

Vascular targeting of adipose tissue as an anti-obesity approach

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Development of obesity is characterized by hypertrophy and hyperplasia of adipocytes in white adipose tissue (WAT). This process relies on concomitant angiogenesis. Results from experimental inhibition or depletion of cells comprising the vasculature in animal models have contributed to the understanding of the mechanisms governing expansion of WAT. Disruption of neovascularization might be potentially useful for obesity prevention. In addition, approaches in which the mature WAT vasculature is disrupted have been sought with the aim of combating obesity after its onset. Other cell types in WAT, including adipose stromal cells, which support angiogenesis, could represent alternative targets for combinatorial WAT treatment. This review discusses recent advances in WAT vascular targeting and implications for the development of new anti-obesity therapeutics.

Obesity: a growing health problem

Obesity is a medical condition affecting all ages and socio-economic groups [1]. Over the past decade, profound changes in nutrition and lifestyle in developed countries have led to a sharp increase in the prevalence of obesity and its complications. Obesity is defined as a body mass index (BMI) of 30 kg/m² or more and is manifested by overgrowth of white adipose tissue (WAT). The normal physiological function of WAT is to assure continuous availability of energy despite highly variable energy supplies in the environment through storage of excess energy as neutral triglycerides in the differentiated cells of WAT (adipocytes), from which stored energy can be rapidly released for use at other sites [2]. WAT has recently been revealed as a potent endocrine organ that is responsible for secretion of numerous hormones, as well as growth and inflammatory factors that are implicated in various diseases including type 2 diabetes and cardiovascular disease. Obesity has also been linked with a number of cancers [3]. One of the key obesity complications is the metabolic syndrome, a constellation of disorders that includes systemic inflammation, insulin resistance and dyslipidemia [4].

Current approaches to the treatment of obesity

Increased physical activity and dieting are the most logical measures against obesity. Unfortunately, these are increasingly less practiced in the lifestyle of the modern world [1]. Gastric banding and bypass surgical interven-

tions are effective anti-obesity measures that reduce the risk of cardiovascular diseases and type 2 diabetes. However, these procedures are reserved for severe forms of obesity, are highly invasive and can result in serious surgical complications. There is, therefore, an urgent need for new approaches to prevent and treat obesity. Advances in pharmacological therapy of obesity have, until recently, been rather limited, with very few drugs available to control pathological WAT accumulation [5]. Anti-obesity drug research has been focused on altering energy balance pathways and by acting on receptors in the brain. An example is Sibutramine (Meridia[®]; Abbott), which acts on the central nervous system (CNS) by reducing energy intake and increasing energy expenditure, resulting in a 3–4% body weight loss over a 2-year period compared with dieting alone. However, drugs of this class have been withdrawn from the market due to side effects including tachycardia and hypertension. The drug remaining approved in the USA for the management of obesity in conjunction with a reduced-calorie or -fat diet is orlistat (Alli[®], Xenical[®]; Roche). This drug inhibits gastric and pancreatic lipases, and thus blocks the absorption of lipids in the gastrointestinal system. The side effects of this drug (fecal urgency or incontinence) and the reduced absorption of a number of vitamins have prevented it from becoming widely accepted. There are new emerging treatments that target processes controlled by gut hormones, such as glucagon-like peptide-1 and ghrelin [6]. However, the current reality is that even with combination dieting, none of the available drugs are satisfactory for long-term weight management [7].

Obesity is the result of WAT overgrowth

It has become apparent that adipose tissue is not a homogeneous organ. WAT develops throughout the mammalian body in areas of loose connective tissue, such as subcutaneous layers between muscle and dermis [8]. In addition, WAT depots also form around the gut, heart, kidneys and other internal organs. Accumulating evidence indicates that different depots of WAT in the body are regulated independently and have different implications in disease. Specifically, it is overgrowth of intra-abdominal (also called visceral) WAT, including intraperitoneal (e.g. omental and mesenteric), retroperitoneal and perigonadal WAT, that is associated with inflammation and the metabolic syndrome. Abdominal adiposity is believed to predominantly account for the poor prognosis of obese patients with cancer and cardiovascular disease.

