogenesis. *Int. J. Obes. (Lond.)* 34(Suppl. 2), S59–S66.

Richard, D., Guesdon, B., and Timofeeva, E. (2009). The brain endocannabinoid system in the regulation of energy balance. *Best Pract. Res. Clin. Endocrinol. Metab.* 23, 17–32.

Richard, D., and Picard, F. (2011). Brown fat biology and thermogenesis. *Front. Biosci.* 16, 1233–1260.

Rodriguez, E., Monjo, M., Rodriguez-Cuenca, S., Pujol, E., Amengual, B., Roca, P., and Palou, A. (2001). Sexual dimorphism in the adrenergic control of rat brown adipose tissue response to overfeeding. *Pflugers Arch.* 442, 396–403.

Rodriguez-Cuenca, S., Pujol, E., Justo, R., Frontera, M., Oliver, J., Gianotti, M., and Roca, P. (2002). Sex-dependent thermogenesis, differences in mitochondrial morphology and function, and adrenergic response in brown adipose tissue. *J. Biol. Chem.* 277, 42958–42963.

Rothwell, N. J., and Stock, M. J. (1982). Effect of chronic food restriction on energy balance, thermogenic capacity, and brown-adipose-tissue activity in the rat. *Biosci. Rep.* 2, 543–549.

Rothwell, N. J., and Stock, M. J. (1983). Luxuskonsumption, diet-induced thermogenesis and brown fat: the case in favour. *Clin. Sci.* 64, 19–23.

Rousseau, C., Bourbouloux, E., Campion, L., Fleury, N., Bridji, B., Chatal, J. F., Resche, I., and Campone, M. (2006). Brown fat in breast cancer patients: analysis of serial (18)F-FDG PET/CT scans. *Eur. J. Nucl. Med. Mol. Imaging* 33, 785–791.

Sacks, H. S., and Fain, J. N. (2011). Human epicardial fat: what is new and what is missing? *Clin. Exp. Pharmacol. Physiol.* 38, 879–887.

Sacks, H. S., Fain, J. N., Holman, B., Cheema, P., Chary, A., Parks, F., Karas, J., Optican, R., Bahouth, S. W., Garrett, E., Wolf, R. Y., Carter, R. A., Robbins, T., Wolford, D., and Samaha, J. (2009). Uncoupling protein-1 and related messenger ribonucleic acids in human epicardial and other adipose tissues: epicardial fat functioning as brown fat. *J. Clin. Endocrinol. Metab.* 94, 3611–3615.

Saito, M., Okamatsu-Ogura, Y., Matsushita, M., Watanabe, K., Yoneshiro, T., Nio-Kobayashi, J., Iwanaga, T., Miyagawa, M., Kameya, T., Nakada, K., Kawai, Y., and Tsujisaki, M. (2009). High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* 58, 1526–1531.

Schoeller, D. A. (2001). The importance of clinical research: the role of thermogenesis in human obesity. *Am. J. Clin. Nutr.* 73, 511–516.

Seale, P. (2010). Transcriptional control of brown adipocyte development and thermogenesis. *Int. J. Obes. (Lond.)* 34(Suppl. 1), S17–S22.

Seale, P., Bjork, B., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scime, A., Devarakonda, S., Conroe, H. M., Erdjument-Bromage, H., Tempst, P., Rudnicki, M. A., Beier, D. R., and Spiegelman, B. M. (2008). PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454, 961–967.

Sell, H., Deshaies, Y., and Richard, D. (2004a). The brown adipocyte: update on its metabolic role. *Int. J. Biochem. Cell Biol.* 36, 2098–2104.

Sell, H., Berger, J. P., Samson, P., Castriota, G., Lalonde, J., Deshaies, Y.,

and Richard, D. (2004b). Peroxisome proliferator-activated receptor gamma agonism increases the capacity for sympathetically mediated thermogenesis in lean and ob/ob mice. *Endocrinology* 145, 3925–3934.

Soderlund, V., Larsson, S. A., and Jacobsson, H. (2007). Reduction of FDG uptake in brown adipose tissue in clinical patients by a single dose of propranolol. *Eur. J. Nucl. Med. Mol. Imaging* 34, 1018–1022.

Song, C. K., Jackson, R. M., Harris, R. B., Richard, D., and Bartness, T. J. (2005). Melanocortin-4 receptor mRNA is expressed in sympathetic nervous system outflow neurons to white adipose tissue. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 289, R1467–R1476.

Song, C. K., Vaughan, C. H., Keen-Rhinehart, E., Harris, R. B., Richard, D., and Bartness, T. J. (2008). Melanocortin-4 receptor mRNA expressed in sympathetic outflow neurons to brown adipose tissue: neuroanatomical and functional evidence. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 295, R417–R428.

Ste, L. Marie, Miura, G. I., Marsh, D. J., Yagaloff, K., and Palmiter, R. D. (2000). A metabolic defect promotes obesity in mice lacking melanocortin-4 receptors. *Proc. Natl. Acad. Sci. U.S.A.* 97, 12339–12344.

Tao, Y. X. (2010). The melanocortin-4 receptor: physiology, pharmacology, and pathophysiology. *Endocr. Rev.* 31, 506–543.

Tatsumi, M., Engles, J. M., Ishimori, T., Nicely, O., Cohade, C., and Wahl, R. L. (2004). Intense (18)F-FDG uptake in brown fat can be reduced pharmacologically. *J. Nucl. Med.* 45, 1189–1193.

Trayhurn, P., Jones, P. M., M. M. McGuckin, and Goodbody, A. E. (1982). Effects of overfeeding on energy balance and brown fat thermogenesis in obese (ob/ob) mice. *Nature* 295, 323–325.

Trayhurn, P., and Richard, D. (1985). Brown adipose tissue thermogenesis and the energetics of pregnancy and lactation in rodents. *Biochem. Soc. Trans.* 13, 826–828.

Truong, M. T., Erasmus, J. J., Munden, R. F., Marom, E. M., Sabloff, B. S., Gladish, G. W., Podoloff, D. A., and Macapinlac, H. A. (2004). Focal FDG uptake in mediastinal brown fat mimicking malignancy: a potential pitfall resolved on PET/CT. *AJR Am. J. Roentgenol.* 183, 1127–1132.

Tseng, Y. H., Kokkotou, E., Schulz, T. J., Huang, T. L., Winnay, J. N., Taniguchi, C. M., Tran, T. T., Suzuki, R., Espinoza, D. O., Yamamoto, Y., Ahrens, M. J., Dudley, A. T., Norris, A. W., Kulkarni, R. N., and Kahn, C. R. (2008). New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454, 1000–1004.

Valle, A., Garcia-Palmer, F. J., Oliver, J., and Roca, P. (2007). Sex differences in brown adipose tissue thermogenic features during caloric restriction. *Cell. Physiol. Biochem.* 19, 195–204.

van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, J. M., Kemerink, G. J., Bouvy, N. D., Schrauwen, P., and Teule, G. J. (2009). Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* 360, 1500–1508.

Virtanen, K. A., Lidell, M. E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N. J., Enerback, S., and Nuutila, P. (2009). Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* 360, 1518–1525.

Wang, Q., Zhang, M., Ning, G., Gu, W., Su, T., Xu, M., Li, B., and Wang, W. (2011). Brown adipose tissue in humans is activated by elevated plasma catecholamines levels and is inversely related to central obesity. *PLoS ONE* 6, e21006. doi:10.1371/journal.pone.0021006

Yamaga, L. Y., Thom, A. F., Wagner, J., Baroni, R. H., Hidal, J. T., and Funari, M. G. (2008). The effect of catecholamines on the glucose uptake in brown adipose tissue demonstrated by (18)F-FDG PET/CT in a patient with adrenal pheochromocytoma. *Eur. J. Nucl. Med. Mol. Imaging* 35, 446–447.

Yoneshiro, T., Aita, S., Matsushita, M., Okamatsu-Ogura, Y., Kameya, T., Kawai, Y., Miyagawa, M., Tsujisaki, M., and Saito, M. (2011a). Age-related decrease in cold-activated brown adipose tissue and accumulation of body fat in healthy humans. *Obesity (Silver Spring)* 19, 1755–1760.

Yoneshiro, T., Aita, S., Matsushita, M., Kameya, T., Nakada, K., Kawai, Y., and Saito, M. (2011b). Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. *Obesity (Silver Spring)* 19, 13–16.

Zhang, Y., Kilroy, G. E., Henagan, T. M., Prpic-Uhing, V., Richards, W. G., Bannon, A. W., Mynatt, R. L., and Gettys, T. W. (2005). Targeted deletion of melanocortin receptor subtypes 3 and 4, but not CART, alters nutrient partitioning and compromises behavioral and metabolic responses to leptin. *FASEB J.* 19, 1482–1491.

Zingaretti, M. C., Crosta, F., Vitali, A., Guerrieri, M., Frontini, A., Cannon, B., Nedergaard, J., and Cinti, S. (2009). The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J.* 23, 3113–3120.

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Assessment of oxidative metabolism in brown fat using PET imaging

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Objective: Although it has been believed that brown adipose tissue (BAT) depots disappear shortly after the perinatal period in humans, positron emission tomography (PET) imaging using the glucose analog ^{18}F-deoxy-d-glucose (FDG) has shown unequivocally the existence of functional BAT in humans, suggesting that most humans have some functional BAT. The objective of this study was to determine, using dynamic oxygen-15 (^{15}O) PET imaging, to what extent BAT thermogenesis is activated in adults during cold stress and to establish the relationship between BAT oxidative metabolism and FDG tracer uptake. **Methods:** Fourteen adult normal subjects (9F/5M, 30 ± 7 years) underwent triple oxygen scans ($H_2{}^{15}O$, $C^{15}O$, $^{15}O_2$) as well as indirect calorimetric measurements at both rest and following exposure to mild cold (16˚C). Subjects were divided into two groups (BAT+ and BAT−) based on the presence or absence of FDG tracer uptake (SUV > 2) in cervical–supraclavicular BAT. Blood flow and oxygen extraction fraction (OEF) was calculated from dynamic PET scans at the location of BAT, muscle, and white adipose tissue (WAT). The metabolic rate of oxygen (MRO_2) in BAT was determined and used to calculate the contribution of activated BAT to daily energy expenditure (DEE). **Results:** The median mass of activated BAT in the BAT+ group (5F, age 31 ± 8) was 52.4 g (range 14–68 g) and was 1.7 g (range 0–6.3 g) in the BAT − group (5M/4F, age 29 ± 6). Corresponding SUV values were significantly higher in the BAT+ as compared to the BAT− group (7.4 ± 3.7 vs. 1.9 ± 0.9; $p = 0.03$). Blood flow values in BAT were significantly higher in the BAT+ group as compared to the BAT− group (13.1 ± 4.4 vs. 5.7 ± 1.1 ml/100 g/min, $p = 0.03$), but were similar in WAT (4.1 ± 1.6 vs. 4.2 ± 1.8 ml/100 g/min) and muscle (3.7 ± 0.8 vs. 3.3 ± 1.2 ml/100 g/min). Moreover, OEF in BAT was similar in the two groups (0.56 ± 0.18 in BAT+ vs. 0.46 ± 0.19 in BAT−, $p = 0.39$). Calculated MRO_2 values in BAT increased from 0.95 ± 0.74 to 1.62 ± 0.82 ml/100 g/min in the BAT+ group and were significantly higher than those determined in the BAT− group (0.43 ± 0.27 vs. 0.56 ± 0.24, $p = 0.67$). The DEE associated with BAT oxidative metabolism was highly variable in the BAT+ group, with an average of 5.5 ± 6.4 kcal/day (range 0.57–15.3 kcal/day). **Conclusion:** BAT thermogenesis in humans accounts for less than 20 kcal/day during moderate cold stress, even in subjects with relatively large BAT depots. Furthermore, due to the large differences in blood flow and glucose metabolic rates in BAT between humans and rodents, the application of rodent data to humans is problematic and needs careful evaluation.

Keywords: brown fat thermogenesis, oxidative metabolism, ^{15}O PET imaging

INTRODUCTION

Brown adipose tissue (BAT or brown fat) is a thermogenic organ that plays a critical role in non-shivering thermogenesis (Foster and Frydman, 1979; Nedergaard et al., 2001). Most mammals, including humans, have abundant BAT during the perinatal period (Dawkins and Scopes, 1965; Blaza, 1983); however, prominent depots disappear shortly after the perinatal period in several species, and it has been widely assumed the same was true for humans. Nevertheless, several investigations have found convincing histological evidence of BAT in postmortem adults (Kortelainen et al., 1993). Recently, numerous papers have appeared in the positron emission tomography (PET) literature identifying symmetric foci of intense uptake of ^{18}F-deoxy-D-glucose (FDG) uptake in humans, and correlative CT clearly indicates that these sites are adipose tissue. Indeed, a very recent series of papers has unequivocally demonstrated the existence of functional BAT in humans, suggesting that most, if not all, humans have some functional BAT (Cohade et al., 2003; Cypess et al., 2009; Lichtenbelt et al., 2009; Virtanen et al., 2009).

It is clear that beta-adrenergic agonists increase fat oxidation and thermogenesis in humans and that activation of BAT in humans (as assessed by FDG uptake) is sensitive to adrenergic activation. However, it is not known whether, and to what extent, the increase in FDG uptake in fact represents an increase in thermogenesis. Indeed, work in rodents has indicated that glucose contributes little to BAT thermogenesis and that most glucose that is taken up in BAT during activation is not oxidized (Ma and Foster, 1986). Therefore, it is critical to directly establish the relationship between BAT oxidative metabolism and FDG uptake, which is a widely used, but indirect and ambiguous measure (Ma and Foster, 1986; Nedergaard et al., 2007). In addition, it is important to determine the quantitative contribution of BAT thermogenesis to total energy expenditure in both resting state and during physiological activation by cold stress in order to judge the potential of BAT thermogenesis in contributing to weight loss in obese patients. Thus, the objective of this study was to determine, using dynamic oxygen-15 (^{15}O) PET imaging, to what extent BAT thermogenesis can be activated in adults during cold stress and to establish the relationship between BAT oxidative metabolism and FDG uptake.

MATERIALS AND METHODS

SUBJECTS

A total of 14 adult normal subjects (9F/5M, 30 ± 7 years) were studied following approval by the Institutional Review Board (IRB) of Wayne State University. The BMI of this study group was 23.7 ± 2.8 (range 19.1–27.4). All subjects underwent triple oxygen scans ($H_2{}^{15}O$, $C^{15}O$, $^{15}O_2$) at rest and following exposure to mild cold (16°C; see **Figure 1**) following a 6-h fasting period. To induce cold stress, subjects rested in the PET scanner room in minimal clothing 20 min prior to the ^{15}O stress scans and during the entire FDG uptake period. The room was held at a temperature of 16°C and fans were used to provide low-level airflow. All subjects reported experiencing cold stress, however, overt shivering was not reported or observed.

PET DATA ACQUISITION

All subjects were scanned on an EXACT HR PET scanner in 2D mode in order to decrease contribution of scatter from outside of the field-of-view (FOV). The EXACT HR scanner (Siemens, Knoxville, TN, USA) generates 47 image planes with a slice thickness of 3.2 mm and an isotropic spatial resolution of 8 mm FWHM for ^{15}O studies. Initially, two venous catheters were established for tracer injection and venous blood sampling. Subsequently, a 10-min indirect calorimetric measurement was performed to obtain baseline total energy expenditure using a MedGraphics VO2000 Portable Metabolic Testing System (St. Paul, MN, USA). The instrument was calibrated against a known gas mixture prior to each experimental day, and auto-calibrated between experimental runs. During the entire baseline protocol, patients were wrapped in warm blankets and rested comfortably either inside the PET gantry or in an armchair in the scanner room. Subjects were positioned inside the PET scanner with the neck/shoulder region in the FOV and a 15-min transmission scan was performed to correct for photon attenuation. Subsequently, subjects inhaled ^{15}O labeled oxygen (80 mCi) through a disposable plastic facemask for 5 s (1–2 deep breaths) which coincided with the start of a 2-min dynamic emission scan (60×2 s). Two venous blood samples (0.4 ml) were obtained at the end of the dynamic scan for determination of blood hematocrit. In addition, arterial oxygen saturation was monitored during the whole dynamic scan using a Dinamap ProCare 400 monitor (GE, Milwaukee, WI, USA). Following a 12-min period to allow for tracer decay, the subjects then inhaled ^{15}O labeled CO gas (50 mCi) in one to two short breaths and after a 2-min equilibration phase, a 3-min static PET scan was initiated. Six venous blood samples (0.4 ml) were taken during the static scan to measure whole-blood radioactivity during the scanning period. Finally, ^{15}O water (80 mCi) was injected as a slow bolus over 45 s and a 2-min dynamic PET scan was obtained (60×2 s). Again, two venous blood samples (0.4 ml) were obtained at the end of the dynamic scan and arterial oxygen saturation was monitored during the dynamic sequence as before. These three scans were then repeated during cold stress, which was induced by removing all blankets from the patient and letting the subjects rest in the PET scanner in minimal clothing exposed to a temperature of 16°C. Following a 20-min transition period to reach stable systemic oxygen consumption, the three ^{15}O PET scans (O_2, CO, and H_2O) were repeated. At the conclusion of the oxygen PET scans (about 60 min after the beginning of cold exposure), another 10 min indirect calorimetric measurement was performed in order to obtain cold stress oxygen consumption. The FDG tracer (5 mCi) was then injected and the subject remained exposed to 16°C temperature during a 50-min uptake period. At the conclusion of the uptake period a 10-min static FDG scan was acquired at the same position as the oxygen scans. Finally, the subject was transported to the nearby PET/CT suite and a whole body PET/CT scan was obtained in order to verify the presence of BAT in adipose tissue and to calculate the BAT volume. The GE Discovery STE PET/CT scanner (GE Medical Systems, Milwaukee, WI, USA) combines a Light-Speed 16-slice CT with an advanced BGO PET system. CT images (acquisition parameters 100 keV, 80 mA) were acquired with an in-plane spatial resolution of ∼1 mm and a reconstructed slice thickness of 3.3 mm. The total dose to the subjects from all acquisitions was 21 mSv (2.1 rem).

IMAGE DATA PROCESSING AND ANALYSIS

Regions of interest (ROIs) were defined in PET/CT images at the location of BAT, white adipose tissue (WAT), and muscle and subsequently transferred to the dynamic ^{15}O scans. BAT was considered present if there were areas of tissue that were more than 5 mm in diameter, had the CT density of adipose tissue (-250 to -50 HU), and had a maximal SUV of FDG of at least 2.0. This cutoff represented more than 2 SD above the maximal SUV seen in typical depots of WAT. BAT volume was determined by thresholding both the CT image volume ($-250 < \text{HU} < -50$) and the FDG volume ($\text{SUV} > 2.0$) and then applying the logical AND operation to the two masks, followed by removal of all areas that were smaller than 0.125 cm^3. The final BAT ROI was chosen at the location of the largest contiguous group of voxels that survived the masking operation. These regions most frequently represented the cervical, supraclavicular, and superior mediastinal depots, superficial and lateral to the sternocleidomastoid muscles (see **Figure 2**). The volume of BAT ROIs (cm^3) was converted into weight (g) by assuming a density of 0.90 g/cm^3. Moreover, WAT ROIs were

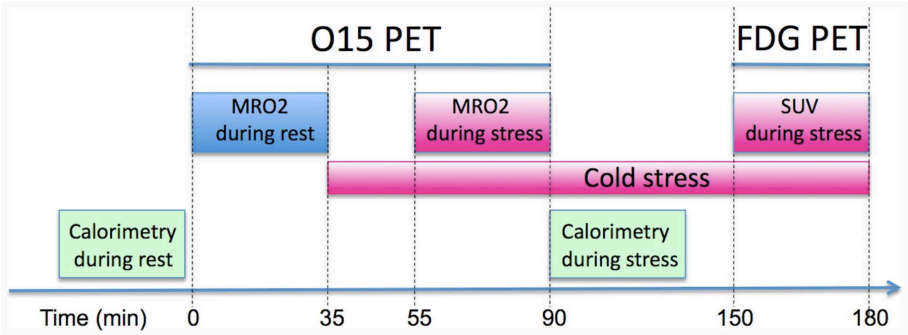

FIGURE 1 | Positron emission tomography protocol used to quantify the metabolic rate of oxygen (MRO_2) in BAT at rest and stress (i.e., mild cold exposure at 16°C). Following the quantitative assessment of oxidative metabolism the patient underwent a FDG PET/CT scan in order to correlate MRO_2 with FDG derived SUV measures.

defined manually on two to three adjacent planes at the location of subcutaneous neck fat as well as muscle ROIs at the location of the shoulder/deltoid area.

Dynamic quantitative PET imaging has been established as gold standard for the *in vivo* determination of metabolic rate of oxygen (MRO_2) in the brain (Frackowiak et al., 1980; Mintun et al., 1984) and skeletal muscle (Ruotsalainen et al., 1997; Kalliokoski et al., 2000). The measurement involves the combined use of ^{15}O-labeled water, carbon monoxide, and molecular oxygen gas to measure the blood flow (F), blood volume (BV), and oxygen extraction fraction (OEF). Data are analyzed based on a three-compartment model that accounts for intravascular ^{15}O hemoglobin and ^{15}O water from systemic and tissue metabolism. The accuracy of this method for the measurement of oxygen OEF in brain tissue has been validated under physiological and pathological conditions over a range of OEF values from 0.05 to 0.80 (Mintun et al., 1984; Hattori et al., 2004). The arterial input function can be derived either from arterial sampling or, in case that the aortic arch is in the FOV of the PET scanner, from a small region at the location of the aorta. The metabolic rate of oxygen (MRO_2; ml/100 g/min) in the tissues of interest is then calculated as the product of blood flow (F; ml/100 g/min), OEF (unitless), and the arterial oxygen concentration (cO_2; mlO_2/100 ml), which is derived from the patient's arterial oxygen saturation (pSat%) and hematocrit (HCT) according to the equation

$$CO_2\,(mlO_2/100ml) = (HCT/3) \times 1.36\,pSat + 0.0031\,pO_2\,(torr)$$

where pO_2 was calculated from the measured pSat according to Severinghaus' (1977) formula and the exact inversion by Ellis (1989). Finally, the daily energy expenditure (DEE; kcal/day) was calculated from the obtained MRO_2 and the weight of BAT according to the formula

$$DEE_{BAT}(kcal/day) = MRO_2(ml/100g/min) \times BAT\ weight(100g) \times 0.0048\,(kcal/ml) \times 1440\,(min/day)$$

Where the conversion factor between kilocalorie and milliliter O_2 was assumed for a respiratory quotient ($RQ = V_{CO_2}/V_{O_2}$) of 0.80 (Leonard, 2010).

STATISTICAL ANALYSIS

Data are reported as mean ± SD and all analysis was performed with the use of the SPSS software version 19. FDG uptake in BAT following cold exposure was highly variable, with a few subjects showing extensive uptake in the cervical–supraclavicular depots, whereas most subjects displayed either no BAT, or only small pockets of activated BAT (**Figure 2**). To account for this skewed distribution, subjects were divided into two groups (BAT+, BAT−), with the threshold being set to 10 g of activated BAT. Normally distributed continuous variables were compared between the two groups using an independent sample *t*-test and non-normally distributed continuous variables using the Mann–Whitney *U*-test. Finally, correlation between variables were assessed using Pearson's *r*. All reported *p*-values are two-tailed and values less than 0.05 were considered to indicate statistical significance.

RESULTS

FDG UPTAKE IN BAT

The amount of detectable BAT in cervical–supraclavicular depots showed a highly skewed distribution (**Figure 3A**). In the BAT+ group (age 31 ± 8), the median mass of activated BAT was 52.4 g (range 14–68 g) and was found to be 1.7 g (range 0–6.3 g) in the BAT− group (age 29 ± 6). The BAT+ group consisted exclusively of women (5F, 0M), whereas the BAT− group was dominated by men (5M, 4F), a result suggesting that women have a greater capacity to increase the mass and activity of their BAT. There was no significant difference in BMI between the BAT+ and BAT− groups (22.4 ± 2.3 vs. 24.9 ± 3.8, $p = 0.38$, **Figure 3B**). In accordance to the experimental design, corresponding SUV values were found to be significantly higher in the BAT+ group as compared to the BAT− group (7.4 ± 3.7 vs. 1.9 ± 0.9; $p = 0.03$, **Figure 3C**).

