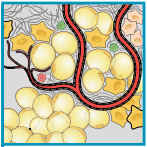


IMPAIRED ADIPOGENESIS AND DYSFUNCTIONAL ADIPOSE TISSUE IN HUMAN HYPERTROPHIC OBESITY

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Hammarstedt A, Gogg S, Hedjazifar S, Nerstedt A, Smith U. Impaired Adipogenesis and Dysfunctional Adipose Tissue in Human Hypertrophic Obesity. *Physiol Rev* 98: 1911–1941, 2018. Published August 1, 2018; doi:10.1152/physrev.00034.2017.—The subcutaneous adipose tissue (SAT) is the largest and best storage site for excess lipids. However, it has a limited ability to expand by recruiting and/or differentiating available precursor cells. When inadequate, this leads to a hypertrophic expansion of the cells with increased inflammation, insulin resistance, and a dysfunctional prolipolytic tissue. Epi-/genetic factors regulate SAT adipogenesis and genetic predisposition for type 2 diabetes is associated with markers of an impaired SAT adipogenesis and development of hypertrophic obesity also in nonobese individuals. We here review mechanisms for the adipose precursor cells to enter adipogenesis, emphasizing the role of bone morphogenetic protein-4 (BMP-4) and its endogenous antagonist gremlin-1, which is increased in hypertrophic SAT in humans. Gremlin-1 is a secreted and a likely important mechanism for the impaired SAT adipogenesis in hypertrophic obesity. Transiently increasing BMP-4 enhances adipogenic commitment of the precursor cells while maintained BMP-4 signaling during differentiation induces a beige/brown oxidative phenotype in both human and murine adipose cells. Adipose tissue growth and development also requires increased angiogenesis, and BMP-4, as a proangiogenic molecule, may also be an important feedback regulator of this. Hypertrophic obesity is also associated with increased lipolysis. Reduced lipid storage and increased release of FFA by hypertrophic SAT are important mechanisms for the accumulation of ectopic fat in the liver and other places promoting insulin resistance. Taken together, the limited expansion and storage capacity of SAT is a major driver of the obesity-associated metabolic complications.

I.	INTRODUCTION	1911
II.	THE ADIPOSE TISSUE	1912
III.	COMMITMENT AND ...	1916
IV.	ADIPOSE TISSUE ANGIOGENESIS	1921
V.	ADIPOSE TISSUE INFLAMMATION	1924
VI.	ECTOPIC LIPID ACCUMULATION—...	1928
VII.	CONCLUSIONS AND PERSPECTIVES	1930

I. INTRODUCTION

The adipose tissue is a good example of the concept that both too much and too little can be harmful! In fact, the metabolic complications of both too much adipose tissue (in obesity) and too little (lipodystrophy/lipodystrophy) are quite similar and increase the risk of type 2 diabetes (T2D), liver disease, cardiovascular disease (CVD), certain cancers, and other disorders. The reduced adipose tissue mass in lipodystrophy precludes safe storage of excess lipids in the best and safest site, i.e., the subcutaneous adipose tissue (SAT) and enhances storage in ectopic sites with negative local and systemic consequences. Similarly, when the lim-

ited storage capacity of SAT is exceeded in obesity, excess lipids accumulate in both the visceral depot as well as in the ectopic sites, promoting the same metabolic and other consequences.

SAT is the largest adipose tissue depot of the body and the least harmful site to store excess calories and release lipids as needed, although differences between body regions are evident. SAT expansion is best accomplished by recruiting new cells rather than merely expanding available adipose cells, which increasingly leads to an inflamed and dysregulated tissue. SAT has a limited ability to recruit new adipose cells and expand. When exceeded, SAT is characterized by hypertrophic adipose cells, the tissue becomes inflamed, and dysfunctional and excess lipids are stored in other more harmful adipose tissue depots (for instance visceral and peri-epicardial fat) and in ectopic sites. Ectopic lipid accumulation occurs in many tissues, including the liver, heart, skeletal muscles, and other sites. This, then, induces several negative consequences for local and systemic insulin sensitivity and inflammation and contributes to incident disease development.

In addition to its lipid storage function, the adipose tissue is also the main provider of free fatty acids (FFA), largely released by the SAT as needed for energy supply in skeletal muscles, heart, liver, and other cells. Excess serum levels of FFA, as in obesity and T2D, reduce peripheral glucose uptake and enhance hepatic glucose production and thus contribute to insulin resistance and glucose intolerance. Additionally, increased FFA levels increase hepatic very low-density lipoprotein-triglyceride secretion and blood levels and thus provide a negative cardio-metabolic profile. The detailed consequences of increased ectopic fat accumulation in different tissues will be reviewed later.

Adipose cell lipolysis and FFA release are increased in obesity as a consequence of the increased amount of body fat and the hypertrophic expansion of the adipose cells, which, in turn, also is associated with local and systemic insulin resistance. Thus the anti-lipolytic effect of insulin is also impaired.

The regulation of human adipose tissue lipolysis has been extensively reviewed elsewhere and will not be included in this review. However, the very unexpected finding that the adipose cells in obesity are characterized by increased basal lipolysis but reduced adrenergic activation is quite puzzling (248), and mechanistic insight is still lacking. Nevertheless, adipose cell FFA release is increased since basal lipolysis is increased and the anti-lipolytic effect of insulin is reduced. Also, it was recently suggested that adipocyte triglyceride lipase, the key regulator of lipolysis in murine adipose cells, may be less important in human adipose cells, while hormone-sensitive lipase is more important in contrast to what has been reported in murine cells (249). These results emphasize the care that must be imposed in directly translating findings in murine animal models to the human situation.

The adipose tissue is not only a key regulator of lipid storage and release in the body, but it is also an endocrine organ secreting many different hormones that cross talk with central and peripheral cells. Leptin was the first identified secreted hormone/adipokine (104) and has been shown to regulate food intake and energy expenditure, and it is an important feedback signal to the brain about the size and state of the adipose tissue. Lack of leptin, or its receptor, leads to massive obesity in both murine models and in humans (75), and leptin deficiency can, in part, be successfully treated with leptin administration. Similarly, leptin treatment improves the massive lipid accumulation in the liver and associated dysregulated lipid and glucose profile in human lipodystrophic states (review by Ref. 200).

Our present knowledge of adipose-tissue secreted adipokines includes over 100 proteins exerting cross talk with other cells/tissues. However, beyond leptin, very few have

been shown to be therapeutically beneficial as replacement therapy or as treatment of disease. There is current interest in adiponectin (197) or its analogs as a potential therapy of insulin resistance/T2D and also interest in developing inhibitors of adipokines with negative metabolic effects, such as retinol binding protein 4 (321) and other proinflammatory molecules, including tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1), and IL-6. Additionally, a novel family of lipids secreted by the adipose tissue and with positive effects on insulin sensitivity, insulin secretion, and inflammation was recently described (326). Adipokine secretion and function have been reviewed extensively, and the interested reader is referred to the many reviews that have been published.

Clearly, a healthy and well-functional adipose tissue is of major importance for whole body metabolism. The objective of this review is to give a comprehensive overview of the literature and discuss concepts relating dysfunctional adipose tissue with metabolic disease. We will discuss the importance of healthy adipose tissue expansion, preadipocyte recruitment and differentiation, and what is currently known about its epi-/genetic regulation. Furthermore, the role of adipose tissue angiogenesis and inflammation for disease development will be reviewed. The final part of this review is focused on the consequences of a dysfunctional adipose tissue, reviewing the current knowledge on ectopic lipid accumulation, its effects in peripheral tissues, and the metabolic consequences.

