Curr Obes Rep
DOI 10.1007/s13679-015-0157-8

OBESITY TREATMENT (CM APOVIAN, SECTION EDITOR)

The Whitening of Brown Fat and Its Implications for Weight Management in Obesity

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Abstract Systemic inflammation resulting from dysfunction of white adipose tissue (WAT) accelerates the pathologies of diabetes and cardiovascular diseases. In contrast to WAT, brown adipose tissue (BAT) is abundant in mitochondria that produce heat by uncoupling respiratory chain process of ATP synthesis. Besides BAT's role in thermogenesis, accumulating evidence has shown that it is involved in regulating systemic metabolism. Studies have analyzed the "browning" processes of WAT as a means to combat obesity, whereas few studies have focused on the impact and molecular mechanisms that contribute to obesity-linked BAT dysfunction—a process that is associated with the "whitening" of this tissue. Compared to WAT, a dense vascular network is required to support the high energy consumption of BAT. Recently, vascular rarefaction was shown to be a significant causal factor in the whitening of BAT in mouse models. Vascular insufficiency leads to mitochondrial dysfunction and loss in BAT and contributes to systemic insulin resistance. These data suggest that BAT "whitening," resulting from vascular dysfunction, can impact obesity and obesity-linked diseases. Conversely, agents that promote BAT function could have utility in the treatment of these conditions.

This article is part of the Topical Collection on *Obesity Treatment*

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Keywords Obesity · Whitening of BAT · Insulin resistance · VEGF-A

Introduction

Obesity has the central role for the development of diabetes and is known to increase the risk of death, predominantly from cardiovascular diseases [1]. Many studies have shown that chronic low-grade adipose inflammation resulting from an imbalance of adipokine production from white adipose tissue (WAT) contributes to systemic metabolic dysfunction [2]. Excessive free fatty acid (FFA) production and release in adipose tissue leads to adipocyte dysfunction and localized inflammatory cell activation. Ultimately, this condition triggers the production of pro-inflammatory adipokines and chemoattractants that increase the infiltration of inflammatory cells, further promoting the inflammatory process. Overall, WAT dysfunction is characterized by an influx of macrophages and other inflammatory cells, the upregulation of pro-inflammatory adipokines and the downregulation of anti-inflammatory adipokines, and it is this adipokine imbalance that confers, in large part, the increased risk of cardiometabolic disease.

In contrast to WAT, brown adipose tissue (BAT) is a highly vascularized organ that is abundant in mitochondria. BAT mitochondria are enriched in uncoupling protein1 (UCP-1) and produce heat by uncoupling the respiratory chain [3]. BAT is abundant in small rodents and newborn humans. BAT was once thought to disappear during postnatal human development, but recent studies have shown that adults also possess active BAT [4–7]. It is now believed that human adults possess 50–80 g of active brown adipose tissue. Acute cold exposure (18 °C for 3 h) increases glucose and fatty acid uptake in the BAT anatomical area, and this is associated with

Published online: 21 April 2015

increased energy expenditure in humans [8]. Chronic exposure to cold temperature is also reported to recruit BAT in humans and increase non-shivering thermogenesis [9]. Even in individuals who have reduced BAT, chronic cold exposure (17 °C for 2 h daily for 6 weeks) was shown to recruit BAT and decrease body fat mass although body weight did not show definite change under this observation period [10•].

It is increasingly realized that BAT contributes significantly to systemic metabolism because of its high energetic expenditure ratio [3, 11–15]. Based upon the growing realization that BAT is a potential regulator of systemic metabolic function, studies have analyzed the "browning" of WAT, i.e., make it more BAT-like, as a strategy to combat obesity [16–18]. However, it has been argued that these actions are quantitatively incapable of significantly impacting systemic metabolism due to the low thermogenic capacity of WAT browning relative to that exhibited by classical BAT depots [19].

