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The brown fat secretome: metabolic functions beyond thermogenesis

Guo-Xiao Wang¹, Xu-Yun Zhao¹, and Jiandie D. Lin^{1,*}

¹Life Sciences Institute and Department of Cell & Developmental Biology, University of Michigan, Ann Arbor, Michigan 48109, USA

Abstract

Brown fat is highly active in fuel oxidation and dissipates chemical energy through uncoupling protein 1 (UCP1)-mediated heat production. Activation of brown fat leads to increased energy expenditure, reduced adiposity, and lower plasma glucose and lipid levels, thus contributing to better homeostasis. Uncoupled respiration and thermogenesis have been considered to be responsible for the metabolic benefits of brown adipose tissue. Recent studies have demonstrated that brown adipocytes also secrete factors that act locally and systemically to influence fuel and energy metabolism. This review discusses the evidence supporting a thermogenesis-independent role of brown fat, particularly through its release of secreted factors, and their implications in physiology and therapeutic development.

Introduction

Brown adipose tissue (BAT) defends against hypothermia in small mammals and newborn infants through thermogenesis. Mature brown adipocytes contain abundant mitochondria and express high levels of uncoupling protein 1 (UCP1), which dissipates the proton gradient across the mitochondrial inner membrane to produce heat [1]. BAT thermogenesis is stimulated by cold exposure through activation of the sympathetic nervous system (SNS) that triggers local catecholamine release and thyroid hormone production [1-3]. The marked increase in glucose and lipid uptake and oxidation is accompanied by an induction of genes involved in mitochondrial biogenesis, fatty acid β -oxidation, and uncoupled respiration [4, 5]. Not surprisingly, BAT thermogenesis also contributes to whole body energy balance. Thus, genetic ablation of brown fat renders mice sensitive to cold and prone to the development of obesity [6], whereas activation of BAT thermogenesis has been linked to increased energy expenditure, reduced adiposity, and lower plasma lipids [7-9].

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* Corresponding Author: Jiandie Lin, Ph.D. 5437 Life Sciences Institute University of Michigan 210 Washtenaw Avenue Ann Arbor, MI 48109 jclin@umich.edu Office: (734) 615-3512 Fax: (734) 615-0495.

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In humans, brown fat is present in newborn infants and has been thought to be absent in adults. Recent studies using positron emission tomography (PET) demonstrated that metabolically active brown fat is present in some adults [10-13]. Human brown fat appears to contain both classical and brown-like adipocytes, cells that have both distinct molecular signatures and developmental origins [14-17]. The latter was also called beige, brite, or inducible brown adipocytes (referred to beige hereafter). In rodents, cold acclimation and the β 3-selective adrenergic agonist CL316,243 promote the formation of beige adipocytes within white adipose tissue (WAT), particularly the inguinal fat depot [18, 19]. WAT browning is also induced by the insulin sensitizing agent rosiglitazone and a growing list of secreted factors [20]. The developmental origin and molecular control of brown and beige fat formation have been discussed in detail in several recent reviews [20-22].

Does brown fat contribute to systemic metabolism via thermogenesis-independent mechanisms?

Mitochondrial uncoupling has been recognized as a central aspect of brown fat biology. Genetic deletion of UCP1 completely abolished uncoupled respiration and thermogenesis in BAT and rendered the null mice cold sensitive [23]. Similarly, diphtheria toxin-mediated ablation of brown fat also severely impaired cold-induced thermogenesis and defense against hypothermia [6, 24]. Mice lacking brown fat were more prone to high-fat diet-induced obesity and its associated metabolic disorders, including insulin resistance and hyperlipidemia. Surprisingly, UCP1-deficiency had a modest effect on diet-induced obesity in mice when housed at ambient room temperature [23, 25, 26]. These paradoxical observations strongly suggest that brown fat contributes to whole body energy homeostasis through additional mechanisms beyond UCP1-mediated thermogenesis.