INDIRECT CALORIMETRY

Due to the fact that the BAT− group was dominated by males (5M, 4F), resting DEE in the BAT− group was higher than in the BAT+ group, which was comprised from females only (1776 vs. 1436 kcal/day). However, after males were removed from the BAT− group, the resting DEE was similar in the two study groups (1453 vs. 1436 kcal/day). As expected from previous work (Orava

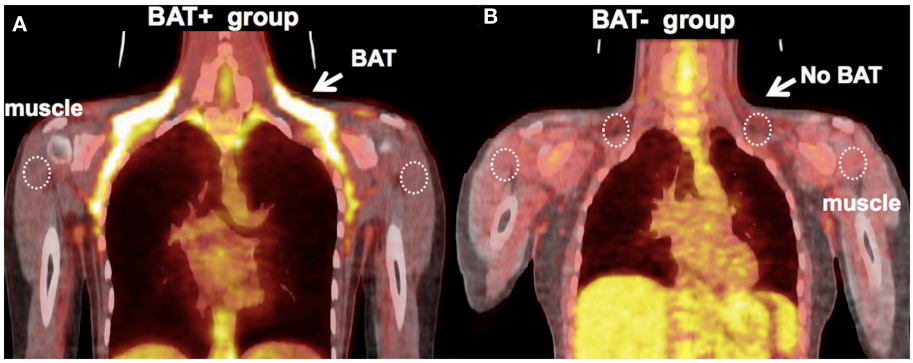

FIGURE 2 | (A) Representative image of a subject with high uptake of FDG in BAT. Five out of 14 subjects had high FDG uptake (BAT+ group, mean ± SD: SUV = 3.6 ± 0.5). ROIs were defined at the location of FDG-defined BAT, abdominal WAT (not shown), and shoulder muscle, which were then transferred to the dynamic ^{15}O sequences for quantification. **(B)** Representative image of a subject from the BAT− group – there is an absence of FDG uptake at the location of BAT following exposure to cold (SUV ∼0.6).

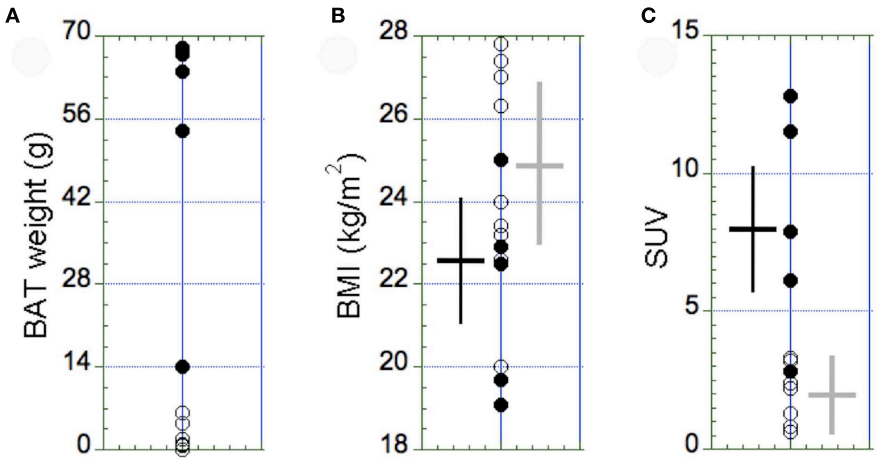

FIGURE 3 | Distribution of the amount of BAT (A), the BMI (B), and the maximal SUV in BAT (C) in the BAT+ group (full circles, *N* = 5) and in the BAT− group (open circles, *N* = 9). (A) The amount of BAT was highly variable, with most of the subjects displaying <10 g of active BAT in small supraclavicular depots. Accordingly, subjects with >10 g of active BAT were assigned to the BAT+ group. **(B)** The BMI was similar between the two groups ($p = 0.38$). **(C)** In contrast, the maximal SUV in BAT observed in the BAT+ group was significantly higher than in the BAT− group ($p = 0.03$). The error bars in **(B,C)** represent SD of the measurements.

et al., 2011; Yoneshiro et al., 2011), individuals with and without detectible BAT responded differently to mild cold exposure. Each subject in the BAT+ group increased DEE by an average of 24 ± 11% (range: 238–492 kcal/day, $p < 0.01$). In contrast, the metabolic response of individuals lacking detectible BAT varied considerably (range: −311 to 345 kcal/day), and overall, cold stress failed to significantly increase DEE in this group (3 ± 19%). Finally, the respiratory quotient (RQ = V_{CO_2}/V_{O_2}) was similar between the two groups (0.83 ± 0.06 in the BAT+ group and 0.91 ± 0.09 in the BAT− group; $p = 0.15$) and was not significantly affected by cold stress.

BLOOD FLOW IN BAT, WAT, AND MUSCLE

Absolute blood flow values were significantly higher in the BAT+ group as compared to the BAT− group only in BAT regions (13.1 ± 4.2 vs. 5.7 ± 1.1 ml/100 g/min, $p = 0.04$), but were similar in WAT (4.1 ± 1.6 vs. 4.2 ± 1.8 ml/100 g/min) and muscle (3.7 ± 0.8 vs. 3.3 ± 1.2 ml/100 g/min) regions (**Figure 4**). Moreover, in the BAT+ group the percent change in blood flow during cold exposure showed a large increase (44 ± 8%) in BAT regions, but remained on average almost unchanged in the BAT− group (5 ± 21%). Smaller increases in blood flow were determined in muscle regions for both groups (11 ± 25% in BAT+ and 8 ± 28% in BAT−), whereas blood flow changes in WAT were mixed. Blood flow in WAT regions decreased in the BAT+ group (−8 ± 15%), whereas these values increased in the BAT− group (13 ± 25%).

OEF AND MRO_2 IN BAT, WAT, AND MUSCLE

The OEF was found to be similar in both groups (BAT+, BAT−) in BAT, muscle, and WAT regions (**Figure 5A**). Specifically, overall BAT OEF was determined as 0.56 ± 0.18 in the BAT+ group and 0.46 ± 0.19 in the BAT− group ($p = 0.39$). Similarly, OEF values in muscle were 0.29 ± 0.08 and 0.28 ± 0.09 in the BAT+ and BAT− groups ($p = 0.63$), and were slightly higher in WAT

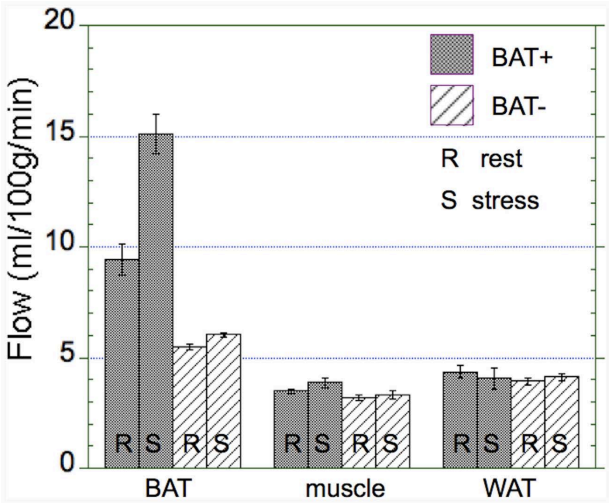

FIGURE 4 | Absolute changes in blood flow in the BAT+ and BAT− groups. Absolute blood flow (ml/100 g/min) in brow adipose tissue (BAT), muscle, and white adipose tissue (WAT) averaged during both rest (R) and cold exposure (S). Error bars represent the SEM. Blood flow in activated BAT is about 50% higher than in non-activated BAT and about threefold higher than in muscle and WAT (3–5 ml/100 g/min). Whereas there was a large increase in blood flow in activated BAT, virtually no increase in blood flow was determined in non-activated BAT, muscle, or WAT.

regions (0.32 ± 0.15 vs. 0.39 ± 0.21, $p = 0.21$). Calculated MRO_2 values in BAT based on corresponding blood flow and OEF values were found to be highest in the BAT+ group during both rest and cold exposure (**Figure 5B**). MRO_2 estimates increased from a mean resting value of 0.95 ± 0.74 ml/100 g/min to a mean value of 1.62 ± 0.82 ml/100 g/min during cold exposure. These MRO_2 values in BAT were significantly higher than those determined in the BAT− group (0.43 ± 0.27 at rest and 0.56 ± 0.24 during cold exposure, respectively). In comparison, MRO_2 estimates in muscle tissue were found to be similar during rest and cold exposure in both groups ($\sim 0.18 \pm 0.09$ ml/100 g/min). Finally, MRO_2 values in WAT were significantly lower in the BAT+ group as compared to the BAT− group at both rest (0.17 ± 0.07 vs. 0.28 ± 0.18 ml/100 g/min) and exposure to cold (0.18 ± 0.09 vs. 0.38 ± 0.26 ml/100 g/min) condition (**Figure 5B**).

ENERGY CONSUMPTION OF BAT

Our data showed a significant correlation between blood flow and the MRO_2 ($r^2 = 0.86$, $p = 0.01$, **Figure 6A**), suggesting that oxidative metabolism is the main determinant of BAT perfusion. Moreover, we also found a significant relationship between the semi-quantitative assessment of glucose uptake in BAT (SUV) and metabolic rate of glucose ($r^2 = 0.63$, $p = 0.02$, **Figure 6B**). Based on the calculated MRO_2 values, the DEE associated with BAT oxidative metabolism was highly variable in the BAT+ group, with an average of 5.5 ± 6.4 kcal/day (range 0.57–15.3 kcal/day). This value was significantly higher than that in the BAT− group, which was determined as only $0.14 + 0.12$ kcal/day (range 0–0.38 kcal/day).

DISCUSSION

Recent work has clearly established the presence of symmetric adipose depots in the cervical and supraclavicular region of humans which, in a subset of individuals, can be induced to greatly increase FDG tracer uptake in response to cold stress. These depots contain UCP1 positive multilocular adipocytes, and the fat cells within these depots express genes that are known to be enriched in brown adipocytes. In rodent models, cold-induced glucose uptake is correlated with a much larger increase in fatty acid-induced thermogenesis (Ma and Foster, 1986), suggesting that the FDG tracer uptake observed in humans might represent significant cold-induced heat production. Indeed, it has been estimated that 50 g of human BAT having the thermogenic activity of cold-adapted rodent BAT, might expend as much as 20% of daily energy intake (Rothwell and Stock, 1983), and thus might be a rational target for anti-obesity therapeutics. Despite these intriguing and potentially promising results, no experiments have directly evaluated oxidative metabolism in human BAT, or its relation to FDG tracer uptake and total energy expenditure.

Our results confirm recent experiments examining cold-induced FDG uptake in human BAT. This study demonstrates that mild cold exposure leads to high FDG tracer uptake in BAT in about a third of young adult subjects, whereas about half of a group of young subjects shows no FDG tracer uptake, with the rest displaying low-level FDG tracer uptake in scattered supraclavicular BAT depots. Similar to previous reports (Cypess et al., 2009), we found higher mass and activity of BAT in females than in males, with a ratio of ∼3:1. Our study group consisted entirely of young adults, so no relationship between BAT activation and age or BMI was observed. Baseline blood flow in BAT of subjects with high FDG tracer uptake was about twice that of subjects with low FDG uptake (10 vs. 5 ml/100 g/min) and increases by about 50% during cold stress (to ∼15 ml/100 g/min). These values are nearly identical to those recently published by Orava et al. (2011). Moreover, semi-quantitative assessment of glucose metabolism in BAT based on FDG SUVs showed a significant correlation with the calculated MRO_2, suggesting that at least part of the glucose transported into adipose tissue might undergo oxidative metabolism. However, direct measurement of oxidative metabolism indicates that human BAT activation contributes little to total energy expenditure, despite the observed (significantly larger than background) FDG tracer uptake in BAT. Tissue oxygen MRO_2 (consumption) is the product of blood flow and OEF. Moreover, the cold-induced increase in human BAT blood flow observed in the present study corresponds closely to that recently reported by Orava et al. (2011). Our data also includes estimates of OEF, allowing estimation of tissue MRO_2 in the range of 1–3 ml/100 g/min during cold stress, which translates into an energy consumption in activated BAT of less than 20 kcal/day. Even if one assumes 100% OEF, blood flow data from both labs indicate that acutely activated human BAT contributes very little to cold-induced energy expenditure. In light of this data it is unclear whether the glucose that is transported into human BAT during cold stress is stored, oxidized, or released as lactate. Regardless, the energy content of the glucose that is taken up is small and is consistent with low overall contribution of human BAT to total energy expenditure. Intriguingly, a significant cold-induced increase in whole body energy expenditure (average of ∼300 kcal/day) was only observed in subjects demonstrating cold-activated BAT. Nonetheless, our quantitative data indicate that other, systemic effects are the likely cause of

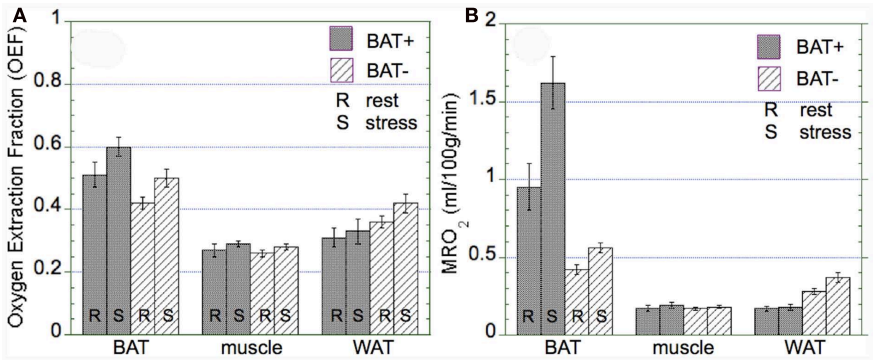

FIGURE 5 | Oxygen extraction fraction (OEF) and absolute metabolic rate of oxygen (MRO_2) in brown adipose tissue (BAT), muscle, and white adipose tissue (WAT) observed in the BAT+ and BAT− groups. Error bars represent the SEM. **(A)** The OEF was similar in both groups in BAT, muscle, and WAT, although OEF in WAT was tended to be lower in the BAT+ group. **(B)** In the BAT+ group, MRO_2 in BAT at rest was about twice as high as that determined in the BAT− group. Moreover, following cold exposure, MRO_2 increased by about 50% in the BAT+ group, but remained at the same level in the BAT− group. In contrast, MRO_2 in WAT was higher in the BAT− group at both rest and cold exposure condition. Finally, MRO_2 in muscle was similar for both groups at rest and following cold exposure.

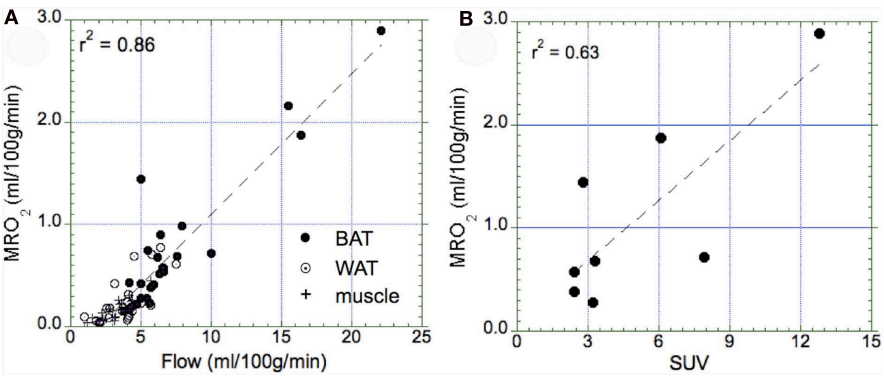

FIGURE 6 | Relationship between the metabolic rate of oxygen and blood flow as well as the maximal standard uptake value (SUV). (A) Correlation between the metabolic rate of oxygen in BAT (full circle), WAT (open circle), and muscle (cross) tissue. We determined a highly significant correlation ($p = 0.01$), indicating that tissue perfusion is the main determinant of oxidative metabolism in all three types of tissue. Highest values of oxidative metabolism was determined in activated BAT tissue (1–3 ml/100 g/min), followed by WAT tissue (0.2–0.5 ml/100 g/min), and muscle tissue (∼0.2 ml/100 g/min). **(B)** Correlation between maximal SUV in BAT and the metabolic rate of oxygen in those subjects who had a SUV > 2.0 (indicative of activated BAT). We found a significant correlation between glucose uptake and oxygen consumption ($p = 0.02$).

increased energy expenditure, and that the elevated FDG uptake in BAT is an epiphenomenon.

Speculation regarding the functional importance of human BAT has often invoked comparisons with rodent models of BAT function. In this regard, the classic experiments by Foster and Frydman (1978, 1979) demonstrated that cold exposure increases BAT blood flow by a factor of 10- to 20-fold, achieving a maximal rate of more than 1000 ml/100 g/min. This rate is 100 times greater than that observed in humans. Similarly, maximal glucose uptake rates in rodent BAT is at least 10-fold greater than that observed in human BAT. As mentioned above, glucose is a minor energy source for rodent thermogenesis. In the case of human BAT, the energy content of the cold-induced glucose uptake reported by Orava et al. (2011) amounts to less than 10 kcal/day, if fully oxidized.

CONCLUSION AND PERSPECTIVES

Our results indicate that BAT thermogenesis in humans accounts for less than 20 kcal/day during moderate cold stress, even in subjects with relatively large BAT depots. Indeed, oxidative metabolism of the imaged BAT depots accounted for less than 2% of the total increase in energy expenditure observed during cold stress in BAT+ subjects. With regard to the potential impact of BAT activation on DEE, we note that moderate physical exercise (∼10 kcal/min) is far more effective than BAT in triggering controlled energy expenditure. The reasons for the relatively small contribution of human BAT, when present, to total thermogenesis is not known. The available histological data indicates that human BAT depots contain a mixture of multilocular brown adipocytes interspersed within a greater volume of unilocular white adipocytes, which have much lower metabolic activity. PET imaging does not have sufficient resolution to localize microscopic patches of brown adipocytes, and thus provides an average metabolic activity of a mixed cell population within the anatomically defined depot. Thus, the low average activity of human BAT depots may reflect the relatively low density of brown adipocytes. If the thermogenic potential of individual brown adipocytes is

similar in humans and rodents, then the abundance of these cells would need to be increased greatly to impact DEE.

Positron emission tomography /CT imaging using the glucose analog FDG has entered clinical routine and although the use of radioactive tracers represents more than minimal risk, the effective dose to participants (in the range of 1–2 rem) is not prohibitive for carefully planned studies. Moreover, because of concerns that some active BAT might not accumulate FDG for unknown reasons, there is a need for other PET tracers that might allow better insight into the mechanisms of BAT activation. In the presented study we directly quantified oxidative metabolism in BAT depots based on independent measurements of both blood flow and oxygen extraction. As any increase in tissue metabolic activity will be necessarily accompanied by increased blood flow and oxygen consumption, we are confident that we were able to accurately assess metabolic activity of BAT, even if FDG accumulation might not have been present. Unfortunately, the applicability of PET imaging using isotopes with short half-life (such as ^{15}O with 2 min) is hindered by the expensive methodology, which requires both an on-site cyclotron as well as sophisticated radio-synthesis modules. As a result, PET studies using short-lived isotopes are likely to be performed only in University research settings, but are ideally suited to study human BAT under various experimental conditions.

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REFERENCES

Blaza, S. (1983). Brown adipose tissue in man: a review. *J. R. Soc. Med.* 76, 213–216.

Cohade, C., Osman, M., Leal, J., and Wahl, R. L. (2003). Direct comparison of (18)F-FDG PET and PET/CT in patients with colorectal carcinoma. *J. Nucl. Med.* 44, 1789–1796.

Cypess, A. M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A. B., Kuo, F. C., Palmer, E. L., Tseng, Y. H., Doria, A., Kolodny, G. M., and Kahn, C. R. (2009). Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* 360, 1509–1517.

Dawkins, M. J., and Scopes, J. W. (1965). Non-shivering thermogenesis and brown adipose tissue in the human new-born infant. *Nature* 206, 201–202.

Ellis, R. K. (1989). Determination of PO2 from saturation. *J. Appl. Physiol.* 67, 902.

Foster, D. O., and Frydman, M. L. (1978). Nonshivering thermogenesis in the rat. II. Measurements of blood flow with microspheres point to brown adipose tissue as the dominant site of the calorigenesis induced by noradrenaline. *Can J. Physiol. Pharmacol.* 56, 110–122.

Foster, D. O., and Frydman, M. L. (1979). Tissue distribution of cold-induced thermogenesis in conscious warm- or cold-acclimated rats reevaluated from changes in tissue blood flow: the dominant role of brown adipose tissue in the replacement of shivering by nonshivering thermogenesis. *Can. J. Physiol. Pharmacol.* 57, 257–270.

Frackowiak, R. S., Lenzi, G. L., Jones, T., and Heather, J. D. (1980). Quantitative measurement of regional cerebral blood flow and oxygen metabolism in man using 15O and positron emission tomography: theory, procedure, and normal values. *J. Comput. Assist. Tomogr.* 4, 727–736.

Hattori, N., Bergsneider, M., Wu, H. M., Glenn, T. C., Vespa, P. M., Hovda, D. A., Phelps, M. E., and Huang, S. C. (2004). Accuracy of a method using short inhalation of (15)O-O(2) for measuring cerebral oxygen extraction fraction with PET in healthy humans. *J. Nucl. Med.* 45, 765–770

Kalliokoski, K. K., Kemppainen, J., Larmola, K., Takala, T. O., Peltoniemi, P., Oksanen, A., Ruotsalainen, U., Cobelli, C., Knuuti, J., and Nuutila, P. (2000). Muscle blood flow and flow heterogeneity during exercise studied with positron emission tomography in humans. *Eur. J. Appl. Physiol.* 83, 395–401.

Kortelainen, M. L., Pelletier, G., Ricquier, D., and Bukowiecki, L. J. (1993). Immunohistochemical detection of human brown adipose tissue uncoupling protein in an autopsy series. *J. Histochem. Cytochem.* 41, 759–764.

Leonard, W. R. (2010). Measuring human energy expenditure and metabolic function: basic principles and methods. *J. Anthropol. Sci.* 88, 221–230.

Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, J. M., Kemerink, G. J., Bouvy, N. D., Schrauwen, P., and Teule, G. J. (2009). Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* 360, 1500–1508.

Ma, S. W., and Foster, D. O. (1986). Uptake of glucose and release of fatty acids and glycerol by rat brown adipose tissue in vivo. *Can. J. Physiol. Pharmacol.* 64, 609–614.

Mintun, M. A., Raichle, M. E., Martin, W. R., and Herscovitch, P. (1984). Brain oxygen utilization measured with O-15 radiotracers and positron emission tomography. *J. Nucl. Med.* 25, 177–187.

Nedergaard, J., Bengtsson, T., and Cannon, B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. *Am. J. Physiol. Endocrinol. Metab.* 293, E444–E452.

Nedergaard, J., Golozoubova, V., Matthias, A., Asadi, A., Jacobsson, A., and Cannon, B. (2001). UCP1: the only protein able to mediate adaptive non-shivering thermogenesis and metabolic inefficiency. *Biochim. Biophys. Acta* 1504, 82–106.

Orava, J., Nuutila, P., Lidell, M. E., Oikonen, V., Noponen, T., Viljanen, T., Scheinin, M., Taittonen, M., Niemi, T., Enerbäck, S., and Virtanen, K. A. (2011). Different metabolic responses of human brown adipose tissue to activation by cold and insulin. *Cell Metab.* 14, 272–279.

Rothwell, N. J., and Stock, M. J. (1983). Luxusconsumption, diet-induced thermogenesis and brown fat: the case in favour. *Clin. Sci. (Lond.)* 64, 19–23.

Ruotsalainen, U., Raitakari, M., Nuutila, P., Oikonen, V., Sipilä, H., Teräs, M., Knuuti, M. J., Bloomfield, P. M., and Iida, H. (1997). Quantitative blood flow measurement of skeletal muscle using oxygen-15-water and PET. *J. Nucl. Med.* 38, 314–319.

Severinghaus, J. W. (1977). Pulmonary vascular function. *Am. Rev. Respir. Dis.* 115, 149–158.

Virtanen, K. A., Lidell, M. E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N. J., Enerbäck, S., and Nuutila, P. (2009). Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* 360, 1553–1556.

Yoneshiro, T., Aita, S., Matsushita, M., Kameya, T., Nakada, K., Kawai, Y., and Saito, M. (2011). Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. *Obesity* 19, 13–16.

