Adipose tissue angiogenesis in obesity

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Summary
Adipose tissue is the most plastic tissue in all multicellular organisms, being constantly remodelled along with weight gain and weight loss. Expansion of adipose tissue must be accompanied by that of its vascularisation, through processes of angiogenesis, whereas weight loss is associated with the regression of blood vessels. Adipose tissue is thus among the tissues that have the highest angiogenic capacities. These changes of the vascular bed occur through close interactions of adipocytes with blood vessels, and involve several angiogenic factors. This review presents studies that are the basis of our understanding of the regulation of adipose tissue angiogenesis. The growth factors that are involved in the processes of angiogenesis and vascular regression are discussed with a focus on their potential modulation for the treatment of obesity. The hypothesis that inflammation of adipose tissue and insulin resistance could be related to altered angiogenesis in adipose tissue is presented, as well as the beneficial or deleterious effect of inhibition of adipose tissue angiogenesis on metabolic diseases.

Keywords
Angiogenesis and inhibitors, obesity, metabolic disorders

Introduction
Adipose tissue, which represents about 20% of the body weight in a normal adult, is the most plastic tissue in all multicellular beings. Plasticity of adipose tissue, i.e. ability to rapidly expand, is crucial for life, as evidenced, for example, by hibernation in mammals, that requires large fat stores. In humans and some domestic animals, obesity is the consequence of overexpansion of adipose tissue and is associated with multiple comorbidities and reduced life expectancy (1). Individuals who repetitively diet and relapse can gain and lose dozens of kilograms over short periods of time and change their fat stores by more than 50%. Expansion of adipose tissue is necessarily accompanied by that of its vascularisation, through processes of angiogenesis, whereas weight loss is associated with the regression of blood vessels. These changes of the vascular bed occur through close interactions of adipocytes with blood vessels, and adipose tissue is thus among the tissues that have the highest angiogenic capacities (Figure 1). Mechanisms of vessel regression have been less well studied, but alteration of the interaction between adipocytes and their blood vessels is an obvious target for the treatment of obesity. Data obtained in animal models of obesity have raised hopes for the treatment of obesity by modulating angiogenesis and antiangiogenesis, i.e. vessel regression.

Epidemiology
According to a recent report by the World Health Organization (WHO), in 2008 more than 1.4 billion adults were overweight (body mass index [BMI] ≥25 kg/m²). Of these over 200 million men and nearly 300 million women were obese (BMI ≥30 kg/m²). Overweight and obesity are the fifth leading risk for global deaths. At least 2.8 million adults die each year as a result of being overweight or obese. Raised BMI is a major risk factor for non-communicable diseases such as cardiovascular diseases which are the leading cause of death but also increase the risk of diabetes; musculoskeletal disorders; and several cancers (endometrial, breast, and colon). Thus 44% of the diabetes burden, 23% of the ischaemic heart disease burden and between 7% and 41% of certain cancer burden (colorectal, breast and prostate cancer) are attributable to overweight and obesity (2).

Structure and function of adipose tissue
Adipose tissue is primarily a site of storage of triacylglycerol, but also secretes hormones, angiogenic factors, growth factors, cytokines, etc. Expression levels of several adipokines including leptin and adiponectin are either positively or negatively correlated with the adipose mass. One of the main function of adipokines is to signal the replenishment status of adipose tissue to the brain and other organs, but they may also participate in the angiogenic process (3-5). Adipose tissue consists of a heterogeneous mix of ma-
ture adipocytes surrounded by a stromal-vascular cell fraction containing preadipocytes, endothelial cells, pericytes, fibroblasts, macrophages, progenitor and mesenchymal stem cells.

Different kind of adipose tissue depots can be distinguished not only by their localisation (subcutaneous, intra-abdominal or peri-visceral, around heart, kidney, muscles etc.), but also by their architecture (white/brown adipose tissues), their metabolic activity and their profile of secretion of adipokines.

