Beyond obesity - Thermogenic adipocytes and cardiometabolic health

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Abstract

The global prevalence of obesity and related cardiometabolic disease continues to increase through the 21st century. Whilst multi-factorial, obesity is ultimately caused by chronic caloric excess. However, despite numerous interventions focussing on reducing caloric intake these either fail or only elicit short-term changes in body mass. There is now a focus on increasing energy expenditure instead which has stemmed from the recent 're-discovery' of cold-activated brown adipose tissue (BAT) in adult humans and inducible 'beige' adipocytes. Through the unique mitochondrial uncoupling protein (UCP1), these thermogenic adipocytes are capable of combusting large amounts of chemical energy as heat and in animal models can prevent obesity and cardiometabolic disease. At present, human data does not point to a role for thermogenic adipocytes in regulating body weight or fat mass but points to a pivotal role in regulating metabolic health by improving insulin resistance as well as glucose and lipid homeostasis. This review will therefore focus on the metabolic benefits of BAT activation and the mechanisms and signalling pathways by which these could occur including improvements in insulin signalling in peripheral tissues, systemic lipid and cholesterol metabolism and cardiac and vascular function.

List of abbreviations: 18F-fluoro-thiaheptadecanoic acid (18FTHA), 2-deoxy-2-[18F]-fluoro-d-glucose (18F-FDG), 2-[1-14C]-deoxyglucose (2-DG), adenosine 3',5'-cyclic monophosphate (cAMP), Adenosine triphosphate (ATP), Adipose Tissue (AT), Adipose triglyceride lipase (ATGL), Angiopoietin-like 4 (ANGPTL4), AMP-activated protein kinase (AMPK), Apolipoprotein E (ApoE), Brown adipose tissue (BAT), Epicardial adipose tissue (EAT), Fibroblast growth factor 21 (FGF21), Free fatty acid (FFA), Glucagon-like peptide 1 (GLP-1), Glucose transporter (GLUT), Guanosine diphosphate (GDP), Hormone sensitive lipase (HSL), Insulin receptor (IR), IR substrates -1 (IRS-1) and -2 (IRS-2), Lipoprotein lipase (LPL), Low-density lipoprotein receptor (Ldlr) Mechanistic target of rapamycin (mTOR), Nicotinamide

adenine dinucleotide phosphate (NAPDH), Norepinephrine (NE), Perivascular adipose tissue (PVAT), Peroxisome proliferator-activated receptor gamma (PPAR-γ), Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), Positron emission tomography-computed tomography (PET-CT) Phosphatidyl-inositol-3, 4, 5-triphosphosphate (PIP3), Phosphoinositide-3-kinase (PI3K), Protein kinase A/B/C (PKA/PKB/PKC), Sirtuin 1 (SIRT1), Triglyceride (TG), Uncoupling protein 1 (UCP1), Very-low-density lipoprotein (vLDL), White adipose tissue (WAT).

Introduction

Our current knowledge of adipose tissue physiology has grown exponentially this century and it is now understood that there are at least three types of adipose tissue namely white, brown and beige which differ in their embryological origin, function, molecular characterisation and anatomical distribution (1-3). White adipose tissue (WAT), traditionally viewed as an energy storage vessel is now considered to be potentially the body's largest endocrine organ. In addition to regulating the storage and release of triglycerides in response to changes in energy demand, WAT modulates an array of physiological processes through the secretion of growth factors, cytokines, peptides and adipokines (4, 5). Brown adipose tissue (BAT), in contrast, is characterised by the possession of the functionally thermogenic mitochondrial uncoupling protein UCP1. It was traditionally thought to be present primarily in the human neonate where activation at birth acted to defend against hypothermia to the cold extra uterine environment (6). Seminal work had previously demonstrated the presence of brown adipocytes in adult autopsies (7); however, the discovery in 2009 that BAT could be activated pharmacologically and environmentally, was associated inversely with body mass index, with age and metabolic health led to it being heralded as a possible anti-obesity target (8-13). Furthermore, the discovery of inducible 'beige' adipocytes in classical WAT depots which are not only thermogenic in vitro (14) but improve metabolic health in vivo (14-17) has led to further interest on 'browning' white AT.

