ETIOLOGY AND PATHOPHYSIOLOGY

The multifaceted roles of the adipose tissue vasculature

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Summary
The prevalence of obesity and its associated pathologies continue to increase, which has led to a renewed interest in our major weight-regulating organ, the white adipose tissue. It has become clear that its development, expansion, and physiological function depend on proper crosstalk between each of its cellular constituents, with a central role for the vascular endothelium lining the blood vessels. Although first considered a mere barrier, the endothelium has emerged as a dynamic unit modulating many critical adipose tissue functions. It not only oversees the uptake of all nutrients to be stored in the adipocytes but also provides an important growth niche for adipocyte progenitors and regulates the expandability of the tissue during overfeeding and obesity. In this review, we describe the reciprocal relationship between endothelial cells, adipocytes, and obesity. We present recent studies that support an important role for endothelial cells as central mediators of many of the physiological and pathological functions of the adipose tissue and highlight several unknown aspects of adipose tissue vascular biology. This new perspective could present exciting opportunities to develop new therapeutic approaches against obesity-related pathologies and is thus of great interest in our increasingly obese society.

KEYWORDS
adipose tissue, angiogenesis, obesity, vasculature

1 | INTRODUCTION

During the last decades, the prevalence of overweight and obesity has progressively increased, having reached pandemic dimensions. Obesity is intimately linked to an increased morbidity rate and reduced life expectancy due to its association with a series of adverse health consequences, including insulin resistance, the metabolic syndrome, type 2 diabetes, liver disease, dyslipidemia, hypertension, cardiovascular disease, osteoarthritis, kidney failure, as well as certain types of cancers. Due to these accompanying comorbidities, obesity is a major public health threat and has, therefore, become a field of intense research with the goal of identifying the underlying molecular mechanisms and developing new potential interventions and therapeutic approaches.

The physiological role of adipose tissue is to maintain whole body energy balance by regulating energy storage and utilization according to systemic energy demands and the fed/fasted state of the individual, while simultaneously protecting peripheral tissues from lipid overloading and lipotoxicity. However, in response to a continued positive caloric balance, adipose tissue continues to expand, leading to weight gain and, with time, to the development of obesity. When a person’s adipose tissue reaches its upper threshold of expansion, its capacity for lipid storage declines, leading to lipid leakage from the tissue, ectopic lipid accumulation in peripheral organs, and a systemic deterioration in metabolic health. Adding to the complexity of obesity, it is becoming clear that it is not only the quantity but also the quality, functionality, and location of the adipose tissue that mediate the regulation of central metabolic health. This is supported by...
lipodystrophic individuals and animal models that lack adequate amounts of adipose tissue and therefore develop a series of metabolic abnormalities resembling type 2 diabetes, including ectopic lipid deposition, hypertriglyceridemia, and insulin resistance.6 On the other end of the spectrum, there are cases of overweight individuals and mouse models that, despite displaying even morbid obesity, seemingly maintain their metabolic health and do not present insulin resistance or dyslipidaemia.7,8 It should however be noted that the prevalence of so-called healthy obesity remains debated and varies greatly based on the clinical parameters used to define it.7,9 Recent year's scientific advances have also underscored that adipose tissue health and function also depend not only on the fat-storing adipocytes but on the intricate crosstalk between the various cell types present within the tissue. It consists, in addition to adipocytes, of a series of other cell types, including committed adipocyte precursor cells (pre-adipocytes), fibroblasts, hematopoietic cells, vessel-associated smooth muscle cells and pericytes as well as vascular endothelial cells (ECs), which together are termed the stromal vascular fraction or SVF. While many studies have explored the contribution of certain parts of this nonadipocyte fraction to the onset of metabolic disease, the role of the adipose endothelium still remains poorly investigated.

ECs are squamous cells that make up the inner lining of all vessels, regulating multiple physiological processes by acting as the barrier between organs and the blood, thus overseeing the delivery of nutrients and oxygen to the underlying tissue. Despite the general term “vasculature,” ECs are a heterogeneous cell population with distinct morphological and functional characteristics, according to the vessel size, anatomical location, and local metabolic demands.10 Microvessels or capillaries, which this review will focus on hereafter, consist only of a thin layer of ECs, sparsely encased by a supporting cell type termed pericytes,11 allowing them to efficiently deliver gasses, fluids, and macromolecules to the parenchyma. Importantly, transport across the microvascular endothelium differs between tissues and capillary types. It can be either paracellular, allowing free unregulated movement of macromolecules between ECs to the underlying tissue, or transcellular, and thereby actively regulated by specific transporters expressed by the endothelium.12,13 Adipose tissue, along with heart, muscle, lung, and skin, has continuous capillaries, where the ECs are tightly aligned and almost impermeable to paracellular leakage of macromolecules and instead funnel all trans-endothelial transport through the ECs via tightly regulated transport systems (Figure 1).10 The endothelial continuity is achieved through the formation of tight junctions between adjacent ECs and a continuous basement membrane along the vessels. Taken together, vascular function and permeability are delicately regulated processes, adapted to the specific functions of each organ, including the adipose tissue. The continuous, nonleaky nature of the adipose tissue vasculature most likely reflects the importance of regulating the quantity and types of macromolecules that are taken up for storage and/or released from the tissue and thus dictate several aspects of adipose tissue function.