II. THE ADIPOSE TISSUE

A. Adipose Tissue and Metabolic Disease

Although the health issues and risk factors of being overweight and obesity are well documented, the prevalence of obesity is increasing and is a major public health issue. The world-wide epidemic of obesity and associated metabolic and other complications have rekindled interest in the adipose tissue and its role in the development of these diseases. It has also become apparent that obesity is remarkably heterogeneous, and not all obesity is associated with immediate risk of metabolic or cardiovascular complications.

Obesity is a well-known risk factor for diseases such as T2D, CVD, and nonalcoholic fatty liver disease/nonalcoholic steatohepatitis (NAFLD/NASH), but links have also been found with cognitive disorders and several cancers (116, 227). Insulin resistance is a common denominator of these disorders, but ~30% of obese individuals do not develop insulin resistance (152), although they still seem to have a higher risk of T2D and CVD in prospective studies (299). Interestingly, however, a similar number of nonobese individuals exhibit metabolic markers as if they were obese (254). Thus body mass

index (BMI) is not a sufficiently sensitive criterion of disease risk at the individual level.

In addition to BMI and total amount of body fat, regional distribution of the adipose tissue, particularly a predominant abdominal distribution, is of importance (314). Interestingly, adipose tissue regional distribution is likely to be of causal importance for disease development, since recent studies of individuals with genetic predisposition for an abdominal obesity have shown that their future development of CVD and T2D is increased around twofold for the same BMI (72). Another example of the limitations of BMI in predicting metabolic consequences is the so-called metabolically obese normal-weight subjects who, despite a normal BMI, exhibit increased risk of the metabolic complications seen in obesity (273).

More than one-half a century ago, the importance of adipose tissue distribution for metabolic complications was recognized, and the so-called android obesity, with adipose tissue preferentially accumulated around the trunk, was identified as the form of obesity more strongly associated with metabolic disease (28, 29, 300). These observations were later substantially buttressed and expanded by several publications showing that body shape and adipose tissue distribution were predictive for CVD and T2D (149, 150, 164, 165). At this point adipose tissue morphology, i.e., adipocyte cell size and number, was introduced as important factors related to metabolic complications (156), and SAT adipose cell size has more recently been identified as an independent predictor of future T2D (185). Furthermore, inappropriate expansion of SAT adipose cells for a given BMI was also found to be a characteristic of individuals with genetic predisposition for T2D [defined as having a close family history of T2D, i.e., being first-degree relatives (FDR)] independent of obesity and body fat distribution (15, 185, 312). Additionally, individuals with genetic risk of insulin resistance are characterized by having a reduced amount of peripheral adipose tissue (187) and probably also likely to have inappropriately expanded SAT cells. These now well-established associations clearly suggest the importance of epi-/genetic factors in regulating SAT adipose cell commitment and/or differentiation, and that reduced SAT adipogenesis is associated with increased risk of the metabolic complications, probably as a consequence of increased ectopic fat accumulation, which will be further discussed later.

Other important factors that influence the risk of metabolic complications are the level of exercise and nutritional status. Clinical studies in patients with different forms of defects in adipose tissue expansion have clearly demonstrated that these factors play an important role for prevention and alleviation of metabolic complications also in this case (186, 255).

B. Differences in Adipocyte Morphology and Plasticity in Adipose Tissue Depots

The adipose tissue expands by a combination of adipocyte hypertrophy of preexisting cells and hyperplasia, i.e., recruitment and differentiation of new preadipocytes. The relationship between cell size and obesity is curvilinear (14), and the increase in cell size reaches a plateau that triggers generation of new adipocytes (156, 194, 269). Elegant studies using ^{14}C dating of adipocytes have shown that ~10% of the fat cells in human SAT are renewed every year (269), and that reduced regeneration capacity is associated with inappropriate adipocyte hypertrophy, i.e., the so-called hypertrophic obesity (14). Hence, inability to recruit new adipocytes on demand is associated with a hypertrophic expansion of the adipose tissue. These, and other recent data, have provided support for the concept that inability to recruit new SAT adipose cells to store excess lipids promotes the metabolic complications of obesity while the ability to increase cell number is protective (247).

Adipocyte cell size and number vary with the anatomical location of the adipose tissue and are also strongly influenced by sex. For instance, women have smaller adipocytes in omental compared with subcutaneous fat, whereas men have similar size in both depots and an overall larger adipocyte size (287). Furthermore, women seem to be able to expand both abdominal and lower body SAT by hyperplasia to a larger extent than can men (288). That sex hormones play an important role for these differences is supported by the fact that, when women reach menopause, sex differences in adipose tissue morphology are reduced, and women adopt a more male pattern (286). This shift is also accompanied by a parallel increase in metabolic risk markers, which become more similar to those in men.

The underlying mechanisms are far from fully understood and involve direct effects of the estrogen receptors in adipocytes, as well as effects of the sympathetic nervous system innervating the adipose tissue depots, as recently reviewed (221). The sex differences in adipose tissue distribution and morphology, with increased plasticity of the SAT and hyperplastic expansion of lower body SAT in women, may contribute to why premenopausal women are more protected from CVD and metabolic disease (331). A lower abdominal/waist-to-hip ratio is protective against future CVD also in women with T2D and a given BMI, further underlining the importance of adipose tissue distribution (69). The protective role of lower body SAT for CVD is also evident in the large Dallas Heart Study, where the protective effect remains after adjustment for BMI and sex (211).

C. Subcutaneous Adipose Tissue Expansion

Consistent with the idea that a healthy hyperplastic SAT expansion is important for preventing insulin resistance and

a dysmetabolic state following weight gain are observations in individuals with lipoatrophy or lipodystrophy. As discussed, they are characterized by marked insulin resistance, reduced glucose tolerance, and ectopic lipid accumulation due to the inability to store fat in the adipose tissue (60, 235). Similar consequences are seen in genetically engineered mouse models of lipoatrophy and lipodystrophy (219).

Adipose tissue expansion occurs through a combination of adipocyte hyperplasia and adipocyte hypertrophy. Recruitment and differentiation of new preadipocytes in SAT when needed to store excess fat is the preferred way of adipose tissue expansion and protects against metabolic disease (98, 161) as illustrated in **FIGURE 1**.

The protective effect of hyperplastic/healthy SAT expansion has also been verified in several animal models. An elegant example is the mouse model overexpressing adiponectin in the adipose tissue. This leads to massive obesity due to a significant hyperplastic expansion of SAT, combined with a metabolic profile that is similar to that of lean control littermates (148). A similar protection was also demonstrated in another example of obesity with normal, or even further improved metabolism, i.e., the mouse model overexpressing glucose transporter-4 (GLUT-4) in the adipose tissue (1). These mice display lower ambient glycemia and insulinemia and enhanced glucose tolerance, in combination with hyperplastic expansion of the adipose tissue. Furthermore, chemically induced hyperplastic expansion of SAT by injecting an adipogenic cocktail has also been reported to improve glucose tolerance and insulin sensitivity (188). Yet another example of how adipose tissue expandability is protective and more important than obesity per se is the recent study where adipose tissue-specific overexpression of Cidea (cell death-inducing DNA fragmentation factor- α -like effector A) improves metabolism through healthy expansion of the adipose tissue (2). That healthy adipose

tissue expansion also improves insulin sensitivity and glucose metabolism in humans is supported by the actions of the insulin sensitizers, the thiazolidinediones (TZD), which improve whole body insulin sensitivity, despite increased body weight, by hyperplastic expansion of SAT (6).

D. Adipocyte Hypertrophy in SAT—A Marker of Dysfunction and Insulin Resistance

Many studies have shown that SAT adipocyte size is related to, and predicts, the metabolic complications of obesity. Correlations between increased SAT adipocyte cell size and insulin resistance have been well documented in both men and women (62, 156, 185, 312) and also in obese children (162). Most studies, but not all (137, 170), have reported that enlarged SAT adipocytes are correlated with insulin resistance in both normal-weight and obese subjects (14, 106, 112, 226, 238, 320). There have also been reports showing that insulin resistance is characterized by a larger population of small adipocytes in SAT, probably reflecting an impaired ability to differentiate newly recruited adipocytes and store fat (199).

