Role of Brown Fat in Lipoprotein Metabolism and Atherosclerosis

Geerte Hoeke, Sander Kooijman, Mariëtte R. Boon, Patrick C.N. Rensen,* Jimmy F.P. Berbée*

Abstract: Atherosclerosis, for which hyperlipidemia is a major risk factor, is the leading cause of morbidity and mortality in Western society, and new therapeutic strategies are highly warranted. Brown adipose tissue (BAT) is metabolically active in human adults, although positron emission tomography-computed tomography using a glucose tracer is the golden standard to visualize and quantify the volume and activity of BAT, it has become clear that activated BAT combats fatty acids rather than glucose. Here, we review the role of brown and beige adipocytes in lipoprotein metabolism and atherosclerosis, with evidence derived from both animal and human studies. On the basis of mainly data from animal models, we propose a model in which activated brown adipocytes use their intracellular triglyceride stores to generate fatty acids for combustion. BAT rapidly replenishes these stores by internalizing primarily lipoprotein triglyceride-derived fatty acids, generated by lipoprotein lipase-mediated hydrolysis of triglycerides, rather than by holoparticle uptake. As a consequence, BAT activation leads to the generation of lipoprotein remnants that are subsequently cleared via the liver provided that an intact apoE–low-density lipoprotein receptor pathway is present. Through these mechanisms, BAT activation reduces plasma triglyceride and cholesterol levels and attenuates diet-induced atherosclerosis development. Initial studies suggest that BAT activation in humans may also reduce triglyceride and cholesterol levels, but potential antiatherogenic effects should be assessed in future studies. (Circ Res. 2016;118:173-182. DOI: 10.1161/CIRCRESAHA.115.306647.)

Key Words: atherosclerosis ■ adipose tissue, brown ■ cholesterol ■ energy metabolism ■ fatty acids ■ lipoproteinlipase ■ triglycerides

Cardiovascular diseases (CVDs) are the leading cause of morbidity and mortality in Western Society.1 An important causal factor is our lifestyle, characterized by excess caloric intake and low physical activity, resulting in, among others, obesity and hyperlipidemia. Hyperlipidemia is represented by high plasma levels of low-density lipoprotein-cholesterol (LDL-C), very-low-density lipoprotein-cholesterol (VLDL-C), and triglycerides, often accompanied by low high-density lipoprotein-cholesterol (HDL-C), and is the main risk factor for atherosclerosis.2 Given the strong evidence that LDL-C is causally involved in CVD development, most current therapies aimed to combat atherosclerosis focus on lowering of LDL-C levels, for example, by using statins that reduce hepatic cholesterol production and concomitantly increase hepatic uptake of LDL. To date, statins are the most widely used drugs to treat hyperlipidemia and they lower cholesterol with ≈30%.3 However, statin treatment only prevents 25% to 45% of all cardiovascular events.4 It has recently been established that elevated plasma triglyceride is an important predictor of CVD as well.5 Hypertriglyceridemia is currently mainly treated by stimulating the clearance of triglycerides by peripheral organs, for example, by fibrates.6 However, lowering of triglycerides cannot prevent CVD without concomitant lowering of cholesterol within lipoprotein remnants.7 Recently, energy-combusting brown adipose tissue (BAT) has been identified as a key player in triglyceride metabolism by mediating triglyceride clearance.8 Furthermore, BAT activation decreases cholesterol levels as well9–11 and might be an interesting new therapeutic target to combat atherosclerosis. This review will focus on the protective role of BAT in hyperlipidemia and atherosclerosis development, based on evidence derived from both preclinical models and human studies.

BAT and Its Role in Lipid Metabolism

Discovery of BAT in Adult Humans

In contrast to white adipocytes that are monolocular cells containing a large lipid droplet and few mitochondria, brown adipocytes are multilocular cells with small lipid droplets and a high content of mitochondria in which uncoupling protein-1 (UCP-1) is present. UCP-1 provides BAT with the ability to generate heat from stored fatty acids (FA),12 as will be discussed below. BAT has long been known to be present in hibernating animals and neonates as an important contributor to nonshivering thermogenesis to defend core body temperature

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in case of exposure to a cold environment. In adult humans, BAT was thought to fully regress with increasing age, although already in 1972, biopsies from adults showed BAT depots surrounding the aorta and neck. In the 21st century, BAT returned in the spotlight when it became evident that BAT is not only present but also still active in adults. More specifically, the use of positron emission tomography-computed tomography (PET-CT) scans in combination with the glucose tracer 18F-fluorodeoxyglucose ([18F]FDG) revealed that on cold induction, symmetrical uptake of [18F]FDG was found in regions that corresponded to BAT, and that is, in the supraclavicular area and along the spine. Indeed, biopsies of these regions showed high expression of UCP-1.