In this review we present current research on the physiological and pathological roles of adipose tissue ECs. We review the multiple physiological functions of the vasculature, the molecular mechanisms that regulate these functions, the interactions between ECs and adipocytes, and how this communication is altered during obesity and might contribute to development of obesity-related diseases. Finally, we summarize key methodological challenges for the future use of the vascular endothelium as a therapeutic target against obesity-induced pathologies.14 Together, current data highlight adipose tissue ECs as a central, but often neglected, cell type within the tissue with a wide-ranging impact on not only tissue function and body weight but also whole body metabolic health.

2 | PHYSIOLOGICAL ROLES OF THE ADIPOSE TISSUE VASCULATURE

Adipose tissue is a highly vascularized organ, with each adipocyte lying adjacent to at least one microvessel in the lean state.15 This allows for close EC-adipocyte crosstalk and most likely enables the continuous regulation of adipose tissue lipid dynamics in response to changes in systemic energy levels, ensuring adequate nutrient storage and release.16,17 In addition to nutrient mobilization, it has been shown that both adipocytes and ECs can impact each other's renewal and remodeling within the tissue, and the vasculature thereby influences the capacity of the adipose tissue to expand during weight gain and obesity.16 Below follows a more in-depth look at some of the physiological roles of the adipose tissue microvasculature that we have chosen to focus on (Figure 2).

2.1 | Trans-endothelial nutrient uptake

Adipose tissue has the unique role of functioning as a buffer for whole body fed/fasted lipid fluxes, much like the liver's role in buffering blood glucose levels.18 Daily net lipid fluxes into adipose tissue, estimated using carbon-14 dating, are roughly 34-g lipid for a lean, weight stable individual (based on 16-kg body fat), and approximately double this for an individual with obesity with 50-kg body fat.19,20 This illustrates the unique challenge for adipose tissue ECs, which experience a high influx of lipids in the fed state, and an outward flux of fatty acids and glycerol between meals. It also highlights the endothelium's potential to regulate the quantity and types of lipid that is stored in the body's fat depots.21
Free fatty acid liberation by lipoprotein lipase

The best-known protein regulating fatty acid uptake from the circulation to adipose tissue is undoubtedly lipoprotein lipase (LpL). Anchored to the luminal side of adipose tissue ECs, it hydrolyzes circulating lipoprotein-associated triglycerides into free fatty acids, which can then be transported through the endothelium. Intriguingly, ECs themselves do not express LpL but instead express an obligate LpL-anchoring protein, Glycosylphosphatidylinositol-Anchored High-Density Lipoprotein-Binding Protein 1 (GPIHBP1), that functions to position LpL at the vascular surface and thus allows its interaction with the triglyceride containing lipoproteins. In contrast, LpL is only expressed by adipocytes and subsequently transported to the endothelial surface with the aid of extracellular heparin-sulfate proteoglycans and GPIHBP1. In this way, the synthesis and release of LpL by adipocytes cooperate with endothelial expression of GPIHBP1, to secure timely uptake of fatty acids to adipose tissue, and thus constitute the most well-described example of paracrine crosstalk that regulates endothelial fatty acid uptake into adipose tissue (as will be discussed below).

The importance of LpL is illustrated by familiar hypachylomicronemia, a condition caused by perturbations in its function. It should be noted that LpL is also strongly expressed in other tissues, especially in muscle, and it long remained a mystery how LpL could partition fatty acid release towards adipose tissue only in the fed state and redirect it to muscle and heart during fasting. The answer came with the discovery that three secreted angiopoietin-like proteins (ANGPTLs) differentially regulate local LpL activity according to nutritional status. ANGPL4, the first to be described, is upregulated in adipose tissue upon fasting and serves as a potent inhibitor of LpL, thereby limiting fatty acid uptake by the adipose tissue in the fasted state. Mice lacking ANGPL4 have low serum triglyceride levels due to LpL overactivity, whereas its overexpression reduces fatty acid storage and causes hypertriglyceridemia. The other two members, ANGPL3 and ANGPL8, cooperate to limit fatty acid uptake by muscle in the fed state. Adding to the complexity, ANGPL8, which is released by adipose tissue and liver upon feeding, also binds to ANGPL4 and suppresses its activity, hence allowing for fatty acid uptake to the adipose tissue specifically in the fed state. Thus, by regulating the relative secretion of these three LpL antagonists according to both location and nutritional status, fatty acid release and uptake are elegantly partitioned between lipid storing and oxidizing organs at the site of the vascular wall. The highly controlled manner by which LpL activity is regulated emphasizes its importance for systemic lipid handling. To date, over 100 mutations in LpL have been identified, of which inactivating mutations most often cause early onset triglyceridaemia, whereas gain-of-function mutations have been associated with a protective phenotype against metabolic disease. In addition, several mutations in LpL-interacting proteins, including GPIHBP1 and the ANGPTLs, lead to the development of dyslipidaemia. Taken together, it is clear that both LpL activity and fatty acid uptake to adipose tissue are pivotal functions for the maintenance of nutritional balance and metabolic health, greatly impacting whole body functions when dysregulated.