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Obesity at the cellular level

Adipocytes in WAT can be remarkably variable in size, which reflects the amount of triglycerides stored in lipid droplets. Recent data indicate that expansion of WAT can result not only from adipocyte hypertrophy (increase in cell size), but also from hyperplasia (increase in cell number), which relies on progenitor cell proliferation [9]. Mild obesity mainly reflects adipocyte hypertrophy, whereas obesity arising in childhood or more severe obesity typically also involves hyperplasia. WAT development and expansion are controlled by concerted actions of a number of extracellular and intracellular signals that together form a highly integrated network designed to maintain energy balance [2]. This homeostatic system links the plethora of environmental, genetic and epigenetic stimuli that control energy balance and regulate preadipocyte differentiation into lipid-laden adipocytes, defined as adipogenesis (Figure 1). The major resources for the biochemical pathways leading to triacylglycerol (TAG) synthesis are sugars and fatty acids [10]. Uptake of glucose and of related hexoses into adipocytes through the cell membrane is assisted by integral membrane glucose carriers coded by genes of the GLUT/SLC2 (solute carrier) family [11]. Compared with regulation of glucose uptake into adipocytes by GLUT4, the molecular control of fatty acid uptake is not as well understood [12]. The program of adipogenesis relies on sequential activation of a cascade of transcription factors [13]. It begins with the transient expression of CCAAT/enhancer binding C/EBP proteins (α, β and δ), which in turn activates a member of the nuclear hormone peroxisome proliferator-activated receptor family, PPAR γ . This protein is induced very early in adipose cell differentiation and is present at higher levels in preadipocytes than other fibroblastic cells. It is primarily responsible for switching

on the broad program of adipogenesis. In addition, PPAR γ exerts positive feedback on C/EBP and acts synergistically to maintain the differentiated state. Sterol-regulatory element binding protein 1c (SREBP1c) responds to insulin and lipids and can activate PPAR γ by inducing its expression and by promoting production of an endogenous PPAR γ ligand. Together, regulation of these factors contributes to the expression of genes that characterize the terminally differentiated phenotype.

Adipocyte differentiation is integrated with lipogenesis in WAT and encompasses the processes of triglyceride synthesis from glucose and subsequent lipid droplet formation. SREBP1c activates the expression of numerous genes of the lipogenic program. This process relies on the activity of fatty acid synthase (FAS) and mitochondrial glycerol-3-phosphate acyltransferase enzymes [14]. PPAR γ and the cooperating factors orchestrate lipogenesis by cooperatively activating the transcription of a cascade of genes coding for proteins selectively active in adipocytes [15]. These include adipocyte fatty acid binding protein aP2, ACC1, FAS, adiponin, pyruvate carboxylase, phosphoenolpyruvate carboxykinase, GLUT4, leptin and other regulators of energy balance. Neutral lipids are packaged into cytosolic lipid droplets, which provide major energy storage for the body. Lipid accumulation in terminally differentiated adipocytes relies on a number of proteins that control lipid droplet formation and TAG utilization, with perilipin the most studied of these [16,17]. Throughout adipocyte differentiation, perilipin family members take turns in coating lipid droplets and subsequently regulate not only lipogenesis, but also TAG lipolysis and the release of glycerol, which is then transported to the liver for metabolism via either gluconeogenesis or glycolysis. This process is deregulated in obesity, which leads to