Over the past decade, human BAT has become a hot research field, not only because it was discovered that BAT is active in human adults but also because it contributes to energy metabolism and its activity inversely correlates with body mass index and body fat mass, suggesting a role for BAT in human energy expenditure. In fact, BAT activation by means of repeated cold exposure enhances nonshivering thermogenesis and lowers fat mass, providing proof of principle that activation of BAT in humans can reduce obesity. In the next paragraphs, we will address physiology and fuel utilization by BAT with special emphasis on its role in lipid metabolism.

**BAT Physiology**

The most important physiological activator of BAT is cold, which is sensed through nerve terminals expressing certain transient receptor potential channels. Activation of these transient receptor potential channels initiates a signal toward the hypothalamic temperature center located in the preoptic area and subsequently enhances sympathetically mediated BAT function. BAT function is reflected by the number of nerve endings in the tissue. Each brown adipocyte is in close proximity to a nerve ending that releases norepinephrine on sympathetic stimulation. Norepinephrine subsequently binds to the β3-adrenergic receptor (β3-AR) on the membrane of the brown adipocyte (Figure 1). This results in activation of adenyl cyclase to produce cyclic AMP that activates protein kinase A to phosphorylate the lipolytic enzymes adipose triglyceride lipase, hormone-sensitive lipase, and monoglycerol lipase, leading to increased intracellular lipolysis. FA that are released subsequently enter the mitochondria where they are broken down into substrates for the citric acid cycle, leading to the generation of heat instead of ATP. Of note, the use of the PET tracer [13C]acetate in rats and humans showed that chronic cold exposure dramatically increases the oxidative metabolic activity of BAT. In addition to being oxidized, FA can allosterically activate UCP-1 by causing a conformational change in UCP-1, thereby enhancing uncoupled respiration.

**Glucose Uptake and Utilization by BAT**

Currently, PET-CT using the glucose tracer [18F]FDG is the golden standard to visualize and quantify (cold-) activated BAT in humans, and is also used to visualize and quantify BAT in mice. After its uptake by the glucose transporter-1 and glucose transporter-4, glucose is used for both de novo lipogenesis and ATP generation that supports general adipocyte function. Indeed, BAT activation increases the expression of genes involved in glycolysis and the pentose phosphate pathway; pathways that provide ATP and reducing equivalents for de novo lipogenesis, respectively.

The importance of BAT in glucose metabolism is reflected by studies in mice demonstrating that BAT transplantation, and cold exposure improves both glucose tolerance and insulin sensitivity. In line with these findings, prolonged cold exposure in humans increases glucose oxidation and insulin sensitivity in BAT-positive, but not in BAT-negative subjects. Likewise, human subjects with a low triglyceride content in the supraclavicular region (ie, indicative for more active BAT) have lower fasting glucose levels and are more insulin sensitive.

Because fat mass is negatively correlated with insulin sensitivity, BAT activation might improve insulin sensitivity via increasing glucose oxidation and reducing body fat mass. In humans, insulin enhances [18F]FDG uptake by BAT and furthermore, uptake of [18F]FDG by BAT is impaired under insulin-resistant conditions and in type 2 diabetics, collectively suggesting that BAT is an insulin-sensitive organ. However, the latter study also showed that BAT oxidative metabolism as assessed by [13C]acetate and uptake of the nonesterified FA-tracer [18F]fluoro-6-thia-heptadecanoic acid (FTHA) were similar between type 2 diabetics and healthy controls. These findings combined thus indicate that despite impaired glucose uptake by BAT during insulin resistance, BAT oxidative metabolism and FA uptake are not impaired. Therefore, a PET-CT using [18F]FDG might not be the most optimal method to determine BAT volume and activity, especially under insulin-resistant conditions, and the use of [18F]
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FTHA or the combination of \( ^{18}F \)FDG with a FA-tracer would be superior than \( ^{18}F \)FDG alone. The FA-tracer \( ^{18}F \)FTHA is taken up by BAT in a lipoprotein lipase (LPL)–independent manner, whereas, at least in mice, an important fraction of the FA that are taken up by BAT is derived from triglyceride in an LPL-dependent manner (see FA Uptake and Utilization by BAT section of this article). Because LPL activity is decreased during insulin resistance in mice, it would be interesting to evaluate the effect of insulin resistance on LPL-dependent FA uptake using a triglyceride-tracer. More dedicated studies are evidently needed to further elucidate the effect of insulin signaling on FA uptake and utilization by BAT.