Secreted factors are important regulators of fuel metabolism and energy balance, as illustrated by the classic endocrine hormones, such as insulin and glucagon. Further, adipose tissue hormones, such as leptin and adiponectin, gut-derived fibroblast growth factors, skeletal myokines, and immune cell-derived factors are emerging to coordinate diverse aspects of metabolic physiology. While WAT has been recognized as an endocrine organ [27, 28], much less is known about the extent to which BAT engages other tissues through its release of protein and non-protein factors [29]. Recent studies demonstrated that BAT transplantation profoundly improves metabolic parameters in mouse models of obesity and diabetes [30-33]. Subcutaneous transplantation of embryonic BAT corrected type 1 diabetes in mice treated with streptozotocin, possibly due to increased serum levels of insulin-like growth factor 1 (IGF-1) and potential activation of the insulin receptor. [31]. Similarly, BAT transplantation improved metabolic parameters in diet-induced obese mice; such beneficial effect required the expression and release of interleukin-6 (IL-6) from the BAT used for transplantation [33]. Interestingly, transplantation of BAT also conferred resistance to high-fat diet-induced obesity through enhanced sympathetic activity, although the nature of factors that increase sympathetic activity in the recipient mice remains unknown [30]. Together, these observations support the emerging concept that the brown fat secretome provides a physiologically significant link between BAT and systemic metabolism.

The brown fat secretome: autocrine, paracrine, and endocrine functions

WAT is known to release important endocrine factors such as leptin and adiponectin [27, 28]. The repertoire of secreted proteins released by brown and beige fat and their physiological functions have not been fully defined. Previous studies have demonstrated that brown fat also synthesizes diverse signaling molecules that alter metabolic physiology via autocrine, paracrine, and endocrine mechanisms. Biologically active molecules, such as thyroid hormone, lipid metabolites, and lactate, may act locally to modulate brown fat development and thermogenesis, whereas secreted factors may enter circulation to exert metabolic effects on other tissues. Recent secretome profiling analysis revealed a distinct set of brown fat-enriched secreted factors [34]. While the existence of a brown fat-specific secreted protein(s) appears unlikely, a growing list of extracellular factors exhibits enriched expression in brown fat and is inducible in response to thermogenic activation. In this section, we will summarize several protein and non-protein factors produced by BAT and discuss their potential role in metabolic signaling and homeostasis.

Neuregulin 4 (Nrg4)

Nrg4 was recently identified as a brown fat-enriched secreted factor in a recent analysis of secretomes across mouse tissues and during brown adipocyte differentiation [34]. Nrg4 expression was strongly induced during brown adipogenesis and further increased by adrenergic receptor activation in brown adipocytes [34, 35]. Acute cold exposure stimulated Nrg4 expression in BAT, whereas cold acclimation elevated Nrg4 mRNA levels in both BAT and inguinal WAT (figure 1). Nrg4 belongs to the epidermal growth factor (EGF) family of extracellular ligands that bind to and activate the receptor tyrosine kinases ErbB3 and ErbB4. Nrg4 is synthesized as a single-span transmembrane protein that contains an extracellular EGF-like domain responsible for receptor binding. A proteolytic site exists between the EGF-like domain and the transmembrane fragment, allowing Nrg4 to be shed from the plasma membrane by one or more matrix metalloproteinases. Using a binding assay, Nrg4 was found to bind to the liver, among a panel of mouse tissues.

It is somewhat unexpected that Nrg4 is largely dispensable for brown fat development and function [34]. In fact, mice lacking Nrg4 were comparable with wild type control littermates in defense against hypothermia caused by cold exposure. These findings suggest that, despite its abundant expression in brown fat, Nrg4 is not required for cold-induced thermogenesis. Adipose tissue Nrg4 expression was reduced in several mouse models of obesity and negatively correlated with body fat mass in humans, suggesting that Nrg4 insufficiency may be a common feature of obesity. The significance of Nrg4 signaling in obesity was demonstrated using Nrg4 knockout and transgenic mice. Nrg4 deficiency exacerbated diet-induced obesity and metabolic disorders in mice, whereas transgenic expression of Nrg4 in adipose tissues had the opposite effects. At the mechanistic level, Nrg4 binds to and activates the receptor tyrosine kinases ErbB3 and ErbB4, leading to STAT5 phosphorylation in hepatocytes. The latter attenuates the induction of *de novo* lipogenesis in response to activation of the LXR/SREBP1c pathway, in a cell-autonomous manner. Previous studies have demonstrated that Nrg4 stimulated neurite outgrowth of

cultured PC-12 cells [35, 39], raising the possibility that this factor may act on extrahepatic tissues to exert its metabolic effects.