Vesicle-mediated transport through the adipose tissue endothelium

After hydrolysis by LpL, free fatty acids need to be transported through the endothelial layer to the adipocytes for uptake and storage. Based on the rapid movement of molecules across the vascular wall, continuous ECs were initially suggested to harbor a system of trans-endothelial pores that facilitated this transport. However, with the emergence of electron microscopy it was discovered that capillary ECs instead have a high number of vesicles on their surface that continuously ferry small volumes of plasma and interstitial fluid across the endothelial barrier via vesicular transcytosis. Endothelial transcytosis in adipose tissue has been visualized by electron microscopy in animals injected intravenously with inert tracer compounds, showing that the tracers were transported across the adipose tissue microvasculature within vesicles, with progressive labeling of the vesicles from the luminal side of the vasculature to the parenchyma and no observations of pores or paracellular tracer leakage.

FIGURE 2 The physiological functions of the adipose tissue vasculature include selective delivery of nutrients for energy usage and storage (represented by blue arrows and secreted vesicles), acting as a differentiation niche and regulating hyperplastic adipose tissue expansion (purple arrow) as well as the release of adipokines and hydrolyzed fatty acids via the vasculature (red) or possibly through the lymphatics (light green)
The nature of such vesicles varies between tissues. In adipose tissue, caveolae predominate and can occupy up to 25% of the surface of ECs, concentrated to regions of high sphingolipid and cholesterol content within the endothelial surface. In addition, caveolae have been shown to impact several other cellular functions throughout the body, including signal transduction, nitric oxide production, and angiogenesis. They are also found in high quantities in adipocytes. For this reason, loss of caveolae is associated with a range of metabolic pathologies in both humans and mice, including the development of hyperlipidemia, lipodystrophy, and type 2 diabetes, and has also been shown to be protective against atherosclerosis in mice. This discrepancy is most likely linked to the multiple roles of caveolae, where their loss in larger arteries and the aorta lowers lipoprotein transcytosis and confers a protective advantage against atherosclerosis, while the simultaneous loss of caveolae in microvessels and adipocytes leads to reduced adipose tissue lipid storage and development of type 2 diabetes. The main proteins regulating caveolae formation in adipose tissue are caveolins 1 and 2 and cavins 1 and 2. Most of their respective knockout mice have distorted caveolae in adipose tissue and lipodystrophic phenotypes, showing that functional caveolae are a prerequisite for proper adipose tissue development and function. Caveolin 1 knockout mice are, for example, resistant to high-fat diet-induced obesity and have poorly differentiated fat pads, leading to the development of severe hyperlipidaemia, despite normal LpL activity. Interestingly, the reduced body weight of the caveolin 1 whole body knockout mice could be normalized by re-expression of caveolin 1 specifically in the endothelium, highlighting the crucial role of endothelial caveolae for adipose tissue function and expansion.

Interestingly, caveolae-type vesicles might also be responsible for mediating the crosstalk between ECs and adipocytes within the tissue, as it was recently identified that ECs and adipocytes exchange both cargo and plasma membrane fragments through secreted extracellular vesicles expressing caveolin 1. This was discovered when the authors tried to knock out caveolin 1 specifically in adipocytes, using the adipocyte-specific cre-driver adiponectin but realized that ECs continuously supplied adipocytes with caveolin-containing vesicles, making adipocyte-specific elimination of caveolin-1 possible only when knockdown was combined with simultaneous inhibition of vesicle formation. The secretion of these EC-derived extracellular vesicles was regulated by glucagon, suggesting that EC-adipocyte crosstalk is hormonally regulated. Although the full spectrum of the proteins and nutrients of these vesicles transport remains to be explored, these data suggest that endothelial caveolae and vesicular transport could be responsible not only for nutrient transport across the endothelium but also for the subsequent transport in to the adipocytes for storage.

### 2.1.3 | Receptor-mediated trans-endothelial transport

In addition to harboring a high density of caveolae, adipose tissue ECs also express several classes of ligand-binding proteins that facilitate nutrient transcytosis. Within other vascular beds, these have been found in high concentrations localized to either caveolae, clathrin-coated pits, or elsewhere within the ECs, but their relative importance and distribution within the adipose tissue vasculature still remains elusive. Endothelially expressed fatty acid transport proteins include the scavenger receptor CD36, fatty acid transport protein (FATP) 3 and 4, as well as some fatty acid binding proteins (FABPs). It is noteworthy that, although all the above mediate fatty acid transport, none of them are believed to be bona fide transporter that facilitate the relocalization of fatty acids over plasma membranes.