Because BAT function is known to decrease with obesity and aging [5, 20], the decline in BAT function may link metabolic dysfunction and weight gain under these conditions. However, the molecular mechanisms that lead to reduced BAT activity in obesity and its physiological implications are largely unknown. Several studies have focused on the role of vascularity in WAT and systemic metabolic dysfunction [21–25]. While these studies have largely correlated capillary rarefaction with WAT dysfunction, relatively few studies have explored the role of BAT vascularity [26]. Recently, it was shown that overnutrition promotes vascular rarefaction and hypoxia in BAT of mice, causing it to whiten through mitochondrial dysfunction and loss [27•]. In this review, we delineate the pathology of BAT whitening associated with vascular dysfunction and discuss its role in systemic metabolic dysfunction.

Molecular Pathways that Maintain BAT Function

BAT is under the control of the sympathetic nervous system. Norepinephrine binds to adrenergic receptors (AR) and activates an AR-adenylyl cyclase-cAMP-protein kinase A (PKA) signaling cascade that leads to the upregulation of thermogenic genes, such as *Pgc-1α* and *Ucp1* [28]. PKA also positively regulates lipase activity, enhancing lipolysis and releasing FFA in brown adipocytes for oxidation and heat production. Although, catecholamine signaling-mediated activation of BAT function could lead to weight loss, in part by promoting an increase in uncoupled mitochondrial respiration, clinical trials targeting catecholamine receptors have been disappointing. Whereas activation of the sympathetic nervous system (SNS) led to weight loss, side effects due to nonselective catecholamine signaling diminished their clinical usefulness [29, 30].

Recently, several other molecules have been shown to have roles in regulating BAT homeostasis. It has been reported that bile acids bind to G-protein-coupled bile acid receptor and upregulate BAT function via the induction of 5′-deiodinase and local induction of triiodothyronine that induces *Ucp-1* gene expression [31]. Bile acids also upregulate BAT activity by the modulation of glucagon-like peptide-1 (GLP1) signaling [32, 33]. Furthermore, a link has been reported between fibroblast growth factor 21 (FGF21) and BAT activation. FGF21, predominantly secreted from liver, binds to the FGF receptor/b-Klotho complex in BAT and induces uncoupled respiration and glucose oxidation [34]. FGF21 promotes the browning of WAT depots and BAT activation in adults; however, it also has been shown to promote bone loss [35, 36]. ANP and BNP secreted from cardiac tissue bind to NP receptors (NPRs) on the brown adipocyte surface to activate cGMP-dependent protein kinase and positively regulate thermogenic gene expression [37], suggesting a mechanism by which the heart can modulate systemic metabolism. BAT secretes bone morphogenetic protein-8b (BMP8b) that acts directly on BAT to sensitize it to SNS stimulation. BMP8b also induces the hypothalamus to modulate sympathetic outflow and influences the activation of BAT [38]. Interestingly, BMP8b selectively activates the SNS outflow toward BAT while sparing SNS flow to the kidney. Finally, alternatively activated (M2) macrophages have been shown to control BAT thermogenesis via local release of catecholamines [39]. Collectively, these recent studies have led to the speculation that noncanonical activation of BAT function may represent promising therapeutic targets for obesity and diabetes (reviewed in [40–43]).

Mechanisms that Contribute to BAT "Whitening" in Obesity

BAT function declines with obesity and aging, giving it a "whitened" appearance, but the mechanisms that contribute to this decline have been incompletely defined. While more than 100 studies have recently analyzed the mechanism of WAT "browning," very few studies have focused on the processes by which BAT whitens [44, 45]. It has been shown in a mouse model that obesity induces the whitening of BAT that is characterized by diminished β-adrenergic signaling, the accumulation of large lipid droplets and mitochondrial dysfunction, and loss [27•]. Notably, this whitening was associated with the preferential loss of vascularity in BAT and the development of hypoxia (Fig. 1). Several lines of evidence suggest that the hypoxic state in BAT results from the loss of vascular endothelial growth factor (VEGF) expression. First, VEGF-loss and capillary rarefaction in BAT was found to precede mitochondrial loss and the development of the whitened BAT phenotype. Second, the genetic ablation of VEGF in adipose tissue (both BAT and WAT) was found to decrease