FA Uptake and Utilization by BAT

Upon BAT activation, intracellular lipolysis causes depletion of intracellular triglyceride stores that subsequently need to be replenished via de novo lipogenesis and via the uptake of FA from the circulation. In the circulation, triglycerides are mainly transported in chylomicrons and VLDL, that is, triglyceride-rich lipoproteins (TRLs). \(^{45}\) TRL–triglyceride–derived FA are the main energy substrate for heart, skeletal muscle, and white adipose tissue (WAT). \(^{46,47}\) These organs take up FA from TRLs in a coordinated fashion involving LPL and the FA transporter cluster of differentiation 36. \(^{46,49}\) Activation of BAT in hypertriglyceridemic mice by cold strongly reduces plasma triglyceride, illustrating the importance of BAT in lipoprotein metabolism. \(^{4}\

A current matter of debate is the mode of action via which BAT takes up TRL–triglyceride–derived FA from the plasma, either after lipolysis of triglycerides, via holoparticle uptake of TRL, or a combination of both. This was first studied in 2011 by Bartelt et al \(^{8}\) through injection of TRL-like particles provided with 2 radiolabels, representing either triglyceride or the particle core, into cold-exposed mice. They showed that BAT takes up both radiolabels to a similar extent, supporting the conclusion that BAT takes up whole TRL. The authors speculated that this was a consequence of remodeling of the endothelial permeability. The distribution of both labels over the whole body, including the liver, was similar, despite increased expression of \( Lpl \) and \( Cluster \ of \ differentiation \ 36 \) in BAT. \(^{8}\) LPL and cluster of differentiation 36 are both classically involved in lipolysis-mediated uptake of FA by heart, skeletal muscle, WAT, \(^{47}\) and BAT. \(^{30}\) Mice that lack the FA transporter cluster of differentiation 36 have impaired uptake of FA by tissues \(^{50}\) and exhibit defective thermogenesis. \(^{8,54}\) Collectively, these data would rather suggest that BAT at least also takes up triglyceride-derived FA via selective delipidation of TRL in a similar manner as heart, skeletal muscle, and WAT. \(^{46,49}\) Recently, Khedoe et al \(^{52}\) injected TRL-like particles in cold-exposed mice and observed a large increase in the selective

Figure 1. Activated brown adipose tissue (BAT) takes up triglyceride (TG)–derived fatty acids after lipolysis. In response to cold, norepinephrine (NE) is released from the sympathetic nervous system and binds to the \( \beta_3 \)-adrenergic receptor on brown and beige adipocytes. This results in activation of adenylyl cyclase to produce cyclic adenosine monophosphate (cAMP) that activates protein kinase A (PKA). PKA phosphorylates and activates cAMP response element-binding protein (CREB) resulting in enhanced transcription of uncoupling protein-1 (\( Ucp-1 \)) and peroxisome proliferator-activated receptor-gamma co-activator (\( Pgc-1 \alpha \)). In addition, activation of PKA enhances the activity of lipolytic enzymes that are associated with the intracellular lipid droplets, leading to release of fatty acids (FA) that enter the mitochondria for \( \beta \)-oxidation. The produced acetyl-CoA is used in the citric acid cycle where reducing equivalents are formed that donate electrons to the electron transport chain. UCP-1 is a proton channel that uncouples respiration, which results in the generation of heat instead of ATP. Upon prolonged activation of brown and beige adipocytes, intracellular lipid droplets become depleted and are replenished via the uptake of TG-derived FA, mainly via selective delipidation of TG-rich lipoproteins (TRLs) through the hydrolyzing action of lipoprotein lipase (LPL). The released FA are taken up by cluster of differentiation 36 (CD36) and FA transport proteins (FATP) and stored in the lipid droplets as TG. Furthermore, glucose is taken up as well via glucose transporter (GLUT) 1 and 4 and used for, among others, de novo lipogenesis. ATGL indicates adipose triglyceride lipase; DAG, diacylglycerol; HSL, hormone-sensitive lipase; MAG, monoacylglycerol; and MGL, monoglyceride lipase (Illustration credit: Ben Smith).
uptake of radiolabeled triglycerides by BAT, whereas uptake of holoparticles by BAT was only slightly increased. Injection of endogenous VLDL, ex vivo radiolabeled, into mice in which BAT was activated by the selective β3-AR agonist CL316243 also resulted in a selectively increased uptake of triglyceride-derived FA by BAT and predominant uptake of the core remnant by the liver (G. Hoeke, MSc, et al, unpublished data, 2014). These data would indicate that holoparticle uptake by BAT takes place to a low extent and that BAT takes up TRL-derived triglycerides mostly after lipolysis of TRLs. Differences in TRL-particle preparation, size, and physiological characteristics (including acquisition of apolipoproteins) may explain the contrasting in vivo kinetics in these studies.\(^5\)\(^2\)