INSERT FIG 1 HERE

There is now a wealth of data from animal models suggesting that the activation of thermogenic adipocytes either environmentally or pharmacologically can elicit substantial reductions in adiposity (18). However, these studies are typically done in chronically cold-acclimated rodents exhibiting large and active BAT depots which does not mimic human physiology (19). Moreover, it is also important that the total amount of UCP1 is determined in each depot, and the tissues relative state

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of differentiation/expansion are also measured (20). These factors therefore not only impact on classical brown fat but also beige adipocytes and the elusive search for a stimulus that can provide a greater stimulus than cold to promote UCP1 and the beiging process (20). Evidence in humans certainly suggests that BAT activation increases energy expenditure, however, there is little to suggest a role of thermogenic adipocytes in combating obesity. In fact, long term cold exposure [19C over 1 month] improved glucose homeostasis without an effect on body weight in healthy young men though adiposity was not studied. At present the role of thermogenic adipocytes in humans suggests a potential role in regulating cardiometabolic health in the absence of changes in weight and therefore these cardiometabolic alterations will be the focus of the current review.

Brown adipose tissue, hyperglycemia and insulin resistance

The dysregulation of insulin signalling and onset of insulin resistance and hyperglycemia is a key feature in obesity and related metabolic syndrome. The mechanisms by which insulin signalling becomes dysregulated is however both complex and tissue specific as the effects of insulin on glucose homeostasis is reliant on numerous downstream intracellular signalling events (21). Typically, the binding of insulin to the α-subunit of the insulin receptor (IR) induces the rapid phosphorylation of its β subunit and the subsequent activation of tyrosine kinase which in turn allows phosphorylation of IR substrates -1 (IRS-1) and -2 (IRS-2). Phosphorylation of IRS-1 and IRS-2 and subsequent activation of phosphoinositide-3-kinase (PI3K) is key and affects numerous downstream signalling pathways by generating phosphatidyl-inositol-3, 4, 5-triphosphate (PIP3), a lipid second messenger. Of these downstream pathways, the Akt/PkB (Protein kinase B) is key and its phosphorylation at the serine 407 and threonine 308 residues is pivotal in driving the metabolic actions of insulin in target tissues.

Importantly, and in the context of this review, recent evidence points to an important role for brown adipocytes in regulating systemic glucose homeostasis with BAT described as a 'glucose sink' (22). It

is essential therefore to understand the mechanisms by which glucose uptake occurs in thermogenic adipocytes (Figure 2). Glucose uptake in brown adipocytes was described as far back as 1985 (23) and this early in-vivo work in mice demonstrated that the uptake of 2-[1-14C]-deoxyglucose (2-DG) into BAT was

a) greater than in both the brain and heart as well as other peripheral tissues (WAT and skeletal muscle) following insulin administration and

b) greater than in the heart following norepinephrine (NE) administration suggestive that there may be divergent, stimulus specific mechanisms by which glucose uptake occurs.

Insulin stimulated glucose uptake in brown adipocytes is now well understood, occurring by the PI3K–Akt pathway and glucose transporter (GLUT) 4 translocation to the plasma membrane as it does in both WAT and skeletal muscle (24). The mechanisms governing adrenergic stimulation of glucose uptake into brown adipocytes were traditionally less well described. Early in-vitro work demonstrated that glucose uptake in brown adipocytes exposed to NE occurs independently of insulin whilst conversely, insulin mediated glucose uptake into brown adipocytes was dependent on NE concentration (25). It was suggested at the time that the effects of NE were likely to be mediated by adenosine 3',5'-cyclic monophosphate (cAMP) and we now know that the increased glucose uptake in response to sympathetic activation occurs independently of GLUT4 translocation (26, 27). This is largely due to both rapid de novo synthesis of the insulin independent GLUT1 in the plasma membrane by cAMP and also the translocation of GLUT1, promoted by mechanistic target of rapamycin (mTOR) complex 2 (26, 27). Whilst both insulin, and NE-mediated glucose uptake initially occur by different mechanisms adrenergic stimulation ultimately activates the cAMP/protein kinase A (PKA) pathway recruiting PI3K and stimulating multiple protein kinase c's (PKCs). This suggests that NE also acts through similar downstream mechanisms as the classic insulin signalling pathway (28) and it is these mechanisms that underpin the potential for BAT to modulate glucose homeostasis.