Adipose tissue angiogenesis and its regulation

Adipose tissue is probably the most highly vascularised tissue in the body, and each adipocyte is surrounded by an extensive capillary network. The adipose vasculature provide nutrients, oxygen, growth factors, hormones and cytokines to adipose tissue, and circulating progenitor cells that are able to differentiate into pre-adipocytes and vascular endothelial cells (6). The vessels also support infiltration of numbers of inflammatory cells (7) and remove waste products. Besides, activated endothelial cells produce various growth factors and cytokines, and fenestrated vessels play an essential part in local or systemic effects of adipokines (3).

The vascular network plays a key role in adipogenesis. During embryonic development, both arteriolar differentiation and differentiation of blood vessel extra-cellular matrix (ECM) precede differentiation of adipocytes (8). However, during later development of adipose tissue, throughout the whole life, it seems that adipocytes themselves drive the development and maintenance of their blood vessels. 3T3-F442A pre-adipocytes injected into severe combined immunodeficient (SCID) mice induce angiogenesis when they differentiate into adipocytes (9). The potential of adipose tissue to promote angiogenesis has been used therapeutically for decades (10, 11), and, indeed adipose-derived stem cells are investigated as potential inducers of angiogenesis in different contexts.

To adapt to changes in the size and metabolic rate of adipose depots, adipose vasculature requires constant regulation by several angiogenic modulators. Adipocytes seem regulate angiogenesis both by cell to cell contact and by adipokine secretion (12). Conditioned media obtained from preadipocytes and tissue homogenates from omentum or subcutaneous fat induce angiogenesis in the chick chorioallantoic membrane and in the mouse cornea (13-16). Both white and brown adipose tissue produce several pro-angiogenic growth factors, including vascular endothelial growth factor A (VEGF-A), acid- and basic fibroblast growth factor (FGF), leptin, etc. (3) as well as antiangiogenic factors, including thrombospondin-1 (TSP-1), or other angiogenic modulators such as plasminogen activator inhibitor or adiponectin, whose expression ratio will determine the angiogenic phenotype in the adipose tissue (17) (Figure 2). During differentiation of 3T3-F442A pre-adipocytes into mature adipocytes, proangiogenic factors are upregulated, whereas TSP-1 and TSP-2 are transiently downregulated (18). In addition to adipocytes, other cell types contribute to angiogenesis modulation, including resident macrophages, other inflammatory cells and stromal cells (19).

Vascular endothelial growth factor/ VEGF-receptor system

The vascular endothelial growth factor/ VEGF-receptor system accounts for most of the angiogenic activity in adipose tissue (17) and is expressed by both stromavascular fraction and mature adipocytes. Among all adipose tissues, omentum expresses the highest level of VEGF (20). Several studies indicate that VEGF-A is critical for physiological and pathological blood vessel formation. VEGF-A acts by signalling via a tyrosine kinase receptor (VEGF-R) (20). The VEGF system is complex and involves several receptors that are activated by several members of the family (Figure 3). With regards to VEGF-A itself, several isoforms are generated by alternate splicing and have different abilities to promote angiogenesis. VEGF-A binds both VEGF-R2 and VEGF-R1. However, only blockade of VEGF-R2, not VEGF-R1 restricts adipose tissue expansion and limit diet-induced fat tissue expansion (21). Other members of the VEGF family may play a role in adipose tissue angiogenesis: VEGF-B is implicated ECM degradation via regulation of plasminogen activation (22), VEGF-C and VEGF-D promote lymphangiogenesis (23, 24). Finally, placental growth factor (PIGF) enhances angiogenesis only in pathological conditions (ischaemic retina, -limb, -heart) (25), but inactivation of PIGF in mice leads to impaired adipose tissue development due to defective angiogenesis (26).