CD36 is best characterized and expressed by both vascular ECs, lymphatic ECs, and parenchymal cells in several tissues including adipose tissue. Humans with genetic CD36 deficiency show symptoms ranging from type 2 diabetes to cardiomyopathy, although it is unclear if these symptoms primarily arise due to reduced fatty acid uptake to the adipose tissue or to other organs. On the cell surface, CD36 is primarily localized to lipid rafts, where it is thought to facilitate the binding and sequestration of lipoproteins for further hydrolysis, and it has been shown to require caveolae for its fatty acid transporting activity. Recently, the molecular mechanism for CD36-mediated fatty acid endocytosis in adipocytes was described. However, its relative importance for endothelial transcytosis in adipose tissue remains obscure, as a recent study deleting CD36 expression specifically in ECs in mice demonstrated an important endothelial role for the protein in the heart vasculature, but not in that of adipose tissue, with no changes in body weight or adipose tissue fatty acid uptake detected upon endothelial CD36 deletion. In contrast, tamoxifen-induced deletion of CD31 in lymphatic ECs disrupted their adherence junctions, impaired lymphatic transport of lipids, and caused spontaneous visceral obesity in mice. Further studies on the role of lymphatic CD36 would therefore be of interest to assess its role in fatty acid export from the adipose tissue (see Section 2.1.4 below).

In comparison with CD36, much less is known about the function of the endothelial FATPs and FABPs, and their mechanisms remain elusive. Although not specifically investigated for the adipose tissue vasculature, endothelial FATP4 has been proposed to trap fatty acids in ECs by acetylating them. Recently, mitochondrial oxidation of glucose and subsequent production of ATP was shown to be required for FATP-mediated acetylation of fatty acids and subsequent trans-endothelial fatty acid transport. Interestingly, the study found that FATP4 localizes to the endoplasmic reticulum of ECs, thus driving FA transcytosis from the EC intracellular compartment and not from the plasma membrane as had been speculated before. Endothelially expressed intracellular fatty acid handling proteins also include FABP4 (also known as aP2), which was previously thought to be expressed within the adipose tissue exclusively by adipocytes and not by other cell types, such as ECs and macrophages, which now seems to be the case. Hence, the use of FABP4 as an adipocyte-specific marker to generate a wide range of adipose-tissue “specific” transgenic mice brings into question the main effector cell type for some of these publications. Interestingly, endothelial expression of most of the above described proteins, including CD36, LpL, caveolin 1, and FATP4, is regulated by the transcription factor peroxisome proliferator activated receptor gamma (PPARγ), and EC-specific
deletion of this master regulator of metabolism leads to decreased fatty acid uptake in multiple tissues, including adipose tissue, suggesting central coordination of the various fatty acid handling pathways within the vasculature.69

In addition to free fatty acids liberated from lipoproteins by LpL, whole lipoproteins are also thought to transverse the endothelial barrier through receptor-mediated transcytosis. The very low density lipoprotein (VLDL) receptor (VLDLR) is involved in peripheral triglyceride uptake to adipose tissue and muscle, especially in the postprandial state, and functions as a receptor for several lipoprotein species. It is expressed by ECs in several tissues including adipose tissue (although not explicitly shown in the original publication).60 On the endothelial surface, the VLDLR is thought to interact with LpL simultaneously promoting both fatty acid release by LpL and vesicular transcytosis of whole lipoproteins to the underlying parenchyma.61 When fed a high fat diet, mice constitutively lacking VLDLR remained lean and were protected from obesity but developed instead severe hypertriglyceridemia, showing the importance of the VLDLR receptor for adipose tissue lipid uptake and storage.62 However, these experiments did not address whether the mouse phenotype was due to the lack of VLDLR on the endothelium or on adipocytes themselves. Similarly, both the low density lipoprotein (LDL) receptor (LDLR) and the scavenger receptor SR-B1 have been suggested to be expressed by adipose tissue ECs but to our knowledge never actually shown to be expressed there. LDLR knockout mice are leaner than their wild-type counterparts when fed a chow diet, but reports on the impact of a high fat diet on their bodyweight vary greatly between different strains and diets, with some LDLR-deficient mice staying lean while others becoming more obese compared with the respective wild-type controls.63,64

Despite the characterization of the proteins involved in endothelial fatty acid uptake described above, the main pathway(s) controlling endothelial fatty acid transcytosis in the adipose tissue vasculature remain elusive. Most importantly, the relative contribution of vesicular versus receptor-mediated transport is still poorly defined, underlining the need for continued, in-depth studies to understand how these distinct mechanisms interact and/or overlap and if they can be targeted to promote healthy adipose tissue expansion during weight gain and obesity.