A relationship between visceral adipocyte cell size and insulin sensitivity has also been shown in a number of studies (170, 214), but not all (191), or that the associations were no longer significant when adjusting for BMI or other anthropometric values (201, 303). However, this is not unexpected, since expanded visceral adipose cells is also associated with increased waist-to-hip ratio, i.e., an abdominal fat distribution, which also is a marker of ectopic fat accumulation in other sites.

Taken together, SAT cell size is negatively correlated with whole body insulin sensitivity. However, after adjusting for markers of the amount of visceral adipose tissue, this relationship is no longer evident, suggesting that SAT adipose

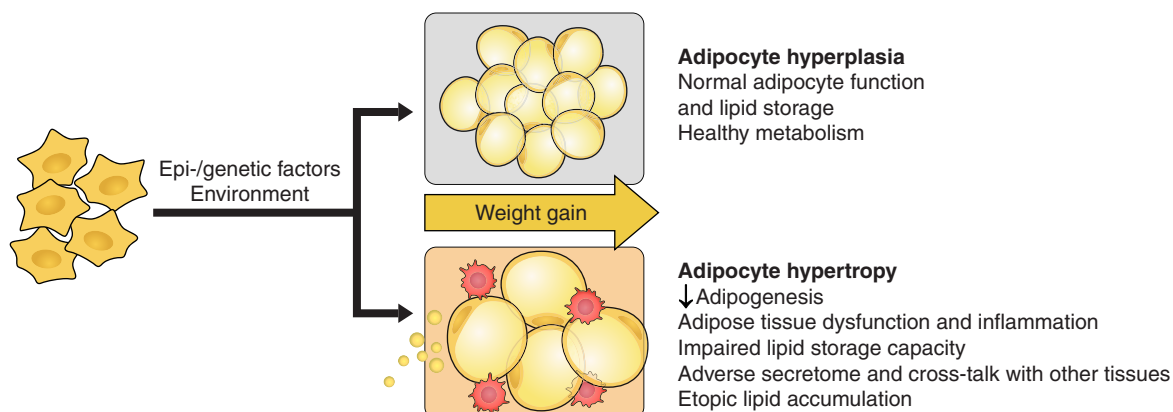


FIGURE 1. Hyperplastic expansion of the adipose tissue protects against metabolic disease. Adipose tissue expansion occurs through a combination of adipocyte hyperplasia and adipocyte hypertrophy and is driven by both epi-/genetic and environmental factors. Adipocyte hyperplasia is the preferred way of adipose tissue expansion and protects against metabolic disease by maintaining normal adipocyte function and sufficient lipid storage capacity within the adipose tissue.

cell size and visceral adiposity are strongly connected, with both representing consequences of a limited hyperplastic expansion and ability to store fat in SAT (22, 161, 265). As expected, significant associations between SAT adipocyte hypertrophy, CVD (112, 120, 126), and liver fat accumulation (11, 230) have also been found and will be further discussed later.

E. Impaired Ability to Recruit SAT Precursor Cells Results in Adipocyte Hypertrophy

SAT adipocyte hypertrophy is an obesity-independent marker of insulin resistance and future risk of developing T2D driven by limited hyperplastic expansion and storage capacity of SAT. As discussed above, FDR are characterized by this inability, leading to inappropriately expanded “obese” SAT cells and reduced insulin sensitivity, even in normal-weight individuals (15, 266).

A likely mechanism for the limited expandability of SAT is reduced recruitment and differentiation of precursor cells. Consistent with this idea, our laboratory has shown in a number of studies that individuals with inappropriate expansion of their SAT adipocytes are characterized by reduced recruitment and adipogenic differentiation of their stromal vascular precursor cells (97, 98, 127). This concept is also supported by a direct study of adipocyte turnover in humans. Arner et al. (14) showed that total adipocyte number and adipocyte size in SAT are negatively correlated, and that the number of new adipocytes generated was significantly reduced in individuals with hypertrophic adipocytes. The mechanism(s) for this is still not clear and under study. However, our laboratory’s recent findings of cross talk between the Wnt-associated molecule WISP2 (WNT1-inducible signaling pathway protein-2), bone morphogenetic protein 4 (BMP-4) and its inhibitors, and zinc finger protein 423 (ZNF423) in regulating adipocyte differentiation strongly support the importance of these factors (87, 95, 107), as discussed later.

The origin of progenitor cells in the adipose tissue remains unclear. The presence of many multipotent stem cells in the adipose tissue stroma is well established (179, 330), and results from animal models suggest that adipocytes may arise from different progenitor populations throughout life (135). Recent studies in mice have also shown that the molecular mechanisms involved in multipotent progenitor cell commitment and differentiation differ between developmental and obesogenic expansion of the adipose tissue and that Akt/PKB activation plays a central role (132). It was also recently shown that another source for adipocyte development in human SAT are cells originating from the bone marrow or peripheral blood stem cells, although the contribution from this source seems to be small in lean individuals. A better understanding of the origin of precursor cells and the mechanisms regulating adipocyte precursor

cell recruitment in human physiology under excess caloric load is an important next step in our understanding of how impaired ability to recruit SAT precursor cells is connected to metabolic complications.

F. SAT Adipose Tissue Expansion Is Genetically Regulated

The individual ability to expand the adipose tissue is likely dependent on epigenetic, genetic, and environmental factors (269). Already in the early 90’s, Bouchard et al. (35) demonstrated that the way the adipose tissue responds to excess energy intake has an important genetic component. However, we are still far from understanding the genetic regulation of adipose tissue expansion.

Very little is known about the importance of epigenetic regulation, but genomewide association studies (GWAS) and other genetic approaches have identified a large number of susceptibility genes associated with obesity (8). Many of these genes have unknown roles, whereas others have been investigated for their functional relevance in adipogenesis and adipocyte biology (24). Knockdown of some of these genes, brain-derived neurotrophic factor, mitochondrial carrier homolog 2, neural growth regulator 1, and transmembrane protein 18, inhibited adipocyte differentiation, whereas others had no effect. Interestingly, transmembrane protein 18 is one of the strongest predictors of obesity, besides the *FTO* gene (253, 270).

Recently, a meta-analysis of genomewide association studies identified 68 loci that associate with body fat distribution, suggesting that the ability to expand different adipose tissue depots is, at least partly, genetically determined through genes located in the vicinity of the identified loci (261). A second study expanded on these findings and demonstrated that many of these candidate genes were involved in adipocyte morphology (adipocyte cell size) and function (67), which eventually contribute to adipose tissue distribution (16, 233).

Genetic variants associated with insulin resistance or specifically with adipogenesis have also been shown to be associated with increased risk of developing T2D. In an extensive study of known peroxisome proliferator-activated receptor- γ (PPAR- γ) variants, it was shown that functional mutations (in contrast to nonfunctional polymorphisms) leading to impaired adipogenesis were associated with around eightfold increase in T2D (192). Furthermore, genetic variants of insulin resistance, including in the insulin receptor substrate-1 (IRS-1), were also associated with “partial lipodystrophy,” implicating a reduced SAT (187). Together, these recent findings strongly support the causal associations between impaired adipogenesis and insulin resistance/T2D.