Other pro-angiogenic factors

Adipose tissue produces several other pro-angiogenic factors. Hepatocyte growth factor (HGF) and basic-FGF (FGF2) promote vascular endothelial cell growth (27, 28) in adipose tissue.
Leptin, one of the main factors that regulate fat stores by acting on the arcuate nucleus, might also directly induce angiogenesis upon binding to its receptor on endothelial cells. Leptin acts also as an indirect angiogenic factor by modulating VEGF, TSP-1 and angiopoietin-2 (29). Leptin regulates VEGF expression via the Jak/Stat 3 pathway suggesting that it might contribute positively to the development of adipose tissue (4, 30) and upregulate TSP-1 expression in vascular smooth muscle cells via JAK2- and MAPK-dependent pathways, thereby acting on vessel stabilization (31).

Neuropeptide Y (NPY) is another peptide acting both centrally and in the periphery as endocrine and paracrine factor to control adipogenesis and obesity. NPY stimulates angiogenesis in vitro and in vivo via activation of the Y2 receptor in vascular endothelial cells, and deletion of the Y2 receptor in mice leads to delayed wound healing (30, 32).

Resistin is another adipose tissue-produced peptide that links obesity to diabetes. Resistin has been reported to promote VSMC proliferation, to stimulate in vitro angiogenesis, both by direct effects and through increased expression of VEGF receptors –R1 and –R2 (33).

Hypoxia-inducible factor 1α (HIF-1α) is a master mediator of hypoxia signal, which is a primary physiological trigger for angiogenesis in both physiological and pathological conditions. HIF-1α has been shown to be increased in the adipose tissue of obese patients and its expression was reduced after surgery-induced weight loss (34).

**Angiogenesis modulators**

The members of angiopoietin family are important functional partners of VEGF. This system involves angiopoietin-1 and -2 (Ang-1 and Ang-2) that are expressed in adipose tissue and bind the 'Tyrosine kinase with Ig and Epidermal growth factor homology domains' (Tie: Tie1 and Tie2) receptor. Functions of the Angiopoietin-Tie system are complex. The Tie1 receptor is inactive, at least with regards to angiogenesis, whereas Ang-1 and Ang-2, both by activation of Tie2 seem to have apparently opposing properties (35): Ang-1 is involved in vessel wall remodelling, maturation and stabilisation, whereas Ang-2 facilitates vessel wall destabilisation, and is involved in the early phases of angiogenesis but might lead to vascular regression in the absence of VEGF-A. The
role of angiopoietins in adipose tissue associated angiogenesis has not been clearly established (36, 37).

Transforming growth factor (TGF)-β is involved in inflammation but also in angiogenesis. TGF-β is increased in adipose tissue of obese mice, and both adipocytes and stromal cells express this factor (38). TGF-β could positively and negatively regulate angiogenesis depending on the concentration and receptor types in endothelial cells (39).

The situation is even more complex for adiponectin, a factor linked to obesity and metabolic syndrome, which has been found to mediate opposite outcomes, both proangiogenic, through activation of adenosine monophosphate kinase in endothelial cells (30) and anti-angiogenic effects (5, 40). It has been shown that adiponectin inhibits endothelial cell proliferation, migration, and survival via activation of the caspase-triggered endothelial cell apoptosis (40). Circulating levels of adiponectin are inversely correlated with BMI and are significantly decreased in obese subjects, but deletion or overexpression of adiponectin in mice does not seem to affect body weight (41).

Adipose tissue also produces several endogenous factors that restrict vessel growth including thrombospondin-1 and plasminogen activator inhibitor (PAI-1). Cold exposure angiogenesis is associated with a down regulation of TSP-1 expression (17) in adipose tissue, but mice deficient in TSP-1 are viable with no severe vasculature-related abnormalities (42). PAI-1 inhibits the fibrinolytic system, whose role is crucial for vessel destabilisation, angiogenesis initiation and adipogenesis, but also in atherosclerosis (43); but PAI-1 can both positively and negatively regulate angiogenesis depending on dosage (44). In human adipose tissue, its expression is positively correlated with BMI (45). However, effect of PAI-1 deficiency on adipose tissue development is not clear (40, 46), but expression of PAI-1 correlates strongly with that of TSP-1 in adipose tissue (47).