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Developments in the imaging of brown adipose tissue and its associations with muscle, puberty, and health in children

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Fusion positron emission and computed tomography (PET/CT) remains the gold-standard imaging modality to non-invasively study metabolically active brown adipose tissue (BAT). It has been widely applied to studies in adult cohorts. In contrast, the number of BAT studies in children has been few. This is largely limited by the elevated risk of ionizing radiation and radionuclide tracer usage by PET/CT and the ethical restriction of performing such exams on healthy children. However, metabolically active BAT has a significantly higher prevalence in pediatric patients, according to recent literature. Young cohorts thus represent an ideal population to examine the potential relationships of BAT to muscle development, puberty, disease state, and the accumulation of white adipose tissue. In turn, magnetic resonance imaging (MRI) represents the most promising modality to overcome the limitations of PET/CT. The development of rapid, repeatable MRI techniques to identify and quantify both metabolically active and inactive BAT non-invasively and without the use of exogenous contrast agents or the need for sedation in pediatric patients are critically needed to advance our knowledge of this tissue's physiology.

Keywords: brown adipose tissue, PET/CT, Hounsfield units, children, MRI, muscle, puberty, white adipose tissue

INTRODUCTION

The recent findings of metabolically active brown adipose tissue (BAT) in adult humans by fusion positron emission and computed tomography (PET/CT) have reinvigorated research toward the tissue's role in energy balance, metabolism, and physiology (Cypess et al., 2009; van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). In contrast to white adipose tissue (WAT), which functions to store fat and consists of cells with large intracellular lipid droplets, BAT is involved in lipid metabolism and both non-shivering and diet-induced thermogenesis, facilitated by its unique uncoupling protein UCP-1 (Rothwell and Stock, 1979; Himms-Hagen, 1989). BAT is also highly vascularized, rich in mitochondria organelles, innervated by the sympathetic nervous system, and most importantly, it is metabolically active and prevalent in newborns and children (Heaton, 1972; Enerback, 2010). In contrast to WAT adipocytes, brown adipocytes are further characterized by multiple smaller intracellular lipid droplets, have lower lipid content by volume, and greater intracellular water (Cannon and Nedergaard, 2004; Cinti, 2006).

While fusion PET/CT has provided the unique ability to non-invasively visualize metabolically active BAT and remains the gold-standard approach, it is now recognized by the scientific community that PET/CT lacks sensitivity and specificity, has key limitations, and that new and alternative imaging techniques need to be developed. First, PET/CT is restricted to oncology patients as a tool for tumor detection and cancer screening. Second, PET/CT is not widely applicable to the general population due to significant radiation exposures, the need to inject a radionuclide tracer, and related prohibitive costs. It is also not suited for longitudinal studies where repeated scans are required. Third, PET/CT can only detect metabolically active BAT that uptakes the injected tracer, which represents only a small percentage of the tissue's total amount in the body. Moreover, tracer uptake by metabolically active BAT is sensitive to factors such as environment and examination room temperature, season, anesthesia, the amount of clothing worn by the patient (Au-Yong et al., 2009; Pfannenberg et al., 2010; Yoneshiro et al., 2011), and the health/disease condition of the subject at exam time. The presence of metabolically active BAT can further be confounded by competing tracer uptake in nearby muscles, organs, and tumors. Perhaps the biggest unmet need in the imaging of BAT by PET/CT is that the endpoint measurement remains a binary visual interpretation of positive or negative depiction of BAT activity, often decided by the reading radiologist. This subjective measure has made systematic comparisons between studies from various investigators difficult with no apparent consensus amongst the literature. Ultimately, the method lacks the ability to generate a quantitative measure of total BAT mass or volume, one however that is critically needed to advance the field. Alternatives such as standalone CT and magnetic resonance imaging (MRI) that can detect BAT based on morphology, regardless of tissue metabolic activity, are necessary to avoid the confounding factors that have limited functional PET/CT.

In this review article, we first survey several unique imaging signatures of BAT that differentiates this tissue from WAT in CT and MRI based solely on morphology. These have been published in recent imaging periodicals. MRI holds promise as the most appropriate non-invasive imaging modality for studying BAT in humans, particularly in newborns and children, since it does not

involve ionizing radiation and can be repeated multiple times with ultra-low risk. In the second part of this review, we summarize the high prevalence of BAT by PET/CT in pediatric patients and findings of BAT associations with muscle volume, puberty, and disease states from studies performed at our institution, Children's Hospital of Los Angeles. Our goal is to inform the reader that (1) due to high BAT prevalence, young cohorts represent an ideal population to study BAT physiology and examine the relationships of BAT to muscle development, puberty, disease states, and body adiposity, and that (2) MRI is the most suitable platform to implement these investigations in pediatric populations.

IMAGING OF BAT

IMAGING SIGNATURES OF BAT IN CT

In PET/CT, standard uptake values (SUVs) have been used as an adjunct to distinguish metabolically active BAT from inactive WAT, based on uptake of a tracer, typically fluoro-deoxyglucose (FDG). By radiology convention CT images of tissues with negative Hounsfield units (HUs) X-ray attenuations have traditionally been interpreted as fat and no additional stratification of the negative HU scale was further considered. In contrast, all other soft tissues and bone occupy the positive HU range. The question of whether there exists a parallel difference in CT HUs and tissue densities between BAT and WAT has only been recently suggested and explored (Carter and Schucany, 2008; Baba et al., 2010; Hu et al., 2011). These studies have found that additional stratification of the negative HU scale is feasible to uniquely differentiate metabolically active BAT and WAT. Namely, metabolically active BAT was characterized by higher, but still negative, HUs than lipid-rich WAT. BAT exhibits higher CT attenuation values than WAT, largely due to its greater intracellular water content and higher degree of vascularization. **Figure 1A** illustrates results from a cohort of pediatric patients who all exhibited positive visual depiction of BAT on their PET/CT exams (Hu et al., 2011). It is important to note that metabolically active BAT and inert WAT data points occupy unique and nearly non-overlapping regions on the CT HU versus PET SUV plot. The use of standalone CT to identify and quantify metabolically active BAT, however, has not yet been corroborated by a large study or validated with histology. Furthermore, whether such HU differences between metabolically inactive BAT and WAT exist has yet to be explored.

IMAGING SIGNATURES OF BAT IN MRI

The use of MRI and magnetic resonance spectroscopy (MRS) to study BAT in rodents was investigated in the 1990s (Sbarbati et al., 1997; Lunati et al., 1999). Inspired by these early works, recent

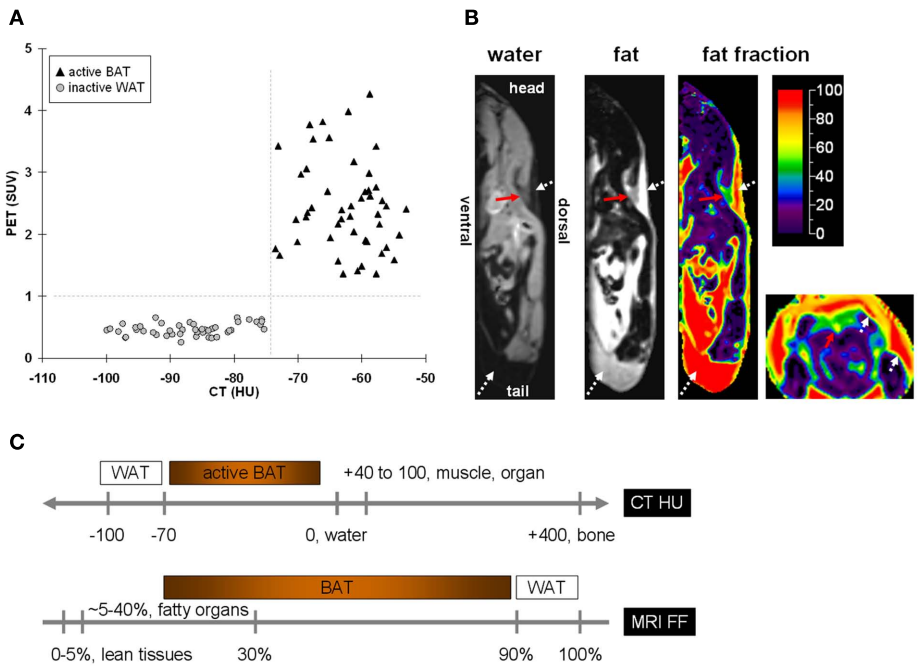

FIGURE 1 | The difference in intracellular water and fat content between BAT and WAT leads to unique signal contrast between the two tissues in (A) PET/CT and (B) MRI. In **(A)** PET/CT, metabolically active BAT and WAT not only differ in their standard uptake values (SUVs) of the radionuclide tracer, but also in their Hounsfield units (HUs) of X-ray attenuation. The greater water content in BAT leads to more positive HUs than WAT. Note that the differences in SUVs and HUs between the two tissues are unique, with no overlap on either axis. In **(B)** MRI, separated water and fat representative images are shown in the sagittal plane of a mouse. Shown additionally is the corresponding fat fraction (FF) map, on a color scale from 0 to 100%. Illustrated to the far right is the fat fraction map in the axial plane from the same animal. The red arrows point to the triangular/trapezoidal interscapular BAT depot. The white dotted arrows denote WAT. Note that BAT has a much lower fat fraction than WAT, caused by BAT's greater water and lower fat content, which can be observed by the opposing grayscale contrast differences in the water and fat images, respectively. **(C)** A schematic of approximate BAT and WAT signal contrasts based on CT HU and MRI FF.

methodological advances in both MRI and MRS methodology have led to several promising literature publications describing more robust and accurate characterizations of rodent BAT. Unlike PET/CT where the functional uptake of a radionuclide tracer was needed to differentiate BAT from WAT, one common theme among these MR-based works was the use of only morphological differences between BAT and WAT for identification and signal contrast. Specifically, the greater vascularity, mitochondria number, and intracellular water in closer proximity to lipid droplets of BAT when compared to WAT have been exploited.

Using chemical-shift water–fat separation MRI techniques that can accurately decompose lean (water-based) and fatty (lipid-based) signals across an imaging volume on a voxel-wise basis, Hu et al. (2010a) demonstrated in mice the feasibility of differentiating BAT and WAT based on a fat fraction metric in wild-type mice. A fat fraction map was computed from the separated water and fat signals. Whereas WAT is predominantly composed of lipids and is thus typically characterized by a fat fraction >90%, interscapular BAT was shown to occupy a much broader and lower fat fraction range. **Figure 1B** illustrates a representative example. **Figure 1C** summarizes the approximate tissue signal contrast between BAT and WAT by CT and chemical-shift MRI. In a follow-up study, the investigators further showed that their technique could detect differences in BAT fat fraction among mice groups that were housed at cold (19˚C) and warm (25˚C) temperatures. Given *ad libitum* access to food, mice exposed to colder temperature consumed significantly more food (∼30%), exhibited lower BAT fat fractions (∼10% difference), but yet maintained the similar body weight, and composition, as determined by quantitative magnetic resonance (QMR), in comparison to warm-housed animals over a 4-week period (Hu et al., 2010b). The lower BAT fat fractions of the cold-exposed animals suggested greater metabolic activity and involvement of the tissue in thermogenesis. One limitation of this chemical-shift MRI approach is the need for high spatial resolution to avoid partial volume effects that can mimic BAT-like fat fractions at fat–lean tissue boundaries.

In another recent study, Branca and Warren (2011a) reported a novel and promising spectroscopy technique utilizing unique intermolecular zero-quantum coherence transitions between water and fat protons, specifically those of the methylene ($–CH_2–$) groups, to differentiate BAT from WAT (Branca and Warren, 2011a). The approach exploited the fact that in BAT, intracellular water and fat are in close physical vicinity to each other, and thus gives rise to distinctive iZQC signal peaks. On the contrary, in WAT, water, and fat are separated by greater distances and thus such iZQC peaks are not observed. Furthermore, the authors demonstrated that the amplitude of the signal peaks were substantially greater in more thermogenically active young mice than in old or obese mice, signifying increased amounts of metabolically active BAT in the former group. However, in BAT, and WAT, iZQC signal peaks from interactions between lipid methylene and olefinic groups were both observed. The critical methodological advancement was that iZQC allowed the investigators to perform a survey-like scan and unambiguously determine whether BAT is simply present in an imaging volume based on the tissue's unique water–fat signal peaks. In a related follow-up study by Branca and Warren (2011b) the researchers used a variant of their spectroscopy technique to determine differences in the chemical composition of adipose tissue, such as triglyceride degree of unsaturation, in mice fed with either standard chow or fatty diets (Branca and Warren, 2011b).

In another spectroscopy study by Hamilton et al. (2011), the authors utilized conventional high-resolution proton MRS to first confirm the finding that BAT has a uniquely lower fat fraction than BAT. The investigators then discovered that the T_1 relaxation time of the water component in BAT was significantly reduced by nearly twofold from that in WAT. Briefly, the MRI/MRS signal arises from hydrogen protons in water and fat. When an object is placed inside a magnetic field, a longitudinal magnetization from the proton ensemble is created. In an MR experiment, radiofrequency pulses are applied repetitively to gather imaging data. The pulses excite the longitudinal magnetization and perturb it away from equilibrium. T_1 is a tissue-specific exponential constant that describes the rate with which the perturbed magnetization recovers between consecutive radiofrequency excitation. The authors speculated that the source of this apparent T_1 contrast was due to the fact that a molecule's T_1 rate is primarily governed by its surrounding chemical environment. In contrast to WAT where intracellular water is confined to a small globule, water molecules in BAT are more interspersed and in greater contact with lipids. Moreover, the researchers were able to quantify an average number of carbon–carbon double bonds from the MR spectra, and reported that BAT was more saturated than WAT, consistent with previous findings (Zingaretti et al., 2009). An extended imaging approach for carbon–carbon double-bond mapping was also recently reported (Bydder et al., 2011) and its application to BAT is under investigation. This MRS approach may not be adequate for the independent identification of BAT since *a priori* knowledge of tissue depot location is required. Nonetheless, the technique should serve as a useful adjunct tool for characterizing BAT properties.

Magnetic resonance is the most appropriate modality to address the shortcomings of PET/CT. It involves no ionizing radiation and requires no radionuclide tracer. It can be safely repeated multiple times with minimal risk in healthy volunteers and is applicable to large study populations and nearly all age groups, including children. The works demonstrated by Hu et al. (2010a,b); Branca and Warren (2011a,b); Hamilton et al. (2011) in identifying unique BAT imaging signatures from MRI and MRS represent the modality's immense versatility. Their results represent exciting new potential from the fact that none of the described MR-based signals of BAT depend on the tissue's metabolic functions, but rather its intrinsic cellular morphology. Validation and application of these MR-based methods to humans remains an area of future research. However, it is unlikely that a single MR-based approach will definitively capture both metabolically active and inactive BAT (Carter and Schucany, 2008). Many additional distinctive features of BAT are known but have not yet been explored by MRI and MRS. For example, BAT's immense vasculature lends itself to well-established MRI perfusion techniques such as arterial-spin-labeling that can quantify local blood flow. MR techniques that are sensitive to T_2^* relaxation as a consequence of fluctuating levels of oxy- and deoxy-hemoglobin in blood are being evaluated in BAT by multiple investigators. Signal contrasts between BAT and WAT, such as diffusion and targeted molecular imaging of the mitochondria, have not been explored. Lastly, recent advances in fusion PET/MR also holds great promise as an additional complementary

tool for imaging BAT and will likely play a critical role in validating a subset of aforementioned standalone MRI techniques in future studies.

BAT IN PEDIATRICS

PREVALENCE OF BAT IN CHILDREN

Based on previous anatomical studies, BAT was known to be present in all neonates but thought to be lost after infancy (Enerback, 2010). However, significant amounts of metabolically active BAT have recently been noticed in a large proportion of pediatric patients undergoing PET/CT examinations (Celi, 2009). While BAT is seen in similar locations in adults and children, a significantly higher prevalence of nearly 10-fold increase in BAT depiction in pediatric PET/CT examinations (Gelfand et al., 2005; Zukotynski et al., 2010; Gilsanz et al., 2011; Hu et al., 2011) has been reported in comparison to the prevalence in adults (Cypess et al., 2009). Based our own experiences, approximately 40% of PET/CT studies in young patients depict metabolically BAT, even when they live in warm climates and when the PET/CT exams are performed under thermoneutral conditions (Gilsanz et al., 2011).

A previous study by Gelfand et al. (2005) found that the incidence of BAT uptake in adolescents was greater than in patients younger than 10 years. More recently, using qualitative and quantitative approaches to analyze PET/CT exams, Gilsanz et al. (2011) reported a higher prevalence and significantly greater amounts of BAT in adolescence than during childhood. In that study, whereas less than 20% of studies in pre-pubertal girls or boys exhibited uptake of FDG by metabolically active BAT, more than 75% of such studies in pubertal teenagers displayed visually identifiable BAT uptake. Additionally, the volume of metabolically active BAT measured using CT HU and PET SUV metrics increased with adolescence in both sexes. The magnitude of the increase in BAT was substantially greater during the late stages of puberty (e.g., Tanner stages) than during the early phases of sexual development and was substantially higher in boys when compared to girls.

Numerous metabolic and hormonal changes occur during puberty, including increases in the production of growth hormone/growth factors, gonadotropins, and sex steroid hormones. Variations in one or more of these important modifiers known to influence muscle development could also account for the increases and gender dimorphism in BAT development during adolescence. Recent data suggest that sex hormones have a marked effect on BAT activity, with corroborating studies demonstrating that associated receptor expression differs in cell-cultured brown adipocytes of male and female rodents, with higher numbers of all receptor types in male BAT (Rodriguez-Cuenca et al., 2007).

ASSOCIATION OF BAT TO DISEASE

In children, like in adults, the depiction of metabolically active BAT by PET/CT has been reported to be dependent on a multitude of physiologic and technical factors including age, sex, body composition, FDG dose, anesthesia, modality acquisition parameters, and season and temperature during examinations (Cohade et al., 2003; Au-Yong et al., 2009; Saito et al., 2009; Lee et al., 2010; Pfannenberg et al., 2010; Yoneshiro et al., 2011). In PET/CT studies of adult cancer patients, the visualization of metabolically active BAT was predominantly thought to be independent of disease status, and to date, no association has been noted between the prevalence of BAT depiction and the presence of metastases. One previous longitudinal investigation assessing changes in BAT in females with breast cancer found no association between tumor response during treatment and activation of the tissue (Rousseau et al., 2006). At our institution, we have recently observed several cases where a patient with a malignant lymphoma exhibited metabolically active BAT only when evidence of the disease was not present on the PET/CT images (**Figure 2**). This prompted the speculation that the prevalence of BAT in pediatric patients with lymphoma is greater when there is no evidence of disease than at diagnosis. In 62 studies to date involving 30 pediatric patients at our institution, we have found that approximately 10% of the exams at diagnosis exhibited uptake of FDG tracer by metabolically active BAT, while a significantly higher 80% of follow-up exams after therapeutic treatment displayed BAT when there was no longer evidence of disease.

The mechanisms responsible for the possible suppression of BAT activity by lymphoma are unknown. However, we speculate that patients with malignant lymphomas have high circulating levels of tumor necrosis factor-alpha (TNF-α; Salles et al., 1996;

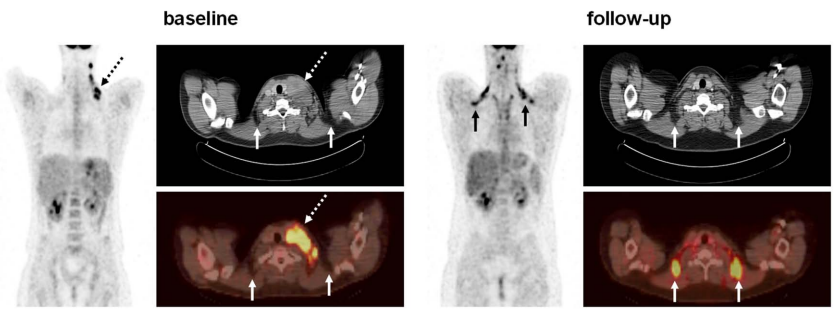

FIGURE 2 | Coronal PET, and registered axial CT and fusion PET/CT of a 16-year-old male patient at baseline and 36 weeks follow-up after chemotherapy treatment. At baseline, the subject exhibits no evident bilateral and symmetrical FDG uptake by interscapular BAT (solid arrows). However, the presence of disease is clearly visualized by strong unilateral FDG uptake of the enlarged cervical lymph nodes (dashed arrows). On the CT image, note that the lymph node mass is characterized by soft-tissue-like attenuation. At follow-up, the subject exhibits clear bilateral FDG uptake indicative of BAT. This is confirmed on the CT images which show negative fat-like attenuation (solid arrows). In contrast, the corresponding BAT depots on the baseline axial images do not exhibit FDG uptake. Note that the enlarged lymph node has been successfully treated.

Warzocha et al., 1997), a pluripotent cytokine reported to elicit a large number of biological effects in different cell systems including the apoptotic degeneration of brown adipocytes (Nisoli et al., 1997). This cytotoxic effect is known to be mediated by the p55 TNF-α receptor subtype, and its deletion has been shown to increase thermogenesis with an associated increase of uncoupling protein-1 expression in BAT (Romanatto et al., 2009). The finding of an apparent association between disease state and BAT depiction is still at an early stage, and additional research is needed to better establish the roles of TNF-α on BAT homeostasis.

ASSOCIATION OF BAT TO MUSCLE

Adipose and musculoskeletal tissues originate from the mesoderm, and the prevailing model has been that a common stem cell gives rise to WAT, BAT, bone, and muscle. In an intermediate developmental step, it was assumed that WAT and BAT share a common precursor because both forms of adipose tissue express a similar array of genes involved in triglyceride metabolism. However, although it is possible to convert WAT precursors into brown adipocyte-like cells, evidence also suggests that brown adipocytes are developmentally close to skeletal muscle. An *in vivo* study by Seale et al. (2008) indicated that some brown adipocytes arise from the same Myf5-expressing progenitor as skeletal muscle cells, whereas white adipocytes did not show such commonality. Consistent with this notion are additional data indicating that brown adipocytes and skeletal muscle cells share many common features: an abundance of mitochondria, energy expenditure via oxidative phosphorylation, and sympathetically mediated adaptive thermogenesis.

In a recent study by Gilsanz et al. (2011) young patients with metabolically active BAT on PET/CT examinations were observed to have significantly greater muscle volume than patients of the same age and body-mass-index with no visibly identifiable BAT on PET/CT. This finding was present in the axial and appendicular skeletons of both male and female patients. Gilsanz et al. (2011) reported that on average, pediatric cancer patients who exhibited visually evident BAT on their PET/CT exams had approximately 50% greater neck volume and approximately 33% greater gluteal muscle volume than patients who did not exhibit BAT FDG uptake. **Figure 3** summarizes some additional results from that study.

Lastly, studies in adults have consistently shown an inverse relation between body mass, body fat, and BAT (van Marken Lichtenbelt et al., 2009; Yoneshiro et al., 2011). However, since BAT decreases while WAT increases with age, attempts to make an unequivocal connection between these two age dependent tissues has been difficult. Establishing whether increased obesity is inversely associated with BAT in normal pediatric populations can have important implications that can lead to potential strategies to combat obesity and its comorbidities (Lee et al., 2011).

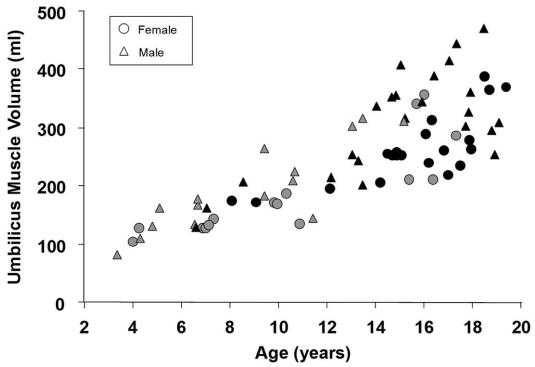

FIGURE 3 | Scatter plot of muscle volume versus age for male and female pediatric patients with (black, BAT+) and without (gray, BAT−) visually depicted metabolically active BAT by PET/CT. For males without visualized BAT (gray triangles), the linear correlation coefficient was $r = 0.88$; for males with visualized BAT (black triangles), the coefficient was $r = 0.72$; for females without visualized BAT (gray circles), the coefficient was $r = 0.85$; for females with visualized BAT (black circles), the coefficient was $r = 0.78$. All correlations were statistically significant with $p < 0.001$.