2-deoxy-2-[18F]-fluoro-d-glucose (18F-FDG) was typically used during positron emission tomography (PET)/computed tomography (CT) scans to quantify metabolic activity of tumours in cancer patients. However, since the discovery that 18F-FDG PET-CT can be used to quantify both the presence and activity of BAT its use in healthy populations has increased. In fasted humans undergoing 18F-FDG PET-CT scans cold exposure increases resting energy expenditure, an effect fuelled by oxidation of plasma glucose and free fatty acids (29). Furthermore, cold exposure shifts glucose disposal to active BAT and away from the heart as earlier described (23, 29). This increase in glucose utilisation by BAT improves whole body insulin stimulated glucose uptake and insulin sensitivity, changes which are potentially mediated by upregulation of GLUT 1/GLUT 4 or the numerous other genes and enzymes that regulate glucose uptake, glycogen turnover and glycolytic flux as discovered by transcriptomic profiling of sympathetically activated BAT (28, 30).

It is these BAT associated changes in glucose homeostasis in humans which have led to the hypothesis that its activation could be a therapeutic target for diabetes.

Long term, intermittent reductions of ambient temperature from 24°C to 19°C for 10 hours/night for a one month period yield no change in body composition but increase diet induced thermogenesis and post prandial insulin sensitivity in association with reduced leptin and increased adiponectin and GLUT4 expression in subcutaneous AT (31). Short-term cold acclimation over 10 days elicits increases of 18F-FDG uptake into BAT concomitant with a ~1.4 fold increase in peripheral insulin sensitivity in type 2 diabetic patients. Given the role of skeletal muscle in regulating glucose homeostasis it is important to note that these effects may be due to the increased translocation of GLUT4 in skeletal muscle which occurred in the absence of changes in insulin signalling and thermogenic genes in WAT (32, 33). Even so, it would seem that BAT regulates glucose-induced thermogenesis during an oral glucose tolerance test and that BAT thermogenesis exhibits a diurnal rhythm which is glucose responsive (34). This may be functionally relevant as humans spend a large

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amount of time in the post-prandial state and should be taken into account when considering dietary interventions that are targeted on response of BAT to nutrient excess. Animal studies allude to numerous other benefits of BAT activation. For instance, in models of type 1 diabetes, BAT transplantation can restore euglycaemia independently of insulin through insulin-like growth factor 1 stimulated adipogenesis (35). The formation of healthy WAT and the secretion of hypoglycaemic adipokines are thought to underlie the effects of transplantation on glycaemia, however, the improvements in metabolic homeostasis are reportedly lost in the absence of interleukin-6 (IL6) suggestive that this pleiotropic cytokine has a pivotal role (36). Whilst the mechanisms governing improvements in glucose homeostasis are not completely understood BAT appears to have a major role in regulating glucose metabolism.

It is feasible that the therapeutic activation of thermogenic adipocytes may be a future treatment for diabetes and related endocrine disorders whilst a number of drugs in use clinically or in development may improve insulin sensitivity in part through their effects on brown and beige adipocytes (37).

1) Metformin

Metformin is a biguanide drug commonly used, alone or in combination with insulin, in the treatment of diabetes. It primarily decreases circulating glucose by inhibiting hepatic gluconeogenesis and by increasing GLUT4-dependent glucose uptake into muscle and WAT through the activation of AMPK and both novel and conventional PKC's (38-40). There is little evidence to date that the improved metabolic homeostasis and modest reductions in body weight occurring with Metformin treatment are due to increased BAT thermogenesis. Indeed, whilst Metformin may increase UCP1 in BAT of lean rats, treatment of both Zucker obese rats and brown adipocytes did not increase BAT UCP1 expression, GDP binding or cellular respiration respectively and importantly no increase in resting energy expenditure is seen in humans (41-45).

Metformin may have a clearer significance in a perinatal setting where administration during early life may be critical in maintaining BAT function in pups overfed during lactation (46). Despite increased size and adiposity, pups exposed to a caloric surplus during lactation had greater basal UCP1 expression in BAT, likely due to increased PPARα activation but were less responsive to cold exposure. After weaning to chow mice exhibited greater body mass, adiposity and insulin resistance at 16 weeks of age concomitant with a reduction in BAT UCP1 and the thermogenic response to both cold and a β3-agonist. Importantly, when pups were given daily metformin injections throughout lactation the adverse effects on BAT function were reversed at 4 weeks of age despite having similar body weight (46). Long-term effects of this early postnatal life treatment and how this intervention may relate to a clinical situation warrant further research.