A further important finding relating genetic inheritance to ability to recruit new adipose cells and expand the SAT came from our studies of individuals with a family history of T2D. FDR were shown to have larger waist circumference and disproportionately larger SAT adipocytes for a given BMI compared with matched individuals without known heredity for T2D or having heredity for being overweight and/or obesity (15, 266). Thus a high genetic risk for T2D, be it FDR or individuals carrying genetic markers for insulin resistance, is associated with evidence of impaired SAT adipogenesis supporting direct causality. Interestingly, a recent study investigating whether a family history of T2D is associated with adipocyte hypertrophy in the abdominal and femoral SAT showed no association for the femoral adipose tissue, whereas there seems to be a sex difference in the association in abdominal adipose tissue after adjusting for age and body fat distribution (13).

Using the population-based METSIM (Metabolic Syndrome in Men) study (275), our laboratory has also shown that a family history of T2D is associated with an increased risk of becoming overweight and obese. Furthermore, it also increases the susceptibility of ectopic lipid accumulation and development of T2D, but, surprisingly, the increased risk was not altered by adjusting for 43 known major diabetes risk genes (50). Thus, although there are clearly important genes contributing to SAT cellular expansion and diabetes risk, they do not seem to be part of the currently identified genetic risk markers for T2D.

III. COMMITMENT AND DIFFERENTIATION OF PREADIPOCYTES ARE CRITICAL FOR ADIPOSE TISSUE DEVELOPMENT AND FUNCTION

A. Commitment and Early Differentiation of Preadipocytes

The stromal vascular fraction from the adipose tissue contains heterogeneous cell populations, such as mesenchymal stem cells (MSC), committed preadipocytes, endothelial cells, T and B cells, mast cells, macrophages, and pericytes. Adipose-derived MSCs are multipotent and, depending on the cell milieu and external/internal cues, have the potential to undergo commitment and differentiation into adipocytes, osteocytes, myocytes, and chondrocytes (reviewed by Ref. 283). The first critical event for progenitor cells to become mature, functional adipocytes is to enter the commitment pathway, which is under the regulation by BMP-4 in human cells, to become committed preadipocytes, followed by their differentiation and maintained state of functional adipose cells.

B. Canonical and Noncanonical WNT Signaling

The wingless-type mouse mammary tumor virus integration site family (WNT) signaling is a highly conserved pathway found in several organisms and regulates cell fate determination, proliferation, and differentiation. WNT ligands, 19 family members in total, are secreted glycoproteins acting in both an autocrine and paracrine manner and signal through the canonical (β -catenin dependent) and noncanonical/alternative (β -catenin independent) WNT pathways. Furthermore, the canonical WNT pathway has been shown to be highly active in MSCs, regulating cell fate by both proosteogenic and anti-adipogenic activities, where the cytosolic β -catenin protein exerts a central role (reviewed by Ref. 167). In the absence of WNT ligands, cytosolic β -catenin is recruited to the degradation complex consisting of AXIN, the adenomatous polyposis coli protein, and glycogen synthase kinase-3 β . This allows the ubiquitination and proteasomal degradation of cytosolic β -catenin, repressing the WNT signaling pathway, and allowing the cells to undergo adipogenic commitment. On binding of WNT ligands to frizzled receptors and low-density lipoprotein-receptor-related protein-5 and -6 (LRP5/6) coreceptors, the degradation complex is inactivated, which results in stabilization and hyperphosphorylation of β -catenin. β -Catenin then translocates into the nucleus, where it binds to the lymphoid enhancer binding factor/T-cell-specific transcription factors to activate WNT target genes, inhibiting adipogenesis by suppressing CCAAT/enhancer binding protein- α and PPAR- γ (reviewed by Ref. 55). Lately, it has become evident that also noncanonical WNT signaling pathways influence the activity of CCAAT/enhancer binding proteins and PPAR- γ in MSCs, but these noncanonical WNT signaling pathways are still poorly defined. However, recently, Park et al. (224) showed that Yes-associated protein (YAP)/transcriptional coactivator with a PDZ-binding domain (TAZ) are important regulators of the WNT pathway, involving both the alternative WNT5A/B and the classic canonical WNT3A ligand. On activation, YAP/TAZ translocate to the nucleus to bind TEAD (TEA domain family member-binding domain) family transcription factors, which will induce the expression of secreted WNT antagonists, such as the BMP-4, Dickkopf 1 (DKK1), and WNT5A/B, inhibiting the WNT/ β -catenin signaling pathway (224).

The canonical WNT ligand, WNT10B, is highly expressed in MSCs and has been shown to rapidly decrease during initiation of differentiation. This favors expression of adipogenic genes, since WNT10B is a potent inhibitor of adipogenesis (reviewed by Ref. 155). DKK proteins inhibit WNT activity and antagonize canonical WNT signaling by binding to LRP5/6, whereas secreted soluble Frizzled-related proteins 1 and 2 and WNT inhibitory factor 1 interact directly with WNT proteins (reviewed by Ref. 167). DKK1 is rapidly induced during differentiation of, and secreted by,

human preadipocytes as an autocrine/paracrine regulator. This inhibits WNT signaling in precursor cells, thereby promoting adipogenesis. However, inhibition of the canonical WNT pathway is impaired in precursor cells from individuals with hypertrophic obesity, at least in part due to an inability of adipose precursor cells to commit to the adipogenic lineage and induce DKK1 (100). DKK1 remains expressed and secreted by mature adipocytes, which implicates an autocrine and paracrine regulation by differentiated adipose cells to antagonize the WNT pathway and favor adipogenesis and differentiation (100). How-

ever, merely inhibiting canonical WNT signaling through DKK1 secretion is not sufficient to induce adipogenic commitment of the precursor cells, since this requires a coordinated activation/inhibition of several signaling pathways (FIGURE 2).

C. WISP2, a Secreted WNT-Associated Protein, Is Regulated by BMP-4

WNT1 inducible signaling pathway protein 2 (WISP2/CCN5) belongs to the connective tissue growth factor/cys-