SUMMARY

With the resurgent discovery of human BAT by PET/CT, new frontiers are now being pursued to study the modulation of this tissue during human growth and development and its involvement in regulating body metabolism, energy expenditure and body composition, especially in children and adolescents. These issues are of high relevance to public healthcare as the obesity epidemic affecting all ages across Western countries continues to spread. MRI represents the most suitable non-invasive imaging modality to overcome the limitations of PET/CT. MRI methods that can detect BAT based on intrinsic tissue morphology are minimally susceptible to environmental and extraneous confounding factors that have thus far restricted functional PET/CT. The demonstration of feasibility in employing such MRI methods in humans, particularly in pediatrics, will be the next major advancement. Progress in knowledge of human BAT physiology will come from MRI methodological developments that will allow the identification and quantification of both metabolically active and inactive BAT with high sensitivity and specificity.

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REFERENCES

Au-Yong, I. T., Thorn, N., Ganatra, R., Perkins, A. C., and Symonds, M. E. (2009). Brown adipose tissue and seasonal variation in humans. *Diabetes* 58, 2583–2587.

Baba, S., Jacene, H. A., Engles, J. M., Honda, H., and Wahl, R. L. (2010). CT Hounsfield units of brown adipose tissue increase with activation: preclinical and clinical studies. *J. Nucl. Med.* 51, 246–250.

Branca, R. T., and Warren, W. S. (2011a). In vivo brown adipose tissue detection and characterization using water-lipid intermolecular zero-quantum coherences. *Magn. Reson. Med.* 65, 313–319.

Branca, R. T., and Warren, W. S. (2011b). In vivo NMR detection of diet-induced changes in adipose tissue composition. *J. Lipid Res.* 52, 833–839.

Bydder, M., Girard, O., and Hamilton, G. (2011). Mapping the double bonds in triglycerides. *Magn. Reson. Imaging* doi: 10.1016/j.mri.2011.07.004. [Epub ahead of print].

Cannon, B., and Nedergaard, J. (2004). Brown adipose tissue: function and

physiological significance. *Physiol. Rev.* 84, 277–359.
Carter, B. W., and Schucany, W. G. (2008). Brown adipose tissue in a newborn. *Proc. (Bayl. Univ. Med. Cent.)* 21, 328–330.
Celi, F. S. (2009). Brown adipose tissue – when it pays to be inefficient. *N. Engl. J. Med.* 360, 1553–1556.
Cinti, S. (2006). The role of brown adipose tissue in human obesity. *Nutr. Metab. Cardiovasc. Dis.* 16, 569–574.
Cohade, C., Mourtzikos, K. A., and Wahl, R. L. (2003). "USA-Fat": prevalence is related to ambient outdoor temperature-evaluation with 18F-FDG PET/CT. *J. Nucl. Med.* 44, 1267–1270.
Cypess, A. M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A. B., Kuo, F. C., Palmer, E. L., Tseng, Y. H., Doria, A., Kolodny, G. M., and Kahn, C. R. (2009). Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* 360, 1509–1517.
Enerback, S. (2010). Human brown adipose tissue. *Cell Metab.* 11, 248–252.
Gelfand, M. J., O'Hara, S. M., Curtwright, L. A., and Maclean, J. R. (2005). Pre-medication to block 18F-FDG uptake in the brown adipose tissue of pediatric and adolescent patients. *Pediatr. Radiol.* 35, 984–990.
Gilsanz, V., Chung, S. A., Jackson, H., Dorey, F. J., and Hu, H. H. (2011). Functional brown adipose tissue is related to muscle volume in children and adolescents. *J. Pediatr.* 158, 722–726.
Hamilton, G., Bydder, M., Smith, D. L. Jr., Nayak, K. S., and Hu, H. H. (2011). Properties of brown and white adipose tissue measured by 1H MRS. *J. Magn. Reson. Imaging* 34, 468–473.
Heaton, J. M. (1972). The distribution of brown adipose tissue in humans. *J. Anat.* 112, 35–39.
Himms-Hagen, J. (1989). Brown adipose tissue thermogenesis and obesity. *Prog. Lipid Res.* 28, 67–115.
Hu, H. H., Chung, S. A., Nayak, K. S., Jackson, H. A., and Gilsanz, V. (2011). Differential computed tomographic attenuation of metabolically active and inactive adipose tissues: preliminary findings. *J. Comput. Assist. Tomogr.* 35, 65–71.
Hu, H. H., Smith, D. L. Jr., Nayak, K. S., Goran, M. I., and Nagy, T. R. (2010a). Identification of brown adipose tissue in mice with fat-water IDEAL-MRI. *J. Magn. Reson. Imaging* 31, 1195–1202.
Hu, H. H., Smith, D. L. Jr., Goran, M. I., Nagy, T. R., and Nayak, K. S. (2010b). Characterization of brown adipose tissue in mice with IDEAL fat-water MRI. *Proc. Int. Soc. Magn. Reson. Med.* 18, 749.
Lee, P., Greenfield, J. R., Ho, K. K., and Fulham, M. J. (2010). A critical appraisal of the prevalence and metabolic significance of brown adipose tissue in adult humans. *Am. J. Physiol. Endocrinol. Metab.* 299, E601–E606.
Lee, P., Swarbrick, M. M., Ting, Z. J., and Ho, K. K. (2011). Inducible brown adipogenesis of supraclavicular fat in adult humans. *Endocrinology*. doi: 10.1210/en.2011-1349. [Epub ahead of print].
Lunati, E., Marzola, P., Nicolato, E., Fedrigo, M., Villa, M., and Sbarbati, A. (1999). In vivo quantitative lipidic map of brown adipose tissue by chemical shift imaging at 4.7 Tesla. *J. Lipid Res.* 40, 1395–1400.
Nisoli, E., Briscini, L., Tonello, C., De Giuli-Morghen, C., and Carruba, M. O. (1997). Tumor necrosis factor-alpha induces apoptosis in rat brown adipocytes. *Cell Death Differ.* 4, 771–778.
Pfannenberg, C., Werner, M. K., Ripkens, S., Stef, I., Deckert, A., Schmadl, M., Reimold, M., Haring, H. U., Claussen, C. D., and Stefan, N. (2010). Impact of age on the relationships of brown adipose tissue with sex and adiposity in humans. *Diabetes* 59, 1789–1793.
Rodriguez-Cuenca, S., Monjo, M., Frontera, M., Gianotti, M., Proenza, A. M., and Roca, P. (2007). Sex steroid receptor expression profile in brown adipose tissue: effects of hormonal status. *Cell. Physiol. Biochem.* 20, 877–886.
Romanatto, T., Roman, E. A., Arruda, A. P., Denis, R. G., Solon, C., Milanski, M., Moraes, J. C., Bonfleur, M. L., Degasperi, G. R., Picardi, P. K., Hirabara, S., Boschero, A. C., Curi, R., and Velloso, L. A. (2009). Deletion of tumor necrosis factor-alpha receptor 1 (TNFR1) protects against diet-induced obesity by means of increased thermogenesis. *J. Biol. Chem.* 284, 36213–36222.
Rothwell, N. J., and Stock, M. J. (1979). A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 281, 31–35.
Rousseau, C., Bourbouloux, E., Campion, L., Fleury, N., Bridji, B., Chatal, J. F., Resche, I., and Campone, M. (2006). Brown fat in breast cancer patients: analysis of serial 18F-FDG PET/CT scans. *Eur. J. Nucl. Med. Mol. Imaging* 33, 785–791.
Saito, M., Okamatsu-Ogura, Y., Matsushita, M., Watanabe, K., Yoneshiro, T., Nio-Kobayashi, J., Iwanaga, T., Miyagawa, M., Kameya, T., Nakada, K., Kawai, Y., and Tsujisaki, M. (2009). High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* 58, 1526–1531.
Salles, G., Bienvenu, J., Bastion, Y., Barbier, Y., Doche, C., Warzocha, K., Gutowski, M. C., Rieux, C., and Coiffier, B. (1996). Elevated circulating levels of TNFalpha and its p55 soluble receptor are associated with an adverse prognosis in lymphoma patients. *Br. J. Haematol.* 93, 352–359.
Sbarbati, A., Guerrini, U., Marzola, P., Asperio, R., and Osculati, F. (1997). Chemical shift imaging at 4.7 Tesla of brown adipose tissue. *J. Lipid Res.* 38, 343–347.
Seale, P., Bjork, B., Yang, W., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scime, A., Devarakonda, S., Conroe, H. M., Erdjument-Bromage, H., Tempst, P., Rudnicki, M. A., Beier, D. R., and Spiegelman, B. M. (2008). PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454, 961–967.
van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, J. M., Kemerink, G. J., Bouvy, N. D., Schrauwen, P., and Teule, G. J. (2009). Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* 360, 1500–1508.
Virtanen, K. A., Lidell, M. E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N. J., Enerback, S., and Nuutila, P. (2009). Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* 360, 1518–1525.
Warzocha, K., Salles, G., Bienvenu, J., Bastion, Y., Dumontet, C., Renard, N., Neidhardt-Berard, E. M., and Coiffier, B. (1997). Tumor necrosis factor ligand-receptor system can predict treatment outcome in lymphoma patients. *J. Clin. Oncol.* 15, 499–508.
Yoneshiro, T., Aita, S., Matsushita, M., Okamatsu-Ogura, Y., Kameya, T., Kawai, Y., Miyagawa, M., Tsujisaki, M., and Saito, M. (2011). Age-related decrease in cold-activated brown adipose tissue and accumulation of body fat in healthy humans. *Obesity (Silver Spring)* 19, 1755–1760.
Zingaretti, M. C., Crosta, F., Vitali, A., Guerrieri, M., Frontini, A., Cannon, B., Nedergaard, J., and Cinti, S. (2009). The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J.* 23, 3113–3120.
Zukotynski, K. A., Fahey, F. H., Laffin, S., Davis, R., Treves, S. T., Grant, F. D., and Drubach, L. A. (2010). Seasonal variation in the effect of constant ambient temperature of 24 degrees C in reducing FDG uptake by brown adipose tissue in children. *Eur. J. Nucl. Med. Mol. Imaging* 37, 1854–1860.

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Recruitment of brown adipose tissue as a therapy for obesity-associated diseases

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Brown adipose tissue (BAT) has been recognized for more than 20 years to play a key role in cold-induced non-shivering thermogenesis (CIT, NST), and body weight homeostasis in animals. BAT is a flexible tissue that can be recruited by stimuli (including small molecules in animals), and atrophies in the absence of a stimulus. In fact, the contribution of BAT (and UCP1) to resting metabolic rate and healthy body weight homeostasis in animals (rodents) is now well established. Many investigations have shown that resistance to obesity and associated disorders in various rodent models is due to increased BAT mass and the number of brown adipocytes or UCP1 expression in various depots. The recent discovery of active BAT in adult humans has rekindled the notion that BAT is a therapeutic target for combating obesity-related metabolic disorders. In this review, we highlight investigations performed in rodents that support the contention that activation of BAT formation and/or function in obese individuals is therapeutically powerful. We also propose that enhancement of brown adipocyte functions in white adipose tissue (WAT) will also regulate energy balance as well as reduce insulin resistance in obesity-associated inflammation in WAT.

Keywords: human, brown adipose tissue, BAT, progenitors, recruitment, therapy

BAT MASS/ACTIVITY CAN BE ENHANCED WITH DRUGS, INDUCING LONG-TERM BODY WEIGHT LOSS AND IMPROVEMENT OF DIABETES IN ANIMALS

The efficacy of increasing brown adipose tissue (BAT) recruitment (BAT mass and expression of UCP1) as a therapeutic approach for obesity and type 2 diabetes has been demonstrated by many groups (Holloway et al., 1992; Himms-Hagen et al., 1994; Kopecky et al., 1995, 1996a; Nagase et al., 1996; Collins et al., 1997; Ghorbani and Himms-Hagen, 1997; Arch, 2002; Kim et al., 2006). Agents that increase BAT recruitment and UCP1 levels (for instance β_3-AR agonists) can effectively treat obesity and insulin resistance/diabetes in all rodent models of obesity (Arch, 2002). Interestingly, in lean animals (which have normal amounts of BAT), enhancement of BAT recruitment or activity (e.g., by drugs or cold exposure) does not affect body weight, and the induced increase in metabolic rate is compensated by an increase in food intake. However, in obese animals enhancement of energy expenditure by BAT recruitment seems to be the most effective (long-term) therapy for decreasing body weight and improving the metabolic status (as compared to treatment with diet drugs). Indeed, increasing or restoring normal levels of BAT mass/UCP1 levels in obese rodents prevents the adaptive drop in metabolic rate invariably observed upon weight loss due to a decrease in food intake (Dulloo and Girardier, 1990; Leibel et al., 1995; Crescenzo et al., 2003; Heilbronn and Ravussin, 2003; Dulloo, 2005, 2007; Major et al., 2007). It seems that increasing BAT mass/UCP1 levels resets the "adipostat" to a lower level (Cannon and Nedergaard, 2009). Weight/fat loss can be maintained long-term only if the adipostat is readjusted to a lower level. The mechanisms participating in this adipostat are not known in detail but BAT (increasing BAT recruitment/activity) seems to play an important role in this system (Cannon and Nedergaard, 2009). Gastric bypass (Roux-en-Y) surgery has been shown to have dramatic effects on body weight and blood glucose homeostasis/glucose metabolism. The molecular mechanisms responsible for this efficacy are not yet fully understood but recent data suggest that BAT recruitment plays a key role (Stylopoulos et al., 2009), again supporting an adipostat-lowering effect of BAT.

Recently, Almind et al. (2007) showed that the capacity to induce BAT/UCP1 expression around muscles confers resistance to obesity in mice. Similarly, Nagase et al. (1996) showed that treatment of obese yellow KK mice with the β_3-AR agonist CL 316243 decreased the body weight and fat of the mice, and induced expression of UCP1 in skeletal muscle [as well as in BAT and white adipose tissue (WAT)]. Even though the authors contend that the β_3-AR agonist induced UCP1 in myofibers, we can speculate that in fact the UCP1 mRNA detected in the muscle mass indeed originated from induced brown adipocytes around the muscle fibers, and that the "UCP" signal identified with an UCP1 antibody in the myofibers was in fact increased levels of UCP3 (Boss et al., 1997). These results support, in a more physiological setting, earlier results showing that overexpression of UCP1 in WAT of mice can prevent the development of obesity in genetic as well as dietary models of the disease (Kopecky et al., 1995, 1996a,b). In contrast, lack of BAT or UCP1 (at or close to thermoneutrality) induces obesity and diabetes in mice (Lowell et al., 1993; Hamann et al., 1996; Feldmann et al., 2009). Initially, it was hoped that β_3-AR agonists would also enhance energy expenditure in humans but these drugs proved to be ineffective probably due to less than optimal bioavailability and/or pharmacokinetic properties of the

compounds (Arch, 2002, 2008). Another likely explanation is that, unlike in rodents, in humans the β_3-AR is expressed at much lower levels than the β_1-AR and β_2-AR in adipose tissues (WAT and BAT; Deng et al., 1996; Arch, 2008). Induction of brown adipocyte formation with drugs in humans, in order to enhance or restore healthy levels of BAT recruitment, is a feasible strategy to enhance energy expenditure but "druggable" molecular targets other than the β_3-AR and PPARγ have yet to be identified (Harper et al., 2004; Ravussin and Kozak, 2004).

RECRUITMENT OF BROWN ADIPOCYTES FROM PROGENITORS

Various studies have shown that primary preadipocytes isolated from white and brown adipose depots differentiate *in vitro* into the corresponding white and brown adipocytes (Kopecky et al., 1990; Rehnmark et al., 1990; Ailhaud et al., 1992). Multilocular fat cells, expressing UCP1 and rich in mitochondria, were initially observed in a WAT depot by Young et al. (1984). The emergence of these so-called ectopic brown adipocytes in WAT could be induced by cold acclimation in rats (Cousin et al., 1992, 1996) and mice (Loncar, 1991a,b; Guerra et al., 1998). This phenomenon is generally referred to as recruitment. The new cells were found to be sympathetically innervated (Giordano et al., 1996) and remained in WAT as long as a sympathetic stimulation persisted (Loncar, 1991b). Subsequent reports have shown that administration of selective β_3-AR agonists like CL 316243 in mice could also induce the emergence of brown adipocytes in WAT depots (Nagase et al., 1996; Collins et al., 1997; Ghorbani and Himms-Hagen, 1997; Guerra et al., 1998; Granneman et al., 2005) and that this phenomenon was strongly dependent on the mouse genetic background (Collins et al., 1997; Guerra et al., 1998; Kozak and Koza, 1999). Interestingly, it was discovered that transgenic overexpression of the human β_1-AR in WAT of mice also induced the appearance of abundant brown adipocytes in this tissue (Soloveva et al., 1997). These results suggested that the β_3-AR might not be the only β-subtype controlling the emergence of brown adipocytes in WAT. These results were recently confirmed using human multipotent adipose-derived stem cells (hMADS; Mattsson et al., 2011). However, administration of β_1-AR agonists would not be appropriate for the treatment of obesity due to the well-known effects of these agents on the heart.

The origin and the true nature of the multilocular cells rich in mitochondria and expressing UCP1 that appeared in WAT upon cold acclimation or β_3-AR stimulation has yet to be determined. The presence of brown adipocyte progenitors in WAT has been hypothesized by studies showing that 10–15% of the precursor cells isolated from mouse WAT differentiate into brown adipocytes in culture (Klaus et al., 1995) and that brown adipocyte progenitors are present in human WAT depots (Digby et al., 1998). Another hypothesis suggests that a few unilocular white adipocytes are indeed "masked" brown adipocytes that can recover a brown phenotype in response to a SNS stimulation such as that induced by cold exposure. Himms-Hagen et al. (2000), studying the effect of CL 316243 in rats, suggested that the multilocular cells expressing UCP1 that appeared in the WAT were different from the classical brown adipocytes and postulated that they might derive, at least in part, from pre-existing unilocular adipocytes. Orci et al. (2004) showed that hyperleptinemia in rats induces the transformation of white adipocytes into so-called post-adipocytes (or fat-oxidizing machines), which have the phenotype of brown adipocytes. Other effectors that enhance brown adipocyte recruitment in white depots include synthetic PPARγ ligands such as thiazolidinediones (Wilson-Fritch et al., 2004; Xue et al., 2005; Vernochet et al., 2009; Petrovic et al., 2010). Overall, it seems very likely that, at least in some WAT depots, brown adipocytes can emerge from differentiation of brown adipocyte precursors/preadipocytes or transdifferentiation of existing white adipocytes (Jimenez et al., 2003; Zingaretti et al., 2009). The precise origins of brown cells in WAT will likely be determined within the very near future since recent studies have started to identify the progenitors of brown as well as white adipocytes. In the case of brown cells, tracing the lineages arising from progenitors expressing the myogenic transcription factor, myf5 have clearly shown that brown adipocytes within the interscapular BAT depot of mice share an origin with skeletal myocytes that arise from the dermomyotome (Seale et al., 2008). In these investigations, the brown cells recruited to WAT in response to the cold were myf5 negative, thus unlikely to share a myogenic origin. Two independent studies employing different procedures have identified white progenitors within the microvasculature of adipose tissue and not of other tissues (Rodeheffer et al., 2008; Tang et al., 2008). These progenitors express markers of mural cells (pericytes) that arise from the sclerotome and give rise to several other cell types of the vasculature. It is conceivable; therefore, that recruitment of WAT brown adipocytes is due to a selective activation of these mural cells to progress along a brown lineage in response to effectors that are activated by the recruitment-associated stimulus. Possible effectors include BMP7, which has been shown to induce the conversion of mesenchymal stem cells to brown adipocytes in culture and is required for BAT formation in mice (Tseng et al., 2008).

HUMAN ADULTS HAVE FUNCTIONAL, ACTIVATABLE BAT

Understanding the importance of BAT in energy balance has entered a new and exciting era of investigation now that the existence and functionality of this tissue in adult humans has convincingly been demonstrated (Heaton, 1972; Rothwell and Stock, 1979; Hany et al., 2002; Cohade et al., 2003; Bar-Shalom et al., 2004; Cannon and Nedergaard, 2004). Early studies by Rothwell and Stock (1979) using infrared thermography resulted in data strongly suggesting that adult humans possess functional BAT that is activated by ephedrine. The authors suggested that if this tissue contributes to diet-induced thermogenesis as it does in animals it could play an important role in energy balance and body weight homeostasis. It is unfortunate that it took almost 30 years, and many studies using more recent techniques, for the research community to accept that adult humans have physiologically significant amounts of BAT, and that defective recruitment or activity of this tissue may contribute to weight gain and insulin resistance.

The new data show that adult humans have appreciable amounts of BAT, that the metabolic activity or rather, glucose uptake activity is activated by cold, and that the amount of BAT in individuals is inversely correlated (independently) to body weight and age (Celi, 2009; Cypess et al., 2009; Farmer, 2009; Saito et al., 2009; Yoneshiro et al., 2011). A role for BAT in regulating human

body weight homeostasis is strongly suggested by the numerous reports that found the amount of BAT (detected by PET) in individuals is inversely correlated to body weight, independent of age, and other factors.

The capacity of BAT to be recruited in humans, as in animals, is convincingly supported by the following data: (a) The existence of a reservoir of brown adipocyte/BAT progenitor cells in human muscle (CD34+) and WAT depots (hMADS; Crisan et al., 2008; Elabd et al., 2009); (b) Cold stimulates BAT activity and energy expenditure (thermogenesis) in humans (Huttunen et al., 1981; Cohade et al., 2003; Garcia et al., 2004, 2006; Christensen et al., 2006; Nedergaard et al., 2007; Saito et al., 2009; Van Marken Lichtenbelt et al., 2009); (c) Pheochromocytoma, a catecholamine-secreting tumor of the adrenal gland, has long been shown to cause increases in BAT mass and metabolic rate as well as leanness (Ricquier et al., 1982; Lean et al., 1986; Cannon and Nedergaard, 2004; Fukuchi et al., 2004; Ramacciotti et al., 2006). When the pheochromocytoma tumor is eliminated the high metabolic rate (BAT) subsides (Ramacciotti et al., 2006).

The recent discovery of brown adipocyte stem/progenitor cells (Crisan et al., 2008; Elabd et al., 2009) in skeletal muscle and subcutaneous WAT of adult humans, further supports a physiological role for BAT in humans. The fact that these progenitor cells have been found in older adults as well as in fetal tissues strongly suggests that enhancing BAT recruitment, in order to restore BAT mass to a healthy level, is feasible in humans of any age.

BROWN ADIPOCYTE PROGENITORS IN HUMANS

A major hurdle in identifying (and validating) novel drug targets for brown adipocyte recruitment is the lack of large quantities of relevant and unmodified (non-immortalized) human brown adipocytes in culture to allow for screening of small molecules as well as other applications. Stroma-vascular cell preparations from human BAT (or other tissues like skeletal muscle or WAT) contain only very limited quantities of cells (if any at all) that can differentiate into brown adipocytes. The recently identified human brown adipocyte stem/progenitor cells, CD34+ in skeletal muscle (Crisan et al., 2008), and hMADs in subcutaneous WAT (Elabd et al., 2009), could provide the means to search for novel molecular targets as well as validate existing candidate targets for the development of therapeutics to increase brown adipocyte/BAT recruitment and UCP1 levels *in vivo*. Both CD34+ and hMADs progenitors have self-renewal capability, and can thus be expanded. They differentiate, in response to specific agents, into functional brown adipocytes that express high levels of UCP1, are rich in mitochondria, and undergo highly uncoupled cell respiration (Crisan et al., 2008; Elabd et al., 2009). The muscle-derived CD34+ cells differentiate exclusively into brown adipocytes, and do not appear to produce white adipocytes (i.e., not expressing UCP1; Crisan et al., 2008). The WAT-derived hMADs, in contrast, differentiate initially into white adipocytes, and only gain a brown phenotype (UCP1 expression) following an extended exposure to PPARγ activators (rosiglitazone; Elabd et al., 2009). These cell types are thus quite distinct, and each have the potential of generating relevant cell models for studying human brown adipocyte biology as well as screening for anti-obesity therapeutics. Such screens could identify agents that induce the differentiation of the cells into brown adipocytes. Validation that the agents have in fact induced brown adipocyte formation will require analysis of several functional features of BAT. These would include quantitative PCR and western blot analysis of brown-selective/specific genes such as UCP1, CIDEA, and PGC-1α as well as measurement of cell respiration

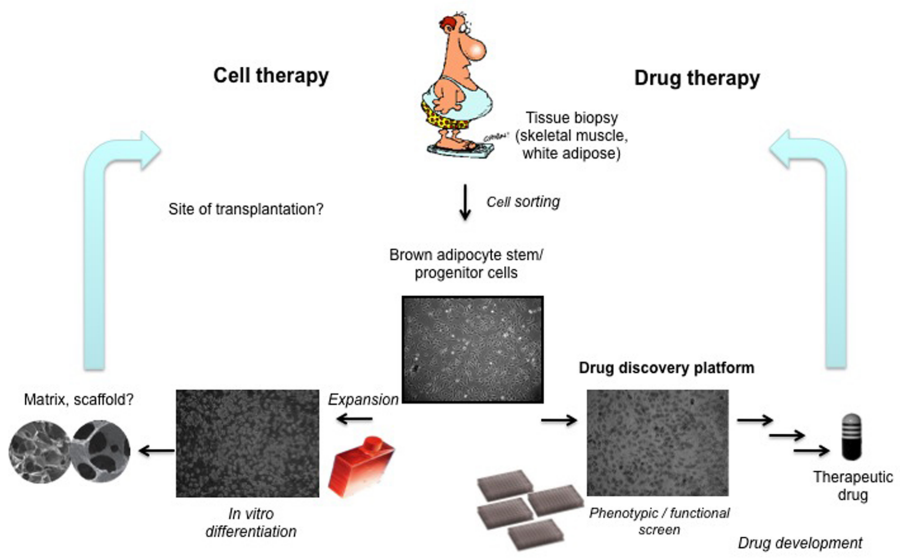

FIGURE 1 | The discovery of brown adipocyte stem/progenitor cells in humans of all ages provides an essential tool for drug discovery. The cells could also be expanded and induced to differentiate in culture, and transplanted back to the patient. Increasing the amount of brown adipose tissue (through recruitment or transplantation) is expected to improve glucose metabolism (diabetes), lower body fat (obesity), and ameliorate dyslipidemia and cardiovascular disease risks.

