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INVITED REVIEW

Role of thermogenic adipose tissue in lipid metabolism and atherosclerotic cardiovascular disease: lessons from studies in mice and humans

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Abstract

Brown adipocytes within brown adipose tissue (BAT) and beige adipocytes within white adipose tissue dissipate nutritional energy as heat. Studies in mice have shown that activation of thermogenesis in brown and beige adipocytes enhances the lipolytic processing of triglyceride-rich lipoproteins (TRLs) in plasma to supply these adipocytes with fatty acids for oxidation. This process results in formation of TRL remnants that are removed from the circulation through binding of apolipoprotein E (ApoE) on their surface to the LDL receptor (LDLR) on hepatocytes, followed by internalization. Concomitantly, lipolytic processing of circulating TRLs leads to generation of excess surface phospholipids that are transferred to nascent HDLs, increasing their capacity for reverse cholesterol transport. Activation of thermogenic adipocytes thus lowers circulating triglycerides and non-HDL-cholesterol, while it increases HDL-cholesterol. The combined effect is protection from atherosclerosis development, which becomes evident in humanized mouse models with an intact ApoE-LDLR clearance pathway only, and is additive to the effects of classical lipid-lowering drugs including statins and proprotein convertase subtilisin/kexin type 9 inhibitors. A large recent study revealed that the presence of metabolically active BAT in humans is associated with lower triglycerides, higher HDL-cholesterol and lower risk of cardiovascular diseases. This narrative review aims to provide leads for further exploration of thermogenic adipose tissue as a therapeutic target. To this end, we describe the latest knowledge on the role of BAT in lipoprotein metabolism and address, for example, the discovery of the β_2-adrenergic receptor as the dominant adrenergic receptor in human thermogenic adipocytes.

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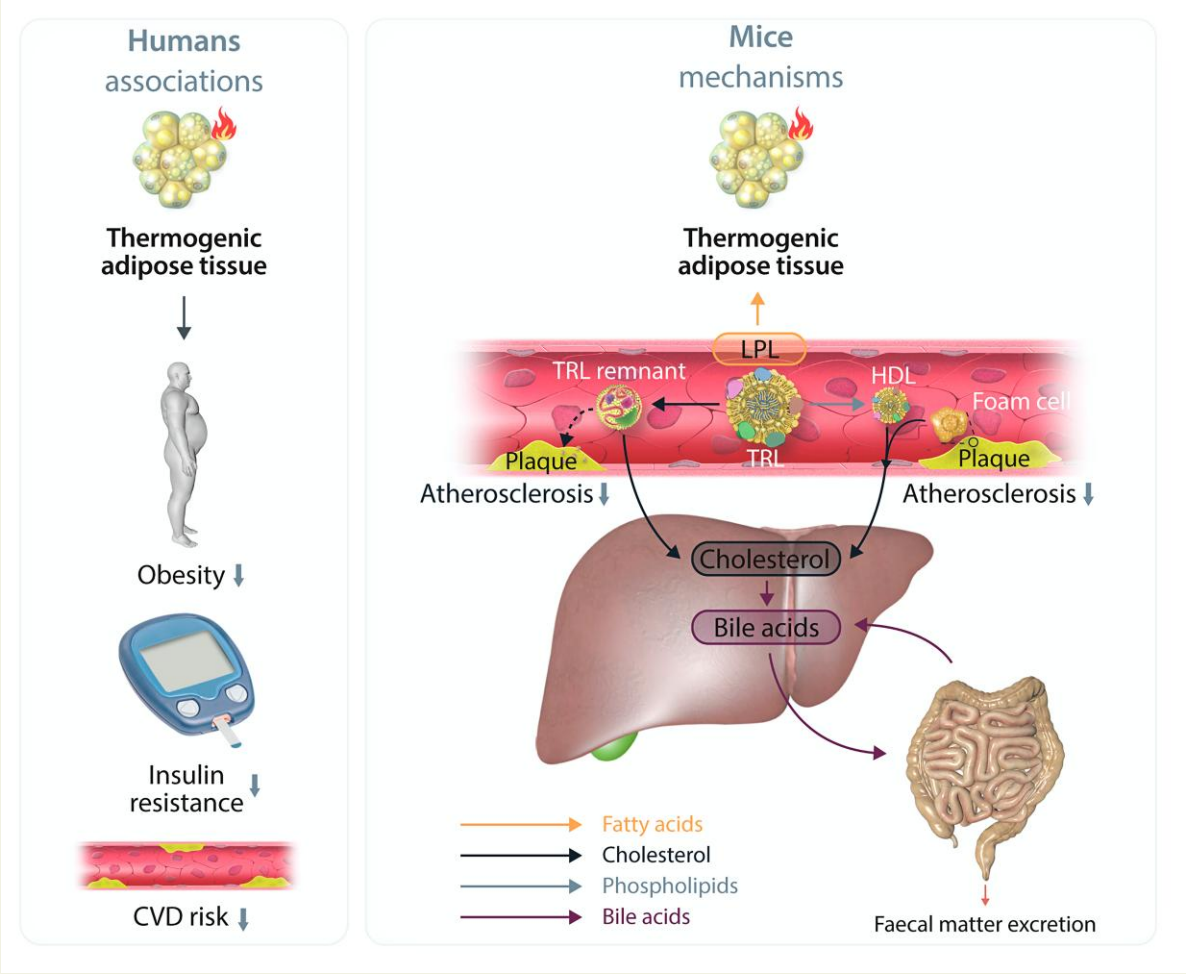
Graphical Abstract



Keywords Atherosclerosis • Adipose tissue • Cardiovascular disease • Dyslipidaemia • Non-shivering thermogenesis

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1. Introduction

Cardiovascular diseases (CVDs) are leading causes of death worldwide. The main underlying cause of CVD is atherosclerosis, which refers to the development of cholesterol-rich plaques in artery walls. Risk factors for atherosclerosis include multiple components of metabolic syndrome, including high blood pressure, obesity, insulin resistance, and dyslipidaemia, the latter being characterized by high plasma levels of triglycerides and non-HDL-cholesterol in the presence of low HDL-cholesterol levels. The (re-)discovery of active brown adipose tissue (BAT) in adult humans opened a new window of therapeutic opportunities for atherosclerotic CVD.[1–4] Studies in mice demonstrated that activated BAT can take up large amounts of fatty acids (FAs) derived from triglyceride-rich lipoproteins (TRLs) and use them as substrates for heat production, which—for reasons outlined in section 4—results in a less atherogenic lipoprotein profile and protection from atherosclerosis development.[5] In humans, the amount and activity of BAT, assessed from the uptake of [^{18}F]fluorodeoxyglucose ([^{18}F]FDG) in positron emission tomography-computed tomography (PET-CT) scans, were found to decrease with age[6,7] and higher BMI,[2,8] and to be dependent on gender (i.e. higher in females)[3,9] and ethnicity (e.g. higher in Europids compared to South Asians).[10] Interestingly, a recent retrospective analysis of as many as 134 529 [^{18}F]FDG PET-CT scans from 52 487 patients associated the presence of [^{18}F]FDG-positive BAT with a lower risk of type 2 diabetes and coronary artery disease,[11] highlighting the potential of BAT as a therapeutic target in cardiometabolic diseases. In this narrative review, we will describe the latest knowledge on the role of murine and humans thermogenic adipocytes in lipoprotein metabolism and atherosclerotic CVD, and discuss recent insights in therapeutic interventions to promote thermogenic activity in adipose tissue.