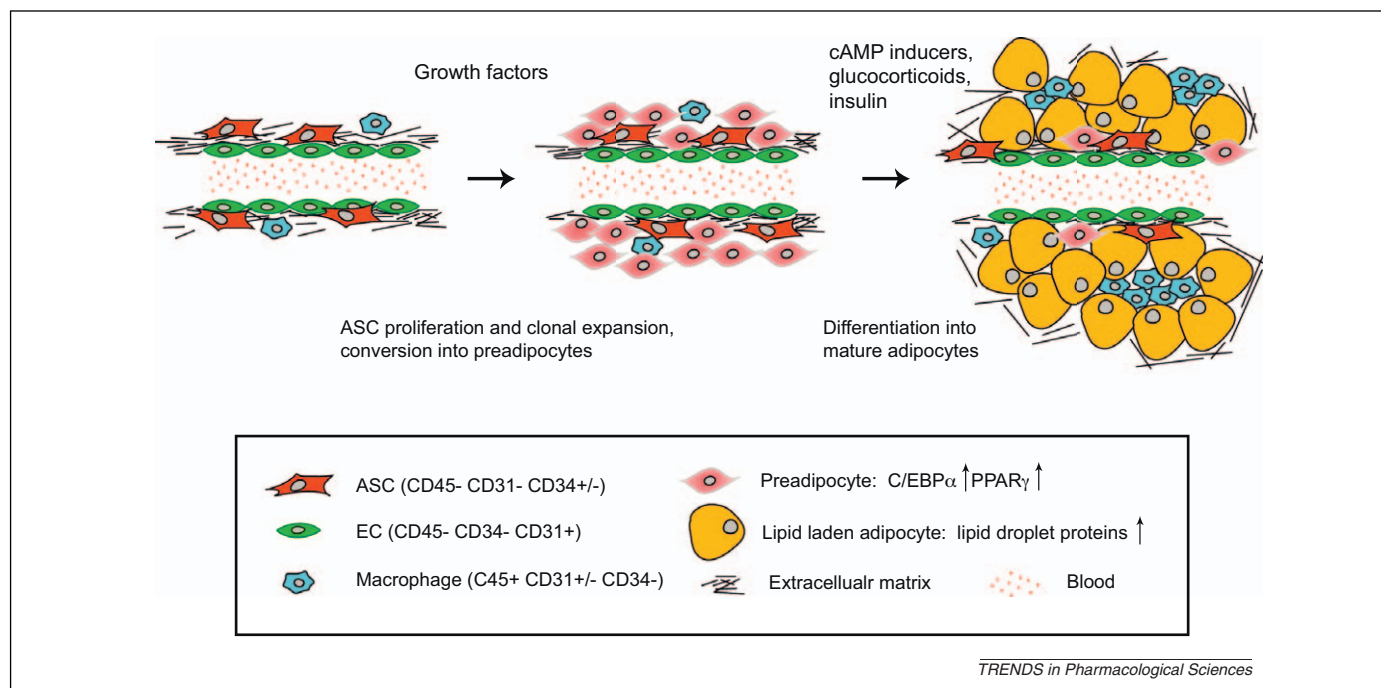


Figure 1. Key cellular and molecular aspects of WAT growth. Growth of WAT involves proliferation and differentiation of pericytic adipose stromal/stem cells (ASCs) into preadipocytes and finally into lipid-laden adipocytes. Adipogenesis relies on growth and differentiation factor stimuli that activate a cascade of transcriptional machinery, including C/EBP α and PPAR γ , which results in lipogenesis. This program is coordinated with angiogenesis and migration or proliferation of other cell types comprising WAT, including endothelial cells (ECs) and macrophages, which can be identified based on their distinct sets of surface markers.

aberrant lipid accumulation in other organs, such as the liver and muscle, and results in lipotoxicity [18]. In addition to their role in energy storage, adipocytes, like other cells, accumulate cholesterol [19]. Cholesteryl ester-rich droplets provide building materials for local membrane synthesis and repair, as well as a source of substrate for steroid hormone synthesis. Although lipogenesis is very active in rodent WAT, its activity is generally lower in human WAT. Nevertheless, pharmacological approaches for manipulation of adipogenesis and lipogenesis are at the forefront of the search for drugs to reduce the burden of obesity and diabetes [20].