**Cytokines involvement in angiogenic factors expression**

Adipose-infiltrated inflammatory cells and adipocytes produce high levels of proangiogenic cytokines such as tumour-necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and IL-8. TNF-α promotes endothelial cell tube formation in vitro and inhibits endothelial cell proliferation (48). In addition to its direct angiogenic activity, TNF-α is a potent inflammatory cytokine that links between inflammation, angiogenesis, and adipogenesis. In humans adipose tissue TNF-α correlates with body mass index, percentage of body fat and hyperinsulinaemia (49). TNF-α is known to inhibit mRNA expression of adiponectin in adipocytes (50). Moreover, IL-6 and Oncostatin M significantly increases VEGF and PAI-1 production in adipose tissue and could contribute to vascularisation preceding adipose tissue growth (51, 52).
Angiogenesis, obesity and insulin sensitivity

We (71) and others (72, 73) have observed that insulin resistance, inflammation and adipocyte hypertrophy are correlated, suggesting that inability to activate angiogenesis notably in subcutaneous adipose tissue may be causal in the determinism of metabolic disorders associated with overnutrition. Remodelling of adipose tissue is accelerated in the weight-gaining phases of obesity, but this does not necessarily associate with metabolic disturbances. Problems occur when the lipid storage capacity of adipose tissue is exceeded, which leads to ectopic deposition of lipids in several organs, including liver and muscle, a process that is highly correlated with insulin resistance (74). This hypothesis is well supported by lipoatrophic subjects, who develop severe insulin resistance. Obese subjects may be either insulin sensitive or insulin resistant, the insulin resistant state being associated with larger adipocytes (71). More evidences sustaining this hypothesis have been proposed by Slawik and Vidal-Puig (75). Recent evidence from mice with deletion of FGF1 in adipose tissue indicates that failure of the ability of adipose tissue to expand in response to metabolic demands leads to metabolic disease (76). Gealeyman has shown a negative correlation between subcutaneous angiogenic capacity and insulin resistance in morbidly obese individuals undergoing gastric bypass surgery (70). However, in a previous report, we found a positive correlation between the subcutaneous angiogenic property and stigma of insulin resistance. In the same way, angiogenesis by sera from polycystic ovary syndrome women, a syndrome that is associated with insulin resistance (71), is significantly increased and this effect is attenuated after six months of metformin treatment (77). Relation between adipocyte size, insulin sensitivity and angiogenesis remains thus uncertain.

A pathway linking adipogenesis, angiogenesis and insulin sensitivity could be hypoxia. Hypoxia resulting from inadequate angiogenesis in patients with adipose tissue expansion (67) may lead to up-regulation of the inflammatory adipokines, including IL-6, TNF-α and monocyte chemotactic protein-1 through increased expression of HIF-1. Hypoxia may also lead to adipose tissue fibrosis and mitochondrial dysfunction (64) and thus lead to insulin resistance (78). However, the role of hypoxia has been challenged by the report that, in obese subjects, insulin resistance is associated with an increase in oxygen tension in adipose tissue (79).
The role of inflammation in both the triggering of angiogenesis and insulin resistance is a hot topic (80). Obese subjects have increased adipose tissue macrophage content, particularly in visceral depots (72). These macrophages are typically form crown-like structures surrounding necrotic adipocytes (81). These are supposed to occur in areas of hypoxia and adipocyte cell death, but to date no conclusive relation has been found between inflammation and vascular density.

Sun et al. propose a concept with two ways of fat pad expansion (80). In one hand, healthy expansion involves recruitment of adipogenic precursor cells that differentiate into small adipocytes, appropriate remodelling of the ECM, subsequent vascularisation, and minimal inflammation. In contrast, pathological expansion is accompanied by massive enlargement of existing adipocytes, limited adipogenesis and angiogenesis and ensuing hypoxia, macrophage infiltration with an inflammatory phenotype. Villar et al. also suggested that the pro-angiogenic and inflammatory phenotype of visceral adipose tissue endothelial cells in obese subjects could be related to premature endothelial cell senescence, as suggested by the over-expression of senescence markers in visceral adipose tissue (62), that could contribute to comorbidities of obesity.