2. BAT morphology and physiology

Whereas white adipocytes store nutritional energy from glucose and FAs as triglycerides, brown adipocytes combust nutrients into heat, a process called non-shivering thermogenesis. This functional distinction between the two types of adipocytes is reflected in their different morphology. White adipocytes are large, unilocular cells, containing a single large lipid droplet and only few small, elongated mitochondria. In contrast, brown

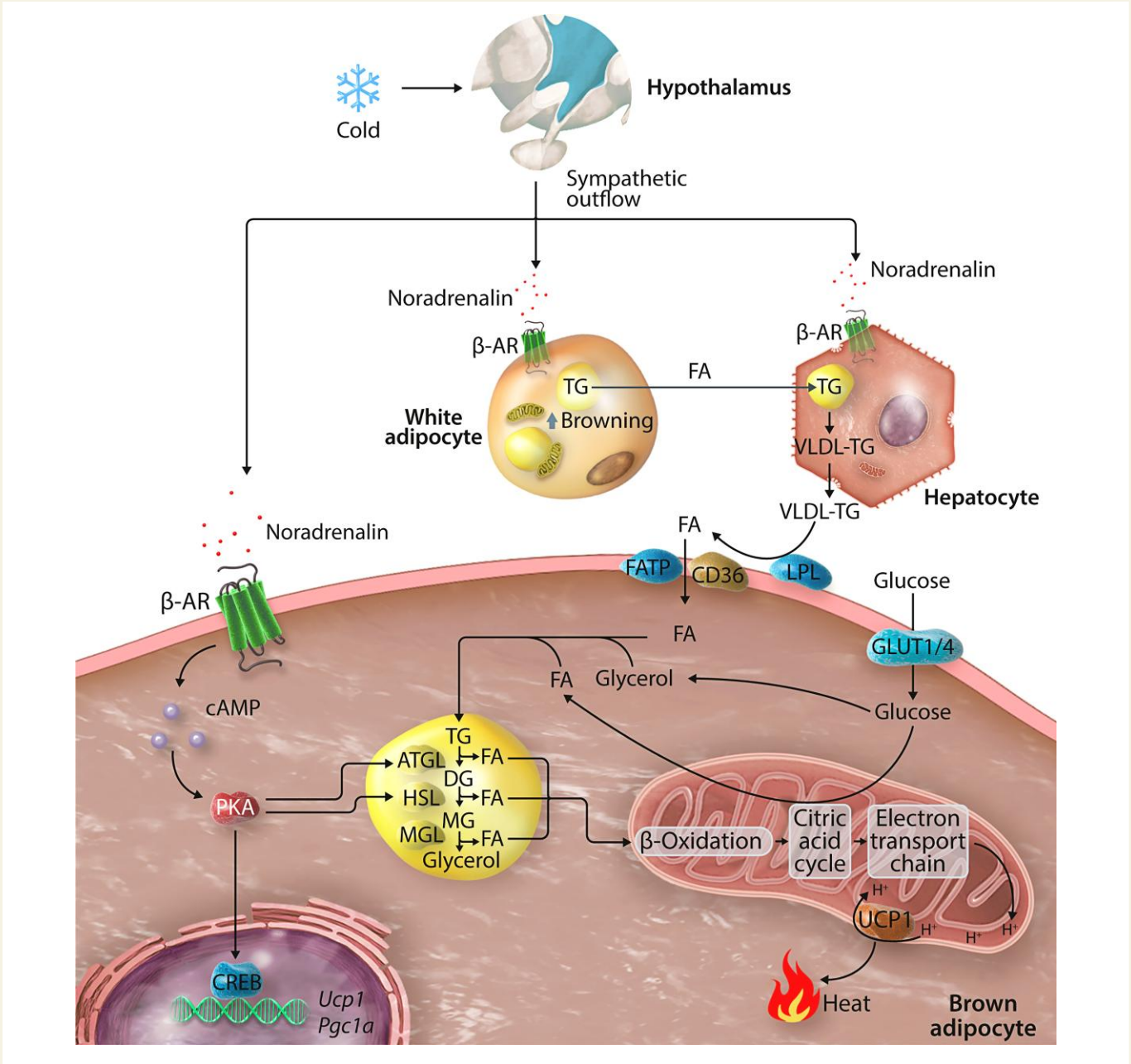

Figure 1 Effect of cold exposure on nutrient partitioning and thermogenic activity in brown adipocytes. Cold stimulates—via the sympathetic nervous system—release of FAs from white adipocytes, which are re-esterified into triglycerides (TGs) by hepatocytes and secreted within VLDLs. At the level of the brown adipocyte, adrenergic stimulation causes a signalling cascade activating the thermogenic gene programme and stimulating intracellular lipolysis. Released FAs are subjected to catabolic processing to fuel the electron transport chain, which generates a proton gradient across the inner mitochondrial membrane. UCP1 disrupts this gradient and releases energy as heat. Intracellular lipid stores are replenished by the uptake of VLDL-TG-derived FAs and glucose. See text for more details. ATGL, adipose triglyceride lipase; CD36, cluster of differentiation 36; CREB, cAMP response element-binding protein; DG, diacylglycerol; FATP1, FA transport protein 1; GLUT1/4, glucose transporter 1/4; HSL, hormone-sensitive lipase; MG, monoacylglycerol; MGl, monoacylglycerol lipase; Pgc1α, peroxisome proliferator-activated receptor-γ coactivator 1-α.

adipocytes are smaller multilocular cells, containing multiple small lipid droplets and numerous mitochondria with high iron and cytochrome content, leading to the brown appearance of BAT compared to white adipose tissue (WAT).[12] In addition, BAT is densely innervated by the sympathetic nervous system and highly vascularized.

While it has long been recognized that small mammals and infants have large quantities of metabolically active BAT, the presence of active BAT in adult humans was only described fifteen years ago[1] and was convincingly established two years later.[2–4] The following sections will describe the processes leading to thermogenic activity in brown adipocytes, which we have also visually summarized in *Figure 1*. From animal studies we have learned that exposure to cold, via activation of thermoreceptor channels on the skin, triggers sympathetic activity from the hypothalamus to release noradrenalin in BAT.[13] Noradrenalin binds to and activates various β-adrenergic receptors (β-ARs) situated on the cell membrane of brown adipocytes. The relative abundance and

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contribution of the three β-AR subtypes (β_{1-3}-AR) are species-dependent, with the β_3-AR being the most potent trigger of thermogenesis in rodents,[14] while a recent study identified the β_2-AR to be the most abundant subtype in human BAT biopsies and the main driver of thermogenesis in brown adipocyte cell cultures of human origin.[15] Interestingly, others have shown that silencing of the β_3-AR in cultured human brown adipocytes did attenuate their thermogenic activity.[16] These studies, together with the finding that the transcription of β_2-AR but not β_1-AR of cultured human brown adipocytes was induced by noradrenalin,[15] imply that both β_2 and $_3$-AR subtypes are involved in thermogenic regulation while the β_2-AR is likely dominant in human brown adipocytes. The possibility to activate BAT via pharmacologically targeting β-ARs or other pathways is further outlined in section 8.