Although it is overgrowth of adipocytes that is manifested as obesity, WAT is a complex organ comprising cell types, interactions among which orchestrate obesity onset [21]. As shown in Figure 1, these cell types include undifferentiated and partially differentiated (preadipocytes) stromal cells, vascular endothelial cells (ECs), pericytes (mural cells) and infiltrating blood cells, including monocytes/macrophages, lymphocytes and mast cells [8,22,23]. Current research implicates invading macrophages as the cell population mainly responsible for low-grade inflammation and metabolic alterations associated with obesity [24]. The stromal/vascular fraction (SVF) of WAT has been identified as a rich source of progenitor cells [25]. Adipose stromal cells (ASCs) comprise the majority of cells in the SVF and display multipotency and proliferation capacity comparable to those reported for bone-marrow-derived mesenchymal stromal cells (MSCs). The ability of MSCs to differentiate into cells of mesenchymal lineage, such as adipocytes, osteocytes and chondrocytes, has resulted in the term mesenchymal stem cells. Although there are reports suggesting that adipocytes can be derived from hematopoietic stem cells [26], there is predominant evidence that bone marrow-derived precursors contribute only to ECs and infiltrating hematopoietic cells, and not to MSCs and adipocytes [27,28]. The transition from adipose progenitors to preadipocytes is still poorly defined, and our understanding of intercellular interactions within WAT is still limited despite recent advances [29,30]. It has been shown that a subpopulation of ASCs, in addition to serving as adipocyte progenitor cells, overlaps with the population of pericytes and cooperates with the endothelium during vascularization [31,32]. ASCs promote EC proliferation and blood vessel formation, at least in part, via trophic effects of secreted vascular endothelial growth factor (VEGF) and other angiogenic molecules [29,31,33]. The molecular mechanisms of interactions between adipose ECs, ASCs and adipocytes that dictate the commitment of progenitor cells to differentiation or mobilization are being investigated [25,31,34]. Adipose progenitors currently occupy the central arena of stem cell biology and regenerative medicine [25,30]. We recently proposed that WAT might contribute to the pool of progenitor cells mobilized in cancer [35]. We have used several mouse tumor graft models in combination with adipose tissue transplantation to demonstrate that cells of WAT are recruited by tumors and that migrating ASCs can promote cancer progression by secreting pro-vasculogenic factors [36]. This previously overlooked phenomenon might partly account for the positive association between obesity and cancer.

Like ASCs, adipose ECs can migrate to tumors [36] and are maintained as a WAT-resident population with only a minor contribution by bone marrow-derived endothelial progenitors [37].

WAT vasculature and angiogenesis: the role in obesity

New blood vessel growth is associated with and is essential for the development of every organ. In adulthood, new blood vessels arise as a result of sprouting of the adjacent resident vasculature (angiogenesis) [38]. Solid cancers, which also require neovascularization, are an example of a setting in which angiogenesis occurs in adulthood in response to factors released by a hypoxic and inflammatory tumor microenvironment. Angiogenesis is associated with extracellular matrix (ECM) remodeling, a process that requires proteolytic activity provided mainly by the fibrinolytic (plasminogen-plasmin) cascade and matrix metalloproteinases [39].

It has recently been appreciated that the vasculature is critical for WAT development as the gatekeeper of blood access to adipocytes [2,21,40]. Because WAT growth underlying obesity proceeds into adulthood, it has been proposed that adipogenesis and angiogenesis feed into each other in obesity [41,42]. ECM remodeling and VEGF [33], the growth factor essential for vessel formation through either angiogenesis or vasculogenesis (progenitor cell recruitment from remote organs), play a critical role in WAT vascularization [43,44]. Recent identification of a close connection between VEGF-B and endothelial fatty acid uptake [45] suggests direct dependence of adipogenesis on angiogenesis. Studies by our research team have contributed to an understanding of how blood vessel formation might be coordinated with ASC differentiation and migration by interaction between the matricellular protein SPARC (secreted protein, acidic and rich in cysteine, also known as osteonectin) and β 1 integrin [34]. This interaction is consistent with the known functions of these proteins in cancer progression. In addition to controlling ASC physiology, SPARC is also likely to decrease WAT deposition through its direct negative effects on angiogenesis [46].