2) FGF21

Fibroblast growth factor (FGF) 21 is an autocrine and endocrine protein secreted primarily from the liver during fasting and starvation, increasing gluconeogenesis and ketogenesis and acting on numerous target tissues including WAT and BAT (47). In addition to having endocrine functions FGF21 can act in an autocrine/paracrine manner. PPARγ induced FGF21 production in WAT does not enter circulation but instead acts on WAT directly activating PPARγ from its inactive, sumoylated, form and, with that, enabling stimulation of adipogenesis, GLUT1 expression, glucose uptake and thermogenesis through browning (48, 49). FGF21 also acts centrally, activating the sympathetic nervous system whereby subsequent NE production directly stimulates UCP1 expression in both BAT and WAT (50). Additionally, in recent years it has been recognised as a potential 'BATokine' as its expression is increased in BAT following cold exposure though the functional relevance of BAT secreted FGF21 and its contribution to circulating concentrations are not yet understood (51).

Obesity increases expression of FGF21 in liver and WAT as well as circulating FGF21 which may be due to reduced function of its receptor and FGF21 resistance (52-54). Since FGF21 expression is

controlled by PPARα and PPARγ, stimulation of these transcription factors through antidiabetic drugs like the fibrate class of hypolipidemic drugs and thiazolidinediones, respectively, will induce FGF21 (55, 56). Direct administration of FGF21 improves glucose tolerance by decreasing both glucose and insulin concentrations, and lowers body weight, plasma triglycerides and non-esterified fatty acids, in both obese humans and mice, an adaptation partly be mediated by increased non-activity energy expenditure alluding to potential increases in BAT thermogenesis (56-58). Indeed, in mice with diet-induced obesity, FGF21 administration induces UCP1 expression and glucose uptake into brown fat, and also promotes a substantial increase in WAT UCP1 (59-61). Furthermore, cultured brown adipocytes directly stimulated with FGF21 also exhibit an upregulation of UCP1 gene expression together with increased oxygen and glucose consumption (62). Data from UCP1 knockout mice suggests the benefits of FG21 are not entirely due to increased thermogenic activity but also possibly due to reduced energy intake (63). Nevertheless, development and optimisation of recombinant forms of FGF21 to be used in therapy of metabolic diseases such as diabetes are still in progress (56, 64).

3) GLP-1 receptor stimulants

Glucagon-like peptide 1 (GLP-1) is a hormone postprandially released from ileum and colon L-cells. GLP-1 stimulates insulin secretion and inhibits glucagon secretion in a glucose-dependent manner, therefore not leading to hypoglycaemia in addition to slowing gastric emptying thus reducing appetite and body weight (65, 66). Endogenous GLP-1 is cleaved by the protease dipeptidyl peptidase 4 (DPP4) within minutes and cleared through the kidney. Synthetic analogues which stimulate the GLP-1 receptor are designed to be resistant to DPP4 degradation and range from short-acting, which are administered twice a day, including liraglutide, to long-acting forms, administered once a week, including albiglutide (67). These analogues are currently in clinical use for the management of hyperglycaemia in type 2 diabetes. In mice it has recently been shown that the metabolic benefits of GLP-1 agonists may occur in part through the activation of BAT and the

browning of WAT depots (41, 68, 69). When delivered through intracerebroventricular injection, GLP-1 (68) and its analogue exendin-4 (69) increase BAT thermogenesis via an increased uptake of TG-derived fatty acids and plasma glucose in addition to browning WAT, effects which may occur by activation of hypothalamic AMPK (41). Similar results have been demonstrated when GLP-1 agonists have been administered peripherally (70-72) with the browning of WAT suggested to occur via upregulation of SIRT1 (73). Whilst these effects remain to be confirmed in humans it is feasible that GLP-1 agonists could be suitable candidates to induce browning of visceral adipose tissues.

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Thermogenic adipocytes regulate lipid and cholesterol metabolism