Independent of the relative involvement of the β-AR subtypes, activation of any β-AR by noradrenalin induces an intracellular cascade, leading to the production of cyclic adenosine monophosphate (cAMP) to activate protein kinase A (PKA). Subsequently, PKA promotes intracellular hydrolysis of triglycerides within the many lipid droplets through phosphorylation and activation of adipose triglyceride lipase and hormone-sensitive lipase. PKA additionally stimulates thermogenic activity by upregulating the transcription of genes involved in thermogenesis via phosphorylation of cAMP response element-binding protein. The intracellularly released FAs are subjected to β-oxidation and further catabolic processing in the citric acid cycle, yielding reduced nicotinamide adenine dinucleotide (NADH) and reduced flavine adenine dinucleotide ($FADH_2$) to fuel the electron transport chain. Uncoupling protein 1 (UCP1), which is uniquely expressed in thermogenic adipocytes, uncouples the electron transport chain from adenosine-5′-triphosphate (ATP) production by disrupting the proton gradient across the mitochondrial inner membrane, leading to heat production.[13] Released long-chain FAs also allosterically activate UCP1, further enhancing thermogenesis.[17] Notably, inhibition of intracellular triglyceride lipolysis by nicotinic acid largely blunted cold-induced increases in BAT oxidative activity and thermogenesis in rats[18] and humans,[19] suggesting a critical role of intracellular triglyceride-derived FAs in BAT thermogenesis. Furthermore, recent studies revealed the existence of UCP1-independent thermogenic mechanisms *in vivo* in mice and *in vitro* in human brown adipocyte cultures, including futile creatine cycling[20,21] and ATP-dependent Ca^{2+} cycling.[22] Nonetheless, the physiological relevance of these mechanisms for thermogenesis in humans, as well as the possibility to target these pathways, warrant further research.

Activation of thermogenesis in brown adipocytes thus depletes intracellular triglyceride stores in BAT of both mice[5] and humans,[23] which are again replenished mainly via the uptake and re-esterification of FAs from the circulation, as demonstrated in mice.[24] To this end, sympathetic outflow to WAT, liver and BAT act in concert. First, sympathetic outflow promotes the hydrolysis of intracellular triglycerides in WAT,[25] explaining the increase in free FAs in the circulation observed in humans upon cold exposure.[26] The vast majority of the released FAs bind to albumin and are taken up by the liver, where the FAs are re-esterified into triglycerides. Although such FA/triglyceride cycling may seem like an inefficient process, released heat during the process could add to thermogenesis in adipose tissue,[27] which may explain the increased extracellular FA/triglyceride cycling seen in cold-challenged humans.[28] Second, sympathetic stimulation of the liver induces the incorporation of triglycerides into very LDLs (VLDLs) and their release into the circulation, as shown in mice.[29] In humans, this is reflected in an increase in large VLDL particles upon cold exposure.[30] Finally, sympathetic stimulation of BAT promotes lipoprotein lipase (LPL)-mediated uptake of triglyceride-derived FAs from TRLs as demonstrated in mice.[31] LPL is expressed by brown adipocytes and bound by endothelial cells towards the lumen of vessels to facilitate triglyceride hydrolysis of TRLs, with its expression and activity being up-regulated upon sympathetic stimulation.[32,33] Interestingly, vascular endothelial cells within BAT also appear to endocytose TRLs as a whole.[34] Internalized TRLs are processed by lysosomal acid lipase in endothelial cells to release FAs for β-oxidation, while generated reactive oxygen species (ROS) stimulate hypoxia-inducible factor 1-α-dependent proliferation and differentiation of thermogenic adipocytes, which further enhances the thermogenic capacity of BAT.[34]

Besides taking up TRL-derived FAs, BAT also extracts glucose from the circulation, a feature that is typically used to determine the presence and activity of BAT in humans through the application of [^{18}F]FDG PET-CT scanning, as mentioned in section 1. The uptake of glucose by brown adipocytes occurs via the noradrenalin-dependent glucose transporter-1 and insulin-dependent glucose transporter-4, albeit their relative contribution to net glucose uptake is still under debate.[13,35] It is also not fully understood for what reasons glucose is utilized by brown adipocytes. At least, glucose feeds *de novo* lipogenesis by providing substrate for acetyl-CoA synthesis, is used to generate the glycerol-3-phosphate backbone of triglycerides, and can enter the pentose phosphate pathway resulting in the synthesis of reduced nicotinamide adenine dinucleotide phosphate (NADPH).[36] *In vitro* studies using ^{13}C-labelled glucose indicated that glucose is fully oxidized upon acute adrenergic activation.[37] However, the conversion of glucose to CO_2 appeared to be dependent on diacylglycerol acyltransferase-2, which is the enzyme responsible for triglyceride synthesis from *de novo* synthesized FAs,[38] indicating that glucose should be converted into triglycerides before combustion.

3. Beige adipocytes and browning of WAT

Although we thus far referred to brown versus white adipocytes, many adipocytes resemble an in-between phenotype.[39] These cells are called 'beige' or 'brown-in-white' adipocytes. Their morphology is comparable to that of brown adipocytes for being multilocular and having a relatively low lipid content and high mitochondrial content. Beige cells are also capable of uncoupled respiration, but to a lesser extent than brown adipocytes. However, cAMP-stimulated uncoupled respiration of mouse-derived beige adipocytes *in vitro* is comparable to or even exceeds that of brown adipocytes.[39] Whilst some studies suggested that human brown adipocytes have a molecular signature that is more comparable to rodent beige adipocytes than 'classical' brown adipocytes,[39,40] others showed that human BAT also expresses classical brown adipocyte markers.[41] These seemingly contradicting results may be explained by differences in the location of human BAT biopsies, as depots deeper in the neck are more similar to classic BAT, while superficial depots are more similar to tissue containing beige adipocytes.[42,43]

The induction of beige adipocyte development in WAT is termed as browning of WAT. Repeated cold exposure was shown to induce browning of subcutaneous WAT in humans, as evidenced by increased protein levels of UCP1 and induction of uncoupled respiration in isolated mitochondria.[44] Two potential ways of inducing WAT browning have been proposed. The first way involves transdifferentiation of existing mature white adipocytes into beige adipocytes, which is supported by several lines of evidence. For example, cannabinoid type 1 receptor blockade with rimonabant of immortalized murine white adipocytes

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Table 1 Overview of studies investigating the effect of stimulation of thermogenic activity in adipose tissue on plasma lipids in relation to atherosclerosis development in mice

Mouse model	Intervention	Diet	Food intake	Triglyceride	Total cholesterol	Non-HDL-cholesterol	HDL-cholesterol	Lesion area	Ref
Models without ApoE-LDLR clearance pathway									
Apoe$^{-/-}$	Cold exposure	15% fat/0.25% chol	↑ +150%	↓ –55%	↑ +130%	↑ +160%[b]	N.D.[a]	↑ +180%	56
Ldlr$^{-/-}$	(4°C vs. 30°C)	15% fat/0.25% chol	N.D.[a]	=	↑ +150%	↑ +140%[b]	N.D.[a]	↑ +90%	
Apoe$^{-/-}$	CL316 243	21% fat/0.2% chol	=	↓ –55%	=	N.D.[a]	N.D.[a]	=	5
Ldlr$^{-/-}$	CL316 243	21% fat/0.2% chol	(pair-fed)	↓ –40%	=	N.D.[a]	N.D.[a]	=	
Apoe$^{-/-}$	Mirabegron	40% fat/1.25% chol	↑ +25%	↓ –55%	↑ +150%	↑ +180%[b]	N.D.[a]	↑ +200%	57
Ldlr$^{-/-}$	Mirabegron	40% fat/1.25% chol	N.D.[a]	↓ –50%	↑ +300%	↑ +260%[b]	N.D.[a]	↑ +400%	
Models with ApoE-LDLR clearance pathway									
E3L.CETP	CL316 243	16% fat/0.1% chol	=	↓ –54%	=	↓ –27%	↑ +50%	↓ –43%	5,58
E3L.CETP	CL316 243	16% fat/0.15% chol	=	↓ –62%	↓ –35%	↓ –30%	↑ +80%	↓ –55%	59
	+atorvastatin		=	↓ –36%[d,e]	↓ –59%[d,e]	↓ –51%[d,e]	↑ +48%[d,e]	↓ –76%[d]	
E3L.CETP	CL316 243	16% fat/0.15% chol	=	↓ –35%	↓ –31%	↓ –45%	↑ +52%	↓ –56%	60
	+colesevelam		=	↓ –35%	↓ –47%[d]	↓ –63%	↑ +36%	↓ –79%[e]	
E3L.CETP	CL316 243	16% fat/0.15% chol	↑ +9%	↓ –45%	↓ –12%	↓ –16%	↑ +45%	↓ –32%	61
	+alirocumab		↑ +10%	↓ –51%	↓ –38%[d,e]	↓ –45%[d,e]	↑ +47%	↓ –72%[d]	
E3L.CETP	CL316 243	16% fat/0.15% chol	N.D.[a]	↓ –34%	↓ –18%	↓ –21%	↑ +36%	↓ –38%	62
	+SRB1-KD[c]		N.D.[a]	↓ –47%[e]	↓ –27%[e]	↓ –34%[e]	↑ +66%[d,e]	↓ –65%[d,e]	