After uptake of TRL-derived FA by BAT, FA are incorporated into triglycerides and stored in intracellular lipid droplets from where they are released on BAT activation. It can be questioned whether TRL-derived FA can also be directly used for mitochondrial β-oxidation and UCP-1 activation without first being incorporated into the cellular triglyceride pool. Because adipose triglyceride lipase-deficient mice exhibit blunted intracellular lipolysis together with defective thermogenesis,\(^5\)\(^1\) it is likely that the intracellular lipid droplets are the predominant source of FA used for oxidation and UCP-1 activation. Indeed, by comparing various tracers for glucose uptake ([\(^18\)F]FDG), FA uptake ([\(^18\)F]FTHA), and oxidative activity ([\(^11\)C]acetate), Labbé et al\(^3\)\(^0\) recently confirmed that internalized FA are first esterified into triglycerides upon which FA can be liberated for mitochondrial β-oxidation. This sequence of events likely represents a control mechanism for unintended uncoupled FA oxidation.

**Phenomenon of Adipose Tissue Browning**

Brown adipocytes are not only present in well-defined fat pads but also lie scattered in other tissues, that is, VAT and skeletal muscle, as so-called recruitable brown adipocytes, or beige or brite cells.\(^1\)\(^2\)\(^5\)\(^4\) Sympathetic innervation of VAT via cold exposure\(^5\)\(^5\) and several other stimuli, such as β3-AR agonism\(^5\)\(^6\) and peroxisome proliferator-activated receptor-γ and -α agonism,\(^5\)\(^7\)\(^8\) orchestrate browning of VAT depots. This process is characterized by the appearance of multilocular adipocytes that express several BAT-related markers such as UCP-1. Although basal UCP-1 gene and protein expression are relatively low in beige adipocytes, both are markedly increased on stimulation, reaching similar levels of UCP-1 expression as in brown adipocytes.\(^3\)\(^4\)

Classical brown adipocytes are derived from myogenic factor 5–positive precursor cells that differentiate toward brown adipocytes under the control of, among others, bone morphogenetic protein 7 and PR domain containing 16.\(^5\)\(^9\)\(^6\)\(^8\) However, the origin and markers of beige cells are less clear. Many efforts have been undertaken to elucidate whether beige cells originate from transdifferentiation of white adipocytes toward beige adipocytes or de novo generation from precursor cells. The first theory, stating that beige cells originate from transdifferentiation, is supported by the fact that constant cell numbers are found in WAT during the process of browning.\(^6\)\(^1\)\(^2\)\(^3\)\(^4\) Furthermore, an elegant in vivo study in transgenic mice using transient and permanent fluorescent adipocyte labeling showed that beige cells that appear in inguinal WAT on cold stimulation can convert into cells that resemble white adipocytes with respect to morphology and gene expression pattern on warm acclimation.\(^6\)\(^5\) On subsequent cold stimulation, these same cells can convert back into beige cells that exhibit multilocular morphology and a beige-specific gene expression profile. This supports the notion that a distinct subset of white adipocytes exists in VAT that has the potential to convert into thermogenic beige cells. The second theory, stating that beige adipocytes may be recruited de novo from precursor cells, is among others supported by a study of Schulz et al.\(^4\) They identified a Sca-1–positive adipocyte precursor cell residing in murine VAT and skeletal muscle that, on stimulation with bone morphogenetic protein 7, has the capacity to differentiate into beige cells. In addition, primary cells isolated from the stromavascular fraction of murine VAT can upregulate Ucp-1 gene expression on stimulation with either noradrenalin or prostaglandin, supporting the notion that in VAT progenitor cells are present that could potentially differentiate into beige cells.\(^6\)\(^2\) Finally, a population of bipotential precursor cells has been identified in mouse VAT that can differentiate into either beige or white adipocytes, depending on the stimulus.\(^6\)\(^6\) Overall, the above studies support that transdifferentiation as well as de novo generation of beige adipocytes likely coexist in VAT.