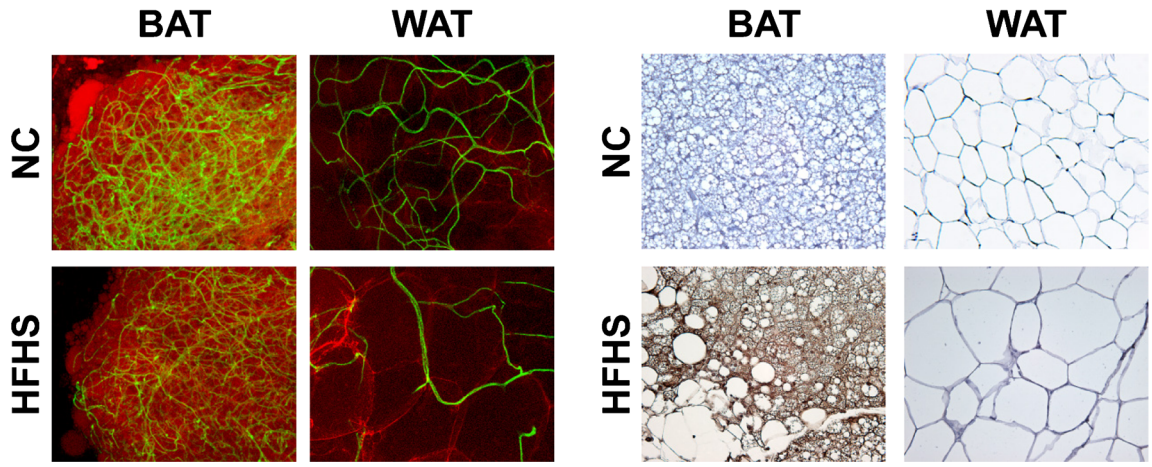

Fig. 1 Adipose tissue vascularity and hypoxia under conditions of diet-induced obesity. **a** Lectin staining (*green*) showing the vascularity of BAT and WAT under normal chow diet (*NC*) and high-fat, high-sucrose (*HFHS*) diet. There is a large difference in vascularity between BAT and WAT. Conditions that promote obesity lead to diminished vascularity both in BAT and WAT. Adipocytes are stained with BODIPY TR (*red*). **b** Histological sections of BAT and WAT stained with pimonidazole, a marker of tissue hypoxia (Hypoxyprobe). High-calorie diet led to small but detectable increases in the reactivity to pimonidazole in WAT, but the extent of signal in BAT was much greater. (With permission from: Shimizu I, Aprahamian T, Kikuchi R, et al. Vascular rarefaction mediates whitening of brown fat in obesity. J Clin Invest. 2014;124(5):2099–112) [54•]

VEGF levels and capillary density in BAT to levels observed in obesity. This genetic manipulation also led to the BAT whitening phenotype and resulted in a reduction in thermogenic function and systemic metabolic dysfunction, even in mice fed a normal chow diet. Third, VEGF rescue experiments provided causal evidence for the role of vascular rarefaction in BAT dysfunction. In these experiments, the *Vegfa* gene was introduced specifically into BAT resulting in the re-browning of the tissue and restored mitochondrial function, both in models of obesity and in the *Vegfa*-KO mice. Notably, VEGF restoration in this model also improved the thermogenic response to cold challenge and partly corrected the insulin-resistant phenotype.

Mechanistically, it was proposed that obesity diminishes VEGF expression in BAT due to interference with β-adrenergic signaling [27•]. β-adrenergic signaling is recognized as a central activator of *Vegfa* expression in brown adipocytes [46]. It was shown that high intracellular fatty acid level and hypoxic stress, conditions that arise from metabolic stress, synergistically inhibit β_1- and β_3-adrenergic signaling and thereby suppressed *Vegfa* expression. These data indicate the existence of a pathological feedback loop in obesity characterized by diminished β-adrenergic signaling in BAT leading to reductions in VEGF and capillary rarefaction. In turn, BAT hypoxia leads to a further impairment of β-adrenergic signaling, greater loss of VEGF expression, and further BAT dysfunction [27•].