Data from studies in *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice were estimated from graphical data.[56,57]
Values represent significant differences compared to vehicle treatment. For studies where combinatorial treatment was evaluated, additional significant differences between groups are indicated with a [d] for the comparison with single CL316 243 treatment, and with a [e] for comparison with the respective other treatment or genotype.
[a]N.D., not determined/reported.
[b]LDL-cholesterol instead of non-HDL-cholesterol.
[c]KD, knock down.

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promoted expression of BAT-specific genes and enhanced oxygen consumption, a read-out of the respiratory chain.[45] In mice, cold exposure increased the number of brown adipocytes across various WAT depots with a parallel reduction in the number of white adipocytes[46] and increased the percentage of brown adipocytes within inguinal WAT without increasing adipocyte proliferation.[47] Moreover, treatment of rats[48] with sympathomimetics increased the number of brown adipocytes within WAT, without changing total cell numbers and without any obvious signs of active proliferation. The most direct evidence was obtained by using transgenic mice to trace the fate of existing mature adipocytes, by which all of ~5200 cold-induced multilocular UCP1-positive adipocytes in inguinal WAT were confirmed to derive from preexisting white adipocytes.[47] Mature human white adipocytes were also shown to transdifferentiate into brown-like adipocytes *in vitro* upon various stimulations, e.g. fibroblast growth factor 21 (FGF21), rosiglitazone, or adenovirus-mediated overexpression of peroxisome proliferator-activated receptor-γ coactivator 1-α, as evident from increased *UCP1* mRNA.[49] The second way involves *de novo* recruitment and/or differentiation from adipogenic progenitors. Adrenergic activation in mice stimulated brown adipocyte differentiation from adipocyte precursor cells in epididymal WAT, which express platelet-derived growth factor receptor alpha.[50] In addition, bone morphogenetic protein 7 induced differentiation of adipose progenitors isolated from interscapular BAT, subcutaneous WAT and skeletal muscle of mice as well as human preadipocytes isolated from subcutaneous WAT into brown adipocytes.[51] Similarly, bone morphogenetic protein 7-treated skeletal muscle-derived adipose progenitors developed into BAT-like adipose tissue after being re-engrafted into skeletal muscle.[51] Rosiglitazone[52] and cyclo-oxygenase 2[53] were also found to induce *de novo* differentiation of brown adipogenic precursors. Although the jury is still out, the two processes probably coexist, take place in different locations and respond to different stimuli.[54,55] To what extent WAT browning contributes to energy expenditure and benefits cardiometabolic health in humans represents another important outstanding question.

4. Activation of thermogenic adipose tissue counteracts dyslipidaemia and atherosclerosis in the presence of an apolipoprotein E-low-density lipoprotein receptor uptake pathway for TRL remnants

During lipolytic processing of TRLs by LPL to liberate FAs for combustion by brown and beige adipocytes, TRLs become smaller, depleted from triglycerides, and relatively enriched in cholesteryl esters. For this reason, BAT activation has been shown to reduce circulating triglyceride levels in many animal studies.[5,56–62] Depletion of triglycerides from TRLs is accompanied by an increased surface curvature, allowing the resulting TRL remnants to acquire (additional) copies of apolipoprotein E (ApoE) in a receptor binding-prone conformation.[63] Thereby the TRL remnants acquire an affinity for mainly the LDL receptor (LDLR) but also the LDLR-related protein 1 (LRP1) on hepatocytes to facilitate their endocytotic internalization.[64,65]

Whereas humans carry plasma cholesterol mainly within (V)LDL, mice carry the majority of cholesterol within HDL, with LDL-cholesterol levels being low, which explains why wild-type mice are resistant to atherosclerosis development. Therefore, genetic mouse models are widely used to study both pathophysiology of atherosclerosis as well as treatment strategies, including hypercholesterolemic *Apoe*$^{-/-}$, *Ldlr*$^{-/-}$ and APOE*3-Leiden.CETP (*E3L.CETP*) mice. These models have also been employed in the past decade to evaluate the effect of BAT-activating strategies on lipoprotein metabolism and atherosclerosis, as summarized in *Table 1*. Throughout various studies with different mouse models, cold exposure and β_3-AR agonism using CL316 243 or mirabegron typically reduced circulating triglycerides by stimulating the uptake of triglyceride-derived FAs by thermogenic adipose tissue.[5,56–62] Interestingly, BAT activation had different and even opposing effects on circulating cholesterol levels between models. In *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice, BAT activation by CL316 243 did not reduce plasma cholesterol,[5] while cold exposure and mirabegron even increased cholesterol.[56,57] The lack of cholesterol-lowering effects for CL316 243 treatment can be explained by an abolished ApoE-LDLR clearance pathway for TRL remnants in these mice that lack either ApoE or LDLR, which precludes efficient coupling of lipolytic processing of TRLs by activated thermogenic adipose tissue with uptake of their remnants by the liver.[5] Cold exposure- and mirabegron-induced increases in circulating cholesterol in *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice are readily explained by a much higher dietary intake of cholesterol in combination with abolished TRL remnant clearance.[56,57] Therefore, cold exposure and mirabegron exacerbated atherosclerosis development in *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice,[56,57] and BAT activation by CL316 243 did not reverse established atherosclerosis in *Ldlr*$^{-/-}$ mice.[66] In favourable contrast, *E3L.CETP* mice express a mutant form of human ApoE3 on top of endogenous mouse ApoE, which attenuates rather than abrogates the binding of TRL remnants to LDLR.[67] Combined with the expression of human cholesteryl ester transfer protein (CETP), which transfers cholesteryl esters from HDL to non-HDL, these mice have a human-like lipoprotein profile and mimic the lipid-lowering and anti-atherogenic response of humans to classic lipid-modulating strategies including statins.[68] For that reason, activation of BAT and browning of WAT by CL316 243 not only strongly enhanced LPL-dependent lipolytic processing of TRLs by thermogenic adipose tissue, but also accelerated the ApoE-LDLR dependent hepatic uptake of TRL remnants, thereby reducing plasma non-HDL-cholesterol and therefore attenuating trapping of lipoproteins in artery walls and atherosclerosis development.[5,58–62]