Fibroblast Growth Factor 21 (FGF21)

FGF21 is an endocrine factor produced by several tissues, including the liver, BAT, and skeletal muscle [40–42]. While the liver provides a major source for FGF21 in the circulation, brown fat also contributes to plasma FGF21 under certain conditions, such as cold exposure and after BAT transplantation [33, 40]. Prolonged cold exposure markedly increased FGF21 mRNA expression in rodent BAT, whereas its expression in the liver was reduced. Arteriovenous flow analysis revealed that FGF21 from activated BAT entered the circulation and contributed to the rise of plasma FGF21 following cold exposure [40]. A similar cold-induced increase of plasma FGF21 levels was also observed in humans [43]. FGF21 is known to exert pleiotropic effects on glucose and lipid metabolism, particularly hepatic fatty acid β -oxidation and gluconeogenesis during starvation [44–46]. Systemic administration of FGF21 corrected obesity in mice and improved metabolic homeostasis in humans [47, 48]. Recent work demonstrated that FGF21 stimulates the thermogenic gene program in brown adipocytes and promotes white fat browning [43, 49]. In addition, FGF21 indirectly regulates brown fat thermogenesis through acting on the central nervous system to stimulate sympathetic outflow to adipose tissues [50] (figure 1). Thus, the inducible release of FGF21 is emerging as an important molecular signal that impinges on systemic fuel metabolism and thermogenesis.

Vascular Endothelial Growth Factor (VEGF)

VEGFs are widely distributed among tissues with both VEGFA and VEGFB expressing at high levels in BAT [51]. Acute cold stress significantly increases VEGFA, but not VEGFB, expression in brown fat [52–54]. Cold-induced VEGFA expression is mediated through the β -adrenergic pathway and can be recapitulated *in vitro* by treating differentiated brown adipocytes with norepinephrine. BAT-derived VEGFA largely functions in an endocrine and paracrine manner, as it directly stimulates proliferation of adipocyte progenitors and the surrounding vascular endothelial cells [55, 56] (figure 1). Fat-specific VEGFA transgenic mice were protected from diet-induced obesity and metabolic disorders as a result of the pleiotropic effects elicited by VEGF signaling, including stimulation of angiogenesis, brown fat thermogenesis, and modulation of macrophage polarization [57]. Overexpressing VEGFA in BAT alone increased thermogenesis in mice during chronic cold exposure, and partly ameliorated the metabolic dysfunction associated with diet-induced obesity [58]. However, plasma VEGFA levels remained unchanged in the transgenic mice, suggesting that the contribution of adipose tissues to systemic VEGFA is modest [57]. Similar to VEGFA, VEGFB exerts local effects on adipose tissues, in part through stimulating endothelial cell proliferation and fatty acid uptake [59, 60].

Bone Morphogenetic Proteins (BMPs)

BMPs are members of the transforming growth factor β (TGF β) superfamily of extracellular signaling proteins initially found to regulate bone and cartilage formation and repair [61]. Several BMP members have been implicated in the regulation of adipocyte differentiation