Origins of WAT vascular cells

Although it is clear that WAT expansion relies on blood vessel formation (neovascularization), the origin of vascular cells is unclear. In the past few years, research has shown that recruitment of circulating vasculogenic progenitors into blood vessels plays an important role in neovascularization [47]. Studies in cancer models have demonstrated that endothelial progenitor cells (EPCs) and stromal progenitor cells derived from bone marrow contribute to neovascularization [48]. Transplantation studies have shown that bone marrow-derived cells also infiltrate WAT vasculature [27,28]. However, it has recently been shown that organs other than bone marrow contain resident progenitor cells (including both EPCs and MSCs) [49,50]. WAT is a striking example of an MSC-rich organ and is also a site of ectopic hematopoiesis [51]. Therefore, vascular cells of WAT could be derived from distinct origins with individual subpopulations that have differential profiles of marker expression and different responses to treatment.

Controlling obesity by targeting the WAT vasculature

Because blood supplies oxygen, nutrients, growth factors and progenitor cells, inhibition of neovascularization has been identified as a powerful approach for control of tissue expansion. Tumors are an illustration of the notion that angiogenesis inhibition can serve as an effective complementary tool to constrain tissue expansion. Over the past decade, vasculature targeting as a cancer therapy has evolved resulting in clinically-approved drugs that have considerably improved the clinical outcome [52]. WAT angiogenesis is regulated through the same mechanisms as tumor neovascularization, such as VEGF-dependent pathways [42,53]. Therefore, like tumors, WAT expansion might be controlled through the vasculature; this has led to new experimental approaches in obesity treatment.

Anti-angiogenesis drugs inhibit WAT expansion

Although WAT is a non-malignant tissue, cells comprising its vasculature have the capacity to quickly proliferate [22,23,54]. The Folkman research team has demonstrated that inhibition of angiogenesis through ECM and proteolytic system disruption has the potential to impair WAT development. Specifically, treatment with angiostatin (kringle 1–4 domains of plasminogen), endostatin (a C-terminal fragment of collagen XVIII), TNP-470, Bay-129566 (a matrix metalloproteinase inhibitor) and thalidomide has demonstrated that inhibition of angiogenesis is potent enough to impede WAT expansion, even in genetically obese leptin-deficient mice [41]. The apelin-APJ signaling system [55] and angiopoietins and their receptors, such as Tie1 and Tie2, are among other promising targets that could result in prospective therapies [56]. A number of recent reports confirm the initial observation that disruption of WAT neovascularization can prevent the onset of obesity in both genetic and diet-induced obesity models [57]. For example, curcumin, the major polyphenol in turmeric spice, suppresses WAT accumulation through its effects on angiogenesis and adipogenesis [58]. Similar results have been obtained with other herbal and synthetic anti-angiogenic compounds, such as Ob-x and fumagillin [59,60]. However, the extent to which the direct effect of these agents on adipocytes (as opposed to their effect on the vasculature) affects accumulation of adipose tissue is uncertain.

Targeting the established WAT vasculature

It is possible that the mature WAT vasculature (on obesity development) could also be amenable to targeted treatment. If so, depletion of the supply of nutrients and oxygen essential for adipocyte maintenance could result in obesity reversal after its onset. Based on the notion that tumor vasculature features differential expression of markers [61], we proposed the existence of cell-surface molecules selectively upregulated in adult WAT blood vessels that could be therapeutically targeted in pathologically expanded WAT. In a proof-of-principle study to identify and use WAT vascular targets for experimental obesity therapy [62], we screened a combinatorial library in mice for peptides systemically homing to WAT. As a result, we isolated a peptide with the sequence CKGGRAKDC that selectively accumulates in WAT. The uptake of CKGGRAKDC by adipose ECs suggested that the internalizing receptor could serve as a target of therapies

directed to WAT. By using WAT membrane protein extracts, we biochemically isolated the vascular receptor of the CKGGRAKDC peptide and identified it as prohibitin. We used a mouse model of diet-induced obesity to test *in vivo* the capacity of the CKGGRAKDC peptide to deliver the cytotoxic (proapoptotic) peptide KLAKLAKKLAKLAK to WAT. Daily subcutaneous injections of the CKGGRAKDC–KLAKLAKKLAKLAK fusion peptide caused rapid obesity reversal [62]. A recent report validated the specificity of the CKGGRAKDC peptide for prohibitin selectively expressed in WAT-derived cultured ECs using nanocarriers [63]. Another study reproduced these results in a rat model [64] and suggested that reduced food consumption in WAT-targeted animals explains the lack of apparent lipo-dystrophic effects. These experiments reveal previously unappreciated crosstalk between the status of the WAT vasculature and central regulation of food intake [64]. Translational studies based on this experimental approach to treat obesity are ongoing.