Besides reducing triglyceride and cholesterol within TRL remnants, activation of adipose tissue thermogenesis in *E3L.CETP* mice through CL316 243[59–61] or in humans by cold exposure,[30] also increased circulating HDL-cholesterol and we have demonstrated that short-term BAT activation was linked to increased reverse cholesterol transport in mice.[58] Mechanistically, this can be explained by the transfer of excessive surface lipids (i.e. mainly phospholipids) from TRLs during LPL-mediated lipolysis to lipid-poor apolipoprotein A1 (ApoA1) via phospholipid transfer protein (PLTP), resulting in the formation of small nascent HDL. These HDL particles acquire cholesterol from peripheral organs, after which the cholesterol is esterified by lecithin:cholesterol acyltransferase (LCAT) into cholesteryl esters that are subsequently selectively taken up by hepatocytes via scavenger receptor class B type 1 (SRB1). In the liver, cholesteryl esters are hydrolysed into cholesterol that can be converted into bile acids to be released into the intestine and partly secreted into the faeces. Such increased reverse cholesterol transport may contribute to the anti-atherogenic properties of promoting adipose tissue thermogenesis,[58] although the overall reduction in atherosclerosis is probably mainly explained by the reduction in non-HDL-cholesterol.[5] Notably, prolonged treatment with a β_3-AR agonist reduced the faecal

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bile acid output in *E3L.CETP* mice, which was explained by enhanced enterohepatic circulation of bile acids as treatment with the bile acid sequestrant colesevelam restored bile acid output to the faeces and enhanced the beneficial effects of BAT activation (see details in Section 5).[60] Interestingly, β_3-AR agonism in *E3L.CETP* mice on top of hepatic SRB1 knockdown was found to further increase plasma HDL-cholesterol and reduce atherosclerosis.[62] Although this may seem counterintuitive, the increase in HDL resulting from SRB1 knockdown provides a larger pool of acceptors for TRL surface remnants thereby facilitating the lipolytic conversion of TRLs and subsequent hepatic removal of TRL remnants, while the presence of human CETP shuttles cholesteryl esters from HDL to non-HDL, providing an alternative pathway for the transport of cholesterol from peripheral tissues to the liver.[62]

The combined mechanisms contributing to the lipid-modulating and anti-atherogenic effect of thermogenesis in adipose tissue as derived from studies mainly in mice as described in this section are graphically summarized in *Figure 2*.

5. Stimulation of thermogenesis in adipose tissue augments the beneficial effects of cholesterol-lowering therapies, and vice versa

Since new CVD-modulating strategies should always be combined with standard lipid-lowering therapy in clinical trials, the question arose if stimulation of thermogenesis further improves lipid metabolism and attenuates atherosclerosis on top of classic cholesterol-modulating agents, which has been evaluated in *E3L.CETP* mice as a relevant mouse model for human-like lipid metabolism and atherosclerosis development (see *Table 1*).

Statins are 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG-CoA) reductase inhibitors that have been developed to treat hypercholesterolaemia and reduce atherosclerotic CVD. Mechanistically, by inhibiting HMG-CoA reductase, statins prevent cholesterol synthesis in the liver, thereby lowering intracellular cholesterol levels and altering hepatic VLDL secretion. Subsequent activation of the Scap/SREBP pathway upregulates hepatic expression of LDLR to increase hepatic uptake of TRL remnants and LDL. Combining statin treatment with β_3-AR agonism in *E3L.CETP* mice significantly reduced non-HDL-cholesterol and increased HDL-cholesterol in the plasma and non-significantly reduced atherosclerotic lesion size relative to statin alone.[59] Similar to statins, the proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors alirocumab[69] and evolocumab[70] reduce hypercholesterolaemia and cardiovascular events in humans. Mechanistically, PCSK9 inhibitors block the PCSK9-induced intracellular transport of LDLR into lysosomes for degradation, thereby decreasing cholesterol via increased hepatic uptake of TRL remnants and LDL.[71] In *E3L.CETP* mice, BAT activation by β_3-AR agonism on top of alirocumab treatment significantly reduced plasma non-HDL-cholesterol, increased HDL-cholesterol and tended to further attenuate atherosclerosis development compared to alirocumab alone.[61]

Bile acid sequestrants bind to bile acids in the small intestine and therefore inhibit intestinal reabsorption. This leads to a reduction of bile acids in the circulation, which is sensed by hepatocytes and in turn the expression of LDLR and cholesterol conversion into bile acids is upregulated, resulting in decreased plasma LDL-cholesterol and reduced CVD risk.[72,73] Whilst short-term activation of adipose tissue thermogenesis promoted the conversion of cholesterol into bile acids due to an increased flux of cholesterol to the liver through accelerated formation of TRL remnants and mature HDL, prolonged BAT activity attenuated this process despite increased hepatic cholesterol. This is possibly due to elevated intestinal reabsorption resulting in an increased bile acid flux to the liver, which consequently downregulates bile acid synthesis.[60] Combining prolonged β_3-AR agonism with the bile acid sequestrant colesevelam restored faecal bile acid excretion and lowered plasma non-HDL-cholesterol levels, therefore leading to improved lesion stability and a trend for reduced atherosclerotic lesion when compared to β_3-AR agonism alone.[60]

6. Human BAT activity inversely relates to CVD incidence

Whilst activation of BAT and browning of WAT creates an anti-atherogenic lipoprotein profile in clinically relevant mouse models on top of classical lipid-lowering agents, studies addressing the role of thermogenic adipose tissue in human lipoprotein metabolism and cardiovascular health are still scarce. In a 5-year follow-up study including 31 healthy subjects, cold-induced BAT activity as determined by [^{18}F]FDG uptake and [^{15}O]H_2O perfusion was shown to correlate with lower carotid intima-media thickness and higher carotid elasticity via vascular imaging.[74] A larger study, using retrospective analysis of BAT activity from [^{18}F]FDG PET-CT scans from a cohort of 443 patients during a follow-up of 4 years, demonstrated that subjects who experienced a CVD event had lower BAT activity and individuals with lower BAT activity had more CVD events.[9] In addition, individuals with lower BAT activity had greater arterial inflammation as measured by [^{18}F]FDG uptake in the aortic wall,[9] which is of interest as arterial inflammation relates to CVD events.[75,76] In this suspected relationship, BAT activity may be representative of perivascular adipose tissue function, which also has BAT-like characteristics.[77] A recent unprecedented large retrospective study, using [^{18}F]FDG PET-CT scans of as many as 52 487 patients during a 9-year follow-up, categorized subjects by presence or absence of detectible BAT activity based on [^{18}F]FDG uptake and also reported a beneficial association between BAT activity and lower CVD risk, as individuals with detectible BAT activity have lower prevalence of various cardiovascular events.[11] After adjusting for confounding factors, including antihypertensive medication, ethnicity, and smoking status, the presence of BAT activity was identified as an independent negative predictor of CVD, coronary artery disease, congestive heart failure as well as hypertension.[11] In addition, individuals with BAT activity had lower plasma triglycerides and higher HDL-cholesterol.[11] Given that mechanistic studies in *E3L.CETP* mice revealed that BAT activation causally reduces plasma triglycerides and increases HDL-cholesterol,[5] a similar relationship is thus likely operative in humans and may explain the inverse relation between BAT activity and CVD risk. A cross-over clinical study demonstrated that short-term cold exposure of young men not only increased the plasma concentration of small HDL particles, but also enhanced ATP-binding cassette A1 (ABCA1)-dependent cholesterol efflux from macrophages to HDL as measured *in vitro*,[30] which is suggestive of higher reverse cholesterol transport in subjects in the presence of BAT activity. Notably, the association between the presence of metabolically active BAT and lower risk of coronary artery disease, congestive heart failure, and hypertension was found stronger in