(i.e., O_2 consumption) to determine the degree of uncoupling of oxidative phosphorylation. Advantages of phenotypic cellular screens (Swinney and Anthony, 2011) are: (a) there is no need to know upfront the identity of a molecular target or how to affect a particular target (activate, inhibit, or modulate); (b) unsuspected, novel targets, or pathways can be identified based on efficacy in the relevant cellular environment; (c) agents toxic to human cells are eliminated early in the screening process. After a phenotypic screen we know that the active agents reach their molecular target(s), whether it is at the cell surface or inside the cell, and they modify their molecular target(s) in the appropriate way to reach efficacy, whether it is through activation, inhibition, or modulation. Very recently, Schulz et al. (2011) identified brown adipocyte progenitors in skeletal muscle, interscapular BAT, and some white adipose depots of mice. It is too early to know whether these cells represent the mouse equivalents of the human CD34+ and hMADs.

USE OF BROWN ADIPOCYTE PROGENITORS FOR TRANSPLANTATION

The existence of brown adipocyte stem/progenitor cells that are readily accessible through biopsy of human tissues such as skeletal muscle and subcutaneous WAT encourages the development of transplantation procedures to treat obese, diabetic patients. Harvested cells from tissue biopsies of insulin resistant individuals could be expanded and induced to differentiate into brown adipocytes prior to their implantation as an autologous transplantation to enhance energy expenditure and improve glucose metabolism in obese, insulin resistant patients. In fact, BAT (tissue rather than cells) implants in mice have recently been shown to robustly improve the metabolic condition of obese, insulin resistant mice (Stanford et al., 2011), and more surprisingly, to restore normoglycemia and glucose tolerance in streptozotocin-induced diabetic mice (Piston and Gunawardana, 2011). In addition to acting as a glucose and energy sink, brown adipocytes are likely to also secrete factors (locally and/or in the circulation) that may have beneficial effects on glucose metabolism/insulin sensitivity and overall energy balance. It is indeed probably through this mechanism that BAT affects the "adipostat." Recent studies suggest that the secretome of BAT is quite different from that of WAT since BAT expresses significantly lower levels of resistin and other adipokines associated with insulin resistance (Kajimura et al., 2008; Vernochet et al., 2009). Additionally, these adipokines are suppressed during the conversion of white adipocytes to brown-like cells in WAT during exposure of mice to synthetic PPARγ ligands (Vernochet et al., 2009).

SUMMARY

From the recent data showing active BAT in adult humans, as well as from animal data, it seems that the most promising strategy for developing therapeutics for obesity and type 2 diabetes is to increase BAT mass, or in fact, restore a healthy level of BAT mass in patients (**Figure 1**). This new approach should allow the development of effective drugs for obesity, diabetes, and the metabolic syndrome that, unlike diet drugs, are devoid of central side effects.

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REFERENCES

Ailhaud, G., Grimaldi, P., and Negrel, R. (1992). Cellular and molecular aspects of adipose tissue development. *Annu. Rev. Nutr.* 12, 207–233.

Almind, K., Manieri, M., Sivitz, W. I., Cinti, S., and Kahn, C. R. (2007). Ectopic brown adipose tissue in muscle provides a mechanism for differences in risk of metabolic syndrome in mice. *Proc. Natl. Acad. Sci. U.S.A.* 104, 2366–2371.

Arch, J. R. (2002). Beta(3)-adrenoceptor agonists: potential, pitfalls and progress. *Eur. J. Pharmacol.* 440, 99–107.

Arch, J. R. (2008). The discovery of drugs for obesity, the metabolic effects of leptin and variable receptor pharmacology: perspectives from beta3-adrenoceptor agonists. *Naunyn Schmiedebergs Arch. Pharmacol.* 378, 225–240.

Bar-Shalom, R., Gaitini, D., Keidar, Z., and Israel, O. (2004). Non-malignant FDG uptake in infradiaphragmatic adipose tissue: a new site of physiological tracer biodistribution characterised by PET/CT. *Eur. J. Nucl. Med. Mol. Imaging* 31, 1105–1113.

Boss, O., Samec, S., Paoloni-Giacobino, A., Rossier, C., Dulloo, A., Seydoux, J., Muzzin, P., and Giacobino, J. P. (1997). Uncoupling protein-3: a new member of the mitochondrial carrier family with tissue-specific expression. *FEBS Lett.* 408, 39–42.

Cannon, B., and Nedergaard, J. (2004). Brown adipose tissue: function and physiological significance. *Physiol. Rev.* 84, 277–359.

Cannon, B., and Nedergaard, J. (2009). Thermogenesis challenges the adipostat hypothesis for body-weight control. *Proc. Nutr. Soc.* 68, 401–407.

Celi, F. S. (2009). Brown adipose tissue – when it pays to be inefficient. *N. Engl. J. Med.* 360, 1553–1556.

Christensen, C. R., Clark, P. B., and Morton, K. A. (2006). Reversal of hypermetabolic brown adipose tissue in F-18 FDG PET imaging. *Clin. Nucl. Med.* 31, 193–196.

Cohade, C., Mourtzikos, K. A., and Wahl, R. L. (2003). "USA-fat": prevalence is related to ambient outdoor temperature-evaluation with 18F-FDG PET/CT. *J. Nucl. Med.* 44, 1267–1270.

Collins, S., Daniel, K. W., Petro, A. E., and Surwit, R. S. (1997). Strain-specific response to beta 3-adrenergic receptor agonist treatment of diet-induced obesity in mice. *Endocrinology* 138, 405–413.

Cousin, B., Bascands-Viguerie, N., Kassis, N., Nibbelink, M., Ambid, L., Casteilla, L., and Penicaud, L. (1996). Cellular changes during cold acclimatation in adipose tissues. *J. Cell. Physiol.* 167, 285–289.

Cousin, B., Cinti, S., Morroni, M., Raimbault, S., Ricquier, D., Penicaud, L., and Casteilla, L. (1992). Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization. *J. Cell Sci.* 103(Pt 4), 931–942.

Crescenzo, R., Samec, S., Antic, V., Rohner-Jeanrenaud, F., Seydoux, J., Montani, J. P., and Dulloo, A. G. (2003). A role for suppressed thermogenesis favoring catch-up fat in the pathophysiology of catch-up growth. *Diabetes* 52, 1090–1097.

Crisan, M., Casteilla, L., Lehr, L., Carmona, M., Paoloni-Giacobino, A., Yap, S., Sun, B., Leger, B., Logar, A., Penicaud, L., Schrauwen, P., Cameron-Smith, D., Russell, A. P., Peault, B., and Giacobino, J. P. (2008). A reservoir of brown adipocyte progenitors in human skeletal muscle. *Stem Cells* 26, 2425–2433.

Cypess, A. M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A. B., Kuo, F. C., Palmer, E. L., Tseng, Y. H., Doria, A., Kolodny, G. M., and Kahn, C. R. (2009). Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* 360, 1509–1517.

Deng, C., Paoloni-Giacobino, A., Kuehne, F., Boss, O., Revelli, J. P., Moinat, M., Cawthorne, M. A., Muzzin, P., and Giacobino, J. P. (1996). Respective degree of expression of beta 1-, beta 2- and beta 3-adrenoceptors in human brown and white adipose tissues. *Br. J. Pharmacol.* 118, 929–934.

Digby, J. E., Montague, C. T., Sewter, C. P., Sanders, L., Wilkison, W. O., O'Rahilly, S., and Prins, J. B. (1998). Thiazolidinedione exposure increases the expression of uncoupling protein 1 in cultured human preadipocytes. *Diabetes* 47, 138–141.

Dulloo, A. G. (2005). A role for suppressed skeletal muscle thermogenesis in pathways from weight fluctuations to the insulin resistance syndrome. *Acta Physiol. Scand.* 184, 295–307.

Dulloo, A. G. (2007). Suppressed thermogenesis as a cause for resistance to slimming and obesity rebound: adaptation or illusion? *Int. J. Obes. (Lond.)* 31, 201–203.

Dulloo, A. G., and Girardier, L. (1990). Adaptive changes in energy expenditure during refeeding following low-calorie intake: evidence for a specific metabolic component favoring fat storage. *Am. J. Clin. Nutr.* 52, 415–420.

Elabd, C., Chiellini, C., Carmona, M., Galitzky, J., Cochet, O., Petersen, R., Penicaud, L., Kristiansen, K., Bouloumie, A., Casteilla, L., Dani, C., Ailhaud, G., and Amri, E. Z. (2009). Human multipotent adipose-derived stem cells differentiate into functional brown adipocytes. *Stem Cells* 27, 2753–2760.

Farmer, S. R. (2009). Obesity: be cool, lose weight. *Nature* 458, 839–840.

Feldmann, H. M., Golozoubova, V., Cannon, B., and Nedergaard, J. (2009). UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab.* 9, 203–209.

Fukuchi, K., Tatsumi, M., Ishida, Y., Oku, N., Hatazawa, J., and Wahl, R. L. (2004). Radionuclide imaging metabolic activity of brown adipose tissue in a patient with pheochromocytoma. *Exp. Clin. Endocrinol. Diabetes* 112, 601–603.

Garcia, C. A., Van Nostrand, D., Atkins, F., Acio, E., Butler, C., Esposito, G., Kulkarni, K., and Majd, M. (2006). Reduction of brown fat 2-deoxy-2-[F-18]fluoro-D-glucose uptake by controlling environmental temperature prior to positron emission tomography scan. *Mol. Imaging Biol.* 8, 24–29.

Garcia, C. A., Van Nostrand, D., Majd, M., Atkins, F., Acio, E., Sheikh, A., and Butler, C. (2004). Benzodiazepine-resistant "brown fat" pattern in positron emission tomography: two case reports of resolution with temperature control. *Mol. Imaging Biol.* 6, 368–372.

Ghorbani, M., and Himms-Hagen, J. (1997). Appearance of brown adipocytes in white adipose tissue during CL 316,243-induced reversal of obesity and diabetes in Zucker fa/fa rats. *Int. J. Obes. Relat. Metab. Disord.* 21, 465–475.

Giordano, A., Morroni, M., Santone, G., Marchesi, G. F., and Cinti, S. (1996). Tyrosine hydroxylase, neuropeptide Y, substance P, calcitonin gene-related peptide and vasoactive intestinal peptide in nerves of rat periovarian adipose tissue: an immunohistochemical and ultrastructural investigation. *J. Neurocytol.* 25, 125–136.

Granneman, J. G., Li, P., Zhu, Z., and Lu, Y. (2005). Metabolic and cellular plasticity in white adipose tissue I: effects of beta3-adrenergic receptor activation. *Am. J. Physiol. Endocrinol. Metab.* 289, E608–E616.

Guerra, C., Koza, R. A., Yamashita, H., Walsh, K., and Kozak, L. P. (1998). Emergence of brown adipocytes in white fat in mice is under genetic control. Effects on body weight and adiposity. *J. Clin. Invest.* 102, 412–420.

Hamann, A., Flier, J. S., and Lowell, B. B. (1996). Decreased brown fat markedly enhances susceptibility to diet-induced obesity, diabetes, and hyperlipidemia. *Endocrinology* 137, 21–29.

Hany, T. F., Gharehpapagh, E., Kamel, E. M., Buck, A., Himms-Hagen, J., and Von Schulthess, G. K. (2002). Brown adipose tissue: a factor to consider in symmetrical tracer uptake in the neck and upper chest region. *Eur. J. Nucl. Med. Mol. Imaging* 29, 1393–1398.

Harper, M.-E., Dent, R., Tesson, F., and Mcpherson, R. (2004). "Targeting thermogenesis in the development of antiobesity drugs," in *Pharmacotherapy of Obesity: Options and Alternatives*, eds K. G. Hofbauer, U. Keller, and O. Boss (Boca Raton, FL: CRC Press), 363–383.

Heaton, J. M. (1972). The distribution of brown adipose tissue in the human. *J. Anat.* 112, 35–39.

Heilbronn, L. K., and Ravussin, E. (2003). Calorie restriction and aging: review of the literature and implications for studies in humans. *Am. J. Clin. Nutr.* 78, 361–369.

Himms-Hagen, J., Cui, J., Danforth, E. Jr., Taatjes, D. J., Lang, S. S., Waters, B. L., and Claus, T. H. (1994). Effect of CL-316243, a thermogenic beta 3-agonist, on energy balance and brown and white adipose tissues in rats. *Am. J. Physiol.* 266, R1371–R1382.

Himms-Hagen, J., Melnyk, A., Zingaretti, M. C., Ceresi, E., Barbatelli, G., and Cinti, S. (2000). Multilocular fat cells in WAT of CL-316243-treated rats derive directly from white adipocytes. *Am. J. Physiol. Cell Physiol.* 279, C670–C681.

Holloway, B. R., Howe, R., Rao, B. S., and Stribling, D. (1992). ICI D7114: a novel selective adrenoceptor agonist of brown fat and thermogenesis. *Am. J. Clin. Nutr.* 55, 262S–264S.

Huttunen, P., Hirvonen, J., and Kinnula, V. (1981). The occurrence of brown adipose tissue in outdoor workers. *Eur. J. Appl. Physiol. Occup. Physiol.* 46, 339–345.

Jimenez, M., Barbatelli, G., Allevi, R., Cinti, S., Seydoux, J., Giacobino, J. P., Muzzin, P., and Preitner, F. (2003). Beta 3-adrenoceptor knockout in C57BL/6J mice depresses the occurrence of brown adipocytes in white fat. *Eur. J. Biochem.* 270, 699–705.

Kajimura, S., Seale, P., Tomaru, T., Erdjument-Bromage, H., Cooper, M. P., Ruas, J. L., Chin, S., Tempst, P., Lazar, M. A., and Spiegelman, B. M. (2008). Regulation of the brown and white fat gene programs through a PRDM16/CtBP transcriptional complex. *Genes Dev.* 22, 1397–1409.

Kim, H., Pennisi, P. A., Gavrilova, O., Pack, S., Jou, W., Setser-Portas, J., East-Palmer, J., Tang, Y., Manganiello, V. C., and Leroith, D. (2006). Effect of adipocyte beta3-adrenergic receptor activation on the type 2 diabetic MKR mice. *Am. J. Physiol. Endocrinol. Metab.* 290, E1227–E1236.

Klaus, S., Ely, M., Encke, D., and Heldmaier, G. (1995). Functional assessment of white and brown adipocyte development and energy metabolism in cell culture. Dissociation of terminal differentiation and thermogenesis in brown adipocytes. *J. Cell Sci.* 108(Pt 10), 3171–3180.

Kopecky, J., Baudysova, M., Zanotti, F., Janikova, D., Pavelka, S., and Houstek, J. (1990). Synthesis of mitochondrial uncoupling protein in brown adipocytes differentiated in cell culture. *J. Biol. Chem.* 265, 22204–22209.

Kopecky, J., Clarke, G., Enerback, S., Spiegelman, B., and Kozak, L. P. (1995). Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity. *J. Clin. Invest.* 96, 2914–2923.

Kopecky, J., Hodny, Z., Rossmeisl, M., Syrovy, I., and Kozak, L. P. (1996a). Reduction of dietary obesity in aP2-Ucp transgenic mice: physiology and adipose tissue distribution. *Am. J. Physiol.* 270, E768–E775.

Kopecky, J., Rossmeisl, M., Hodny, Z., Syrovy, I., Horakova, M., and Kolarova, P. (1996b). Reduction of dietary obesity in aP2-Ucp transgenic mice: mechanism and adipose tissue morphology. *Am. J. Physiol.* 270, E776–E786.

Kozak, L. P., and Koza, R. A. (1999). Mitochondria uncoupling proteins and obesity: molecular and genetic aspects of UCP1. *Int. J. Obes. Relat. Metab. Disord.* 23(Suppl. 6), S33–S37.

Lean, M. E., James, W. P., Jennings, G., and Trayhurn, P. (1986). Brown adipose tissue in patients with pheochromocytoma. *Int. J. Obes.* 10, 219–227.

Leibel, R. L., Rosenbaum, M., and Hirsch, J. (1995). Changes in energy expenditure resulting from altered body weight. *N. Engl. J. Med.* 332, 621–628.

Loncar, D. (1991a). Convertible adipose tissue in mice. *Cell Tissue Res.* 266, 149–161.

Loncar, D. (1991b). Development of thermogenic adipose tissue. *Int. J. Dev. Biol.* 35, 321–333.

Lowell, B. B., V, S. S., Hamann, A., Lawitts, J. A., Himms-Hagen, J., Boyer, B. B., Kozak, L. P., and Flier, J. S. (1993). Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. *Nature* 366, 740–742.

Major, G. C., Doucet, E., Trayhurn, P., Astrup, A., and Tremblay, A. (2007). Clinical significance of adaptive thermogenesis. *Int. J. Obes. (Lond.)* 31, 204–212.

Mattsson, C. L., Csikasz, R. I., Chernogubova, E., Yamamoto, D. L., Hogberg, H. T., Amri, E. Z., Hutchinson, D. S., and Bengtsson, T. (2011). Beta1-adrenergic receptors increase UCP1 in human MADS brown adipocytes and rescue cold-acclimated beta3-adrenergic receptor-knockout mice via nonshivering thermogenesis. *Am. J. Physiol. Endocrinol. Metab.* 301, E1108–E1118.

Nagase, I., Yoshida, T., Kumamoto, K., Umekawa, T., Sakane, N., Nikami, H., Kawada, T., and Saito, M. (1996). Expression of uncoupling protein in skeletal muscle and white fat of obese mice treated with thermogenic beta 3-adrenergic agonist. *J. Clin. Invest.* 97, 2898–2904.

Nedergaard, J., Bengtsson, T., and Cannon, B. (2007). Unexpected evidence for active brown adipose tissue in adult humans. *Am. J. Physiol. Endocrinol. Metab.* 293, E444–E452.

Orci, L., Cook, W. S., Ravazzola, M., Wang, M. Y., Park, B. H., Montesano, R., and Unger, R. H. (2004). Rapid transformation of white adipocytes into fat-oxidizing machines. *Proc. Natl. Acad. Sci. U.S.A.* 101, 2058–2063.

Petrovic, N., Walden, T. B., Shabalina, I. G., Timmons, J. A., Cannon, B., and Nedergaard, J. (2010). Chronic peroxisome proliferator-activated receptor gamma (PPARgamma) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. *J. Biol. Chem.* 285, 7153–7164.

Piston, D. W., and Gunawardana, S. C. (2011). "Reversal of type 1 diabetes by brown adipose tissue transplant," in *American Diabetes Association Scientific Sessions*, San Diego, CA.

Ramacciotti, C., Schneegans, O., Lang, H., Lindner, V., Claria, M., Moreau, F., Chenard, M. P., Pinget, M., and Kessler, L. (2006). Diffuse uptake of brown fat on computed-tomography coupled positron emission tomoscintigraphy (PET-CT) for the exploration of extra-adrenal pheochromocytoma. *Ann. Endocrinol. (Paris)* 67, 14–19.

Ravussin, E., and Kozak, L. (2004). "Energy homeostasis," in *Pharmacotherapy of Obesity: Options and Alternatives*, eds K. G. Hofbauer, U. Keller, and O. Boss (Boca Raton, FL: CRC Press), 3–23.

Rehnmark, S., Nechad, M., Herron, D., Cannon, B., and Nedergaard, J. (1990). Alpha- and beta-adrenergic induction of the expression of the uncoupling protein thermogenin in brown adipocytes differentiated in culture. *J. Biol. Chem.* 265, 16464–16471.

Ricquier, D., Nechad, M., and Mory, G. (1982). Ultrastructural and biochemical characterization of human brown adipose tissue in pheochromocytoma. *J. Clin. Endocrinol. Metab.* 54, 803–807.

Rodeheffer, M. S., Birsoy, K., and Friedman, J. M. (2008). Identification of white adipocyte progenitor cells in vivo. *Cell* 135, 240–249.

Rothwell, N. J., and Stock, M. J. (1979). A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 281, 31–35.

Saito, M., Okamatsu-Ogura, Y., Matsushita, M., Watanabe, K., Yoneshiro, T., Nio-Kobayashi, J., Iwanaga, T., Miyagawa, M., Kameya, T., Nakada, K., Kawai, Y., and Tsujisaki, M. (2009). High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* 58, 1526–1531.

Schulz, T. J., Huang, T. L., Tran, T. T., Zhang, H., Townsend, K. L., Shadrach, J. L., Cerletti, M., Mcdougall, L. E., Giorgadze, N., Tchkonia, T., Schrier, D., Falb, D., Kirkland, J. L., Wagers, A. J., and Tseng, Y. H. (2011). Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. *Proc. Natl. Acad. Sci. U.S.A.* 108, 143–148.

Seale, P., Bjork, B., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scime, A., Devarakonda, S., Conroe, H. M., Erdjument-Bromage, H., Tempst, P., Rudnicki, M. A., Beier, D. R., and Spiegelman, B. M. (2008). PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454, 961–967.

Soloveva, V., Graves, R. A., Rasenick, M. M., Spiegelman, B. M., and Ross, S. R. (1997). Transgenic mice overexpressing the beta 1-adrenergic receptor in adipose tissue are resistant to obesity. *Mol. Endocrinol.* 11, 27–38.

Stanford, K. I., Middelbeek, R. J. W., An, D., Townsend, K. L., Hitchcox, K. M., Jung, D. Y., Lee, Y. J., Kim, J. K., Hirshman, M. F., Tseng, Y. H., and Goodyear, L. J. (2011). "Transplantation of brown adipose tissue exerts beneficial effects on glucose homeostasis," in *American Diabetes Association Scientific Sessions*, San Diego.

Stylopoulos, N., Hoppin, A. G., and Kaplan, L. M. (2009). Roux-en-Y gastric bypass enhances energy expenditure and extends lifespan in diet-induced obese rats. *Obesity (Silver Spring)* 17, 1839–1847.

Swinney, D. C., and Anthony, J. (2011). How were new medicines discovered? *Nat. Rev. Drug Discov.* 10, 507–519.

Tang, W., Zeve, D., Suh, J. M., Bosnakovski, D., Kyba, M., Hammer, R. E., Tallquist, M. D., and Graff, J. M. (2008). White fat progenitor cells reside in the adipose vasculature. *Science* 322, 583–586.

Tseng, Y. H., Kokkotou, E., Schulz, T. J., Huang, T. L., Winnay, J. N., Taniguchi, C. M., Tran, T. T., Suzuki, R., Espinoza, D. O., Yamamoto, Y., Ahrens, M. J., Dudley, A. T., Norris, A. W., Kulkarni, R. N., and Kahn, C. R. (2008). New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454, 1000–1004.

Van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, J. M., Kemerink, G. J., Bouvy, N. D., Schrauwen, P., and Teule, G. J. (2009). Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* 360, 1500–1508.

Vernochet, C., Peres, S. B., Davis, K. E., Mcdonald, M. E., Qiang, L., Wang, H., Scherer, P. E., and Farmer, S. R. (2009). C/EBPalpha and the corepressors CtBP1 and CtBP2 regulate repression of select visceral white adipose genes during induction of the brown phenotype in white adipocytes by peroxisome proliferator-activated receptor gamma agonists. *Mol. Cell. Biol.* 29, 4714–4728.