Despite the short-term effectiveness in experimental animal models, obesity relapses owing to WAT regrowth on cessation of treatment with EC-targeting agents [41,62]. Although proliferating ECs serve as building blocks for growing vessels, this process relies on a trophic contribution and mechanical support from stromal cells [31]. Indeed, whereas ECs and adipocytes undergo cell death under ischemic conditions, ASCs are resistant to hypoxia [65]. Therefore, resistance to vascular-targeting agents seems to result from the pro-angiogenic action of surviving ASCs that quickly re-wire the tissue. In the future, ASC targeting in parallel with EC targeting in WAT might provide an approach to long-term control of WAT mass (Figure 2).

Targeting adipocyte differentiation

Because white adipocytes are the major unit defining WAT, approaches in which preadipocyte differentiation into adipocytes is inhibited have been the focus of numerous groups [20]. The PPAR γ pathway has been identified

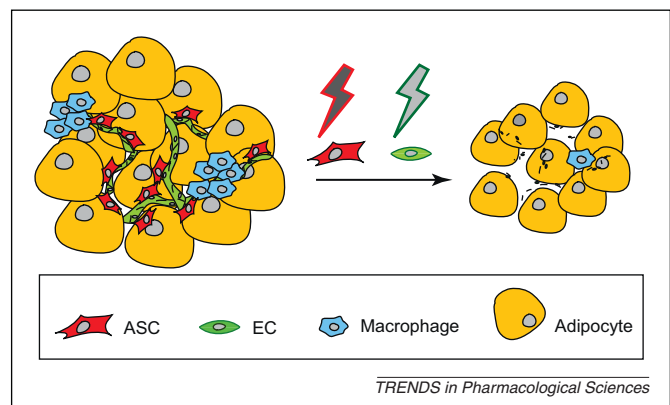


Figure 2. Vascular targeting of WAT as an approach to controlling WAT mass. Targeted depletion of adipose ECs, which are the integral component of the vasculature delivering nutrients and oxygen to adipocytes, results in rapid WAT resorption in rodent models. Because the WAT mass reduction achieved is transient and obesity relapses on discontinuation of treatment, non-endothelial components of WAT might present a complementary target. Depletion of ASCs, the pro-angiogenic adipocyte progenitors, in parallel with ECs is proposed as a potential approach for prevention of WAT regrowth on discontinuation of EC-targeting therapy.

as a clinically effective target. Drugs of the thiazolidinedione (TZD) class that activate the PPAR γ pathway, such as rosiglitazone, can be used for adipogenesis modulation in addition to insulin sensitization. However, its effects have remained somewhat paradoxical. Treatment of obese mice with TZD leads to an unexpected decrease in adipocyte size and normalized insulin action [66], whereas in insulin-resistant patients, TZD treatment can conversely cause enlargement of adipocytes [67]. Identification of other druggable targets that regulate adipogenesis, as well as adipolysis of mature adipocytes, might lead to more effective approaches to regulate the size of individual adipocytes, and thus expansion of WAT and the accompanying inflammation.