Alterations in systemic lipid and cholesterol metabolism during obesity play a major role in the onset of associated cardiometabolic disease. Insulin and the classical insulin signalling pathways regulate lipid metabolism in both organs and peripheral tissues and dysregulation of insulin signalling effects WAT lipolysis, free fatty acid (FFA) and triglyceride (TG) synthesis and lipid uptake from blood in addition to both hepatic cholesterol synthesis and very-low-density lipoprotein (vLDL) formation (74). Whilst changes in lipid metabolism following BAT activation may relate to improvements in the insulin signalling, BAT utilises lipids as a fuel source stimulating whole body metabolism (Figure 2) (75). During sympathetic stimulation the release of NE from sympathetic nerves results in the activation of adenylyl cyclase and production of cyclic AMP which in turn activates protein kinase A (PKA) . PKA then phosphorylates adipose triglyceride lipase (ATGL) and hormone sensitive lipase (HSL), triggering lipolysis and the release of FFA from intracellular lipid droplets. These FFA enter the mitochondria and activate UCP1 uncoupling oxidative phosphorylation from ATP synthesis to result in the dissipation of heat (76). Ongoing stimulation of BAT requires a replenishment of intracellular lipid, a process mediated by both de novo lipogenesis (77) and the uptake of FA from TG-rich lipoproteins by lipoprotein lipase (LPL) and CD36 (78). Other regulators of lipid mobilisation in BAT

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exist. For example, angiopoietin-like 4 (ANGPTL4), a regulator of LPL activity during both fasting and exercise was recently shown to modulate lipid shuttling in BAT during cold exposure preferentially driving uptake of TG-rich lipoprotein derived FFA, an effect which is regulated by AMP-activated protein kinase (79).

The use of animal models to determine how BAT regulates lipid metabolism has been invaluable. They have been used to demonstrate that activation of BAT, through the above mechanisms can correct hypercholesterolemia and attenuate atherosclerosis (75, 80). In contrast, it has also been suggested that BAT activation may be involved in the progression of atherosclerosis due to cold-induced alterations in lipid profile (81). However, it is important to note that the adverse effects of BAT activation are limited to apolipoprotein E K/o (ApoE −/−) and low-density lipoprotein receptor K/o (Ldlr −/−) mice, which both lack a functional ApoE-Ldlr pathway, a requirement for the physiological regulation of lipid metabolism and hepatic clearance of lipoprotein remnants such as LDL and VLDL (80). When exposed to cold the increased metabolic activity of BAT and upregulation of thermogenic genes in WAT of these animals leads to unfavourable changes in total cholesterol, LDLc, intermediate LDL and in particular VLDL in addition to enhanced atherosclerotic lesions and plaque instability (81). This, however, contrasts with the effects of BAT activation seen in APOE*3-Leiden.CETP mice. These mice have the human cholesterol ester transfer protein knocked-in and thus have a functional ApoE-Ldlr pathway and 'human-like' lipid metabolism (82). In these animals' administration of the β3-adrenergic receptor agonist CL316-243 to activate BAT markedly reduces plasma TG, total cholesterol and VLDL concomitant with increased hepatic clearance of lipoprotein remnants and attenuation of atherosclerosis (80). Whilst common murine models of atherosclerosis closely resemble human pathology their use for studies investigating BAT physiology is limited, in this case the APOE*3-Leiden.CETP mice have a greater translational value and suggest BAT may be a therapeutic target combating CAD in humans (83).

Relative to glucose metabolism there is less evidence for a role of BAT in human lipid metabolism primarily due to the routine use of FDG tracer uptake in PET-CT studies. Individuals with higher amounts of active BAT, however, exhibit lower fasting TG and higher HDL whilst the expression of LPL and the acetyl-CoA dehydrogenase family of enzymes in UCP-1 containing epicardial adipose tissue is correlated with both circulating TG and HDL (84, 85). Using the FFA tracer 18F-fluoro-thiaheptadecanoic acid (18FTHA) acute cold exposure (2 hours) increased both fractional uptake of 18FTHA and uptake of FFA in BAT compared to subcutaneous AT and muscle (86). In BAT positive subjects (based on 18F-FDG disposal) during fasting and acute cold, prolonged cold exposure (5-8h) increases FFA oxidation and contributes to ~70% of the increase in resting energy expenditure. This is likely due to induction of UCP1, together with type 2 deiodenase, β3-adrenoreceptor and PGC1α which have been shown in rodent studies to be essential for BAT thermogenesis (29). BAT activity also correlates with cold-induced lipolysis and whilst glucose uptake is reduced, cold-induced increases in FFA uptake are not impaired in individuals with type 2 diabetes or age matched controls (87). This suggests that cold exposure may be able to modulate lipid metabolism independent of ageing and insulin resistance. More recently BAT activation was shown to correlate with cold-induced changes in whole body FFA oxidation, lipolysis and TG-FFA cycling with BAT demonstrating a 45-fold higher respiration rate (e.g. 45 fold higher capacity for heat generation) compared to WAT (88). Interestingly, whilst cold did not affect plasma TG, cholesterol or lipoproteins reductions in TG and VLDL were seen the following day suggestive that lipid metabolism may be modified after the cessation of cold exposure. These systemic effects are likely to be mediated by cold-induced increases in the expression of genes involved in both thermogenesis and lipid metabolism.