and energy expenditure [62]. BMP7 treatments enhanced Ucp1 gene expression and mitochondrial biogenesis during brown adipocyte differentiation and promoted differentiation of mesenchymal progenitors toward the brown adipocyte lineage [63]. BMP7 also increased the formation of brown-like adipocytes derived from skeletal muscle and subcutaneous white fat [64]. However, the cellular sources of BMP7 within the adipose tissues remain to be clarified. BMP4 also promotes adipogenesis and the formation of beige adipocytes [66] (figure 1). Transgenic expression of BMP4 increased energy expenditure and protected mice from diet-induced obesity, whereas BMP4 null mice developed more severe insulin resistance. Consistent with a direct role of BMP signaling on brown fat development, mice lacking type 1A BMP receptor had significantly reduced brown fat mass that was associated with a compensatory activation of white fat browning [65]. Beyond the local effects of BMPs in brown fat, BMP8b appears to also act on the hypothalamus to regulate energy expenditure [67]. In this case, BMP8b may serve an important role in mediating the feedback from adipose tissues to the sympathetic nervous system (figure 1).

Interleukin-6 (IL-6)

IL-6 is a cytokine produced by multiple tissues in the body, including adipose tissues, skeletal muscle, and immune cells. Beyond its role in inflammatory response, IL-6 exerts profound and pleiotropic effects on glucose metabolism and energy balance [68]. Mice lacking IL-6 developed mature-onset obesity and diet-induced glucose intolerance. Because plasma IL-6 levels were significantly increased by exercise, IL-6 has been proposed as a major myokine that contributes to the metabolic benefits of physical activity. Interestingly, the anti-inflammatory immunomodulator amlexanox, an inhibitor of the serine/threonine protein kinase TBK1 and of NF- κ B Kinase Subunit Epsilon (Ikk-e), induced IL-6 gene expression in subcutaneous adipose tissue and strongly increased plasma IL-6 concentrations [69, 70]. In this case, IL-6 engaged the signal transducer and activator of transcription 3 (STAT3) signaling pathway to acutely repress hepatic gluconeogenic gene expression and glucose output. Chronic activation of IL-6 signaling promoted white fat browning and increased energy expenditure in the context of cancer-associated cachexia [71]. Recent studies have implicated BAT-derived IL-6 as a key mediator of the improvement of glucose homeostasis and insulin sensitivity following brown fat transplantation [33]. It would be of interest to establish the significance of IL-6 released by brown fat in physiological regulation of metabolic homeostasis.

Nerve growth factor (NGF)

Nerve growth factor (NGF) is a growth factor that promotes the survival and proliferation of neurons, including the peripheral sensory and sympathetic neurons [80]. There are two structurally different types of receptors identified for NGF, the high affinity receptor tyrosine kinase A (TrkA) and the low-affinity neurotrophin receptor p75 [81, 82]. Upon binding to TrkA, NGF activates the PI3K/AKT and Ras/MAPK signaling pathways and promotes cell survival and proliferation. NGF also binds to p75 and initiates c-Jun amino-terminal kinase (JNK) activity. Interestingly, NGF is also produced by brown fat, along with its receptor p75, and by cultured brown adipocytes [83]. Its expression was reduced in response to cold exposure or treatments with norepinephrine [84]. NGF signals through TrkA to promote sympathetic axon growth and the innervation of target tissues [85, 86]. It is

possible that NGF signaling may establish and/or remodel sympathetic innervation of brown adipose tissues.

Adiponectin

Adiponectin was initially identified as a secreted factor released by white adipocytes and later found to be also expressed in BAT [72-74]. Adiponectin expression in WAT and its plasma concentrations negatively correlate with obesity. In mice, adiponectin deficiency exacerbated diet-induced insulin resistance [75], while acute increase in circulating adiponectin levels lowered hepatic glucose production [76]. In addition to its role in hepatic metabolism, adiponectin also stimulates fatty acid oxidation and glucose uptake in skeletal muscle [77], and regulates energy expenditure by acting on the hypothalamus [78]. Recent work demonstrated that bone marrow adipose tissue is a major source of circulating adiponectin during caloric restriction [79], and as such, the contribution of brown fat to endocrine signaling by adiponectin is likely minor compared to other fat depots.