Wilson-Fritch, L., Nicoloro, S., Chouinard, M., Lazar, M. A., Chui, P. C., Leszyk, J., Straubhaar, J., Czech, M. P., and Corvera, S. (2004). Mitochondrial remodeling in adipose tissue associated with obesity and treatment with rosiglitazone. *J. Clin. Invest.* 114, 1281–1289.

Xue, B., Coulter, A., Rim, J. S., Koza, R. A., and Kozak, L. P. (2005). Transcriptional synergy and the regulation of Ucp1 during brown adipocyte induction in white fat depots. *Mol. Cell. Biol.* 25, 8311–8322.

Yoneshiro, T., Aita, S., Matsushita, M., Kameya, T., Nakada, K., Kawai, Y., and Saito, M. (2011). Brown adipose tissue, whole-body energy expenditure, and thermogenesis in healthy adult men. *Obesity (Silver Spring)* 19, 13–16.

Young, P., Arch, J. R., and Ashwell, M. (1984). Brown adipose tissue in the parametrial fat pad of the mouse. *FEBS Lett.* 167, 10–14.

Zingaretti, M. C., Crosta, F., Vitali, A., Guerrieri, M., Frontini, A., Cannon, B., Nedergaard, J., and Cinti, S. (2009). The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J.* 23, 3113–3120.

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Therapeutic prospects of metabolically active brown adipose tissue in humans

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The world-wide obesity epidemic constitutes a severe threat to human health and wellbeing and poses a major challenge to health-care systems. Current therapeutic approaches, relying mainly on reduced energy intake and/or increased exercise energy expenditure, are generally of limited effectiveness. Previously believed to be present only in children, the existence of metabolically active brown adipose tissue (BAT) was recently demonstrated also in healthy human adults. The physiological role of BAT is to dissipate chemical energy, mainly from fatty acids, as heat to maintain body temperature in cold environments. Recent studies indicate that the activity of BAT is negatively correlated with overweight and obesity, findings that raise the exciting possibility of new and effective weight reduction therapies based on increased BAT energy expenditure, a process likely to be amenable to pharmacological intervention.

Keywords: brown adipose tissue, thermogenesis, energy expenditure, obesity, overweight, weight loss, metabolic syndrome

INTRODUCTION

The prevalence of overweight and obesity has increased worldwide during the recent decades, constituting a severe threat to public health and causing enormous health-care costs. Obesity is a major risk factor for type II diabetes mellitus, cardiovascular disease, osteoarthritis, and certain forms of cancer (Haslam and James, 2005), ultimately leading to increased mortality (Pischon et al., 2008; Berrington De Gonzalez et al., 2010; Zheng et al., 2011). The causes of obesity are complex in terms of physiology, but are quite straightforward in terms of physics: obesity stems from a long-term imbalance of energy intake and energy expenditure. While lipid deposition in adipose tissue is a normal and vital physiologic process not necessarily causing disease, excess triglyceride accumulation strains the capacity of adipose depots and often leads to ectopic lipid deposition in skeletal muscle and liver. This perturbs a multitude of metabolic pathways, which leads to insulin resistance and type II diabetes mellitus (Qatanani and Lazar, 2007).

Fortunately, the adverse metabolic consequences of obesity can be reversed by weight loss (Petersen et al., 2005). However, this is often difficult to achieve with currently available therapies.

LIMITATIONS OF CURRENT THERAPIES AGAINST OBESITY

Presently, the only scientifically proven, effective therapy of morbid obesity, both in terms of weight loss and decreased morbidity and mortality, is bariatric surgery (Sjostrom et al., 2004, 2007; Adams et al., 2007). However, this therapeutic approach is invasive and it is available only to a subgroup of morbidly obese subjects.

A mainstay of current medical management of overweight and obesity is caloric restriction, i.e., limiting the amount of calories ingested to a level below the patient's estimated or measured total energy expenditure (Eckel, 2008). While this may seem to be the most logical approach, it faces several obstacles: the sensation of hunger is recognized as very unpleasant and drugs acting on the CNS to suppress appetite such as Sibutramine or Rimonabant can cause serious adverse events. Use of sibutramine, a substance targeting the central monoamine system, was associated with increased incidence of non-fatal myocardial infarction and stroke (James et al., 2010), which led to its withdrawal. Another promising appetite suppressant, Rimonabant, a cannabinoid receptor 1 blocker that was effective in inducing and maintaining weight loss as well as improving insulin sensitivity, failed to get approval from the Federal Drug Administration in the United States and was withdrawn in Europe due to increased incidence of depression and suicide (Christensen et al., 2007).

Additionally, caloric restriction as the sole method of weight reduction is accompanied by both metabolic and behavioral compensations leading to reduced basal metabolic rate (BMR) and reduced physical activity level (Martin et al., 2007). These adaptations commonly lead to unwanted weight regain, often predominantly as fat mass, once the caloric restriction ends (Dulloo et al., 1997).

In comparison these compensatory changes were not observed in a combination therapy of caloric restriction and exercise (Redman et al., 2009). This is in line with results from other studies, identifying energy expenditure through physical activity as a main determinant of successful weight reduction and long-term weight control (Wing and Hill, 2001; Weinsier et al., 2002). Moreover, a

Abbreviations: BAT, brown adipose tissue; BMR, basal metabolic rate; DIO2, type 2 deiodinase; FDG PET–CT, ^{18}F-fluorodeoxyglucose positron emission tomography combined with computed tomography; FGF, fibroblast growth factor; SNS, sympathetic nervous system; THR, thyroid hormone receptor; UCP1, uncoupling protein 1; WAT, white adipose tissue.

combination of caloric restriction and structured exercise led to greater weight loss and a more favorable cardio-metabolic profile (Goodpaster et al., 2010). Unfortunately, increasing physical activity often proves difficult for obese patients due to musculoskeletal and cardiovascular disease, causing a vicious circle of reduced exercise, reduced energy expenditure, increased body weight and even further reduced activity. Alternative ways to increase energy expenditure could expand the therapeutic options for treating obese patients and ultimately lead to more successful treatment.

BROWN ADIPOSE TISSUE

BROWN ADIPOSE TISSUE PHYSIOLOGY

Adipose tissue exists in two distinct forms: white adipose tissue (WAT) and brown adipose tissue (BAT). WAT is the main energy storage compartment of the mammalian body. It is characterized by adipocytes containing a single large lipid droplet storing excess energy in the form of triglycerides. White adipocytes possess relatively few mitochondria and a peripherally located nucleus. In addition, WAT constitutes a large endocrine organ that secretes hormones such as leptin and adiponectin which are involved in the regulation of satiety and insulin sensitivity (Kershaw and Flier, 2004). BAT is a densely innervated and highly perfused tissue consisting of brown adipocytes, a cell type characterized by small multilocular lipids droplets, a central nucleus, and a high number of mitochondria. The major differences between WAT and BAT are summarized in **Table 1**.

Within the mitochondrial matrix acetyl-CoA derived from carbohydrates, fatty acids, or proteins is fed into the citric acid cycle. The chemical energy released by the stepwise oxidation of acetyl-CoA is then transferred to the electron transport chain in the inner mitochondrial membrane building up a proton gradient across the membrane. Usually, the flow of protons back to the mitochondrial matrix through a channel in the ATP-synthase complex powers the synthesis of ATP (Berg et al., 2007), thereby coupling the oxidation of nutrients to the generation of the cell's energy currency. Brown adipocytes are unique in their expression of UCP1, the gene encoding *uncoupling protein 1*. Activation of UCP1 short-circuits or uncouples the proton gradient from ATP generation, thereby dissipating the energy stored in the gradient as heat (**Figure 1A**).

Table 1 | Characteristic of white vs. brown adipose tissue.

White adipose tissue	Brown adipose tissue
White adipocytes contain single large lipid droplet, few mitochondria	Brown adipocytes contain multiple small lipid droplets, rich in mitochondria
Secretes adipose derived hormones, that regulate insulin sensitivity and satiety	Densely vascularized and innervated by sympathetic nerve endings
Stores excess energy as triglycerides, releases fatty acids during fasting periods	Expression of uncoupling protein 1
	Dissipates chemical energy (mainly from fatty acids) to generate heat

The physiological function of BAT is thus to convert chemical energy, mainly from lipids, into thermal energy. This capability is very important for small and hibernating mammals as well as for newborn humans to maintain body temperature in cold environments. In the mammalian body heat is generated as a by-product of basic cellular and organ functions (BMR), of digestion and absorption of food, and of voluntary and involuntary movement. If this amount of thermal energy does not suffice to keep the body temperature within the normal range in a cold environment, *adaptive thermogenesis* sets in. Even before core body temperature drops cold is sensed by receptors in the skin. The afferent information is integrated in the hypothalamus leading to disinhibition of its thermogenesis-promoting neurons, which in turn increases sympathetic output to BAT (Nakamura and Morrison, 2007). Acute cold stress in non-cold adapted animals causes muscle shivering as a first mechanism to defend body temperature. During chronic cold exposure the amount of shivering abates over the course of days to weeks as *non-shivering thermogenesis* increases (Cannon and Nedergaard, 2004). The signal from the sympathetic nervous system (SNS) is relayed by noradrenergic neurons, which stimulate G-protein coupled β3-adrenoreceptors (β3-AR) in BAT. Stimulation of β3-ARs leads to lipolysis via activation of the G_s-adenylyl cyclase-PKA pathway. The fatty acids released by lipolysis do not only represent the main substrate of thermogenesis, but they also activate UCP1 allosterically (**Figure 1B**; Cannon and Nedergaard, 2004).

Thyroid hormone is an important endocrine modulator of thermogenesis. It potentiates the actions of catecholamines on the β3-AR and adenylyl cyclase and increases the transcription of UCP1 through thyroid hormone receptor (THR) response elements located in the UCP1 promoter (Silva and Rabelo, 1997). Moreover, BAT expresses high levels of type 2 iodothyronine deiodinase (DIO2), an enzyme converting tetraiodothyronine (T_4) into the active triiodothyronine (T_3). Type 1 iodothyronine deiodinase (DIO1) is mainly expressed in liver and kidney and is the main source of plasma T_3 in hyperthyroid patients. While DIO1 is under tight transcriptional control of thyroid hormones, DIO2 expression is induced by catecholamines acting through the cAMP signaling cascade. Its effects in promoting T_3 production are limited to BAT and do not affect plasma T_3 levels (Bianco and Kim, 2006).

About 30 years ago Rothwell and Stock described that *ad libitum* feeding of a high calorie diet rich in fat and carbohydrates, casually dubbed "cafeteria diet," led to recruitment and activation of BAT in rats and thus increased energy expenditure. They concluded that BAT was a major site of diet induced thermogenesis (DIT), the increase in energy expenditure that follows food intake and might thus protect against diet induced obesity (Rothwell and Stock, 1979). In contrast to cold-induced thermogenesis, which is generally undisputed, the proposal of DIT as a function of BAT is more controversial (Kozak, 2010; Fromme and Klingenspor, 2011).

ORIGINS AND DIFFERENTIATION OF BROWN ADIPOSE TISSUE

In contrast to earlier assumptions of a common origin of brown and white adipocytes, recent research clearly demonstrates that brown and white adipocytes have distinct developmental origins.

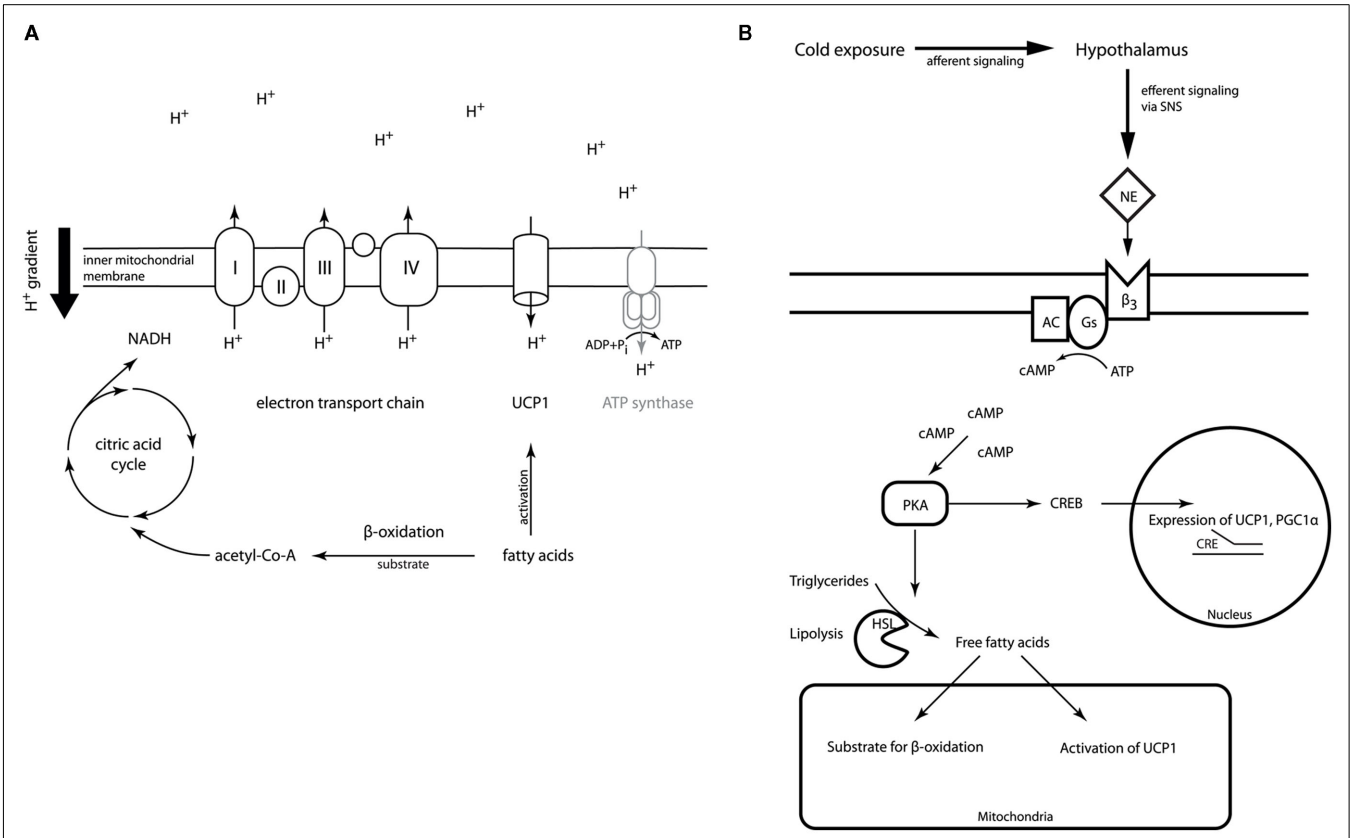

FIGURE 1 | (A) Outline of UCP1 action in brown adipocyte mitochondria. The electron transport chain generates a proton gradient across the inner mitochondrial membrane. This electromotive force is usually drives ATP-synthase restoring the cell's energy currency ATP. Activation of UCP1 by fatty acids in brown adipocytes dissipates the proton gradient without coupling to ATP regeneration releasing the energy as heat. (Details of the electron transport chain have been omitted for reasons of simplicity). **(B)** Outline of BAT regulation. Upon cold exposure afferent signals from cold receptors are integrated in the hypothalamus. The efferent signal is transmitted via the sympathetic nervous system (SNS) and norepinephrine (NE) acting on the β_3-adrenoreceptor located on the brown adipocyte surface, which is coupled to the cAMP second messenger pathway. Phosphorylation of hormone sensitive lipase and perilipin activates lipolysis. The resulting free fatty acids provide the fuel for thermogenesis and activate UCP1 allosterically. Additionally, transcription of UCP1 and PGC-1α is activated via the CREB/CRE pathway.

Atit et al. (2006) proposed a common origin of skeletal muscle and BAT, based on their finding that specific cells of the dermomyotome positive for the transcription factor engrailed-1 develop into both skeletal myocytes and brown, but not white adipocytes. In parallel with this finding, lineage tracing revealed that both brown adipocytes located in the interscapular BAT and myocytes derive from precursors positive for the myogenic transcription factor *Myf5* (Timmons et al., 2007).

Seale et al. (2008) delineated the existence of two different types of brown adipocytes: those found in "classic" BAT (e.g., interscapular BAT), deriving from *Myf5* positive precursors, and a second population, deriving from *Myf5* negative precursors, that is interspersed in WAT. Because these interspersed brown adipocytes appear upon stimulation with cold or β3-AR agonists, we chose to call them "recruitable" brown adipocytes to discriminate them from the invariably present "classic" brown adipocytes, which are located in typical BAT fat pads.

Our knowledge of BAT development and differentiation has advanced considerably during recent years, as several transcriptional regulators essential to BAT have been discovered.

PPARγ coactivator-1α (PGC-1α) is highly induced in BAT upon cold exposure, mediated via SNS stimulation and the cAMP-PKA second messenger pathway. It interacts with PPARγ and is an important regulator of mitochondrial biogenesis and oxidative phosphorylation. Ectopic overexpression of PGC-1α in white adipocytes induces UCP1 and other thermogenic genes, while its genetic ablation severely reduces the animals' capacity for adaptive thermogenesis (Seale et al., 2009). The importance of the cAMP-PKA pathway for brown adipocyte development is highlighted by the fact that FOXC2, a member of the forkhead transcription factor family, sensitized adipocytes to the actions of β3-AR agonists by inducing the expression of the RIα subunit of PKA (Cederberg et al., 2001; Dahle et al., 2002). Overexpression of FOXC2 in adipose tissue led to a "browning" of white fat pads and protected mice against diet induced obesity and insulin resistance.

In conjunction with the discovery of a common lineage of BAT and muscle PRDM16 was identified as a molecular switch between brown adipocyte and myocyte development. Ectopic expression of PRDM16 in myoblasts induced differentiation into brown adipocytes possessing the full molecular machinery necessary for

thermogenesis. In contrast, loss of PRDM16 in BAT pre-cursor cells led to a loss of the brown adipocyte phenotype and myocyte differentiation (Seale et al., 2008). When PRDM16 was transgenically expressed in the subcutaneous WAT of mice, brown adipocyte like cells appeared in this fat pad, rendering the mice less obesity prone and more glucose tolerant (Seale et al., 2011).

IMPLICATIONS OF BAT IN METABOLISM AND BODY WEIGHT IN RODENTS

During the last three decades the effects of active BAT on body weight and metabolism in rodents have been carefully studied.

Several different models of BAT disruption clearly demonstrated its importance for adaptive thermogenesis and also highlighted its relevance for body weight regulation. Surgical denervation or excision of BAT in mice led to increased body weight as compared with sham operated animals (Dulloo and Miller, 1984). Biochemical disruption of BAT by expression of cholera toxin A under the control of the UCP1 promoter induced obesity and insulin resistance in mice (Lowell et al., 1993). A complete knock-out of the UCP1 gene did, somewhat unexpectedly, not result in obesity, when mice were housed at usual animal facility temperatures (i.e., around 22˚C; Enerback et al., 1997). However, keeping them at conditions of thermoneutrality (i.e., 30–33˚C for mice) rendered the animals obese (Feldmann et al., 2009).

Expansion of BAT on the other hand resulted in opposite effects on body weight and metabolism. Transgenic mice overexpressing UCP1 in WAT displayed a reduced amount of subcutaneous fat at 3 months of age and when the transgene was expressed in a genetically obese mouse strain, it protected against obesity (Kopecky et al., 1995). Overexpression of the forkhead transcription factor FOXC2 in adipose tissue led to a "browning" of WAT fat pads, with expression of UCP1 and increased vascularization. Accordingly, the animals were protected from diet induced obesity, as well as insulin resistance and hypertriglyceridemia (Cederberg et al., 2001; Kim et al., 2005).

Pharmacological stimulation of the β3-adrenoreceptor (β3-AR) with the specific agonist CL316,243 in Zucker fa/fa rats led to the appearance of brown adipocytes in WAT regions, increased BMR, and reversed diet induced obesity. Upon treatment with the agonist obese rats showed a more pronounced increase of BMR than lean controls and partial reversion of hyperphagia, while food intake remained constant in lean controls. Additionally, the hyperinsulinemia and hyperglycemia of the Zucker fa/fa rats was ameliorated, indicating reduced insulin resistance (Ghorbani and Himms-Hagen, 1997).

Similar experiments were performed in mice. However, the ability of β3-AR agonists to induce brown adipocytes in WAT regions varied enormously between different mouse strains, as did the body weight after high fat feeding. Importantly, the variation of UCP1 expression in the classic interscapular BAT fat pad was rather small between the different mouse strains, indicating that the differences in body weight were largely conferred by recruitable brown adipocytes in WAT regions (Collins et al., 1997; Guerra et al., 1998). This genetic variability of UCP1 expression in different adipose tissue compartments could not be explained by variation in the expression of specific transcription factors, but rather suggests different developmental mechanisms for the two brown adipocyte populations (Xue et al., 2007).

Bartelt et al. investigated the implications of BAT activation on lipid metabolism. They demonstrated that clearance of triglyceride-rich lipoproteins from plasma in BAT drastically increased upon short-term cold exposure thus correcting hyperlipidemia and ameliorating insulin resistance in mice (Bartelt et al., 2011). As hyperlipidemia is a leading risk factor for cardiovascular disease, the authors speculated that activation of BAT might be a valuable therapeutic approach to correct hypertriglyceridemia and associated diseases in humans.

In conclusion, in rodents an impressive amount of scientific evidence highlights the beneficial consequences of active BAT for regulation of normal body weight, insulin sensitivity, and lipid metabolism. Given the implications of these findings, BAT had already been considered an attractive therapeutic target in humans suffering from overweight and obesity decades ago. However, the recent proof of its presence and activity in human adults has sparked new interest in this possibility.

BROWN ADIPOSE TISSUE IN HUMANS

EVIDENCE AND PREVALENCE OF ACTIVE BAT IN HUMAN ADULTS

While the existence of BAT in rodents and human infants has been known for decades and its physiological function was described as early as the 1960s, it was only recently that several researchers could unequivocally identify active BAT in human adults using ^{18}F-fluorodeoxyglucose positron emission tomography combined with computed tomography (FDG PET–CT; Cypess et al., 2009; Van Marken Lichtenbelt et al., 2009; Virtanen et al., 2009). For a summary of recent studies investigating BAT in humans see **Table 2**.

The FDG PET–CT is currently the only imaging technique that can visualize active BAT. Due to the ionizing radiation associated with its use, it has up to now not been possible to conduct large studies in healthy individuals to test for the presence of active BAT. Instead researchers have often made use of already available FDG PET–CT scans, most commonly obtained during routine diagnostic studies for staging of cancer. The total proportion of subjects displaying active BAT in these studies was relatively low, generally below 10% (Cypess et al., 2009; Jacene et al., 2011; Ouellet et al., 2011).

Using BAT as a therapeutic target to combat obesity and associated diseases does, however, only make sense, if it is present in the vast majority of patients and not just an occasional remnant of BAT present at birth. While the data obtained from cross-sectional retrospective analysis of FDG PET–CT scans performed for other diagnostic purposes suggests that BAT is present only in a minority of adults the true prevalence is probably significantly higher.

The activity of BAT is highly variable and, among other factors, dependent on outdoor temperature at the time of diagnostic imaging (Cypess et al., 2009; Saito et al., 2009; Ouellet et al., 2011). Moreover, radiologists had noted bilateral symmetric glucose uptake in FDG PET scans since the early 1990s. As, understandably, such "artifacts" interfered with the main intention of the diagnostic studies (predominantly detection of tumor and metastases), they tried to suppress this unwanted PET tracer uptake by using pre-medication with beta blockers, benzodiazepines and by

Table 2 | Recent studies on BAT in humans, sorted by study type.