2.1.4 Export of fatty acids and adipokines from the adipose tissue

Adipose tissue differs from muscle and heart as it not only takes up but also releases large quantities of fatty acids between meals. Despite the massive quantity of research published on the mechanisms controlling adipocyte lipolysis and lipid release, few publications have studied the export of fatty acids and other macromolecules from the interstitial fluid surrounding the adipocytes to the blood stream. One should remember that the adipose tissue vasculature is continuous and thus differs from that of endocrine glands, which have fenestrated capillaries that allow rapid secretion of large quantities of peptides directly to the blood. For tissues with continuous capillaries such as the adipose tissue, secreted molecules can leave the tissue either directly through trans-endothelial export to the blood or via the lymphatics. Trans-endothelial export would most likely employ some of the same mechanisms detailed above. However, at least for the femoral-gonadal subcutaneous fat depot, the lymphatics have also been implicated in fatty acid release from adipose tissue, with reduced lymphatic function in human study subjects correlating to reduced plasma concentrations of free fatty acids.65 Substantial quantities of free fatty acids and glycerol, the two products of lipolysis, could be measured in the afferent lymph of the leg, whereas it contained no lipoproteins or triglycerides (which are secreted by the liver), confirming the lymphatics to be a true secretory route.66 In addition, many adipokines and adipose-derived cytokines can be measured in high quantities in leg lymph, with larger adipokines such as tumor necrosis factor alpha (TNFa) and interleukin 6 (IL-6) being more readily exported from adipose tissue through the lymphatics than the blood, although both vascular pathways contributed to adipokine export to some extent.66 This finding of a lymphatic route for adipose tissue fatty acid and adipokine export suggests that caution should be taken when evaluating adipose tissue secretion through solely measuring arterial–venous differences over the tissue, as this would miss the lymphatic component. The results also open potential novel research avenues, using the lymphatics to limit excessive lipid leakage from adipose tissue to liver during obesity. However, the export mechanism might vary significantly between different fat depots and anatomical locations, and therefore, the results from leg-fat should be interpreted with some caution.

2.1.5 Transport of glucose and other macromolecules

In addition to lipids, hydrophilic nutrients and proteins such as glucose, insulin, and amino acids must also transverse the endothelial barrier, although their trans-endothelial transport mechanisms have been less studied than for fatty acids.43 Adipose tissue takes up a significant proportion of meal-derived glucose, which it uses to synthesize glycerol and other biomolecules.67 Indeed, knocking out the main insulin-sensitive glucose transporter, GLUT4, specifically in mouse adipose tissue reduced whole body glucose disposal by approximately 50% during euglycemic clamp studies and caused hyperglycaemia.68 Glucose is both transported through the ECs and used by the ECs as their main metabolic fuel, since they rely primarily on aerobic glycolysis due to their low mitochondrial content and their role in facilitating the diffusion of as much oxygen as possible to the parenchymal tissue. In contrast to most other cell types, endothelial glucose uptake has been shown to be insulin-independent and mainly mediated by the glucose transporter GLUT1.13 Interestingly, whereas the insulin-sensitive glucose transporter GLUT4 is not highly expressed by ECs, ECs still express the insulin receptor, highlighting insulin’s multiple roles as both mitogen and hormone. The endothelial insulin receptor has been shown in vitro, using human adipose tissue microvascular
cells, to activate receptor-mediated transcytosis of insulin itself. This remains controversial, and alternative nonreceptor mediated routes of insulin transport have been suggested, including caveolae- and clathrin-mediated vesicular transport. These discrepancies might be explained by differences in the microvascular cell types studied or by the use of supra-physiological levels of insulin. Multiple parallel trans-endothelial pathways might also exist to assure quick delivery of insulin to parenchymal cells, such as adipocytes and myocytes, in order to promote efficient postprandial insulin-mediated glucose uptake. In fact, while still debated, delays in endothelial transcytosis of insulin have been suggested to induce insulin resistance.

Endothelially expressed micro RNAs (miRNAs) have also been shown to control insulin signaling and function within the adipose tissue ECs. Taken together, it is clear that the understanding of receptor-mediated nutrient uptake into adipose tissue is only rudimentary, and many details remain to be explored, most importantly whether uptake mechanisms differ from that of muscle and other tissues, given the unique role of adipose tissue in lipid storage.

### 2.2 Paracrine adipocyte-EC communication

For muscle and heart, which in contrast to adipose tissue, depend on a constant supply of fatty acids for proper function and cannot tolerate lipid overloading, several mechanisms have been described whereby the tissue parenchyma regulates endothelial transcytosis through paracrine signaling. Examples include the vascular endothelial growth factor B (VEGF-B) and the branched amino acid derivate 3-hydroxyisobutyrate (3-HIB). These pathways have recently been reviewed elsewhere, and their relative importance within adipose tissue still remains elusive.