Several studies have associated adipose tissue vascularity with systemic metabolic homeostasis [21–25, 47, 48•]. Most of these studies have largely focused on WAT vascularity, and the role of BAT vascularity in system metabolic control has not been investigated in detail. In a side-by-side comparison, VEGF ablation in adipose tissues has been shown to predominantly affect BAT, yet have minimal effects on WAT [27•]. VEGF ablation in adipose tissues was found to profoundly reduce oxygen content in BAT, yet have a modest effect on this parameter in WAT. Similarly, there was a marked reduction in mitochondrial marker expression in BAT under these conditions, but no detectable impact on the expression of these markers in WAT. Thus, it would appear that diminished vascularity has a minimal impact on WAT, likely due to its minimal oxidative capacity, whereas BAT vascularity is absolutely essential for the proper functioning of this highly metabolically active tissue. In light of these findings, one can speculate that the contradictory findings of earlier studies on the systemic impact of alterations in adipose tissue vascularity [21–23, 47] might be explained by taking the status of BAT function into account.

BAT whitening resulting from obesity or VEGF ablation involved mitochondrial dysfunction as indicated by the loss of mitochondria-associated transcripts, elevated reactive oxygen species production, and mitochondrial membrane depolarization. These conditions were also associated with an increase in mitochondria loss due to autophagy [27•]—a process that is referred to as "mitophagy". A basal level of autophagy is generally accepted to have a crucial role for removing and renewing dysfunctional organella and maintaining cell homeostasis. However, the role of stress-induced autophagy is more complex and can contribute to pathological conditions. Autophagy is also known to have both protective and detrimental roles in metabolic disorders. A reduction in hepatic autophagy is observed in a murine dietary obese model and the restoration of this response inhibited metabolic dysfunction [49]. In contrast, the inhibition of autophagy improves

insulin sensitivity by inducing the browning of WAT [50, 51]. It has been shown that the hypoxic microenvironment in BAT, due to obesity or VEGF ablation, leads to an accelerated loss of mitochondria [27•]. Presumably, loss of mitochondria under these conditions is a compensatory response to the mitochondrial ROS production and dysfunction that results from the hypoxic stress. As oxidative capacity is lost, lipid droplets accumulate, giving BAT the "whitened" appearance.

Several antiobesity drugs increasing energy expenditure have been developed and tested in clinical trials (reviewed in [52]). Among them, compounds targeting the activation of sirtuin1 (SIRT1) are broadly studied. SIRT1 is one of the sirtuin family proteins involved in the regulation of cellular energy homeostasis and mitochondrial biogenesis. The activation of SIRT1-signaling extends lifespan in several species including mammals (reviewed in [53]). It is also known to regulate systemic metabolic homeostasis. A high level of resveratrol, a well-described SIRT1 inducer, inhibits weight gain and improves systemic metabolic dysfunction in diet-induced obese mice. Resveratrol treatment increases mitochondrial size and mitochondrial DNA content in the BAT of mice, inhibits the whitening of BAT by promoting SIRT1-PGC1α signaling, resulting in the increased energy expenditure and possibly contributing to suppress weight gain upon dietary obesity [54, 55]. Considering that SIRT1 is involved in angiogenic responses [56, 57], further studies are needed to show whether SIRT1 promotes vessel network formation in BAT. Taken together, the modulation of SIRT1 signaling could be a promising therapy to combat obesity via the maintenance of BAT homeostasis.

Conclusions and Future Directions

Besides being a critical regulator of thermogenesis, accumulating evidence has shown the importance of BAT in regulating systemic metabolism. Although "whitening" of BAT is easily recognizable under conditions of obesity, few studies have focused on the molecular mechanisms that contribute to this phenotypic change and the physiological implications of this phenomenon. Recently, vascular rarefaction due to VEGF deficiency was shown to be causal for BAT whitening associated with obesity. Therefore, cardiovascular conditions that lead to diminished vascular function could contribute to metabolic disease via impaired BAT function. In turn, diminished BAT function could exacerbate obesity and further promote vascular disease.

Acknowledgments This work was supported by Manpei Suzuki Diabetes Foundation, Kanae Foundation for the Promotion of Medical Science, and Novartis Research Foundation (to IS) and National Institutes of Health (NIH) grants HL081587, HL116591, HL120160, and 126141 (to KW).

Compliance with Ethics Guidelines

Conflict of Interest Ippei Shimizu and Kenneth Walsh declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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