**Human BAT: Beige Instead of Brown?**

[\(^18\)F]FDG PET-CT scans have shown that adult humans primarily exhibit BAT depots in the supraclavicular and neck region as well as along the spine.\(^1\)\(^6\) After isolation from these regions, differentiated adipocytes have higher oxygen consumption rate than white adipocytes and respond to cyclic AMP by increasing oxygen consumption rate, indicating that these cells have functional characteristics of thermogenic adipocytes.\(^6\)\(^7\)\(^8\)

To date, it remains elusive whether the adipocytes in these depots are either brown, beige, or both. Murine beige cells express high levels of Cd137, Tbx1, and Tmem26, which are practically not expressed in brown and white adipocytes.\(^5\)\(^4\) Using this beige signature, one study demonstrated that superficial BAT depots in humans are mainly composed of beige adipocytes, while the deeper depots contain mostly brown adipocytes.\(^6\)\(^7\) Yet, biopsies from the supraclavicular region of humans showed that human BAT most closely resembles murine beige adipocytes.\(^5\)\(^4\) Likewise, the gene signature of human differentiated clonal brown adipocyte cultures also presented more similarity to mouse beige adipocytes,\(^6\)\(^8\) indicating that human BAT, in general, mostly consists of beige adipocytes.

**BAT Activation and Browning as a Tool to Alleviate Hypertriglyceridemia**

**Mice**

It was previously established that BAT takes up FA from the plasma to be combusted toward heat.\(^1\)\(^2\) In fact, we\(^2\)\(^1\) and others\(^8\) have recently shown that FA uptake by activated BAT can reach such extent that BAT activation lowers plasma triglyceride levels. BAT activation through cold exposure largely reduces plasma triglyceride levels in hypertriglyceridemic ApoA5−/− mice as well as in normolipidemic C57Bl/6J mice, suggesting that BAT activation is a promising target to combat hypertriglyceridemia. Furthermore, metformin,\(^1\)\(^1\) salsalate,\(^6\)\(^9\)
rimonabant, and vascular growth factor A overexpression reduce plasma triglyceride levels accompanied by BAT activation in mice. Although BAT activation is probably causal to their triglyceride-lowering effects, alternative pathways may contribute, that is, reduction of hepatic VLDL production or intestinal triglyceride absorption. Metformin and rimonabant do not affect hepatic VLDL production, and the effects of salutary and vascular growth factor A overexpression on VLDL production are still unknown. The effect of these compounds on intestinal lipid absorption remains to be elucidated. Rimonabant enhances FA oxidation in WAT and liver, which could be partly responsible for the lipid-lowering effect of rimonabant.

As discussed above, beige adipocytes have a similar phenotype as classical brown adipocytes and also generate heat after burning of FA derived from intracellular lipid droplets. Moreover, browning can have beneficial metabolic effects in mice. Transgenic overexpression of PR domain containing 16 in subcutaneous WAT protects mice from diet-induced obesity and insulin resistance, further supporting beneficial metabolic effects of browning. Several studies showed that cold exposure or β3-AR agonism increases browning in WAT, and thereby enhances the delivery of triglycerides to WAT in mice. This indicates that beige cells take up triglyceride-derived FA from the circulation and may, on activation, play a role in alleviation of hypertriglyceridemia. However, dedicated studies on the beneficial effects of browning upon hypertriglyceridemia are yet to be performed.