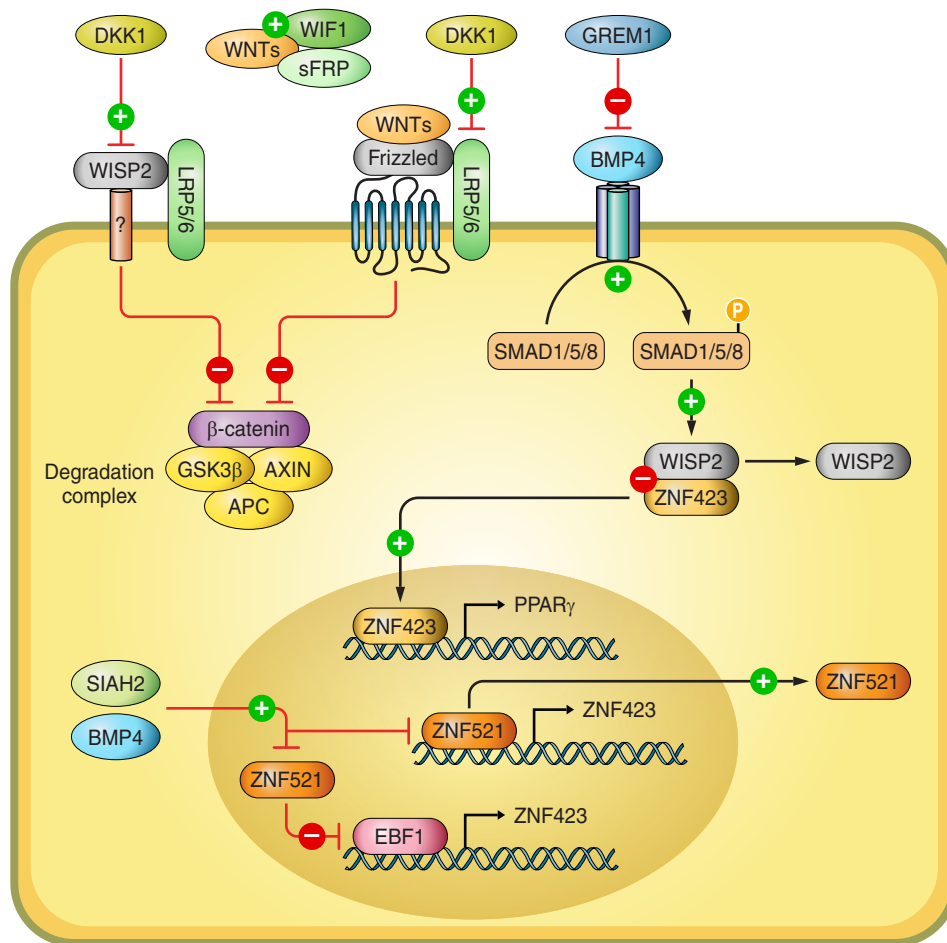


FIGURE 2. Molecular regulation of adipogenic commitment and early differentiation of mesenchymal progenitor cells. A coordination of different signaling molecules and pathways, both pro- and anti-adipogenic, is required for a progenitor cell to enter the adipogenic lineage. Important for commitment is dissociation of the inhibitory complexes zinc finger protein 521 (ZNF521)/early b-cell factor (EBF1) and WNT1-inducible signaling pathway protein 2 (WISP2)/ZNF423, since these complexes prevent ZNF423 and EBF1 from entering the nucleus and activating the transcription of peroxisome proliferator-activated receptor (PPAR)_γ, the master regulator of adipogenic differentiation. Bone morphogenetic protein-4 (BMP-4), the effect of which is regulated by the BMP-inhibitor gremlin-1 (GREM1) in human cells, is a key regulator of adipogenic commitment and induces SMAD1/5/8 phosphorylation, which leads to dissociation of the WISP2/ZNF423 complex and nuclear entry of ZNF423. Recently, SIAH2 was shown to promote early commitment by influencing ZNF521 protein stability and, thereby, transcriptional activation of ZNF423. The proadipogenic factors Dickkopf 1 (DKK1), WNT inhibitory factor 1 (WIF1), and soluble Frizzled-related protein (sFRP) inhibit the WNT/WISP2 signaling pathway by allowing proteasomal degradation of β-catenin, a necessity for the cells to undergo adipogenic commitment. Thus it is the balance between these opposing signals that determines the fate of a progenitor cell. Green circles represent positive effects on adipogenic commitment and early differentiation; red circles represent inhibitory effects on adipogenic commitment and early differentiation. APC, adenomatosis polyposis coli; GSK-3β, glycogen synthase kinase-3β; LRP5/6, lipoprotein-receptor-related protein-5 and -6.

teine-rich 61/nephroblastoma overexpressed (CCN) family and is induced by the canonical WNT signaling pathway (reviewed by Ref. 239). Current data strongly indicate that WISP2 has an important role in the regulation of adipogenesis. Waki et al. (308) showed that the WNT3a-induced expression of WISP2 was repressed in 3T3-L1 by harmine, a small molecule that targets PPAR- γ in preadipocytes and reversed the inhibitory effect of WNT3a on adipocyte differentiation. Recently, our laboratory identified WISP2 as a novel secreted adipokine, which was markedly upregulated in human SAT characterized by hypertrophic obesity and insulin resistance (107). In a secretome analysis of human SAT, comparing lean and obese patients, WISP2 came out as a top candidate being upregulated in obesity (66). WISP2 is most highly expressed in SAT, primarily in MSCs, fibroblasts, and preadipocytes, and acts both intra- and extracellularly (87, 313). In addition, our laboratory has found that secreted WISP2 has the potential to enhance the proliferation of MSCs in both white (WAT) and brown adipose tissue (BAT) (88). Secreted WISP2 is an atypical WNT ligand, which activates the canonical WNT pathway through an unknown cellular signaling pathway involving LRP5/6. This allows the cells to proliferate and remain in an uncommitted state. However, the secreted WNT antagonist DKK1 counteracts this effect of WISP2, and activation of BMP-4 signaling directly inhibits WISP2 transcriptional activation and secretion and enhances adipogenic commitment (87).

In MSCs, cytosolic WISP2 forms a complex with ZNF423 (known as Zfp423 in mouse), a nuclear protein belonging to the family of Kruppel-like C2H2 zinc finger proteins, which is highly expressed in the stromal vascular fraction of the adipose tissue, including preadipocytes (92, 107). Interestingly, ZNF423 has been shown to regulate PPAR- γ expression in undifferentiated preadipocytes via the BMP/SMAD signaling pathway, a key regulator of adipogenic commitment of the precursor cells (92). The WISP2/ZNF423 complex is sequestered in the cytosol and the transcriptional activity of ZNF423 is inhibited (107). However, BMP-4, a member of the transforming growth factor- β superfamily and a key regulator of MSCs adipogenic commitment and differentiation, was found to dissociate the WISP2/ZNF423 complex via SMAD1/5/8 activation, thereby allowing nuclear translocation of ZNF423 and the induction of PPAR- γ (38, 100, 107). Furthermore, BMP-4 is highly expressed in, and secreted by, mature adipocytes, probably as a feedback regulator aimed at recruiting new precursor cells into the adipogenic lineage to prevent hypertrophic cell expansion (95). Surprisingly, we found that mRNA, protein levels, and secretion of BMP-4 are markedly increased in SAT adipose cells in hypertrophic obesity, suggesting that the precursor cells are resistant to secreted BMP-4 (95). The effect of BMP-4 is regulated by endogenous BMP-inhibitors, including gremlin-1 (GREM1), chordin, chordin-like-1, and noggin. We identified GREM1 to be a secreted and powerful inhibitor of BMP-4 in human

preadipocytes, the secretion of which was highly upregulated in SAT from individuals with hypertrophic obesity (95). Based on this finding and associated mechanistic results, our laboratory suggested that obese, hypertrophic SAT is characterized by a BMP-4 resistance, which prevents its positive feedback recruitment of new adipose cells (95). Our laboratory has also provided evidence for BMP-4 resistance in the adipose tissue of obese mice, but, in mice, it is a consequence of increased noggin (119), rather than GREM1, as in humans (FIGURE 2).

Interestingly, ZNF423 was recently shown to also be important in differentiated adipose cells, preserving the white phenotype of WAT by binding to early b-cell factor (EBF) 2 and inhibiting the white-to-beige phenotypic switch (256). However, also the EBF2/ZNF 423 complex was dissociated by both BMP-4 and BMP-7, which allowed the cells to assume a beige/brown rather than the classic white phenotype (256). Our laboratory has also previously shown that maintained BMP-4 signaling during adipogenic differentiation favors the precursor cells to assume a beige/brown oxidative phenotype (95), as later discussed.

D. ZNF 521—A Repressor of Commitment

ZNF521, like ZNF423, is a transcription cofactor with 30 zinc finger motifs and is shown to be highly abundant in hematopoietic stem and early progenitor cells and decreasing rapidly on differentiation (34). However, ZNF521 is also highly expressed in murine adipogenic progenitor cells, such as MSCs, and has been shown to act upstream of the preadipocyte commitment factor ZNF423 (142). ZNF521 directly represses the expression of ZNF423 by binding to its promoter and intronic enhancer regions (4) and/or by binding to the proadipogenic factor EBF1 inhibiting its transcriptional activity and associated repression of ZNF423 (7, 136, 142). A negative feedback loop was also identified in which EBF1 binds to an intronic enhancer of ZNF521, thereby inhibiting its expression. Recently, the *Drosophila* seven-in-absentia homolog 2, an E3 ubiquitin protein ligase, was shown to promote early steps of the commitment via degradation of ZNF521 in a BMP-4-dependent manner, influencing ZNF423 gene expression (147). However, the role and importance of ZNF521 in human precursor cell commitment remains to be characterized (FIGURE 2).