In addition to LpL discussed above, several recent studies have described pathways facilitating paracrine adipocyte-EC crosstalk. Prohibitin and annexin2 were recently shown to control fatty acid uptake to the adipose tissue and knocking out annexin 2 reduced adipose tissue lipid uptake and adipocyte size. The authors suggested that prohibitin, annexin 2, and the fatty acid scavenger receptor CD36 form a complex on the cell surface of adjacent ECs and adipocytes upon fatty acid exposure, which promotes endothelial fatty acid transcytosis and the subsequent funneling of fatty acids to the adipocytes for uptake. Another signaling molecule proposed to facilitate paracrine adipocyte-EC crosstalk is apelin, an atheroprotective protein which enhances glucose utilization and promotes whole body insulin sensitivity by binding to the apelin receptor expressed on ECs. Apelin signaling downregulated endothelial FABP4 expression and subsequent fatty acid transport. Moreover, in the subcutaneous adipose tissue of mice, but not in visceral fat, angiopteoitin-2 released by adipocytes signals to integrin α5β1 on the adipose tissue vasculature, inducing the expression of FATP3 and subsequent trans-endothelial fatty acids transport. The authors also showed that the so-called healthy obesity in humans was associated with higher adipose tissue expression of angiopteoitin-2 and increased subcutaneous lipid uptake, confirming the importance of subcutaneous fat as a whole body lipid buffer.

### 2.3 The vasculature as a growth niche for adipogenesis

Whereas the size of most organs varies very little during adulthood, adipose tissue is highly plastic, and its mass can significantly increase or decrease throughout life, especially in response to changes in energy consumption. Adipogenesis and hyperplastic expansion of fat are tightly coordinated with the outgrowth of new blood vessels (angiogenesis), both during embryonic and postnatal adipose tissue development and during diet-induced tissue expansion. This has been elegantly shown in mice, where the establishment of a local vascular tree precedes the maturation of fat cells during fat pad development, with new adipocytes subsequently differentiating and growing alongside the newly formed vascular branches. We recently showed that the presence of vasculature also enhances adipocyte differentiation and maturation in vitro. The vasculature is thought to facilitate adipogenesis by serving as a local stem cell niche, where committed pre-adipocytes reside prior to differentiation and subsequently mature into fully differentiated adipocytes. Some studies even suggest that the adipose tissue endothelium could be a direct source of pre-adipocytes. Although the trans-differentiation of mature ECs to adipocytes is less likely, many pre-adipocyte markers are common with those of vessel-associated pericytes. Pericytes, which wrap themselves around the microvasculature, have previously been shown to also serve as progenitors for, among others, myocytes, neural cells, and fibroblasts. It is thus tempting to speculate that pericytes could also transdifferentiate into adipocytes during adipogenesis, at least in mice. Whether some pre-adipocytes are indeed derived from pericytes remains unknown, single-cell technologies are likely to answer this question in the near future. Nevertheless, the fact that the vasculature serves as growth niche for differentiating pre-adipocytes highlights the close interactions between the vasculature and parenchymal cells in adipose tissue and is likely to explain, at least in part, why the endothelium has
such profound effects on adipose tissue function and morphology during the onset of obesity.

2.4 | Angiogenic regulation of adipose tissue expansion

Over 20 years ago, Judah Folkman, a pioneer in endothelial biology, showed that inhibition of angiogenesis in mice prevents the expansion of adipose tissue, similar to its prevention of tumor growth. At that time, the findings were celebrated as the new pathway to reducing obesity, and it was only later discovered that reducing angiogenesis during adipose tissue expansion actually aggravates metabolic pathologies instead of improving them. In fact, angiogenesis, which otherwise is a relatively rare process in normal adult physiology, is considered an absolute requirement for adipogenesis and promotes adiposity during obesity. To increase vessel density, adipocytes secrete pro-angiogenic factors, most notably members of the VEGF family, leading to the sprouting of new vessels. VEGF-A is the predominant regulator of angiogenesis, and genetic ablation or overexpression of VEGF-A in murine fat showed that enhanced VEGF-A-signaling can protect against obesity-related comorbidities by increasing vessel density, thereby lowering adipose tissue hypoxia. VEGF-A also activates thermogenesis and energy expenditure in both brown and white fat, leading to reduced body weight. It should, however, be noted that adipose tissue angiogenesis can have either beneficial or detrimental consequences for tissue function depending on context and obesity status. During weight gain, anti-angiogenic therapy has adverse effects by restricting adipose tissue expansion, thereby inducing ectopic lipid accumulation and whole body insulin resistance. Instead, when the same therapy was given to animals with already established obesity, it reduced body weight and ameliorated metabolic complications. This can be reproduced using pro-apoptotic nanoparticles specifically targeting the adipose tissue vasculature, which reduce adiposity and ameliorate obesity-associated dysfunctions. This implies that limiting access to blood vessels in already inflamed, dysfunctional fat can lead to clearance of pro-inflammatory cells with beneficial effects, but giving the therapy to relatively healthy subjects could have detrimental consequences.