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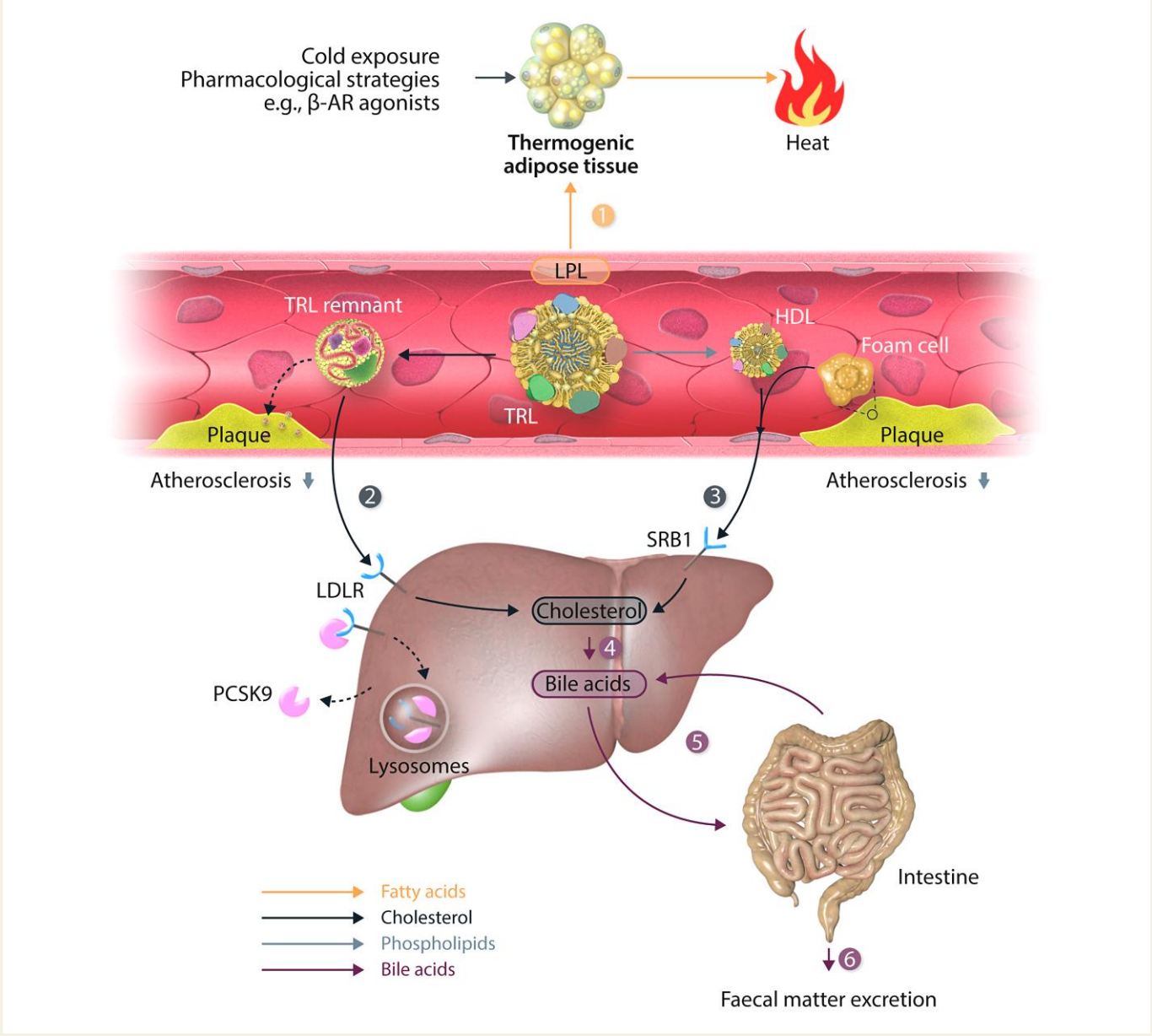

Figure 2 Schematic model detailing how thermogenic adipose tissue activation attenuates dyslipidaemia and protects from atherosclerosis development. Activated thermogenic adipose tissue (i.e. BAT and browned WAT) burns FAs liberated from intracellular lipid droplets for heat production. To replenish intracellular triglyceride stores, thermogenic adipose tissue takes up FAs from circulating TRLs after liberation by LPL-mediated lipolysis (pathway 1), leaving TRL core remnants and TRL surface remnants, mainly phospholipids, in the circulation. The core remnants are then efficiently taken up by the liver after binding of ApoE on the particle surface to mainly the LDLR on hepatocytes (pathway 2), protein levels of which are regulated by proprotein convertase subtilisin/kexin type 9 (PCSK9), resulting in a reduction in cholesterol-enriched lipoproteins and therefore attenuating trapping of lipoproteins in artery walls and atherosclerosis progression. The surface remnants are sequestered into HDL to improve HDL's cholesterol efflux-inducing capacity from e.g. macrophages in atherosclerotic plaques, with subsequent esterification of the acquired cholesterol by LCAT and selective delivery of the cholesteryl esters via SRB1 to hepatocytes (pathway 3), which can contribute to the atheroprotective effect of thermogenic adipose tissue activation. The uptake of cholesterol-enriched TRL remnants and cholesteryl esters from HDL combined leads to the accumulation of cholesterol in the liver, which drives cholesterol conversion into bile acids (pathway 4) with increased bile acid excretion via faeces (pathway 6), an effect that is attenuated upon prolonged stimulation of thermogenesis in adipose tissue due to enhanced bile acid re-uptake via enterohepatic circulation (pathway 5). See text for more details.

individuals who are overweight or obese as compared to lean individuals,[11] possibly suggesting that populations at high risk for CVD may benefit more from BAT-targeted therapy.

Reassuringly, although obese individuals showed blunted expression of thermogenic genes in BAT[78] and decreased glucose uptake by the tissue,[79] adipocyte progenitors isolated from BAT of obese individuals can differentiate into thermogenic adipocytes at an equal frequency as those isolated from lean individuals, and the resulting differentiated brown adipocytes displayed comparable basal and noradrenalin-stimulated mitochondrial respiration.[78] Similarly, applying an ice pack 30 min per day for 10 days to one side of the thighs induced protein expression of UCP1 and the beige adipocyte marker transmembrane protein 26 in

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subcutaneous WAT of the cold-exposed thigh in both lean and obese subjects.[44] This response even extended to the contralateral thigh, which is likely explained by activation of the sympathetic nervous system.[44] Furthermore, individuals who had less BAT activity based on [^{18}F]FDG PET-CT scan before a 27-day treatment with the β_3-adrenergic receptor agonist mirabegron gained larger BAT activity and volume than those who started with higher BAT activity,[80] again supporting the opportunity for BAT-targeted therapy.

7. Human BAT activation by cold exposure attenuates risk factors of CVD

Besides the observed beneficial relation between the presence of BAT and CVD in humans (see Section 6), cold exposure has been shown to beneficially affect several risk factors for CVD, including adiposity and insulin resistance.

Adiposity results from excessive energy intake relative to energy expenditure, or alterations in nutrient partitioning. Acute cold exposure increased resting energy expenditure in both lean[81–83] and obese[84] participants, and notably such increases were only evident[82] or more pronounced[83] in BAT-positive individuals (i.e. with detectible [^{18}F]FDG uptake by BAT depots). Even though BAT activity is generally assessed using the glucose tracer [^{18}F]FDG, the cold-induced increase in energy expenditure was mainly explained by an increase in lipid oxidation.[81,82] Subjecting healthy lean humans to daily 2 h cold exposure at 17°C for 6 weeks increased BAT activity with a parallel increase in whole-body energy expenditure and a modest reduction in body fat mass.[83] Despite these encouraging data, cold acclimatization at 19°C for at least 10 h each night for a month enhanced resting energy expenditure but did not affect body fat mass in another study with a healthy lean population.[85] Similarly, a single dose of the β_3-AR agonist mirabegron enhanced BAT activity (i.e. [^{18}F]FDG uptake) and increased energy expenditure in humans,[86,87] but long-term treatment with either mirabegron or other β_3-AR agonists (i.e. L-796 568 and TAK-677) did not reduce body fat mass.[88–90] Therefore, it remains to be determined under what specific conditions cold exposure or pharmacological therapies can be employed to efficiently promote BAT activity and attenuate fat mass. Alternatively, it is well possible that BAT activity simply improves overall metabolic health, rather than reducing adipose tissue mass *per se*. In line with this notion, a very recent study has suggested that after correcting for BMI, the presence of active BAT, as measured by [^{18}F]FDG uptake, was associated with decreased visceral adipose tissue and increased subcutaneous adipose tissue,[91] a phenotype that is typically associated with better metabolic health.