E. Adipocyte Maturation

PPAR- γ is the master regulator of adipogenesis and required for adipocyte differentiation, insulin sensitivity, lipogenesis, as well as adipocyte survival. The process of in vitro adipocyte differentiation from preadipocytes has been extensively studied, and results from these studies show a complex picture involving many different transcription fac-

tors and coactivators that finally lead to PPAR- γ activation (74, 242, 283). The most commonly used model to study preadipocyte differentiation is the 3T3-L1 mouse cell line. These cells are induced by a differentiation cocktail activating the insulin-like growth factor-1, glucocorticoid, and cAMP pathways simultaneously, eventually resulting in lipid-accumulating and insulin-sensitive cells. The early steps involve several rounds of mitosis, so-called clonal expansion, where induction of the transcription factor CCAAT-enhancer-binding protein (C/EBP)- β by cyclic AMP response element-binding protein and phosphorylation by mitogen-activated protein kinase are crucial steps (284, 332). Later, C/EBP- β is also phosphorylated by glycogen synthase kinase-3 β driving the adipogenic process forward (282). Although C/EBP- β is critical for *in vitro* adipogenesis, C/EBP- β knockout in mice has little effect on adipose tissue development *in vivo*, and only double knockouts of C/EBP- β and its family member C/EBP- δ result in a significant phenotype, suggesting redundancy of these two transcription factors (281). C/EBP- α , together with PPAR- γ , functions as a pleiotropic transcriptional activator promoting further adipogenic differentiation. The promoter of these two genes contains C/EBP regulatory elements through which C/EBP- β coordinately induces their expression (56, 57). When activated, C/EBP- α and PPAR- γ induce and maintain the expression of key adipogenic genes, such as GLUT-4, fatty acid binding protein (FABP)-4, and adiponectin, which are central for proper adipocyte function, including their insulin responsiveness (summarized in **FIGURE 3**).

The importance of PPAR- γ for adipose tissue formation *in vivo* has been studied in mouse models. Whereas whole body PPAR- γ knockout is embryonically lethal, it has been

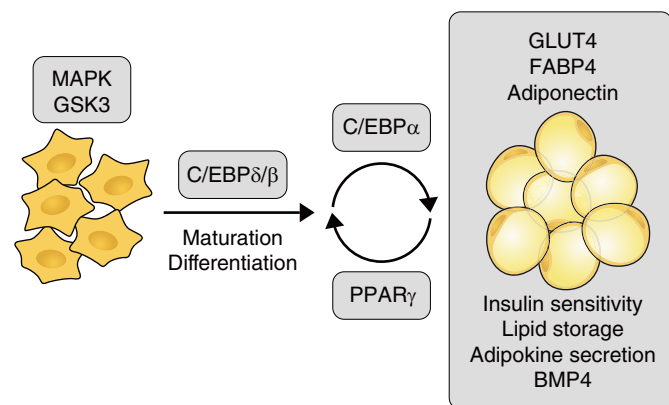


FIGURE 3. Adipocyte differentiation is a complex and tightly regulated process. *In vitro* adipocyte differentiation is a complex process involving many different signaling pathways that finally lead to the activation of CCAAT-enhancer-binding protein (C/EBP)- α and peroxisome proliferator-activated receptor (PPAR)- γ . These transcription factors induce and maintain the expression of key adipogenic genes, such as glucose transporter-4 (GLUT-4), fatty acid binding protein (FABP)-4, and adiponectin, which are central for proper adipocyte function. BMP-4, bone morphogenetic protein-4; GSK-3, glycogen synthase kinase-3.

shown that embryonic stem cells lacking PPAR- γ cannot contribute to adipose tissue formation (20, 241). In line with these findings, adipose tissue-specific deletion results in reduced adipose tissue formation, reduced adipogenic gene expression, and ultimately ectopic lipid accumulation. In addition, mutations of PPAR- γ in human cells support the important role of this transcription factor also for human adipose tissue function (294). In rare families with lipodystrophy, loss-of-function mutations of the PPAR- γ gene, which impair adipocyte differentiation *in vitro*, have been found to segregate with lipodystrophy, associated insulin resistance, and T2D (5, 21). Majithia et al. (192) identified several gene variants of PPAR- γ in the normal population that impair adipocyte differentiation *in vitro* and that are associated with a substantial risk of developing T2D, further supporting the importance of PPAR- γ for adipocyte differentiation and whole body insulin sensitivity.

Given the central role of PPAR- γ in adipogenesis, it is not surprising that ligands activating this transcription factor promote adipogenesis. The search for an endogenous ligand for PPAR- γ is ongoing, but so far this remains an unresolved question. The most well-known synthetic PPAR- γ ligands are the anti-diabetic drugs, the TZDs. There are potentially several mechanisms by which TZDs improve insulin sensitivity and lower blood glucose, but most evidence suggests that adipose tissue is the primary tissue responsible for their therapeutic effects (51, 111). By activating PPAR- γ , TZDs stimulate differentiation of existing committed preadipocytes, increasing the body's capacity to store excess fat in the SAT, while reducing ectopic lipid accumulation in other tissues, such as liver and skeletal muscle (294). In parallel to the effects on adipocyte differentiation, TZDs also affect secretion of certain adipokines, such as adiponectin, from the adipose tissue known to enhance insulin sensitivity in other tissues (218, 329).

Although PPAR- γ is crucial for adipose tissue formation and function, it has been shown that C/EBP- α activation is necessary for adipocyte insulin sensitivity and not merely for maintaining PPAR- γ expression (74). The importance of C/EBP- α for *in vivo* adipose tissue function has been demonstrated in different mouse models. Similar to PPAR- γ , global C/EBP- α knockout is lethal due to severe effects on hepatic glucose production. C/EBP- α depletion in all tissues, except liver, has shown that C/EBP- α is necessary for WAT, but not BAT, adipose tissue formation (180).

F. Adipose Tissue Hypertrophic Expansion and Dysfunction Are Characterized by Impaired Adipocyte Differentiation

Adipocyte hypertrophy is a marker of adipose tissue dysfunction and perturbation of many biological functions. Several investigations have shown alterations in biological pathways related to hypoxia, inflammation, and angiogen-

esis, but also pathways related to adipocyte differentiation and function are impaired (94). The main function of the WAT is to store and release lipids as needed, and alterations of either process are associated with metabolic complications. In obesity, the basal rate of lipolysis in SAT is increased and a determinant of variations in insulin sensitivity across a wide range of fat mass (81). The increased basal lipolysis is suggested to be a consequence of adipocyte hypertrophy-associated inflammation, which also involves endoplasmic reticulum (ER) stress and hypoxia, at least in mouse models. In human SAT, two important regulators of lipolysis and the ability of insulin to inhibit lipolysis are TNF- α and IL-6. IL-6 is highly secreted by human SAT cells, is increased in hypertrophic obesity, and is an important obesity-associated insulin antagonist (245), enhancer of lipolysis, and liver fat accumulation (228). TNF- α also plays an important role, both through its antagonistic cross talk with insulin, as well as by enhancing the release of other cytokines, including IL-6 (16, 245). It has also been shown that genetic or pharmacological antagonism of the adipose tissue lipolytic enzyme, the hormone-sensitive lipase, results in reduced accumulation of lipids in ectopic tissues and improved insulin sensitivity (58, 81).

Adipocyte hypertrophy is associated with reduced expression of adipocyte differentiation markers in both nonobese and obese insulin-resistant individuals (152, 202, 322) and also with alterations in expression and secretion of important adipocytokines (264). For instance, secretion of the insulin-sensitizing adipokine, adiponectin, has consistently been reported to show a negative association with subcutaneous adipocyte hypertrophy (11, 106, 264). Furthermore, the key insulin-stimulated effector protein regulating glucose uptake, GLUT-4, is reduced in adipose tissue, but not in muscle, in subjects with impaired insulin sensitivity and T2D (139, 240) and associated with adipocyte hypertrophy (47).