As opposed to WAT (which stores excess energy), brown adipose tissue (BAT) is responsible for energy dissipation in the form of heat [68]. In humans, BAT is clearly present and functional in newborns, whereas it was thought that adults lack BAT. A number of groups recently reported the presence of functional BAT in adults [69]. The fine balance between WAT and BAT is an important issue to take into consideration in designing vasculature-targeting therapies. A number of results from rodent models indicate that BAT has a protective effect against the pathological consequences of obesity. The significance of discovering BAT in adults [69] lies in possible new approaches to treatment of obesity and associated disorders [30]. The ontogeny of brown adipocytes [70] at least partly accounts for the metabolic benefits of BAT. It has been demonstrated that brown adipocytes located within residual WAT are derived from Myf5-negative progenitors, which indicates that ASCs are an alternative origin of BAT (as opposed to adipocytes arising from Myf5-positive cells in developing muscle). Indeed, ASCs can be differentiated into brown adipocytes with PPAR γ agonists [71]. More importantly, white adipocytes can be directly converted to a BAT-like phenotype both in culture and *in vivo* [72]. This conversion is driven by sympathetic nervous system stimuli, such as cold temperature, and signal transduction cascades triggered by activation of β 3-adrenergic receptors in WAT. In mice, expansion of residual BAT 'patches' within WAT can lead to virtually all adipose depots becoming BAT-like at the expense of WAT. The apparent dependence of adipocyte physiology on the status of the vasculature suggests that vascular-targeting agents could be designed to convert WAT into BAT, rather than destroying tissue altogether, as a more physiological and safer anti-obesity treatment. The development of pharmacological approaches to activate proliferation, vascularization and/or metabolism of existing residual BAT could, in theory, tilt the WAT–BAT balance and could be used to treat obesity (Figure 3).

Unresolved issues in WAT vascular targeting

Types of WAT: possible depot-specific issues

Consistent with depot-specific roles of WAT in pathology, the content and properties of cells comprising WAT differ between visceral and subcutaneous WAT depots [4]. Visceral WAT is susceptible to inflammation in severely obese individuals as a result of insufficient oxygenation of grossly enlarged adipocytes, which is the underlying cause of

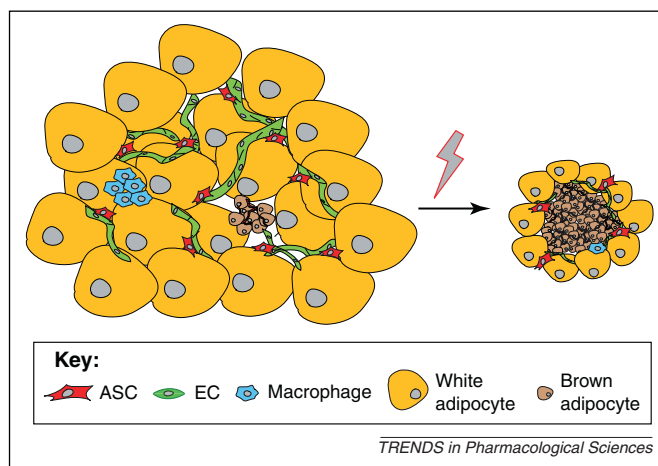


Figure 3. Prospective therapies converting WAT to BAT. ASCs can differentiate into white or brown adipocytes. The development of agents that selectively target ECs of WAT but not of residual BAT could make it possible to ablate WAT and spare beneficial BAT. Alternatively, the development of agents that selectively target ECs of BAT could make it possible to induce expansion of brown adipocytes at the expense of white adipocytes. Both of these compartment-specific targeting strategies for adipose tissue could lead to new obesity treatment approaches. ASCs and adipocytes could also represent a direct target for this approach.

lipotoxicity and the associated pathological consequences [73]. By contrast, subcutaneous WAT, which typically remains sufficiently vascularized, has the potential to benefit metabolism by improving glucose homeostasis and increasing energy consumption [4]. A recent comparison of angiogenesis in human visceral and subcutaneous WAT did not detect significant differences [74]. However, separate assessment of the physiological consequences of targeting the vasculature in distinct WAT depots might still be important. Interestingly, visceral adipose progenitors seem to have a comparatively high self-renewal capacity, coincident with abdominal WAT remaining in old age when subcutaneous depots are depleted [75]. Such an apparent distinction between 'good' subcutaneous WAT and 'bad' intraperitoneal WAT could correspond to currently unknown differences in the physiology and markers expressed in the endothelia of these depots. Although the prohibitin-targeting peptide does not seem to discriminate between WAT depots [62], future studies might reveal agents useful for selective targeting of vasculature in a WAT depot of interest.

Targeting lymphatic vasculature for obesity treatment?