BAT has also been implicated in glycaemic control. In healthy lean humans, acute cold stimulation (18°C)[92] or 1-month cold acclimation (i.e. ~ 10 h at 19°C each night)[85] increased uptake of glucose by BAT and improved whole-body insulin sensitivity, albeit no effects on fasting plasma glucose levels were observed. Of note, prolonged (5–8 h) cold exposure at ~19°C in healthy overweight humans enhanced both basal and insulin-stimulated glucose disposal in subjects with detectable BAT, while not affecting either basal or insulin-stimulated glucose disposal in those individuals without detectable BAT.[82] This was accompanied by a selectively increased [^{18}F]FDG uptake by BAT but not by other organs, including skeletal muscle.[82] Another study in healthy overweight individuals showed that 4-week treatment with mirabegron also selectively promoted glucose uptake by BAT, while improving insulin sensitivity and insulin-independent glucose metabolism.[80] In patients with type 2 diabetes, cold acclimation (14–15°C, ~5.5 h/day for 10 days) increased insulin sensitivity and whole-body glucose disposal, as evidenced by increased glucose infusion rate during the hyperinsulinemic-euglycemic clamp.[93] Still, it should be noted that this improved glucose disposal was explained by increased [^{18}F]FDG uptake by BAT as well as skeletal muscle, the latter also contributing to cold-induced shivering.[93] In addition to directly taking up glucose, a variety of brown adipokines were identified to be secreted by human brown adipocytes upon adrenergic stimulation, and were shown to improve glucose tolerance and insulin sensitivity in preclinical models.[94,95]

Thus, studies have unequivocally demonstrated that cold exposure activates BAT, enhances energy expenditure, and improves glycaemic control. The relative contribution of BAT and other metabolic organs needs to be better understood, but at the very least it seems that the presence of (cold-) activate(d) BAT is associated with metabolic health.

8. Therapeutic interventions to recruit BAT and promote BAT activity

In 2015, Cypess *et al.*[86] were the first to show that human BAT can be activated by the β_3-AR agonist mirabegron, which seemed to nicely corroborate the effects of the β_3-AR agonist CL316 243 in mice,[5] although with a concomitant increase in heart rate.[86] A more recent study, however, revealed that such a BAT-activating effect was only observed at a supra-pharmacological dose of 200 mg, as the pharmacological dose of 50 mg applied in the treatment of hyperactive bladder appeared ineffective,[15] which suggested that mirabegron-induced activation of BAT resulted from cross-reactivity with other β-ARs. Indeed, transcriptomic analysis of human BAT biopsies showed that abundance of β_2-AR far exceeds that of β_3-AR, while β_3-AR is the dominant AR in mouse BAT.[15] An *in vitro* study using primary human brown adipocytes corroborated that thermogenic activation by noradrenalin and mirabegron is predominately mediated via β_2-AR.[15] On the other hand, chronic treatment (10 weeks; 50 mg/day) with mirabegron promoted thermogenic gene expression in WAT of insulin-resistant, obese humans.[44] In addition, Trp64Arg polymorphism in the *ADRB3* gene encoding for the β_3-AR is associated with dyslipidaemia and therefore might represent a genetic risk factor for CVD.[96] For these reasons we should not fully discard the β_3-AR as a therapeutic target.

The recent finding regarding the prominent role of β_2-AR in human BAT activation, however, opened up new opportunities for BAT as a therapeutic target in (cardio)metabolism. Interestingly, the amino acid sequence of human β_2-AR is highly polymorphic.[97] The Thr|le164 variant has lower affinity for β_2-AR agonists[98] and individuals with this variant are characterized by early onset of coronary artery disease.[99] Besides, the β_2-AR agonist formoterol stimulates fat oxidation in humans, a feature of BAT activation,[10,81,82] without increasing heart rate.[100] This suggests that β_2-AR agonism may provide an efficient and possibly safe option to activate human BAT and improve (cardio)vascular health. These findings thus imply that β_2-AR agonism may be the way forward in adrenergic BAT activation, and further studies are warranted to assess whether this can effectively and safely activate human BAT *in vivo*.

Besides cold-mediated sympathetic stimulation and pharmacological β-AR agonism, stimulation of two hormonal pathways also potently activate BAT and are worth noting as they lower atherosclerosis in

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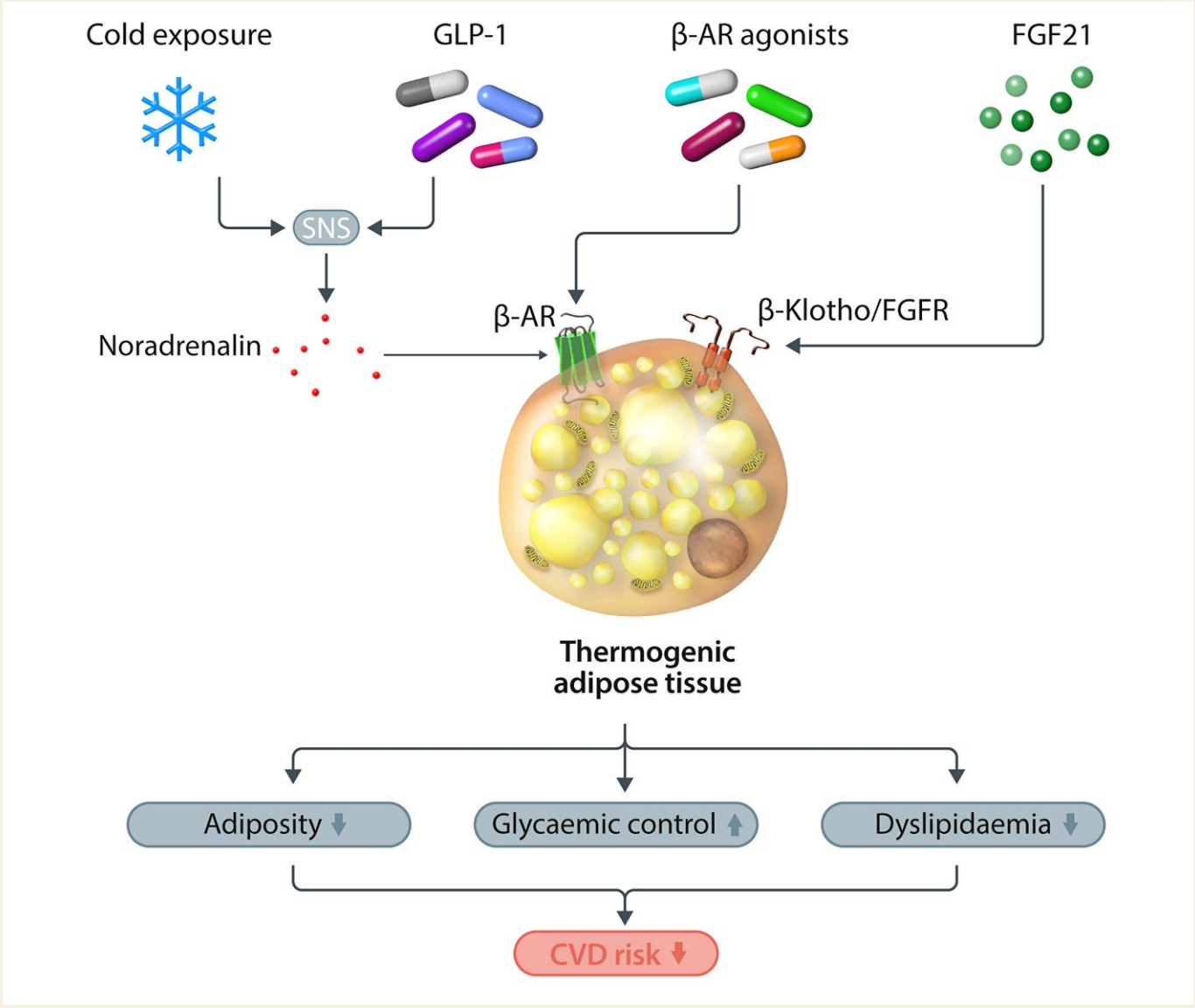