Humans

Because [18F]FDG PET-CT is still the golden standard to detect and quantify BAT, studies in humans have focused mostly on the effect of BAT activation on glucose metabolism rather than on triglyceride metabolism. However, several studies suggest that, also in humans, BAT contributes to triglyceride metabolism. BAT-positive subjects have lower plasma triglyceride and higher HDL-C levels. Furthermore, LPL expression in perivascular adipose tissue, a BAT depot surrounding the aorta, is negatively correlated with plasma triglyceride levels. Studies using the FA tracer [18F]FITHTA in combination with [18F]FDG have shown increased uptake of FA by BAT after acute cold stimulation. Short-term cold exposure, however, does not seem to be accompanied by decreased plasma triglyceride levels. It is possible that acute cold exposure causes a rapid increase in hepatic VLDL-triglyceride production that can mask a potential BAT-mediated decrease in plasma triglyceride. Indeed, acute cold activation increases hepatic VLDL-triglyceride secretion in rats and possibly also in humans (G. Hoeke, MSc, et al, unpublished data, 2015).

Until present, no clinical studies have been reported that focus on the role of browning in human metabolic health. However, recent studies reported that stem cells from human WAT treated with the peroxisome proliferator-activated receptor-γ agonist rosiglitazone or bone morphogenetic protein 7 easily differentiate toward beige adipocytes and markedly enhance oxygen consumption. In addition, bone morphogenetic protein 7–driven browning of adipocytes results in higher glucose and FA uptake than white adipocytes. Browning of WAT has therapeutic potential, as humans have large amounts of WAT that may be susceptible to browning. This is nicely illustrated by a markedly increased brown fat mass and energy expenditure in patients who have a phaeochromocytoma, a catecholamine-producing neuroendocrine tumor. Patients have increased uptake of [18F]FDG in the classical brown fat depots, as well as in other fat depots including perirenal fat and omental fat. This increased uptake of [18F]FDG is abolished after removal of the tumor. These studies indicate that an adrenergic environment can drive browning of various WAT depots in human adults. However, the susceptibility of the different human WAT depots to browning and the estimated percentage of WAT that can be browning are still unknown, and, therefore, the amount of WAT browning that contributes to metabolic health remains to be elucidated.

**BAT as a Novel Target to Treat Hypercholesterolemia and Atherosclerosis**

**Mice**

After lipolysis of triglyceride from TRLs by BAT through LPL, the triglyceride content in the TRLs decreases, whereas the relative content of cholesterol increases. In addition, the particles become enriched with apoE. The resulting remnant particles are transported to the liver and taken up by hepatocytes by means of an interaction of apoE and potentially also apoB100 with mainly the LDL-receptor (LDLR) in addition to the LDLR-related protein-1 and proteoglycans. VLDL remnants can also be further converted to LDL that is even richer in cholesterol and carries apoB100 as its main apolipoprotein. LDL delivers cholesterol to peripheral tissues including the adrenals for, eg, steroid hormone synthesis or to the liver through recognition of apoB100 by the LDLR. The importance of the apoE–LDLR interaction for recognition and uptake of TRL remnants by the liver is best illustrated by the fact that defects in apoE or LDLR expression and/or recognition result in hyperlipidemia.

Although it is currently well established that BAT activation can alleviate hypertriglyceridemia in mice, the effects of BAT activation on hypercholesterolemia that especially underlies the development of atherosclerosis are less well known. Two recent studies have investigated this issue. Berbée et al activated BAT using the β3-AR agonist CL316243 in hyperlipidemic APOE*3-Leiden.CETP (E3L.CETP) mice, which express a natural occurring mutant form of human apoE3 with attenuated binding affinity for the LDLR and found that this resulted in reduced plasma cholesterol levels, explained by reduced VLDL/LDL-C levels. Mechanistically, they showed that BAT activation accelerated plasma clearance and uptake of triglyceride-derived FA by BAT and subsequent uptake of cholesterol-rich remnants by the liver. In contrast to E3L.CETP mice, β3-AR agonism did not reduce plasma cholesterol levels in Ldlr−/− and ApoE−/− mice, suggesting that an intact apoE–LDLR pathway, which is present in E3L.CETP mice, is needed for the uptake of the formed TRL remnants. As a result, β3-AR agonism reduced atherosclerotic lesion area and lesion severity in E3L.CETP mice, but not in Ldlr−/− and ApoE−/− mice. Dong et al also studied the effect of BAT activation on hypercholesterolemia and atherosclerosis...
development in Ldlr<sup>−/−</sup> and Apoe<sup>−/−</sup> mice, but used cold as a trigger for BAT activation. In contrast to β3-AR agonism, cold exposure even aggravated hypercholesterolemia and atherosclerosis development in these mice. This aggravated phenotype when compared with β3-AR agonism can at least partially be explained by an elevated hepatic cholesterol synthesis and upregulation of genes important in cholesterol metabolism, likely resulting in increased VLDL-C secretion.<sup>89</sup> Although not yet demonstrated in Ldlr<sup>−/−</sup> and Apoe<sup>−/−</sup> mice, cold exposure indeed increases hepatic VLDL production in rats.<sup>78</sup> Thus, the available data suggest that an intact apoE–LDLR pathway is crucial for the cholesterol-lowering effect of BAT activation and show that BAT activation in mice might be a potent tool to combat hypercholesterolemia and atherosclerosis development. However, in line with the data of Dong et al.,<sup>89</sup> when activated by cold, the beneficial effects of BAT activation on plasma cholesterol levels may be dampened because of a concomitant increase in hepatic cholesterol synthesis.