Glucose uptake and metabolism are crucial for normal adipocyte function and tightly coupled to insulin sensitivity by regulating *de novo* lipogenesis (DNL) and endocrine function, as shown in animal studies (1, 114, 259, 326). Overexpression of GLUT-4 selectively in adipose tissue results in enhanced whole body glucose uptake and metabolism (259), and adipose tissue-specific deletion of GLUT-4 results in a similar degree of insulin resistance as deleting GLUT-4 in the skeletal muscle (1). This finding was surprising, since the amount of glucose taken up by the adipose tissue is minor compared with that taken up by skeletal muscle, which is ~70% of postprandial glucose uptake. The metabolic effects resulting from modulation of GLUT-4 in the adipose tissue is, at least in part, orchestrated by the transcription factor carbohydrate-responsive element-binding protein (114), which regulate DNL, and production of a novel family of insulin-sensitizing lipids, the branched fatty acids of hydroxy-fatty acids (FAHFAs)

(326). Reduced DNL has also been shown in the adipose tissue of patients with insulin resistance and adipocyte hypertrophy (238), but alterations in hepatic DNL have also been observed, as further discussed later (10).

G. Beige and Brown Adipocytes

In addition to the classic WAT, active BAT, which is present in specific sites and of which a prevailing amount is negatively correlated to obesity, was identified in adult humans by four independent publications in 2009 (64, 251, 301, 306). Furthermore, studies designed to increase BAT activity in humans suggest that there is increased peripheral glucose disposal and insulin sensitivity (31, 173). The improved metabolic response under these conditions can be attributed to both BAT and skeletal muscle, in which the importance of BAT seems to increase with cold acclimation (30). Due to its energy-dissipating properties and effects on metabolism, human BAT has been a major focus for metabolic and anti-obesity research in recent years (27). However, since much of our knowledge about BAT is based on mouse studies, a better understanding of BAT in human physiology is necessary. Although BAT must here be mentioned as an important part of the adipose tissue, the current understanding of the role of BAT is not the focus of this review, and the interested reader is referred to several recent publications (183, 305, 310).

In addition to the classic BAT, brown-like/oxidative (beige) adipocytes have also been found in WAT depots. Brown and beige adipocytes share many characteristics, including energy dissipation capabilities. They do, however, originate from distinct cellular lineages. While brown adipocytes originate from precursor cells with common ancestry to myocytes, beige adipocytes seem to develop from similar precursor cells as white adipocytes (262, 310). Several studies have also suggested that mature white adipocytes are plastic and may transdifferentiate into beige adipocytes (77, 117, 243). Oxidative beige adipocytes can be induced by several means, including chronic cold exposure and certain types of exercise, but also by hormones and metabolites, as summarized in several reviews (140, 262, 315).

In addition to these factors, recent studies have identified the BMPs as of particular interest, since they are essential for the recruitment and commitment of mesenchymal precursor cells into adipocyte lineage. While BMP-7 is a regulator of brown adipogenesis (298), BMP-4 is particularly important for white adipogenesis (38, 100). However, our laboratory has recently shown that maintained BMP signaling during adipogenic differentiation of human precursor cells, either through maintained exposure to BMP-4 itself, or by silencing the important secreted BMP-4 antagonist GREM1, induced the cells to assume a beige/brown oxidative phenotype (95). GREM1 is secreted by human preadipocytes and upregulated in hypertrophic obesity (95).

These findings suggest that increased GREM1 can contribute to both the development of hypertrophic obesity and the associated reduced browning of the SAT. In support of this concept, our laboratory recently showed that BMP-4 gene therapy in fully mature mice, leading to increased circulating BMP-4 levels, prevented high-fat-diet-induced obesity and increased energy expenditure by pronounced browning of SAT (119). Taken together, these results show that BMP-4 and its signaling pathways play a critical role in regulating mesenchymal precursor cells to both undergo adipogenic commitment and to assume a mainly white or beige/brown oxidative phenotype. The latter requires maintained BMP-4 signaling during differentiation, while the early intermittent increase leads to precursor cell commitment and white adipose cell development.

The inducible nature of beige adipocytes and its metabolic and weight-reducing effects have recently attracted attention as a new therapeutic target for obesity and T2D. Turning the large SAT into a beige, energy-dissipating tissue may certainly have an important impact on body weight, whole body glucose, and lipid metabolism. So far, however, most evidence of the effect of SAT being on whole body phe-

notype is derived from animal studies, and the physiological importance in humans need to be further addressed.

IV. ADIPOSE TISSUE ANGIOGENESIS

A. Angiogenesis—A Critical Regulator of Adipose Tissue Expansion and Function

Normal adipose tissue remodeling and expansion must be accompanied by parallel changes in its large and dynamic capillary network. These changes occur through close cross talk between the vascular components (endothelial cells, pericytes and smooth muscle cells) and the adipose tissue components (pre-/adipocytes, mesenchymal stromal precursor cells, fibroblasts, macrophages, and other inflammatory cells). Appropriate cross talk should promote the recruitment, commitment, and differentiation of new preadipocytes, together with the formation of new vessels, which will supply the cells with oxygen and nutrients (44, 54, 61) (FIGURE 4).

It is known that angiogenesis is under the tight control of several proadipogenic and angiogenic factors, such as the

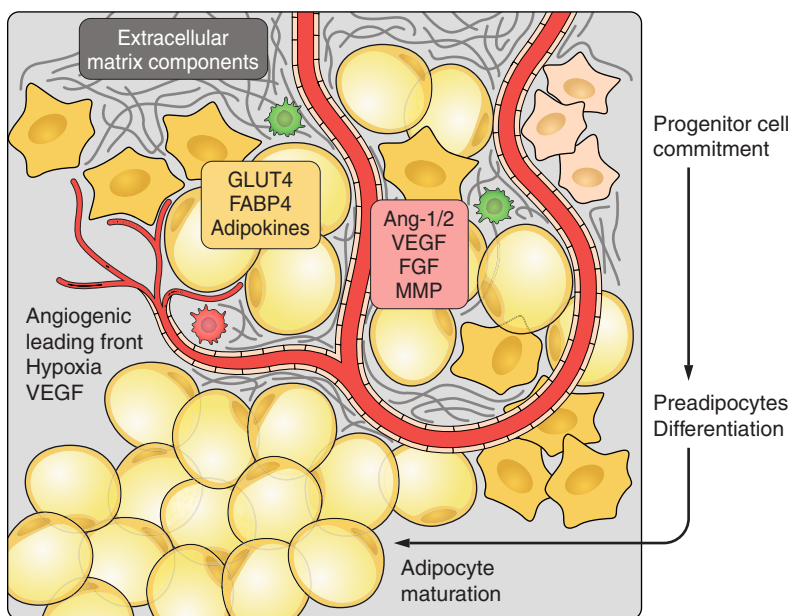


FIGURE 4. Adipose tissue remodeling. Adipose tissue remodeling requires the concomitant expansion of its capillary network and the coordination of adipogenesis and angiogenesis. Adipogenesis relies on the presence of precursor cells that have undergone adipogenic commitment, allowing them to differentiate into new functional mature adipocytes. These events are highly regulated by a combination of different factors, including zinc finger protein 423 (ZNF423), bone morphogenetic protein-4 (BMP-4), and peroxisome proliferator-activated receptor (PPAR)- γ . The induction of adipose tissue angiogenesis is also regulated by several factors, including the VEGF family of growth factors and angiopoietins, as well as the production of extracellular matrix (ECM) components. Endothelial cells proliferate in response to VEGF and angiopoietins, resulting in the expansion of a new capillary. The signals that coordinate the regulation of angiogenesis and adipogenesis can originate from the adipocytes or other nonadipocyte cells, as well as cells from the preexisting vessels. Cross talk between angiogenesis and adipogenesis is fundamental for the normal expansion of the adipose tissue, but detailed sequences and mechanisms are still under debate. FABP-4, fatty acid binding protein-4; FGF, fibroblast growth factor; GLUT-4, glucose transporter-4; MMP, matrix metalloproteinase. [Parts of these images are from Servier; Servier Medical Art by Servier is licensed.]