Despite the suggested benefits of cold adaptation (89, 90), FFA uptake into BAT during acute cold exposure accounts for less than 1% of total FFA turnover whilst there is no change in circulating TG (86). This points to a minimal role for BAT in the acute regulation of lipid metabolism though increased hepatic VLDL-TAG secretion may confound studies over such a short time-frame (91). It

has long been speculated since the seminal work of Rothwell and Stock that BAT activation may play a role in diet-induced thermogenesis (92). Whilst there is data to suggest both diet and dietary factors play a role in a compensatory activation of thermogenic tissues, evidence for this in humans is sparse as human BAT studies using PET-CT are typically done in the fasted state (93). Importantly, glucose uptake is impaired following prolonged fasting suggestive that the general fasting state may not be appropriate for assessing the ability of BAT to regulate systemic metabolism (94), although uptake by BAT as measured using PET-CT is much lower than in skeletal muscle after feeding (95). There is now increasing evidence that BAT function is stimulated by feeding

Following 8 weeks of overfeeding in humans there is no increase in BAT thermal activity as measured by taking only two thermal images before feet immersion into cold water, and then two more two minutes later (96). Not surprisingly given the acute nature of this test there was no correlation between thermal activity and metabolic adaptation or body weight (96). It is important to note that instead of using a subscapular reference point, the reference region used includes the background area behind the torso which is cooler than skin temperature and so the results may not be indicative of BAT function. Furthermore, the use of 18FTHA pre and post cold acclimation demonstrates that despite a 2.6-fold increase in BAT oxidative capacity there is no increase in the uptake of dietary fatty acids in the post-prandial state (97). Furthermore, dietary fatty acid uptake by BAT was comparable to abdominal WAT and skeletal muscle and significantly lower than both the heart (~55%) and liver (~83%) accounting in total for only ~0.3% dietary fatty acid uptake. It is important to note here however that the post-prandial cold-exposure period of 240 minutes may not have been sufficient to deplete intracellular lipid in BAT. These stores must normally be depleted before replenishment from circulating lipids can take place with a longer 5-8 hour protocol speculated to be required (88). Methodological differences between the various studies make it difficult to reach a definite conclusion on the potential for BAT to modulate lipid metabolism and in particular this may

be negligible in short exposure periods. More studies with increased exposure time are needed to determine if the positive effects of BAT activation on lipid metabolism can be replicated (88).

Vascular brown adipocytes and atherosclerosis

Whilst the main focus in rodents has been on interscapular BAT and 'browning' in recent years there has also been an increased recognition of the physiological relevance of thermogenic adipocytes elsewhere, especially located around the heart (epicardial) and vasculature (perivascular)(98, 99). This is because their contiguity with the myocardium and major vessels respectively implies they might be capable of regulating cardiomyocyte and vascular function in a paracrine and/or endocrine fashion (100). Epicardial AT (EAT) originates from the splanchnic mesoderm and/or the epicardium (101, 102) and is primarily located between the myocardium and visceral pericardium where it sits along the myocardium, the atrio- and inter-ventricular grooves and is vascularised by the coronary arteries. EAT provides mechanical protection to the coronary vasculature during contraction (98, 103), modulates coronary vascular tone through the secretion of numerous bioactive peptides (e.g. nitric oxide (104) and angiotensinogen 1-7 (105)) and perhaps protects the myocardium from lipotoxicity through the uptake of intravascular FFA (106, 107). Importantly, EAT has also been proposed to protect the myocardium by acting as a source of heat during hypothermia (108) however to date there is no direct evidence to suggest that the UCP1 expressing cells in this depot are thermogenically active. Similar to EAT, perivascular AT (PVAT) is the adipose tissue located around the major vasculature with its main role being to maintain and regulate vascular tone/remodelling and endothelial function/dysfunction (109, 110). It is important to note that these tissues are characteristically similar to BAT at birth and also, to some degree throughout adulthood and that the transition from BAT to white-like AT may be due primarily to maternal and early life factors such as under/over-nutrition and diet composition (100, 111, 112).