We propose a mechanism by which BAT activation increases lipolytic processing of TRLs, thereby enhancing the formation of cholesterol-rich remnants that acquire apoE and are taken up by the liver, mainly via the LDLR, collectively resulting in reduced plasma cholesterol levels (Figure 2).

The precise mechanism by which BAT-mediated processing of TRLs results in enhanced hepatic remnant uptake remains to be elucidated. Increasing the expression of hepatic LDLR may be expected to even further enhance the hepatic uptake of TRL remnants resulting from BAT activation. This implies that BAT-activating strategies should ideally be combined with LDLR-enhancing therapies such as statins<sup>90</sup> or proprotein convertase subtilisin/kexin type 9 inhibitors.<sup>91</sup> Of note, because statins also reduce hepatic cholesterol synthesis, a possible compensatory increase in hepatic cholesterol synthesis.

**Humans**

The effect of BAT activation on hypercholesterolemia and atherosclerosis development in humans is still largely unknown. To date, only few studies investigated the effect of BAT activation on plasma cholesterol levels. One study reported that subjects with detectable BAT have lower plasma total cholesterol and LDL-C levels than subjects without detectable BAT.<sup>72</sup> Moreover, De Lorenzo et al.<sup>89</sup> showed that prolonged, daily cold exposure of 20 minutes for 90 days reduced total cholesterol, LDL-C, and body mass in hypercholesterolemic individuals, without differences in physical activity and food intake.<sup>10</sup> These data suggest that BAT activation may alleviate hypercholesterolemia in humans, thus decreasing atherosclerosis.

Interestingly, South-Asians, a population with increased risk for developing type 2 diabetes mellitus, metabolic syndrome, and atherosclerosis have lower energy expenditure, nonshivering thermogenesis, and BAT volume than whites, which may contribute to the increased cardiometabolic risk.<sup>77</sup> However, whether decreased BAT activity is causally related to the development of CVDs in humans and whether BAT activation might prevent atherosclerosis development remains to be elucidated.

**Potent BAT Activators to Combat Hyperlipidemia and Atherosclerosis**

BAT has received increasing attention as a potential target to combat various metabolic diseases, such as hyperlipidemia, obesity, and diabetes mellitus. Therefore, studies focus both on the mechanism by which known compounds can activate BAT and the identification of novel compounds that can be used to activate BAT. In this section, BAT activators that have shown promising plasma-lowering results in mice and might be potential BAT activators in humans as well will be discussed.

Although in humans the β3-AR is mostly expressed by BAT and urinary bladder,<sup>93,94</sup> until recently it was unknown whether this subtype of the AR family controls BAT activation in humans as it does in mice. Currently available β3-AR agonists have high efficacy in murine cells transfected with human β3-AR and can activate UCP-1 in human-derived differentiated brown adipocytes.<sup>95</sup> However, treatment of humans with a β3-AR agonist showed no effects<sup>96</sup> or minor effects<sup>97,98</sup>
on metabolic parameters or caused adverse side effects in the cardiovascular system because of cross-reactivity with the β3-AR. Lack of effect of β3-AR agonism could be because of low bioavailability or low specificity of the agonists. Interestingly, a recent study showed that single administration of the highly specific β3-AR agonist mirabegron indeed increased BAT activity and increased energy expenditure in healthy subjects. These data show the importance of the β3-AR as therapeutic target to activate BAT in humans. Additional studies with chronic BAT activation are evidently needed to illuminate the role of β3-AR agonism on obesity, hyperlipidemia, and other risk factors for atherosclerosis development.