Healthy, hyperplastic expansion of adipose tissue requires coordinated vessel growth and increased secretion of pro-angiogenic factors such as VEGFs, fibroblast growth factor 2 (FGF2), and insulin-like growth factor (IGF), all secreted by mature adipocytes. Indeed, both the VEGF-A and VEGF-B genes were identified by genome wide studies to reside within loci associated with healthy obesity and an uncoupling between adiposity and metabolic disease. However, adipose tissue vessel growth needs to be tightly regulated, as aberrant vascular sprouting can lead to vascular dysfunction, whereas inadequate angiogenesis during weight gain can cause hypoxia to develop (see more below). Adipocyte VEGFA expression has been proposed to be regulated by estrogen receptor 1, but it remains to be seen if other regulatory mechanisms also exist. Recently, it was shown that endothelial TWIST1-SLIT2-mediated signaling is reduced in adipose tissue of individuals with obesity and suggested that this reduction decreases vascular formation in the obese state. Taken together, it is clear that adipogenesis is tightly intertwined with angiogenesis and vascular density, underlining the importance of continuing to expand our understanding of the many physiological roles of the adipose tissue vasculature.

3 | PATHOLOGICAL EFFECTS OF OBESITY ON ENDOTHELIAL FUNCTION

3.1 | Capillary rarefaction and adipose tissue hypoxia

One of the direct consequences of obesity is capillary rarefaction, as the distance between neighboring microvessels increases significantly when adipocytes become larger, thereby lowering the vascular density (Figure 3). There seems to be a stepwise reduction in capillary density with progressive obesity and glucose intolerance in both subcutaneous and visceral human fat, while maintaining the same percentage of ECs per tissue, suggesting the net number of capillaries remain similar despite an overall increase in the amount of adipose tissue mass and angiogenesis being activated. When the capillary bed becomes too sparse, especially in combination with obesity-induced reductions in blood flow, it leads to a lower local oxygen pressure and the development of hypoxia, with detrimental health consequences. Hypoxia can, among other effects, trigger inflammatory...
responses in parenchymal cells, lower endothelial permeability, and further weaken the tissue’s angiogenic potential and vascular tone through alterations in the vasodilation/vasoconstriction balance. Knocking out hypoxia-sensing pathways in rodent adipose tissue improves overall metabolism and insulin sensitivity, providing proof of concept that capillary rarefaction and reduced vascular density has severe metabolic consequences.97

Interestingly, while adipose tissue hypoxia seems to be a well-established phenomenon in rodent obesity, the occurrence and implications of hypoxia in human obesity remain debated.98 For example, one investigation of adipocyte oxygen consumption in human subcutaneous adipose tissue did not provide any evidence for hypoxia and hypoxia-driven dysfunctions in obesity,99 whereas several others have shown that obesity and insulin resistance are associated with adipose tissue hypoxia.100,101 It should be noted that the oxygen consumption in adipose tissue is lower than in many other tissues and is further reduced during obesity due to the accompanying development of adipocyte mitochondrial dysfunction, raising the question to what extent hypertrophic adipocytes are impacted by the reduced capillary supply of oxygen.98 Nevertheless, capillary rarefaction might have multiple other effects on adipose tissue by also impairing fatty acid uptake and disrupting adipocyte-EC crosstalk, thereby greatly affecting overall tissue functionality in obesity.

3.2 | Endothelial dysfunction

In response to obesity, adipose tissue undergoes major structural and functional reprogramming, including increased unstimulated lipid release, tissue inflammation, and fibrosis.102 The increase in the levels of free fatty acids and cytokines released by the adipocytes also triggers the development of local endothelial dysfunction.103,104 This is characterized by perturbations in normal EC reactivity, gene expression, permeability, and structure and leads to endothelial secretion of an array of pro-inflammatory signaling mediators and adhesion molecules that can further impair tissue functions.105 For example, it was shown that exposing adipocytes to conditioned medium from ECs isolated from subjects with obesity decreases adipocyte insulin sensitivity and lipolytic activity and increases their pro-inflammatory secretion.106 Similarly, EC-specific deletion of nuclear factor-κB (NFkB), involved in oxidative stress and inflammation, in obese mice was sufficient to reduce adipose tissue macrophage infiltration and increase whole body insulin sensitivity and metabolic health.107 The capillary basement membrane of visceral adipose tissue microvessels has also been found to progressively thicken with increased levels of obesity and metabolic complications, a sign of emerging microangiopathy.95 Some of these alterations have been connected to the induction of senescence, or premature aging, in the adipose tissue vasculature.108-109 Importantly, inducing endothelial senescence either genetically in mice or through serial passaging of human ECs impaired adipose tissue insulin sensitivity and PPARγ-dependent fatty acid uptake, leading to the development of ectopic lipid accumulation in peripheral tissues.108,109 To date, most studies have found ECs from visceral fat to be more susceptible to endothelial dysfunction than those from subcutaneous fat,106 which is in line with the visceral fat being considered more prone to inflammation. More research is needed to understand the spatial and temporal development of adipose tissue inflammation and if subcutaneous adipose tissue ECs also become dysfunctional albeit at a slower pace. Importantly, the effects of endothelial dysfunction on vascular nutrient uptake to the adipose tissue remain incompletely understood. Taken together, the evidence so far support that development of vascular defects during obesity negatively impacts adipose tissue functionality, with severe consequences on systemic metabolism.