With ageing and overnutrition adipocytes accumulate excess lipid, becoming hypertrophic and hypoxic and, after outstripping the vasculature, the hypoxic and inflammatory microenvironment drives the downregulation of mitochondrial function and adrenergic signalling with insulin resistance and atherogenesis ensuing (113-115). It has also been demonstrated in mice that the thermogenic properties of PVAT are essential to optimal cardiovascular health. The complete ablation of PVAT in PDGRFα k/o mice renders animals hypertriglyceridemic (116) suggestinga major role of PVAT in lipid homeostasis. Mice lacking PVAT are also unable to regulate intravascular temperature suggestive that the thermogenic properties of this depot are essential to maintenance of adequate core temperature (116). The role of PVAT in regulating lipid homeostasis is evident in humans where the association between UCP1 expression in human EAT and circulating HDL and triglycerides suggests that this depot plays a role in systemic lipid metabolism (85).

Given the role of dyslipidemia in atherosclerosis the ability for cardiac and vascular adipocytes to regulate intravascular lipid metabolism may be a clinically significant (117). Current evidence also suggests that the brown-to-white transition can modulate redox state (118) with this supported by human data demonstrating excess reactive oxygen species in CAD patients with reduced expression of thermogenic genes (119). Given that components of the mitochondrial electron transport chain in PVAT are critical regulators of vascular tone it would suggest that the adipocytes present in EAT and PVAT may modulate vascular and myocardial redox state (112) and that 'whitening' of these may drive disease partly through excess reactive oxygen species. In fact, BAT regulates vascular function through production of the anti-contractile factor hydrogen peroxide which is regulated by NADPH Oxidase 4 and alters vascular contractility through activation of protein-kinase G1α (120). Importantly, mechanisms regulating contractiliy differ in mesenteric PVAT (which resembles WAT) and browning this depot increases the anti-contractile effect through hydrogen peroxide dependant mechanisms in a manner similar to BAT. Thus, the anti-contractile effects of brown adipose tissue may be lost in the transition to WAT that occurs with ageing and obesity but may be restored in the

presence of browning. Whilst manipulating the phenotype of adipocytes local to the vasculature is clinically challenging they offer a novel target to alleviate obesity associated vascular disease and it is clear that cross-talk between these adipocytes, the myocardium and vascular wall are of great importance.

Conclusion and Outlook

It is clear that activating BAT is an attractive target for the prevention of metabolic and cardiovascular disease. To date there is clear evidence that BAT can improve glucose and insulin homeostasis through insulin-dependent and independent mechanisms (90). Furthermore, BAT activation may be a means of manipulating systemic lipid metabolism in humans but will require further work to validate the promising results shown in rodents (80, 88). Future work will no doubt further target the manipulation of thermogenic adipocytes by pharmacological and environmental means whilst the development of new tracers and imaging methods will greatly enhance our understanding of both BAT and 'beige' adipocyte biology.

Highlights

- **Rodent data provides clear evidence of a role for BAT in reducing adiposity.**
- **To date no human study has shown that activating BAT leads to reductions in adiposity.**
- **Active brown adipose tissue plays a key role in glucose and insulin homeostasis in humans.**
- **Activation of BAT may play a key role in human triglyceride metabolism but further studies are needed.**
- **Brown adipocytes surrounding the heart and vasculature may play a key role in attenuating atherosclerosis.**