Targeting angiogenesis as treatment for obesity and metabolic diseases

Non pharmacological management of obesity, including lifestyle changes and physical activity is recommended as first-line treatment of obesity. Surgical procedures provide alternative options for reducing the life-threatening complications, but are restricted to severe obesity (82, 83). Modulation of angiogenesis has the potential to impair the development of obesity. These findings have paved avenues for possible therapeutic intervention of obesity and obesity associated disorders by targeting angiogenesis (84).

Systemic administration of endogenous (angiostatin, endostatin [60]) or pharmacological (TNP-470 [85], VEGFR2 specific inhibitors [21]) specific anti-angiogenic agents in different mouse obesity models has been shown to prevent diet-induced and genetic obesity in mice, in a dose-dependent and reversible manner. Barnhart et al. recently reported that treatment of obese monkeys with a ligand-directed peptidomimetic called “adipotide” induced apoptosis of white adipose tissue blood vessels, resulting in rapid weight loss and improved insulin sensitivity (86). These results include dose dependent weight reduction and loss of fat mass, partly explained by reduced food intake (85-87) and is associated with a reduction in vascular density, a decreased number of proliferating endothelial cells, and increased of endothelial cell apoptosis. That anti-angiogenic agents targeting specifically adipose tissue trigger decreased food intake is very intriguing.

Recently, a peptide designed to cause apoptosis of endothelium only in white adipose tissue has been shown to selectively inhibits angiogenesis and to improve glucose tolerance in a weight- and food intake-independent manner (88). However, a too strong inhibition of adipose tissue expansion, by impairing angiogenesis, may lead to lipotoxicity and metabolic dysfunction because of ectopic fat accumulation. In fact, adipose specific ablation of VEGF decreased fat tissue vascularity with a concurrent fat mass reduction in white adipose tissue (89) and induced metabolic defects and insulin resistance on a high-fat diet (HFD). Conversely, overexpression of VEGF-A specifically in adipose tissue of diet-induced obese mice results in enhanced vascularisation with improved insulin sensitivity and higher energy expenditure (85, 87, 89). Thus, enhancing adipose tissue angiogenesis, preferably in the subcutaneous depot, should promote fat storage in the appropriate tissue and subsequently protect against metabolic dysfunction. This may explain part of the favourable effects of thiazolidinediones in patients with type 2 diabetes: these drugs promote adipogenesis and fatty acid uptake in subcutaneous adipose tissue (90). This associates with increased number of small adipocytes and increased capillary density, by a direct PPARγ activation (91).

Thus, it is uncertain whether a positive, a negative, or a combination of angiogenesis modulator could be used to treat obesity, as it depends on the metabolic status of a given individual.

Given that obesity and almost all obesity related disorders, including complications of diabetes, cardiovascular disorders and malignancies, are associated with vascular dysfunctions and pathological angiogenesis (92, 93), there is much uncertainty with regards to overall effects of an anti-angiogenesis approach for the treatment of obesity (84). For example, anti-angiogenic agents used for cancer treatment have triggered cardiovascular and renal disorders (94), they also impair the process of wound healing and tissue repair. On the other side, pro-angiogenic therapy can lead to development of benign or malignant tumours, and progression of atherosclerosis (95, 96).

Conclusion

Recent evidence indicates that angiogenesis modulation may offer a tremendous opportunity to treatment of obesity and metabolic disorders. This way of treatment remains an intriguing approach, highlighting the complexity of interfering with adipose tissue angiogenesis. A better understanding of the regulation of the expression of pro- and anti-angiogenic components, their interplay with adipokines, the interactions of endothelial cells and adipocytes and the contribution of inflammatory cells will be instrumental in the development of specific targeting approaches.

Conflicts of interest

None declared.

References


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