Hormones and metabolites

Brown adipocytes are known to produce biologically active molecules that modulate thermogenesis, such as thyroid hormone (triiodothyronine, T3), retinaldehyde, and retinoic acid. Thyroid hormone has been recognized as an important regulator of thermogenesis. The expression of type II thyroxine 5'-deiodinase (Dio2), which converts thyroxine to T3, is restricted to BAT and strongly induced during BAT activation [87]. Mice with targeted ablation of Dio2 suffered hypothermia upon cold exposure despite normal plasma T3 levels, suggesting that BAT-derived T3 likely acts primarily locally to stimulate thermogenesis [88]. Both retinaldehyde and retinoic acid serve as agonists for retinoic acid receptor and appear to modulate BAT thermogenesis through their effects on brown fat development and thermogenic gene expression [89]. Brown adipocytes also express 11 β -hydroxysteroid dehydrogenase 1, an enzyme responsible for the conversion of biologically inert cortisone to active cortisol [90], suggesting that endogenously produced glucocorticoids may regulate brown fat biology through an autocrine mechanism.

Cold exposure strongly stimulates adipose tissue lipolysis, resulting in increased release of fatty acids from adipocytes. In addition to providing an important source of fuel, free fatty acids also engage a unique class of G protein-coupled receptors called free fatty acid receptors (FFARs) [91, 92]. Because FFAR expression is highly tissue-specific, it is conceivable that activation of FFARs by free fatty acids likely elicit pleiotropic metabolic responses in different tissues. Among fatty acids released by adipocytes, the branched fatty acid esters of hydroxyl fatty acids represent a new class of biologically active lipids that improve glucose tolerance and reduce adipose tissue inflammation in obesity [93]. Brown adipose tissue releases significant amounts of lactate derived from glycolysis during cold exposure. Recent work demonstrated that adipose tissue expresses high levels of Gpr81, a cognate receptor for lactate, and that activation of Gpr81 by lactate attenuates adrenergic receptor signaling and inhibits lipolysis in adipocytes [94]. Thus, it is likely that lactate released by brown fat during cold-induced thermogenesis may provide a brake for lipolysis in response to adrenergic receptor activation.

Metabolic adaptation associated with cold-induced thermogenesis

Acute cold stress stimulates sympathetic nerve activity, leading to increased adipose tissue lipolysis and thermogenesis, whereas chronic cold exposure increases sympathetic innervation and promotes BAT hypertrophy and WAT browning. Beyond adipose tissues, metabolic activities in other tissues also undergo significant changes under thermogenic conditions. Cold exposure induces the activities of hepatic gluconeogenic enzymes, leading to increased hepatic glucose production [95]. Similarly, mitochondrial oxidation and ketone production are also elevated in the liver following cold acclimation. Elevated hepatic glucose and ketone production likely contributes to fuel homeostasis during cold-induced thermogenesis. Recent work demonstrated that skeletal muscle also contributes to non-shivering thermogenesis through stimulating sarcoplasmic Ca^{2+} futile cycling [96]. Accordingly, cold exposure significantly increased the activities of key enzymes involved in glycolysis and citrate acid cycle in skeletal muscle [97]. In addition, oxidative muscles adapted to cold by increasing angiogenesis in non-hibernating rodents, potentially augmenting the supply of oxygen to the tissue. These observations illustrate that cold-induced thermogenesis is tightly linked to a coordinated program of metabolic adaptation in other tissues to maintain fuel and energy homeostasis.

The factors responsible for coordinating the metabolic adaptations in the liver and skeletal muscle during thermogenesis remain unknown. Stress hormones, such as norepinephrine and glucocorticoids, are released by the adrenal gland and significantly elevated in plasma after both short-time cold exposure and long-time cold acclimation [98]. It is likely that cold-induced changes of endocrine signaling contribute to metabolic adaptation. What has been much less known is whether brown and beige adipocytes may play a more direct role in coordinating metabolic adaptation through their release of secreted factors. In this regard, it is notable that the expression and secretion of these factors by brown fat are highly responsive to cold exposure. Interestingly, adipose tissues appear to contain afferent sensory innervation that may relay information to the central nervous system, which in turn modulates thermogenic response [99]. Future studies are needed to unravel the molecular nature of the neural and endocrine networks that are engaged to maintain whole body fuel homeostasis during thermogenesis.