Figure 3 Graphical summary of promising therapeutic interventions to promote thermogenic adipose tissue activity and their effects on risk factors for CVD in humans. SNS, sympathetic nervous system; β-Klotho/FGFR, β-Klotho/fibroblast growth factor receptor complex.

preclinical models and improve risk factors for CVD in humans (see also the graphical summary in *Figure 3*). Firstly, treatment of mice with recombinant human FGF21 enhanced the uptake of glucose[101] and triglyceride-derived FAs from TRLs[102] by BAT and promoted WAT browning,[101,102] which normalized glycaemia[101] and reduced plasma triglycerides.[102] Furthermore, FGF21-stimulated lipolysis of TRLs by BAT consequently stimulated TRL-remnant uptake by the liver, therefore decreasing plasma non-HDL-cholesterol and protecting from atherosclerosis in *E3L.CETP* mice.[103] In humans, serum FGF21 levels correlate with BAT activity[104] and treatment of primary adipocytes isolated from human neck beige adipocyte depots with FGF21 stimulated thermogenic gene/protein expression and noradrenalin-induced heat production,[105,106] accompanied by increased lipid oxidation.[106] Clinical studies in subjects with type 2 diabetes showed that administration of an FGF21 analogue remarkably improved dyslipidaemia, including decreases in plasma LDL-cholesterol and triglycerides and an increase in HDL-cholesterol, while the much-anticipated glucose-lowering effect did not reach statistical significance.[107,108] These lipid-manipulating effects of FGF21 treatment in humans suggest that FGF21-based therapies may be effective to combat human dyslipidaemia and atherosclerotic CVD, as shown in our preclinical study.[103] Whether and to what extent BAT plays a role in the beneficial effects of FGF21 in humans still remains to be investigated.

Similar to FGF21, studies of glucagon-like peptide 1 receptor (GLP-1R) agonism have also shown promising results. In lean mice, intracerebroventricular administration with the GLP-1R agonist liraglutide activated BAT thermogenesis as evident from decreased intracellular lipid content in combination with increased interscapular temperature[109]. In both lean and diet-induced obese mice, another GLP-1R agonist, exendin-4, was shown to increase UCP1 protein content in BAT.[110] In parallel, exendin-4 increased BAT uptake of glucose and triglyceride-derived FAs, accompanied by lowered plasma glucose and triglyceride levels.[110] Similarly, in healthy humans, GLP-1R agonism with exenatide increased BAT volume and glucose uptake, accompanied by lower

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circulating triglyceride and total cholesterol levels.[111] These glucose and lipid-lowering actions are indicative of atheroprotective effects of GLP-1R agonism. Indeed, patients with type 2 diabetes using liraglutide showed less death from cardiovascular causes and a lower frequency of nonfatal myocardial infarction and stroke.[112] Several studies in *Apoe*$^{-/-}$, *Ldlr*$^{-/-}$ and *E3L.CETP* mice have shown that GLP-1R agonists reduced atherosclerosis development via reducing inflammation in atherosclerotic plaques.[113–115] To what extent GLP-1R agonism can also attenuate atherosclerosis development via regulating lipid metabolism through BAT activation remains to be studied. Furthermore, glucose-dependent insulinotropic polypeptide receptor (GIPR) agonism was proposed to enhance the metabolic effects of GLP-1R agonism. Consistent with this hypothesis, a recent phase 2 clinical trial demonstrated that the dual GLP-1R/GIPR agonist LY3298176 not only produced superior benefits regarding glucose control, but also in weight loss in patients with type 2 diabetes as compared to single GLP-1R agonism by dulaglutide.[116] Mechanistically, while both agonists promote glucose-stimulated insulin secretion, glucose-dependent insulinotropic polypeptide has also been shown to promote lipolysis in white adipocytes *in vitro*.[117] In theory, released FAs may fuel BAT activated by GLP-1R agonism and therefore lead to superior metabolic benefits, although this should still be confirmed in experimental studies. As with FGF21 analogue treatment, whether combined GLP-1R/GIPR agonism can be employed to prevent or treat dyslipidaemia and atherosclerotic CVD, and to what extent BAT activation plays a role, still have to be revealed.

9. Concluding remarks and future directions

Taken together, there is compelling evidence for a relationship between the presence of metabolically active BAT in humans and lower CVD risk. The still unresolved question, however, is to what extent the observed associations imply causality or merely reflect overall metabolic health. Cold interventions have been shown to activate BAT activity and thermogenesis, and large prospective intervention studies applying cold interventions will be needed to prove causality. In addition, genetic polymorphisms determining the thermogenic capacity of adipose tissue may be identified to allow proof of causality between adipose tissue thermogenesis and CVD risk in large Mendelian-randomization studies.

Experimental studies in mice have convincingly shown that thermogenic activity in adipose tissue enhances lipolytic processing of TRLs, resulting in FA uptake by adipocytes and consequently promotes liver uptake of TRL remnants provided that an intact human-like ApoE-LDLR pathway is present (*Table 1*). Together, these result in combined attenuation of hypertriglyceridaemia and hypercholesterolaemia and reduce atherosclerosis development. This anti-atherosclerotic effect is likely further enhanced by elevated reverse cholesterol transport, which is driven by enhanced cholesterol efflux capacity of HDL as a consequence of increasing lipid transfer from TRLs to HDL during lipolytic processing. In humans, BAT activity inversely correlates with circulating triglyceride and HDL-cholesterol levels and CVD prevalence and seems to protect against additional risk factors for CVD including adiposity and insulin resistance. Combined with the findings from preclinical studies that thermogenic adipose tissue activation adds to the lipid-lowering and antiatherogenic effects of classical lipid-lowering strategies (i.e. HMG-CoA reductase inhibition and PCSK9 inhibition), these findings highlight the potential of activating BAT to further prevent/treat dyslipidaemia and atherosclerotic CVD in humans.

Obviously, further research is needed to reveal whether promotion of BAT activity or browning of WAT can be used to treat dyslipidaemia and atherosclerotic CVD in humans, especially in those individuals who are at high risk for CVD. FGF21 and GLP-1R agonism (likely in combination with GIPR agonism) activate BAT and promote browning of WAT in mice and are promising therapeutic strategies to treat human atherosclerotic CVD. Further clinical studies are warranted to assess their efficacy to reduce atherosclerotic CVD, as well as the involvement of BAT activation therein. Also, the recent discovery that human brown adipocytes are mainly activated via β_2-AR stimulation, in contrast to mouse brown adipocytes that are activated mainly through the β_3-AR,[15] provides a unique opportunity to assess both the efficacy and safety of β_2-AR agonism in human BAT activation in relation to (cardio)metabolic health.

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Data availability

No new data were generated or analyzed in support of this manuscript.

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