Another important clinical observation worth considering is the connection between lymphatic vasculature abnormalities and obesity. Lymphedema is associated with obesity, which could be related to the fact that WAT surrounds collecting lymphatic vessels and lymph nodes [53]. Interestingly, the bulk of WAT depots seem to lack lymphatic capillaries; however, no careful investigation has been performed to reach an unequivocal conclusion. It is possible that the lymphatic vasculature does infiltrate WAT but is modified in a way that makes it seem different from that in the rest of the body. Obesity resulting from lymphatic malfunction in mice [76] further suggests that WAT lymphatic vasculature could represent a target for anti-obesity intervention potentially superior to that of blood vessels.

Box 1. Unanswered questions in obesity treatment through tissue targeting.

- Would it be possible to target WAT and spare BAT?
- Are there vascular markers that will help to discriminate between visceral and subcutaneous WAT?
- What are the effects of vascular cell depletion on homeostasis?
- Could the efficacy of treatment be improved by targeting non-endothelial cells in WAT, such as ASCs?
- Could blood cells that infiltrate WAT be targeted for therapeutic purposes?

Potential drawbacks or limitations to WAT targeting as an obesity therapy

Although animal treatment with anti-angiogenic agents has consistently resulted in inhibition of WAT accumulation, there is much preclinical work to be done before this approach can be considered clinically feasible. Although generally mild, side effects of angiogenesis inhibition have been reported in clinical trials [77]. For example, VEGF inhibition can result in off-site proteinuria, hypertension and internal bleeding [78]. Moreover, studies in mouse models have shown that chronic blockade of certain endothelial pathways can backfire, with pathological activation of ECs, perturbed organ physiology and even vascular tumorigenesis, which emphasizes the need for safety concerns [79]. The side effects of anti-angiogenesis treatments relate to the fact that neovascularization does occur, albeit at levels much lower than in development, outside pathological tissues in adulthood [39]. New blood vessel formation generally accompanies tissue remodeling that takes place either on injury or during certain normal physiological processes. Because angiogenesis accompanies BAT formation [80], inhibition of neovascularization in a non-specific manner is expected to have a negative impact on BAT and therefore on energy expenditure, which defeats the purpose of the treatment. Thus, the development of approaches in which neovascularization is targeted to WAT might be critical.

An equally important point is that tissue targeting as a therapeutic approach in obesity must take into account the biological role of WAT and the well-described pathologies associated with its deficiency [81,82]. The primary role of WAT is to store excess ingested calories in the form of lipid droplets that can be mobilized for use during fasting. WAT lipid storage defects can lead to increased circulating fatty acids that are ectopically deposited in muscle, liver and other tissues, which leads to insulin resistance and metabolic disease, as observed in genetic or pharmacologically induced lipodystrophies. Therefore, any therapy aimed at reducing adipose tissue size could result in lipodystrophy in the absence of decreased caloric intake, which also applies to anti-angiogenesis approaches. The lack of apparent steatosis in observed in animals with ablated WAT vasculature is likely to be attributable to the small but detectable decrease in food consumption [62]. The extent to which the anorexic effects of other experimental compounds targeted at the vasculature are responsible for weight loss is not easy to assess. However, it is certain that anti-angiogenesis drugs, if they are to be used for amelioration of obesity without pathological consequences, must be accompanied by a concomitant reduction in food intake.

Concluding remarks

Careful analysis of preclinical models will be necessary to establish whether vascular targeting indeed represents a viable approach for the treatment of obesity. The field of vascular targeting and its application to obesity treatment are evolving, so a number of questions remain to be answered. Despite many unknowns (Box 1), treatment of obesity using anti-vascular agents holds great promise. Systemic deregulation of angiogenesis is a hallmark of obesity-associated pathologies including cardiovascular disease, diabetes and cancer. Therefore, it is possible that targeting of the neovasculature not only in WAT, but also at other sites of ectopic angiogenesis might have a combined therapeutic benefit. However, recent findings indicate that systemic inhibition of angiogenesis could be dangerous. There might be a great advantage to minimizing the effects of anti-obesity treatment outside WAT and affecting CNS and gut physiology at the lowest level possible. Therefore, a goal of the future is to develop WAT vasculature specificity for emerging new obesity therapeutics.

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