Reference	Study population, characteristics	Main findings
RETROSPECTIVE ANALYSIS OF FDG PET–CT SCANS PERFORMED FOR ROUTINE DIAGNOSTIC STUDIES IN PATIENTS		
Cypess et al. (2009)	1972 Patients (3640 consecutive scans)	FDG PET positive BAT in 76 of 1013 women (7.5%) and 30 of 959 men (3.1%), inverse correlation of BAT activity with age, beta-blocker use, and BMI among older patients.
Jacene et al. (2011)	908 Patients; 445 female, 463 male; mean age ± SD 58 ± 15 years	FDG PET positive BAT in 56 of 908 patients (6.2%), patients without active BAT had higher risk for hyperglycemia in univariate analysis.
Lee et al. (2010)	2934 Patients; female 1848, male 1086; mean age 36 years; age range 18–87 years	Active BAT identified in 250 patients (8.5%); 145 patients scanned more than once, showing a high intra-individual variability of BAT activity. Active BAT associated with female sex. Inversely associated with age, BMI, and fasting glucose.
Pfannenberg et al. (2010)	3604 Patients screened, 260 patients analyzed; 98 BAT positive, 162 study-date matched control subjects; 136 female, 124 male; mean age ± SE, 48 ± 1 years; age range 11–82 years; mean BMI 24.5 ± 0.3 kg/m^2	BAT activity was associated with female sex; inverse correlation of BAT activity with age in men but not in women; inverse correlation with BMI.
Gilsanz et al. (2011)	71 Children and adolescents; 26 female, 45 male; age range 6–20 years	30 of 71 children displayed active BAT (42%); prevalence of active BAT was positively correlated with muscle volume. No differences in age, BMI, or sex were detected.
Ouellet et al. (2011)	4842 Patients; 2370 female, 2472 male; mean age ± SE, 62 ± 0.2 years; age range 2–94 years	328 of 4842 patients with active BAT. Prevalence of active BAT was negatively associated with BMI, diabetes mellitus, outdoor temperature, and BMI. Men were less likely than women to display active BAT.
Yilmaz et al. (2011)	3666 Consecutive scans in 1832 patients screened; 30 patients positive for BAT, 90 age and sex matched controls	30 of 1832 patients (10 male, 20 female) displayed active BAT (prevalence 2%); non-alcoholic fatty liver disease was significantly more prevalent among patients negative for active BAT.
PROSPECTIVE STUDIES USING FDG PET–CT AND COLD EXPOSURE PROTOCOLS IN HEALTHY VOLUNTEERS		
Saito et al. (2009)	56 Healthy volunteers; 25 female, 31 male; 23–65 years of age	Upon cold exposure active BAT was observed in 17 of 32 subjects aged 23–35 years and in 2 of 24 subjects aged 38–65 years. Inverse correlation of BAT activity vs. BMI, total and visceral body fat, respectively.
Virtanen et al. (2009)	5 Healthy volunteers, 20–50 years of age; PET–CT guided surgical biopsies of adipose tissue taken in cervical region of 3 subjects	Cold exposure induced FDG uptake in all five subjects; presence of BAT proven by immunohistochemistry and molecular analysis (qRT-PCR) for BAT markers.
Van Marken Lichtenbelt et al. (2009)	24 Healthy volunteers; age range 20–32 years, male, BMI < 25 in 10 subjects, BMI > 25 in 14 subjects; energy expenditure measured at thermoneutrality and during cold exposure	BAT activity observed in 23 of 24 subjects (96%) after cold exposure, but not under conditions of thermoneutrality. BAT activity negatively correlated with BMI and percentage body fat and positively correlated with basal metabolic rate.
Vijgen et al. (2011)	15 Morbidly obese subjects, 2 male, 13 female, BMI 35–48 kg/m^2	Cold-induced BAT activity detected in 3 out of 15 morbidly obese subjects.
Yoneshiro et al. (2011)	162 Healthy volunteers; 59 female, 103 male; mean age ± SD 32.0 ± 12.1; BMI 22.1 ± 3.0 kg/m^2	Cold activated BAT detected in 41% of subjects; inverse correlation of active BAT with BMI, body fat content, abdominal fat, and age.
ANALYSIS OF TISSUE SAMPLES OBTAINED DURING SURGERY IN THE NECK REGION		
Zingaretti et al. (2009)	35 Patients; 8 male, 27 female; age range 18–82 years, BMI range 18–37 kg/m^2; thyroid surgery for goiter and papillary carcinoma	Histologic evidence of BAT in 10 of 35 patients; presence of BAT inversely correlated with BMI and age.
Lee et al. (2011b)	Prospective cohort study in 17 patients; underwent preoperative FDG PET/CT for staging of head and neck-malignancies prior to surgery; analysis of BAT markers in cervical fat biopsies compared to subcutaneous WAT	FDG PET/CT positive for BAT in 3 of the 17 patients. However, molecular markers of BAT could be detected in cervical fat biopsies from both PET positive and PET negative patients.
OTHER STUDIES		
Wang et al. (2011)	FDG PET/CT in 14 patients with pheochromocytoma; 14 matched healthy controls	BAT activity as assessed by FDG PET correlated with total plasma metanephrine.

warming up to a comfortable room temperature before injecting the tracer substance (Christensen et al., 2006; Soderlund et al., 2007).

Studies specifically designed to detect BAT indicate a much higher prevalence in adult humans. Van Marken Lichtenbelt et al. (2009) used a specific cold stimulation protocol prior to FDG PET scanning and were able to detect active BAT in 23 out of 24 healthy male subjects (96%). Using a different approach Lee et al. examined 17 patients that underwent staging for head and neck malignancy. While only 3 of 17 had indications of BAT in the FDG PET–CT scan molecular analysis of samples taken from the supraclavicular fat revealed the presence of molecular markers of BAT (UCP1, PRDM16) in all subjects (Lee et al., 2011b). Of the 2934 patients examined with FDG PET–CT in a large Australian study 145 were scanned more than once. The probability to identify active BAT in an individual increased considerably with the number of scans, allowing the authors to estimate the true prevalence of BAT in their cohort to be 64% (Lee et al., 2010).

ASSOCIATION OF ACTIVE BAT AND OBESITY IN HUMANS

While the body of evidence is far from being as robust as in rodents, several studies also link the activity of BAT in humans to obesity and associated diseases.

Studies using retrospective analysis of routine FDG PET–CT scans identified a negative correlation of BAT activity and body mass index (BMI) in humans (Cypess et al., 2009; Lee et al., 2010; Pfannenberg et al., 2010; Ouellet et al., 2011). The results of these publications are corroborated by two trials in healthy volunteers that underwent FDG PET–CT scanning after cold exposure to activate BAT (Saito et al., 2009; Van Marken Lichtenbelt et al., 2009). Zingaretti et al. (2009) observed similar results when studying the presence of brown adipocytes in samples of adipose tissue collected in the cervical region of 35 patients undergoing thyroid surgery. Remarkably, active BAT was present in only 3 out of 15 morbidly obese subjects (mean BMI 42.1 kg/m^2) upon cold stimulation as assessed with FDG PET–CT (Vijgen et al., 2011). However, the presence of active BAT in 20% of the subjects indicates that BAT activation might be a possible therapeutic intervention in at least a part of morbidly obese patients.

The prevalence of PET positive BAT seems to decrease with age, as assessed both in retrospective observational studies (Cypess et al., 2009; Pfannenberg et al., 2010; Ouellet et al., 2011) and in prospective studies in healthy volunteers (Saito et al., 2009; Yoneshiro et al., 2011). The declining presence of active BAT with age might be one explanation for the increase of overweight and obesity with age.

In retrospective PET studies the prevalence and activity of BAT was unanimously higher in women than in men (Cypess et al., 2009; Ouellet et al., 2011). Evaluating the FDG PET–CT scans of 260 subjects, 98 of which displayed active BAT, Pfannenberg et al. (2010) described that while in male subjects BAT activity and mass declined with age, this was not the case in female subjects.

Although there are currently no studies prospectively addressing the question, whether active BAT might protect against insulin resistance and diabetes, there is indirect evidence from retrospective cross-sectional studies of patients that underwent FDG PET–CT. Using routine scans Jacene et al. (2011) investigated, whether the presence of active BAT was predictive of elevated fasting glucose levels at the time of the examination. Of the 908 patients 6.2% had FDG uptake in BAT and in the univariate analysis patients without active BAT had a significantly increased risk to have a blood glucose level of more than 100 mg/dl (>5.6 mmol/l). After adjusting for age, BMI, and sex this difference was not statistically significant. In a larger Australian cohort with 2934 patients, 250 of which displayed active BAT in FDG PET–CT, a significant correlation between fasting glucose and BAT activity was observed. The authors also analyzed data from patients who had been studied repeatedly at different time points and observed an inverse correlation of blood glucose levels and BAT activity in this subset (Lee et al., 2010). These findings are in line with another study describing a reduced prevalence of active BAT in patients with known diabetes (Ouellet et al., 2011).

Non-alcoholic fatty liver disease (NAFLD) is a manifestation of the metabolic syndrome with ectopic lipid deposition in the liver. It is often associated with insulin resistance or type II diabetes mellitus, hyperlipidemia, and hypertension (Vuppalanchi and Chalasani, 2009). In another retrospective evaluation of FDG PET–CT data the presence of active BAT in adults was independently associated with a lower likelihood of NAFLD (Yilmaz et al., 2011).

Recently, Gilsanz et al. published results of a retrospective analysis of FDG PET–CT scans performed in 71 children and adolescents. As expected, the overall prevalence of active BAT was higher (42%) than in an adult population. Intriguingly, an association of BAT activity and muscle mass was observed. Contrary to studies performed in adults, no associations with age, BMI, or adiposity could be detected (Gilsanz et al., 2011). However, a major limitation of this study is – as with most of the studies using FDG PET–CT in adult humans – the fact that the study population consisted only of subjects suffering from neoplastic disease.

In some adult patients suffering from pheochromocytoma, a catecholamine secreting tumor of the adrenal medulla, BAT had been discovered and characterized ultrastructurally and biochemically already in the 1980s (Ricquier et al., 1982). It was speculated that the weight loss seen in many patients suffering from pheochromocytoma was due to the activation or recruitment of BAT through high levels of catecholamines (Lean et al., 1986). Not unexpectedly, a more recent study was able to describe a significant correlation of plasma catecholamine levels and BAT activity in patients with pheochromocytoma (Wang et al., 2011).

Correspondingly, the use of beta blockers was inversely correlated to the presence of active BAT in a retrospective, cross-sectional study with FDG PET–CT (Cypess et al., 2009). In a controlled prospective trial using cold exposure to activate adaptive thermogenesis, however, administration of the β adrenoreceptor antagonist propranolol led to suppression of cold-induced thermogenesis in muscle (presumably β2 AR mediated) but did not alter cold-induced thermogenesis in BAT (presumably β3 mediated; Wijers et al., 2011).

Additionally, the pre-treatment with diazepam – a common practice meant to suppress FDG uptake of BAT during PET scanning – did not result in reduced BAT activity in a randomized controlled trial (Sturkenboom et al., 2009).

PHARMACOLOGICAL TARGETING OF THERMOGENESIS AND BAT

A calculation performed on the basis of glucose uptake and estimated usage of fatty acids in BAT of healthy individuals yielded the result that full activation of the present BAT would lead to a potential consumption of 4.1 kg of adipose tissue over the course of a year (Virtanen et al., 2009). Increasing thermogenesis pharmacologically could therefore provide a promising approach to prevent weight gain and facilitate weight loss in humans.

PHARMACOLOGIC ACTIVATION OF THERMOGENESIS

About eight decades ago 2,4-dinitrophenol (DNP), an artificial uncoupler of mitochondrial respiration, was used to induce weight loss in humans. It is a small lipophilic molecule that acts as a protonophore, as it can carry protons across membranes (Berg et al., 2007).

The substance was introduced as a drug for weight reduction in the 1930s and a series of studies demonstrated it to cause increased metabolic rate and considerable weight loss without dietary restriction. Since nitrogen excretion remained stable upon treatment with DNP, it was assumed that weight loss was due to fat loss and that muscle was spared. The efficacy of the substance led to a widespread use even as non-prescription drug. Due to the narrow therapeutic range of DNP several cases of fatal hyperthermia occurred, but given the large number of patients who had taken it (more than 100,000 by 1934), the number of severe adverse events was remarkably low. In 1935 several cases of cataract were reported in women taking DNP, which finally resulted in its withdrawal from the market in 1938 (Harper et al., 2001). DNP does of course not selectively uncouple BAT mitochondria, but it nevertheless provides an important proof of principle, as it demonstrates the efficacy of mitochondrial uncoupling for weight loss in humans.

Central stimulation of BAT via the SNS is believed to contribute to the weight reducing effect of sibutramine (Liu et al., 2002). A popular "herbal" weight loss drug, ephedrine, also increases SNS activity and activates BAT, thereby increasing oxygen consumption and thermogenesis (Dulloo et al., 1991; Baba et al., 2007). Yet, stimulation of the SNS can lead to adverse effects such as tachycardia, hypertension, and cardiac arrhythmias, all of which are highly undesirable, especially in obese patients already suffering from cardiovascular disease.

A more selective activation of BAT could be achieved through β3-AR agonists. In fact, this strategy has been shown to be effective in rodents (Nagase et al., 1996; Ghorbani and Himms-Hagen, 1997; Umekawa et al., 1997). The orally available selective β3-AR agonist L-796568 acutely increased energy expenditure and lipolysis in obese men (Van Baak et al., 2002), but no effects on thermogenesis or body fat were observed after extended treatment over 28 days, probably due to β3-AR down-regulation (tachyphylaxis; Larsen et al., 2002). Another study in young, lean men demonstrated increased insulin sensitivity and fat oxidation after 8 weeks of treatment with the β3-AR agonist CL316,243, while 24 h-energy expenditure and body weight after 8 weeks remained unchanged as compared to baseline (Weyer et al., 1998).

It must be noted that these studies measured energy expenditure to assess the activation of adaptive thermogenesis. Using FDG PET–CT to visualize BAT activity could possibly detect smaller changes induced by β3-AR agonists and should therefore be used for future trials testing similar compounds.

The thermogenic effect of thyroid hormones is well known. Not surprisingly levothyroxine and triiodothyronine have been used to facilitate weight loss in obese patients. Unfortunately, administration of excess thyroid hormone results in overt hyperthyroidism, a condition associated with increased BMR, tachycardia, atrial arrhythmia, muscle wasting, and osteoporosis. The CNS effects of excess thyroid hormone include increased irritability, nervousness, and anxiety (Baxter and Webb, 2009). However, the effects of thyroid hormones are mediated by different isoforms of the THR in a tissue specific fashion. THRα conveys most of the effects on bone development, cardiac gene expression, heart rate, and catecholamine potentiation, while activation of THRβ1 in mice increased UCP1 expression in BAT (Melmed and Conn, 2005; Ribeiro et al., 2010). Accordingly, the thyroid hormone mimetics GC-1 and KB141, which are selective for THRβ1, reduced diet induced obesity and normalized serum lipids via increased metabolic rate while not affecting heart rate (Grover et al., 2003, 2004; Villicev et al., 2007). In diabetic ob/ob mice KB141 improved glucose tolerance and insulin sensitivity (Bryzgalova et al., 2008). Thus selective thyroid hormone mimetics might also be of therapeutic benefit in humans.

Activation of the G-protein coupled receptor TGR5 by bile acids increased the expression of DIO2 in BAT and resulted in increased energy expenditure and reduced body weight in mice (Watanabe et al., 2006). Stimulation of DIO2 expression in BAT is particularly interesting as this approach increases triiodothyronine availability locally but not systemically thus obviating the deleterious effects of hyperthyroidism. The co-expression of TGR5 and DIO2 in BAT makes bile acids and derived compounds promising drug candidates for the activation of thermogenesis.

EXPANSION OF BROWN ADIPOSE TISSUE

As outlined above, the prevalence of active BAT, as determined by FDG PET–CT, is lower in overweight and obese subjects than in lean controls. To make use of the energy dissipating effect of BAT in obese persons, it might thus be necessary to expand the amount of BAT. Recently, brown adipocyte progenitors that could be differentiated into UCP1 expressing cells *in vitro* have been discovered in human WAT (Elabd et al., 2009). Additionally, Lee et al. obtained fat biopsies from the supraclavicular fossa guided by PET–CT. They were able to differentiate brown adipocyte precursors to UCP1 expressing brown adipocytes, regardless whether PET analysis had shown active BAT or not (Lee et al., 2011a). These findings provide a rationale for therapeutic expansion of BAT in humans.

In rodents several small molecules and hormones have been found to promote the expansion of BAT. Fibroblast growth factors (FGF) are a family of polypeptide growth factors that regulate cell proliferation, migration, and differentiation during embryonic development and function as homeostatic factors in tissue repair and response to injury in the adult organism (Ornitz and Itoh, 2001). Several members of the family have been implied in BAT physiology.

Cold exposure induces the expression of basic fibroblast growth factor (bFGF = FGF-2) in BAT and this growth factor seems to

stimulate BAT growth through autocrine feedback (Yamashita et al., 1994; Asano et al., 1999).

While FGF-16 mainly seems to be responsible for growth of BAT during embryonic development (Konishi et al., 2000), FGF-19 and FGF-21 demonstrated effects on thermogenesis in adult mice. Intra-cerebroventricular administration of FGF-19 in mice increased metabolic rate, prevented diet induced obesity and improved insulin sensitivity (Fu et al., 2004). It is an especially attractive molecule since it specifically binds to FGF-receptor 4 and does not appear to be mitogenic.

In newborn mice FGF-21 expression in the liver is reduced in response to fasting and induced by suckling via PPARα dependent mechanisms. Injection of FGF-21 into newborn fasting mice induced genes involved in thermogenesis in BAT and increased body temperature. *In vitro* FGF-21 induced expression of thermogenic genes and increased total and uncoupled respiration (Hondares et al., 2010). Interestingly, FGF-21 expression is induced in BAT itself by cold exposure or adrenergic stimulation via β-adrenergic pathways (Chartoumpekis et al., 2011; Hondares et al., 2011).

Fibroblast growth factors are attractive from a pharmacological point of view: like other peptides they might be produced recombinantly or substituted by small molecules that specifically activate FGF receptors and could easily be produced in large scale.

BMP-7, a member of the TGF-β superfamily was originally identified as a bone inducer, but recent evidence suggests an important role in brown fat development as well. BMP-7 can activate the full program of brown fat differentiation both *in vitro* and *in vivo*. Tseng et al. (2008) injected mice with hepatotropic adenoviruses expressing BMP-7 resulting in hepatic overexpression and secretion of the protein. In comparison to control mice those injected with the BMP-7 expressing retroviruses showed increased thermogenesis, higher body temperature and reduced body weight.

An alternative strategy could be to transdifferentiate adipocyte precursors obtained from liposuction into brown adipocytes *ex vivo* and then re-transplant the thermogenically active cells. This could be achieved through overexpression of PRDM16, FOXC2, or PGC-1α in the cells. Compared to a conventional pharmacological approach, however, such a method would be elaborate and expensive and questions of optimal location for implantation, of blood supply and of innervation remain to be solved.

Currently, FDG PET–CT is the sole imaging modality to investigate BAT activity directly. This is a major obstacle in the investigation of BAT activity and potential pharmaceuticals that increase BAT activity. Recent developments in nuclear magnetic resonance imaging (MRI; Hu et al., 2010; Branca and Warren, 2011) could facilitate the evaluation of BAT activity without exposure to ionizing radiation thus giving the research in the field an enormous boost.

Apart from the search for drugs that increase BAT thermogenesis, it will also be important to further investigate the consequences of thermoneutrality and cold exposure. Humans in the developed world spend most of their time in a thermoneutral zone maintained by advanced heating systems and protective clothing (Van Marken Lichtenbelt and Schrauwen, 2011). As BAT is activated by sensing cold long prior to a drop of core temperature and at ambient temperatures around 16°C, deliberately lowering ambient temperatures in buildings could be a feasible approach to increase BAT activity and to prevent obesity.

CONCLUSION

Given the growing world-wide prevalence and increasing burden of obesity and associated diseases and the current lack of effective treatment strategies, new therapeutic approaches in the field of anti-obesity therapies are clearly needed.

During the recent years our knowledge of BAT physiology and development and its implications in metabolic disease has increased dramatically. Most importantly, there is robust evidence that BAT is present in a majority of human adults. Furthermore, a growing body of literature indicates that active BAT is inversely associated with obesity and metabolic disease. In rodent models several pharmacological approaches which increase BAT activity, have been proven to effectively prevent obesity, facilitate weight reduction, and ameliorate insulin resistance. Deducing from the animal model these approaches might work in humans as well.

Many questions regarding therapeutic activation or expansion of BAT remain to be solved. It is yet unclear how effective an increase of thermogenesis would be to reduce obesity and ameliorate insulin resistance. Compensating mechanisms, such as increased appetite, could reduce the benefits of this approach. Not least, the infamous history of the uncoupling agent DNP reminds us to pay close attention to contingent risks of drugs that activate thermogenesis. Considering the disappointing results of pharmacological approaches targeting energy intake and appetite, stimulation of BAT activity and increasing energy expenditure would provide exciting new options in obesity therapy.

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REFERENCES

Adams, T. D., Gress, R. E., Smith, S. C., Halverson, R. C., Simper, S. C., Rosamond, W. D., Lamonte, M. J., Stroup, A. M., and Hunt, S. C. (2007). Long-term mortality after gastric bypass surgery. *N. Engl. J. Med.* 357, 753–761.

Asano, A., Kimura, K., and Saito, M. (1999). Cold-induced mRNA expression of angiogenic factors in rat brown adipose tissue. *J. Vet. Med. Sci.* 61, 403–409.

Atit, R., Sgaier, S. K., Mohamed, O. A., Taketo, M. M., Dufort, D., Joyner, A. L., Niswander, L., and Conlon, R. A. (2006). Beta-catenin activation is necessary and sufficient to specify the dorsal dermal fate in the mouse. *Dev. Biol.* 296, 164–176.

Baba, S., Tatsumi, M., Ishimori, T., Lilien, D. L., Engles, J. M., and Wahl, R. L. (2007). Effect of nicotine and ephedrine on the accumulation of 18F-FDG in brown adipose tissue. *J. Nucl. Med.* 48, 981–986.

Bartelt, A., Bruns, O. T., Reimer, R., Hohenberg, H., Ittrich, H., Peldschus, K., Kaul, M. G., Tromsdorf, U. I., Weller, H., Waurisch, C., Eychmuller, A., Gordts, P. L., Rinninger, F., Bruegelmann, K., Freund, B., Nielsen, P., Merkel, M., and Heeren, J. (2011). Brown adipose tissue activity controls triglyceride clearance. *Nat. Med.* 17, 200–205.

Baxter, J. D., and Webb, P. (2009). Thyroid hormone mimetics: potential applications in atherosclerosis, obesity and type 2 diabetes. *Nat. Rev. Drug Discov.* 8, 308–320.

Berg, J. M., Tymoczko, J. L., and Stryer, L. (2007). *Biochemistry*. New York, NY: W. H. Freeman.

Berrington De Gonzalez, A., Hartge, P., Cerhan, J. R., Flint, A. J., Hannan, L., Macinnis, R. J., Moore, S. C., Tobias, G. S., Anton-Culver, H., Freeman, L. B., Beeson, W. L., Clipp, S. L., English, D. R., Folsom, A. R., Freedman, D. M., Giles, G., Hakansson, N., Henderson, K. D., Hoffman-Bolton, J., Hoppin, J. A., Koenig, K. L., Lee, I.-M., Linet, M. S., Park, Y., Pocobelli, G., Schatzkin, A., Sesso, H. D., Weiderpass, E., Willcox, B. J., Wolk, A., Zeleniuch-Jacquotte, A., Willett, W. C., and Thun, M. J. (2010). Body-mass index and mortality among 1.46 million white adults. *N. Engl. J. Med.* 363, 2211–2219.

Bianco, A. C., and Kim, B. W. (2006). Deiodinases: implications of the local control of thyroid hormone action. *J. Clin. Invest.* 116, 2571–2579.

Branca, R. T., and Warren, W. S. (2011). In vivo brown adipose tissue detection and characterization using water-lipid intermolecular zero-quantum coherences. *Magn. Reson. Med.* 65, 313–319.

Bryzgalova, G., Effendic, S., Khan, A., Rehnmark, S., Barbounis, P., Boulet, J., Dong, G., Singh, R., Shapses, S., Malm, J., Webb, P., Baxter, J. D., and Grover, G. J. (2008). Anti-obesity, anti-diabetic, and lipid lowering effects of the thyroid receptor beta subtype selective agonist KB-141. *J. Steroid Biochem. Mol. Biol.* 111, 262–267.

Cannon, B., and Nedergaard, J. (2004). Brown adipose tissue: function and physiological significance. *Physiol. Rev.* 84, 277–359.

Cederberg, A., Gronning, L. M., Ahren, B., Tasken, K., Carlsson, P., and Enerback, S. (2001). FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. *Cell* 106, 563–573.

Chartoumpekis, D. V., Habeos, I. G., Ziros, P. G., Psyrogiannis, A. I., Kyriazopoulou, V. E., and Papavassiliou, A. G. (2011). Brown adipose tissue responds to cold and adrenergic stimulation by induction of FGF21. *Mol. Med.* 17, 736–740.