Concluding remarks and future perspectives

The recognition that functional brown/beige fat is present in adult humans raises the prospect that brown fat abundance and/or function may be augmented to restore energy balance in obesity and treat obesity-associated metabolic disorders. In support of this, total brown fat mass appears to inversely correlate with body mass index and body fat content [12]. In mice, cold-induced thermogenesis ameliorates weight gain and improves plasma glucose and lipid profiles. As such, a potential strategy to harness the beneficial effects of brown fat is to develop pharmacological agents that enhance the total mass and/or activity of BAT. While $\beta 3$ -adrenergic receptor agonists have been shown to potently stimulate brown fat thermogenesis and browning of white fat in mice, therapeutic development targeting this receptor in humans has encountered significant challenges due to species-specific biology of the $\beta 3$ -adrenergic receptor and undesirable side effects [63]. Recent work has uncovered a

growing number of circulating factors that promote brown and beige fat development. While the biology underlying the action of these factors in whole body energy balance remains to be elucidated, it is conceivable that they may offer molecular targets for the development of biologic agents to promote thermogenesis and energy expenditure in humans.

A potentially fruitful but much less appreciated avenue toward developing therapies based on brown fat thermogenesis is through targeting the extracellular factors released by brown fat. These secreted factors may participate in coordinating various aspects of metabolic adaptation during thermogenesis and exert metabolic benefits on adipose tissues (autocrine and paracrine) and other peripheral tissues (endocrine). For example, the expression of FGF21 is strongly induced in brown fat in response to cold exposure. Elevating FGF21 levels promotes hepatic fat oxidation and improves glucose homeostasis, in part through its stimulation of WAT browning and thermogenesis. Nrg4 is a brown fat-enriched secreted factor that attenuate hepatic lipogenic signaling and preserves metabolic homeostasis during diet-induced obesity [34]. Defining the brown fat secretomes and elucidating their functions are a critical first step toward understanding the metabolic crosstalk between BAT and other tissues during thermogenesis. We should witness in the coming years whether this approach succeeds in yielding new therapies for metabolic disorders.

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Abbreviations

BAT	brown adipose tissue
FGF21	fibroblast growth factor 21
BMP	bone morphogenesis protein
Nrg4	neuregulin 4
EGF	epidermal growth factor
ErbB	epidermal growth factor receptor
VEGF	vascular endothelial growth factor
IL-6	interleukin-6
NGF	nerve growth factor
FFA	free fatty acids
Rald	Retinaldehyde
RA	Retinoic acid

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Highlights

1. Brown fat contributes to systemic metabolism via thermogenesis dependent and independent mechanisms
2. The brown fat secretome includes Nrg4, FGF21, and BMPs, among others
3. The brown fat secretome may coordinate metabolic adaptation during thermogenesis
4. Developing novel therapies based on brown fat thermogenesis may target the extracellular factors released by brown fat.

Figure 1. Secreted factors released by brown fat

Brown adipose tissue is a source of protein and non-protein signaling factors that influence diverse metabolic processes. While brown fat is not the exclusive source of these extracellular signaling factors, many of them exhibit enriched and/or inducible expression in BAT upon thermogenic stimulation. Nrg4 is an EGF-like endocrine factor that is enriched in brown fat. Nrg4 binds to ErbB receptors in the liver and preserves metabolic homeostasis in obesity through attenuating hepatic lipogenesis. FGF21 is induced in brown fat by cold exposure and exerts pleiotropic effects on hepatic metabolism, white fat browning, sympathetic outflow, and BAT thermogenesis. BMP promotes brown and beige fat formation and also acts on the central nervous system to regulate thermogenesis. VEGFA and VEGFB are expressed at high levels in brown fat and regulate angiogenesis, thermogenesis, and macrophage function. T3, Rald, and RA exert effects locally to promote thermogenesis. Additional secreted factors include IL-6, Adiponectin, and metabolites released upon thermogenic activation, such as FFA and lactate.