Acknowledgements

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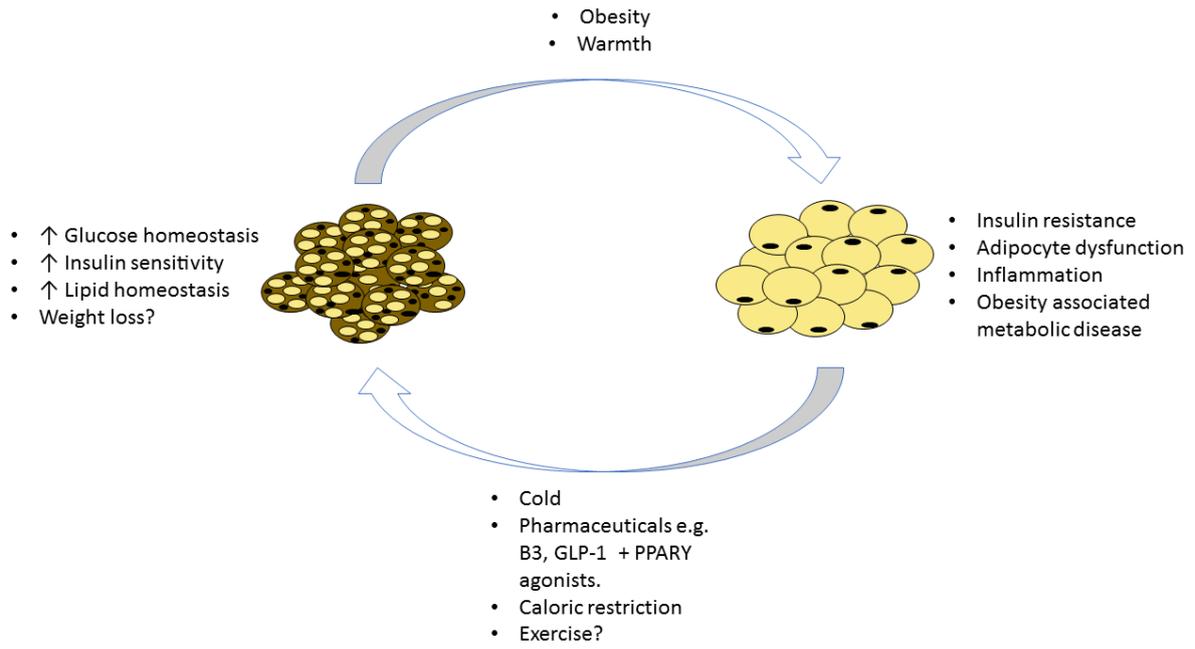
Figure 1. Overview of brown-to-white adipocyte transition, associated metabolic alterations and regulatory factors.
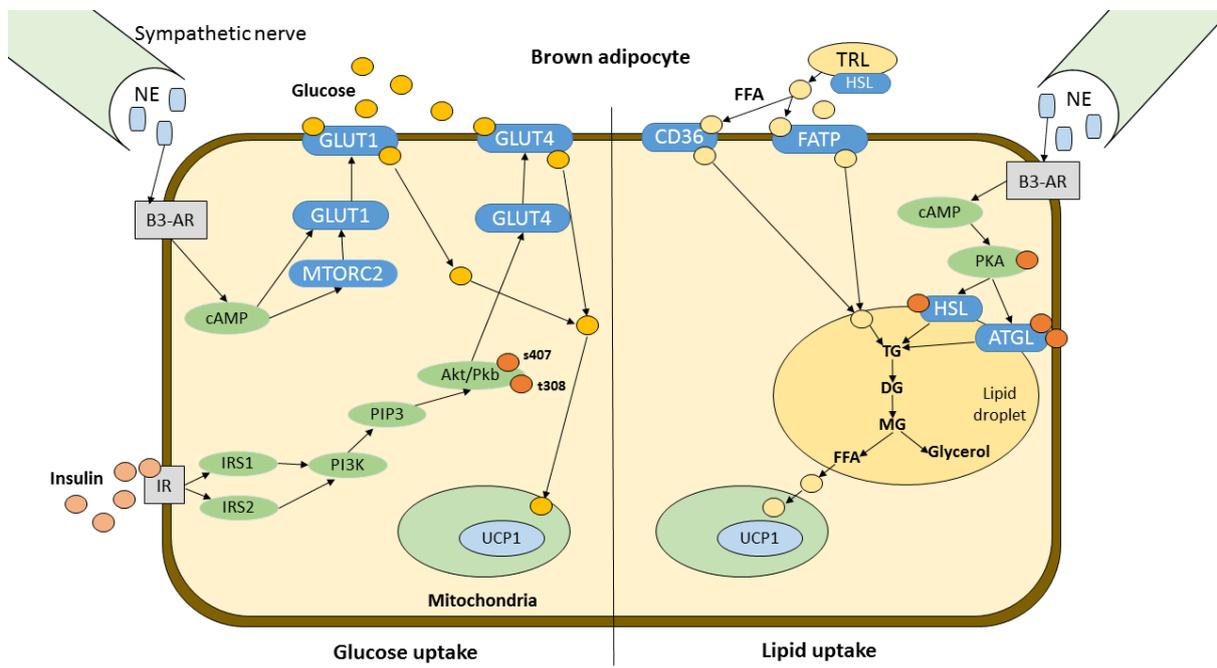
Figure 2. Overview of mechanisms regulating glucose and lipid metabolism in brown adipocytes. Discussed in detail in text.