Christensen, C. R., Clark, P. B., and Morton, K. A. (2006). Reversal of hypermetabolic brown adipose tissue in F-18 FDG PET imaging. *Clin. Nucl. Med.* 31, 193–196.

Christensen, R., Kristensen, P. K., Bartels, E. M., Bliddal, H., and Astrup, A. (2007). Efficacy and safety of the weight-loss drug rimonabant: a meta-analysis of randomised trials. *Lancet* 370, 1706–1713.

Collins, S., Daniel, K. W., Petro, A. E., and Surwit, R. S. (1997). Strain-specific response to β 3-adrenergic receptor agonist treatment of diet-induced obesity in mice. *Endocrinology* 138, 405–413.

Cypess, A. M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A. B., Kuo, F. C., Palmer, E. L., Tseng, Y. H., Doria, A., Kolodny, G. M., and Kahn, C. R. (2009). Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* 360, 1509–1517.

Dahle, M. K., Gronning, L. M., Cederberg, A., Blomhoff, H. K., Miura, N., Enerback, S., Tasken, K. A., and Tasken, K. (2002). Mechanisms of FOXC2- and FOXD1-mediated regulation of the RI alpha subunit of cAMP-dependent protein kinase include release of transcriptional repression and activation by protein kinase B alpha and cAMP. *J. Biol. Chem.* 277, 22902–22908.

Dulloo, A. G., Jacquet, J., and Girardier, L. (1997). Poststarvation hyperphagia and body fat overshooting in humans: a role for feedback signals from lean and fat tissues. *Am. J. Clin. Nutr.* 65, 717–723.

Dulloo, A. G., and Miller, D. S. (1984). Energy balance following sympathetic denervation of brown adipose tissue. *Can. J. Physiol. Pharmacol.* 62, 235–240.

Dulloo, A. G., Seydoux, J., and Girardier, L. (1991). Peripheral mechanisms of thermogenesis induced by ephedrine and caffeine in brown adipose tissue. *Int. J. Obes.* 15, 317–326.

Eckel, R. H. (2008). Clinical practice. Nonsurgical management of obesity in adults. *N. Engl. J. Med.* 358, 1941–1950.

Elabd, C., Chiellini, C., Carmona, M., Galitzky, J., Cochet, O., Petersen, R., Penicaud, L., Kristiansen, K., Bouloumie, A., Casteilla, L., Dani, C., Ailhaud, G., and Amri, E. Z. (2009). Human multipotent adipose-derived stem cells differentiate into functional brown adipocytes. *Stem Cells* 27, 2753–2760.

Enerback, S., Jacobsson, A., Simpson, E. M., Guerra, C., Yamashita, H., Harper, M. E., and Kozak, L. P. (1997). Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* 387, 90–94.

Feldmann, H. M., Golozoubova, V., Cannon, B., and Nedergaard, J. (2009). UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab.* 9, 203–209.

Fromme, T., and Klingenspor, M. (2011). Uncoupling protein 1 expression and high-fat diets. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 300, R1–R8.

Fu, L., John, L. M., Adams, S. H., Yu, X. X., Tomlinson, E., Renz, M., Williams, P. M., Soriano, R., Corpuz, R., Moffat, B., Vandlen, R., Simmons, L., Foster, J., Stephan, J. P., Tsai, S. P., and Stewart, T. A. (2004). Fibroblast growth factor 19 increases metabolic rate and reverses dietary and leptin-deficient diabetes. *Endocrinology* 145, 2594–2603.

Ghorbani, M., and Himms-Hagen, J. (1997). Appearance of brown adipocytes in white adipose tissue during CL 316,243-induced reversal of obesity and diabetes in Zucker fa/fa rats. *Int. J. Obes. Relat. Metab. Disord.* 21, 465–475.

Gilsanz, V., Chung, S. A., Jackson, H., Dorey, F. J., and Hu, H. H. (2011). Functional brown adipose tissue is related to muscle volume in children and adolescents. *J. Pediatr.* 158, 722–726.

Goodpaster, B. H., Delany, J. P., Otto, A. D., Kuller, L., Vockley, J., South-Paul, J. E., Thomas, S. B., Brown, J., Mctigue, K., Hames, K. C., Lang, W., and Jakicic, J. M. (2010). Effects of diet and physical activity interventions on weight loss and cardiometabolic risk factors in severely obese adults. *JAMA* 304, 1795–1802.

Grover, G. J., Egan, D. M., Sleph, P. G., Beehler, B. C., Chiellini, G., Nguyen, N. H., Baxter, J. D., and Scanlan, T. S. (2004). Effects of the thyroid hormone receptor agonist GC-1 on metabolic rate and cholesterol in rats and primates: selective actions relative to 3,5,3′-triiodo-L-thyronine. *Endocrinology* 145, 1656–1661.

Grover, G. J., Mellstrom, K., Ye, L., Malm, J., Li, Y. L., Bladh, L. G., Sleph, P. G., Smith, M. A., George, R., Vennstrom, B., Mookhtiar, K., Horvath, R., Speelman, J., Egan, D., and Baxter, J. D. (2003). Selective thyroid hormone receptor-beta activation: a strategy for reduction of weight, cholesterol, and lipoprotein (a) with reduced cardiovascular liability. *Proc. Natl. Acad. Sci. U.S.A.* 100, 10067–10072.

Guerra, C., Koza, R. A., Yamashita, H., Walsh, K., and Kozak, L. P. (1998). Emergence of brown adipocytes in white fat in mice is under genetic control. Effects on body weight and adiposity. *J. Clin. Invest.* 102, 412–420.

Harper, J. A., Dickinson, K., and Brand, M. D. (2001). Mitochondrial uncoupling as a target for drug development for the treatment of obesity. *Obes. Rev.* 2, 255–265.

Haslam, D. W., and James, W. P. (2005). Obesity. *Lancet* 366, 1197–1209.

Hondares, E., Iglesias, R., Giralt, A., Gonzalez, F. J., Giralt, M., Mampel, T., and Villarroya, F. (2011). Thermogenic activation induces FGF21 expression and release in brown adipose tissue. *J. Biol. Chem.* 286, 12983–12990.

Hondares, E., Rosell, M., Gonzalez, F. J., Giralt, M., Iglesias, R., and Villarroya, F. (2010). Hepatic FGF21 expression is induced at birth via PPARalpha in response to milk intake and contributes to thermogenic activation of neonatal brown fat. *Cell Metab.* 11, 206–212.

Hu, H. H., Smith, D. L. Jr., Nayak, K. S., Goran, M. I., and Nagy, T. R. (2010). Identification of brown adipose tissue in mice with fat-water IDEAL-MRI. *J. Magn. Reson. Imaging* 31, 1195–1202.

Jacene, H. A., Cohade, C. C., Zhang, Z., and Wahl, R. L. (2011). The relationship between patients' serum glucose levels and metabolically active brown adipose tissue detected by PET/CT. *Mol. Imaging Biol.* 13, 1278–1283.

James, W. P., Caterson, I. D., Coutinho, W., Finer, N., Van Gaal, L. F., Maggioni, A. P., Torp-Pedersen, C., Sharma, A. M., Shepherd, G. M., Rode, R. A., and Renz, C. L. (2010). Effect of sibutramine on cardiovascular outcomes in overweight and obese subjects. *N. Engl. J. Med.* 363, 905–917.

Kershaw, E. E., and Flier, J. S. (2004). Adipose tissue as an endocrine organ. *J. Clin. Endocrinol. Metab.* 89, 2548–2556.

Kim, J. K., Kim, H. J., Park, S. Y., Cederberg, A., Westergren, R., Nilsson, D., Higashimori, T., Cho, Y. R., Liu, Z. X., Dong, J., Cline, G. W., Enerback, S., and Shulman, G. I. (2005). Adipocyte-specific overexpression of FOXC2 prevents diet-induced increases in intramuscular fatty acyl CoA and insulin resistance. *Diabetes* 54, 1657–1663.

Konishi, M., Mikami, T., Yamasaki, M., Miyake, A., and Itoh, N. (2000). Fibroblast growth factor-16 is a growth factor for embryonic brown adipocytes. *J. Biol. Chem.* 275, 12119–12122.

Kopecky, J., Clarke, G., Enerback, S., Spiegelman, B., and Kozak, L. P. (1995). Expression of the mitochondrial uncoupling protein gene from the aP2 gene promoter prevents genetic obesity. *J. Clin. Invest.* 96, 2914–2923.

Kozak, L. P. (2010). Brown fat and the myth of diet-induced thermogenesis. *Cell Metab.* 11, 263–267.

Larsen, T. M., Toubro, S., Van Baak, M. A., Gottesdiener, K. M., Larson, P., Saris, W. H., and Astrup, A. (2002). Effect of a 28-d treatment with L-796568, a novel beta(3)-adrenergic receptor agonist, on energy expenditure and body composition in obese men. *Am. J. Clin. Nutr.* 76, 780–788.

Lean, M. E., James, W. P., Jennings, G., and Trayhurn, P. (1986). Brown adipose tissue in patients with phaeochromocytoma. *Int. J. Obes.* 10, 219–227.

Lee, P., Greenfield, J. R., Ho, K. K., and Fulham, M. J. (2010). A critical appraisal of the prevalence and metabolic significance of brown adipose tissue in adult humans. *Am. J. Physiol. Endocrinol. Metab.* 299, E601–E606.

Lee, P., Swarbrick, M. M., Zhao, J. T., and Ho, K. K. (2011a). Inducible brown adipogenesis of supraclavicular fat in adult humans. *Endocrinology* 152, 3597–3602.

Lee, P., Zhao, J. T., Swarbrick, M. M., Gracie, G., Bova, R., Greenfield, J. R., Freund, J., and Ho, K. K. (2011b). High prevalence of brown adipose tissue in adult humans. *J. Clin. Endocrinol. Metab.* 96, 2450–2455.

Liu, Y. L., Heal, D. J., and Stock, M. J. (2002). Mechanism of the thermogenic effect of metabolite 2 (BTS 54 505), a major pharmacologically active metabolite of the novel anti-obesity drug, sibutramine. *Int. J. Obes. Relat. Metab. Dis.* 26, 1245–1253.

Lowell, B. B., V, S. S., Hamann, A., Lawitts, J. A., Himms-Hagen, J., Boyer, B. B., Kozak, L. P., and Flier, J. S. (1993). Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. *Nature* 366, 740–742.

Martin, C. K., Heilbronn, L. K., De Jonge, L., Delany, J. P., Volaufova, J., Anton, S. D., Redman, L. M., Smith, S. R., and Ravussin, E. (2007). Effect of calorie restriction on resting metabolic rate and spontaneous physical activity. *Obesity (Silver Spring)* 15, 2964–2973.

Melmed, S., and Conn, P. M. (eds). (2005). *Endocrinology: Basic and Clinical Principles.* Totowa, NJ: Humana Press.

Nagase, I., Yoshida, T., Kumamoto, K., Umekawa, T., Sakane, N., Nikami, H., Kawada, T., and Saito, M. (1996). Expression of uncoupling protein in skeletal muscle and white fat of obese mice treated with thermogenic beta 3-adrenergic agonist. *J. Clin. Invest.* 97, 2898–2904.

Nakamura, K., and Morrison, S. F. (2007). Central efferent pathways mediating skin cooling-evoked sympathetic thermogenesis in brown adipose tissue. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 292, R127–R136.

Ornitz, D. M., and Itoh, N. (2001). Fibroblast growth factors. *Genome Biol.* 2, reviews3005.3001–3005.3012.

Ouellet, V., Routhier-Labadie, A., Bellemare, W., Lakhal-Chaieb, L., Turcotte, E., Carpentier, A. C., and Richard, D. (2011). Outdoor temperature, age, sex, body mass index, and diabetic status determine the prevalence, mass, and glucose-uptake activity of 18F-FDG-detected BAT in humans. *J. Clin. Endocrinol. Metab.* 96, 192–199.

Petersen, K. F., Dufour, S., Befroy, D., Lehrke, M., Hendler, R. E., and Shulman, G. I. (2005). Reversal of nonalcoholic hepatic steatosis, hepatic insulin resistance, and hyperglycemia by moderate weight reduction in patients with type 2 diabetes. *Diabetes* 54, 603–608.

Pfannenberg, C., Werner, M. K., Ripkens, S., Stef, I., Deckert, A., Schmadl, M., Reimold, M., Haring, H. U., Claussen, C. D., and Stefan, N. (2010). Impact of age on the relationships of brown adipose tissue with sex and adiposity in humans. *Diabetes* 59, 1789–1793.

Pischon, T., Boeing, H., Hoffmann, K., Bergmann, M., Schulze, M. B., Overvad, K., Van Der Schouw, Y. T., Spencer, E., Moons, K. G. M., Tjønneland, A., Halkjaer, J., Jensen, M. K., Stegger, J., Clavel-Chapelon, F., Boutron-Ruault, M.-C., Chajes, V., Linseisen, J., Kaaks, R., Trichopoulou, A., Trichopoulos, D., Bamia, C., Sieri, S., Palli, D., Tumino, R., Vineis, P., Panico, S., Peeters, P. H. M., May, A. M., Bueno-De-Mesquita, H. B., Van Duijnhoven, F. J. B., Hallmans, G., Weinehall, L., Manjer, J., Hedblad, B., Lund, E., Agudo, A., Arriola, L., Barricarte, A., Navarro, C., Martinez, C., Quirós, J. R., Key, T., Bingham, S., Khaw, K. T., Boffetta, P., Jenab, M., Ferrari, P., and Riboli, E. (2008). General and abdominal adiposity and risk of death in Europe. *N. Engl. J. Med.* 359, 2105–2120.

Qatanani, M., and Lazar, M. A. (2007). Mechanisms of obesity-associated insulin resistance: many choices on the menu. *Genes Dev.* 21, 1443–1455.

Redman, L. M., Heilbronn, L. K., Martin, C. K., De Jonge, L., Williamson, D. A., Delany, J. P., and Ravussin, E. (2009). Metabolic and behavioral compensations in response to caloric restriction: implications for the maintenance of weight loss. *PLoS ONE* 4, e4377. doi:10.1371/journal.pone.0004377

Ribeiro, M. O., Bianco, S. D., Kaneshige, M., Schultz, J. J., Cheng, S. Y., Bianco, A. C., and Brent, G. A. (2010). Expression of uncoupling protein 1 in mouse brown adipose tissue is thyroid hormone receptor-beta isoform specific and required for adaptive thermogenesis. *Endocrinology* 151, 432–440.

Ricquier, D., Nechad, M., and Mory, G. (1982). Ultrastructural and biochemical characterization of human brown adipose tissue in pheochromocytoma. *J. Clin. Endocrinol. Metab.* 54, 803–807.

Rothwell, N. J., and Stock, M. J. (1979). A role for brown adipose tissue in diet-induced thermogenesis. *Nature* 281, 31–35.

Saito, M., Okamatsu-Ogura, Y., Matsushita, M., Watanabe, K., Yoneshiro, T., Nio-Kobayashi, J., Iwanaga, T., Miyagawa, M., Kameya, T., Nakada, K., Kawai, Y., and Tsujisaki, M. (2009). High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* 58, 1526–1531.

Seale, P., Bjork, B., Yang, W., Kajimura, S., Chin, S., Kuang, S., Scime, A., Devarakonda, S., Conroe, H. M., Erdjument-Bromage, H., Tempst, P., Rudnicki, M. A., Beier, D. R., and Spiegelman, B. M. (2008). PRDM16 controls a brown fat/skeletal muscle switch. *Nature* 454, 961–967.

Seale, P., Conroe, H. M., Estall, J., Kajimura, S., Frontini, A., Ishibashi, J., Cohen, P., Cinti, S., and Spiegelman, B. M. (2011). Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. *J. Clin. Invest.* 121, 96–105.

Seale, P., Kajimura, S., and Spiegelman, B. M. (2009). Transcriptional control of brown adipocyte development and physiological function – of mice and men. *Genes Dev.* 23, 788–797.

Silva, J. E., and Rabelo, R. (1997). Regulation of the uncoupling protein gene expression. *Eur. J. Endocrinol.* 136, 251–264.

Sjostrom, L., Lindroos, A. K., Peltonen, M., Torgerson, J., Bouchard, C., Carlsson, B., Dahlgren, S., Larsson, B., Narbro, K., Sjostrom, C. D., Sullivan, M., and Wedel, H. (2004). Lifestyle, diabetes, and cardiovascular risk factors 10 years after bariatric surgery. *N. Engl. J. Med.* 351, 2683–2693.

Sjostrom, L., Narbro, K., Sjostrom, C. D., Karason, K., Larsson, B., Wedel, H., Lystig, T., Sullivan, M., Bouchard, C., Carlsson, B., Bengtsson, C., Dahlgren, S., Gummesson, A., Jacobson, P., Karlsson, J., Lindroos, A. K., Lonroth, H., Naslund, I., Olbers, T., Stenlof, K., Torgerson, J., Agren, G., and Carlsson, L. M. (2007). Effects of bariatric surgery on mortality in Swedish obese subjects. *N. Engl. J. Med.* 357, 741–752.

Soderlund, V., Larsson, S. A., and Jacobsson, H. (2007). Reduction of FDG uptake in brown adipose tissue in clinical patients by a single dose of propranolol. *Eur. J. Nucl. Med. Mol. Imaging* 34, 1018–1022.

Sturkenboom, M. G., Hoekstra, O. S., Postema, E. J., Zijlstra, J. M., Berkhof, J., and Franssen, E. J. (2009). A randomised controlled trial assessing the effect of oral diazepam on 18F-FDG uptake in the neck and upper chest region. *Mol. Imaging Biol.* 11, 364–368.

Timmons, J. A., Wennmalm, K., Larsson, O., Walden, T. B., Lassmann, T., Petrovic, N., Hamilton, D. L., Gimeno, R. E., Wahlestedt, C., Baar, K., Nedergaard, J., and Cannon, B. (2007). Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. *Proc. Natl. Acad. Sci. U.S.A.* 104, 4401–4406.

Tseng, Y. H., Kokkotou, E., Schulz, T. J., Huang, T. L., Winnay, J. N., Taniguchi, C. M., Tran, T. T., Suzuki, R., Espinoza, D. O., Yamamoto, Y., Ahrens, M. J., Dudley, A. T., Norris, A. W., Kulkarni, R. N., and Kahn, C. R. (2008). New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* 454, 1000–1004.

Umekawa, T., Yoshida, T., Sakane, N., Saito, M., Kumamoto, K., and Kondo, M. (1997). Anti-obesity and anti-diabetic effects of CL316,243, a highly specific beta 3-adrenoceptor agonist, in Otsuka Long-Evans Tokushima Fatty rats: induction of uncoupling protein and activation of glucose transporter 4 in white fat. *Eur. J. Endocrinol.* 136, 429–437.

Van Baak, M. A., Hul, G. B., Toubro, S., Astrup, A., Gottesdiener, K. M., Desmet, M., and Saris, W. H. (2002). Acute effect of L-796568, a novel beta 3-adrenergic receptor agonist, on energy expenditure in obese men. *Clin. Pharmacol. Ther.* 71, 272–279.

Van Marken Lichtenbelt, W. D., and Schrauwen, P. (2011). Implications of nonshivering thermogenesis for energy balance regulation in humans. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 301, R285–R296.

Van Marken Lichtenbelt, W. D., Vanhommerig, J. W., Smulders, N. M., Drossaerts, J. M., Kemerink, G. J., Bouvy, N. D., Schrauwen, P., and Teule, G. J. (2009). Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* 360, 1500–1508.

Vijgen, G. H., Bouvy, N. D., Teule, G. J., Brans, B., Schrauwen, P., and Van Marken Lichtenbelt, W. D. (2011). Brown adipose tissue in morbidly obese subjects. *PLoS ONE* 6, e17247. doi:10.1371/journal.pone.0017247

Villicev, C. M., Freitas, F. R., Aoki, M. S., Taffarel, C., Scanlan, T. S., Moriscot, A. S., Ribeiro, M. O., Bianco, A. C., and Gouveia, C. H. (2007). Thyroid hormone receptor beta-specific agonist GC-1 increases energy expenditure and prevents fat-mass accumulation in rats. *J. Endocrinol.* 193, 21–29.

Virtanen, K. A., Lidell, M. E., Orava, J., Heglind, M., Westergren, R., Niemi, T., Taittonen, M., Laine, J., Savisto, N. J., Enerback, S., and Nuutila, P. (2009). Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* 360, 1518–1525.

Vuppalanchi, R., and Chalasani, N. (2009). Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: selected practical issues in their evaluation and management. *Hepatology* 49, 306–317.

Wang, Q., Zhang, M., Ning, G., Gu, W., Su, T., Xu, M., Li, B., and Wang, W. (2011). Brown adipose tissue in humans is activated by elevated plasma catecholamines levels and is inversely related to central obesity. *PLoS ONE* 6, e21006. doi:10.1371/journal.pone.0021006

Watanabe, M., Houten, S. M., Mataki, C., Christoffolete, M. A., Kim, B. W., Sato, H., Messaddeq, N., Harney, J. W., Ezaki, O., Kodama, T., Schoonjans, K., Bianco, A. C., and Auwerx, J. (2006). Bile acids induce energy expenditure by promoting intracellular thyroid hormone activation. *Nature* 439, 484–489.

Weinsier, R. L., Hunter, G. R., Desmond, R. A., Byrne, N. M., Zuckerman, P. A., and Darnell, B. E. (2002). Free-living activity energy expenditure in women successful and unsuccessful at maintaining a normal body weight. *Am. J. Clin. Nutr.* 75, 499–504.

Weyer, C., Tataranni, P. A., Snitker, S., Danforth, E. Jr., and Ravussin, E. (1998). Increase in insulin action and fat oxidation after treatment with CL 316,243, a highly selective beta3-adrenoceptor agonist in humans. *Diabetes* 47, 1555–1561.

Wijers, S. L., Schrauwen, P., Van Baak, M. A., Saris, W. H., and Van Marken Lichtenbelt, W. D. (2011). Beta-adrenergic receptor blockade does not inhibit cold-induced thermogenesis in humans: possible involvement of brown adipose tissue. *J. Clin. Endocrinol. Metab.* 96, E598–E605.

Wing, R. R., and Hill, J. O. (2001). Successful weight loss maintenance. *Annu. Rev. Nutr.* 21, 323–341.

Xue, B., Rim, J. S., Hogan, J. C., Coulter, A. A., Koza, R. A., and Kozak, L. P. (2007). Genetic variability affects the development of brown adipocytes in white fat but not in interscapular brown fat. *J. Lipid Res.* 48, 41–51.

Yamashita, H., Sato, Y., Kizaki, T., Oh, S., Nagasawa, J., and Ohno, H. (1994). Basic fibroblast growth factor (bFGF) contributes to the enlargement of brown adipose tissue during cold acclimation. *Pflugers Arch.* 428, 352–356.

Yilmaz, Y., Ones, T., Purnak, T., Ozguven, S., Kurt, R., Atug, O., Turoglu, H. T., and Imeryuz, N. (2011). Association between the presence of brown adipose tissue and nonalcoholic fatty liver disease in adult humans. *Aliment. Pharmacol. Ther.* 34, 318–323.

Yoneshiro, T., Aita, S., Matsushita, M., Okamatsu-Ogura, Y., Kameya, T., Kawai, Y., Miyagawa, M., Tsujisaki, M., and Saito, M. (2011). Age-related decrease in cold-activated brown adipose tissue and accumulation of body fat in healthy humans. *Obesity (Silver Spring)* 19, 1755–1760.

Zheng, W., Mclerran, D. F., Rolland, B., Zhang, X., Inoue, M., Matsuo, K., He, J., Gupta, P. C., Ramadas, K., Tsugane, S., Irie, F., Tamakoshi, A., Gao, Y.-T., Wang, R., Shu, X.-O., Tsuji, I., Kuriyama, S., Tanaka, H., Satoh, H., Chen, C.-J., Yuan, J.-M., Yoo, K.-Y., Ahsan, H., Pan, W.-H., Gu, D., Pednekar, M. S., Sauvaget, C., Sasazuki, S., Sairenchi, T., Yang, G., Xiang, Y.-B., Nagai, M., Suzuki, T., Nishino, Y., You, S.-L., Koh, W.-P., Park, S. K., Chen, Y., Shen, C.-Y., Thornquist, M., Feng, Z., Kang, D., Boffetta, P., and Potter, J. D. (2011). Association between body-mass index and risk of death in more than 1 million Asians. *N. Engl. J. Med.* 364, 719–729.

Zingaretti, M. C., Crosta, F., Vitali, A., Guerrieri, M., Frontini, A., Cannon, B., Nedergaard, J., and Cinti, S. (2009). The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J.* 23, 3113–3120.

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