Other BAT activators are fibroblast growth factor 21 (FGF21) and metformin. FGF21 increases energy expenditure, enhances uncoupling in BAT, and lowers plasma triglyceride and (diet-induced) obesity in mice. Furthermore, stimulation of isolated human preadipocytes with FGF21 induces the formation of beige cells, and FGF21 concentrations correlate with BAT activity in humans. Moreover, administration of FGF21 in humans improves hyperlipidemia by lowering plasma triglyceride and LDL-C levels, while increasing levels of HDL-C. Treatment with the AMP-activated protein kinase activator metformin, already implemented for the treatment of type 2 diabetes mellitus, increases BAT activity in mice and decreases plasma levels of triglyceride and LDL-C in mice and to a lesser extent in humans.

The UK Prospective Diabetes Study (UKPDS) group showed in a subset of the UKPDS patient population that metformin treatment reduces the risk of macrovascular diseases and myocardial infarction when compared with nontreated patients. A more recent study showed that metformin treatment also reduces levels of remnant lipoprotein cholesterol. Ohira et al showed that metformin treatment, without affecting LDL-C levels, reduced oxidized LDL that is involved in the development of atherosclerosis.

Although FGF21 and metformin have beneficial effects on (cardio)metabolic health in humans, it is possible that these effects are mediated via mechanisms additional to BAT activation. Hence, further research is warranted to investigate whether the effects of these compounds on BAT activity and plasma lipids in mice can be translated to humans.

Summary

With the discovery of active BAT in human adults, recent studies have focused on elucidating the contribution of this metabolically active tissue to energy expenditure and lipoprotein metabolism. Studies in mice showed that BAT activation results in lipolysis of intracellular triglycerides to generate FA for oxidation, uncoupled respiration, and heat production. As a consequence, intracellular triglyceride is replenished by mainly the selective uptake of TRL–triglyceride–derived FA, resulting in the accelerated generation of TRL remnants in plasma. By these mechanisms, activated BAT has a massive capacity to clear triglyceride-derived FA from plasma and prevents plasma TRL–triglyceride–derived FA to be stored in WAT. Accordingly, activation of BAT in mice lowers plasma triglyceride and obesity. Recently, it has also been shown that BAT activation lowers plasma cholesterol levels and attenuates atherosclerosis development provided that an intact hepatic apoE–LDLR clearance pathway for generated lipoprotein remnants is present. It would be interesting to study the effect of BAT activation combined with therapies that increase hepatic LDLR expression, that is, statins or proprotein convertase subtilisin/kexin type 9 inhibitors. Such a combined strategy may even further reduce plasma cholesterol levels and atherosclerosis development when compared with BAT activation or cholesterol lowering alone.

The next step is to assess whether these promising effects of BAT activation on lipid metabolism and atherosclerosis can be translated to humans. Although FA uptake by BAT is increased on acute cold exposure in humans, this effect is not simply accompanied by lower plasma triglyceride levels. This is likely because of increased hepatic VLDL–triglyceride production, which masks the plasma triglyceride lowering because of triglyceride uptake by BAT. Although cold acclimation of patients with hypercholesterolemia results in lowering of plasma cholesterol levels, more studies are needed to draw definite conclusions on the involvement of BAT in human lipid metabolism. Because hypercholesterolemia, rather than hypertriglyceridemia, is the main risk factor for CVD, future efforts should focus on the effects of BAT activation on plasma cholesterol levels within remnants and LDL. Preferably, plasma measurements should be combined with tracer techniques that monitor FA uptake by BAT or measure triglyceride content in BAT by proton magnetic resonance spectroscopy.

Moreover, novel tools to activate BAT in humans are evidently needed. With the recent discovery that the β3-AR is indeed responsible for BAT activation in humans as it is in mice, future research may focus on the development of more selective and potent β3-AR agonists and the effect of FGF21 and metformin on BAT activity in humans.

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Disclosures

None

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Brown Fat, Lipoproteins, and Atherosclerosis


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