4 | UNDERSTANDING THE VASCULAR ENDOTHELIUM: FUTURE METHODOLOGICAL CHALLENGES

The growing evidence for an active role of the vasculature in the pathophysiology of obese adipose tissue has highlighted the potential of ECs to act as therapeutic targets.110 However, the existence of ECs in every tissue throughout the body brings an important limitation to this aim, and multiple pieces of the puzzle are still missing. Thanks to the powerful single-cell technologies that are now emerging, we have for the first time an opportunity to dissect the factors that define and distinguish microvascular heterogeneity between different organs. Below we have listed this and some of the other limitations and/or opportunities that still exist within the vascular research field and which could allow us to target adipose tissue ECs in the future to improve health in obesity and associated diseases:

- **Utilization of high-throughput technologies to characterize ECs in-depth.** This will result in further unbiased insight into factors that are linked to abnormal endothelial function and the development of disease states. Approaches include bulk, single-cell, and spatial transcriptomics, (quantitative) proteomics, and metabolomics, as well as whole genome genetic screens.111,112 The rapid publication of such data by the research community will serve as a powerful tool for identification of organ-specific endothelial disease mechanisms and drug targets and key to the success of many of the other points below.113

- **Identification of organ-specific endothelial biomarkers,** both phenotypic (mRNA/protein) and metabolic (lipid profile, energy usage).113,114 Based on sequencing data, an open-access online atlas should ideally be constructed to enable identification of both mouse and human organ-specific endothelial markers. This would allow the wider research community to perform tissue-specific endothelial targeting and thus greatly limit the off-target effects of future pharmacological interventions.

- **Novel animal models.** Wider utilization and development of new EC-specific conditional and/or inducible animal models,115 especially models based on new tissue-specific endothelial markers, will increase our knowledge of the vascular endothelium, help to dissect EC-autonomous effects, and examine the systemic
consequences of EC manipulation in research animals in vivo and thus be of great preclinical benefit.

- Development of improved delivery systems for in vivo transfer of biomolecules such as nucleic acids and proteins to ECs would enhance the possibilities of targeting the vasculature in humans. Furthermore, achieving the delivery of specific cargos to specific vascular beds would represent significant progress. It would eliminate many of the potential negative effects of targeting molecules systemically and in the future allow for truly targeted interventions.

- Improved culturing systems with the standardization of existing and the development of new endothelial in vitro/ex vivo systems that could accurately mimic the in vivo state. This includes usage of ECs originating from the correct vascular bed (as not all ECs are the same), developing new types of cell systems for co-culture to understand tissue-specific EC interactions with other cell types, and a more general use of nontransformed, primary ECs for such work. With such tools and improved practices, we will be able to better identify the specific pathways/factors that mediate EC dysfunction during obesity.

Taken together, the refinement of existing methods and development of new techniques to assess EC function both in adipose tissue and other organs are of utmost importance and could then be used to complement the existing noninvasive technologies measuring EC function, as well as circulating biomarkers, to provide a comprehensive picture of vessel function during health and disease. This will allow us to better detect and characterize the molecular alterations that mediate EC dysfunction, so as to develop novel diagnostic and therapeutic targets for the prevention of obesity-associated pathologies.

5 | CONCLUDING REMARKS AND FUTURE DIRECTIONS

The vascular endothelium is progressively gaining increased attention within the obesity research field. From previously being considered a mere structural barrier, it has emerged as a crucial mediator of multiple physiological functions and pathological changes within adipose tissue during obesity. The many regulatory functions of the endothelium also underline its fundamental role in maintaining adipose tissue functionality. However, many questions remain about the vasculature's role in supporting nutrient uptake and adipogenesis and the conditions that lead to vascular rarefaction and EC dysfunction during obesity, highlighting the need for continued in-depth investigation. For example, can we develop tools that could specifically increase endothelial lipid uptake and transport to the adipose tissue and would this have beneficial effects on metabolic health or detrimental consequences for body weight—or both? Understanding and revealing new cascades and mediators linking EC function to systemic organ physiology will open novel paths to the development of prognostic and therapeutic approaches to the major pathologies linked to the ongoing obesity pandemic.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

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