vascular endothelial growth factor A and B (VEGF-A, VEGF-B), fibroblast growth factor-2, angiopoietins 1–2 (Ang-1 and Ang-2), leptin, adiponectin, and hypoxia inducible factor-1 (HIF-1). Angiogenesis is also regulated by anti-angiogenic factors, including thrombospondin-1, angiostatin, or other modulators, such as plasminogen activator inhibitor-1. These products are secreted by both adipocytes and stromal-vascular cells, which reciprocally control their functions via paracrine/autocrine mechanisms (79, 80). However, the detailed coordination of these mechanisms is largely unknown (FIGURE 5).

Angiogenesis and adipogenesis are viewed as parallel processes, where the expansion of the capillary bed occurs in conjunction with expanding regional adipose depots as a result of the cell component interactions. However, it is also possible that one of these two processes precedes the other. Is the adipose tissue expansion dependent on angiogenesis, or is it rather the adipose tissue expansion that drives the recruitment of vessels?

B. Angiogenesis—A Consequence of Hypoxia in the Adipose Tissue?

The adipose tissue expands by a combination of adipocyte hypertrophy of preexisting adipocytes and hyperplasia. Adipocyte hyperplasia requires the formation of new vessels, which, in turn, also promotes adipocyte differentiation. However, in hypertrophic expansion, capillaries primarily undergo remodeling and expansion.

The hypertrophic growth of the adipose tissue is not accompanied by a similar expansion rate of angiogenesis, generating an imbalanced production of overlapping angiogenic factors and inhibitors contributing to a dysfunction of the tissue (43, 54) (FIGURE 5).

It has been proposed that, as the adipose tissue expands and exceeds the vascular supply, clusters of hypertrophic adipocytes become distant from the vasculature, creating local hypoxic regions and influencing adipose tissue function (122, 225, 296).

Hypoxia has been shown to be an important stimulus of vascular growth due to the stabilization of the HIF-1 (122, 234, 246). In the presence of an adequate oxygen supply, HIF-1 α is hydroxylated in proline residues and degraded, while, in hypoxia, proline-hydroxylases are inhibited, leading to stabilization of HIF-1 α and activation of genes required for angiogenesis, including VEGFs, Ang-1 and Ang-2, matrix metalloproteinases, leptin, and plasminogen activators (45, 54, 83, 122, 319).

Although hypoxia response alone is not enough to promote angiogenesis, several studies in animal models confirm that hypoxia is one of the initiators of angiogenesis in vitro and in vivo (122, 323). However, the evidence of WAT hypoxia in human obesity is controversial. Some publications support that WAT becomes hypoxic as it expands in obesity (138, 226), whereas others did not find this (118, 296), or even suggest that the adipose tissue was hyperoxic (84, 296). There is no clear explanation for these divergent re-

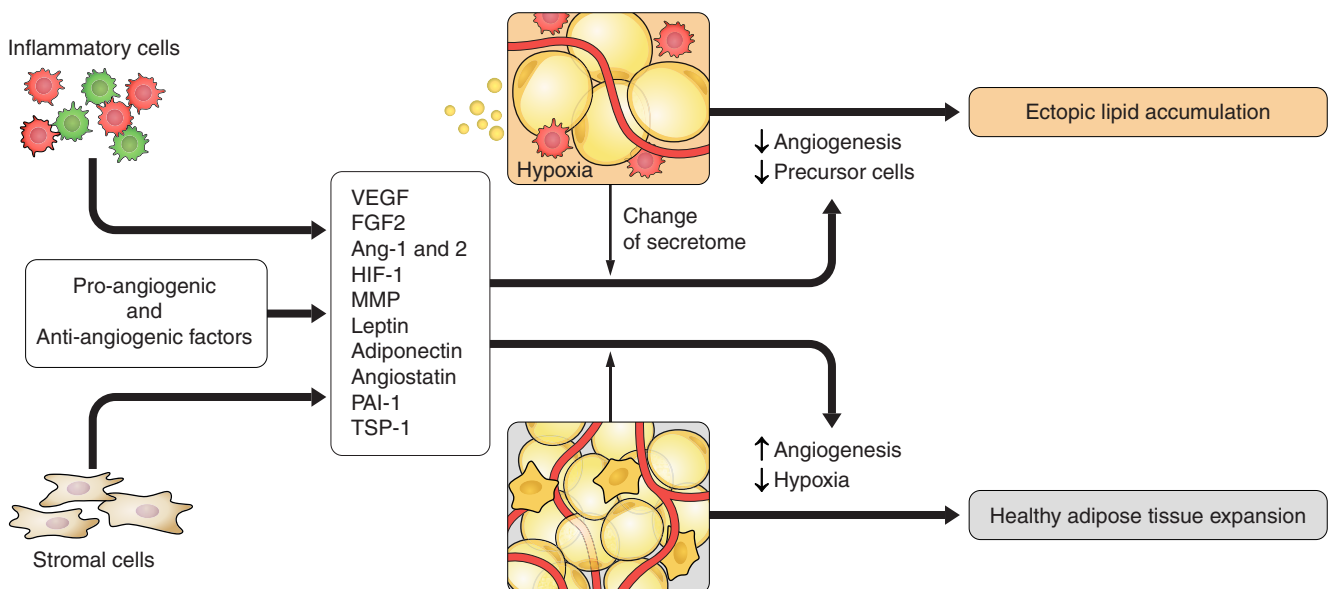


FIGURE 5. Coordination of adipose tissue angiogenesis and tissue expansion. Adipose tissue hyperplastic expansion (*bottom*) is coordinately regulated with angiogenesis, leading to a healthy adipose tissue expansion and low hypoxia. In contrast, hypertrophic expansion of the adipocytes (*top*) is not coordinately regulated, leading to excessive hypoxia, changes in the secretome, metabolic stress, and ectopic fat accumulation. Ang-1 and -2, angiopoietins 1 and 2; FGF-2, fibroblast growth factor-2; HIF-1, hypoxia inducible factor-1; MMP, matrix metalloproteinase; PAI-1, plasminogen activator inhibitor-1; TSP-1, thrombospondin-1.

sults, but methodological differences are likely to be important issues. However, on balance, it appears that the low oxygenation theory is too simple, and that the regulation of the microvasculature by other factors, at least in human obesity, is essential.

In fact, this also seems to be the case in mouse models. Overexpression of a constitutively active form of HIF-1 α did not induce an angiogenic response, but promoted a fibrotic response and increased local inflammation (105). Furthermore, disruption of HIF-1 α or HIF-1 β in adipose tissue reduced lipid accumulation, protected from high-fat-diet-induced obesity and insulin resistance (134, 172). These studies indicate that adipocyte hypoxia and induction of HIF-1 α primarily lead to fibrosis and an inflammatory response rather than a compensatory proangiogenic response, and that activating the classic hypoxia response alone is not sufficient to improve angiogenesis in obesity.

Hypoxia-induced activation of inflammatory pathways in the adipose tissue in obesity promotes insulin resistance and lipolysis (78, 82, 90, 246, 293). Activation of inflammation changes the secretion pattern of the tissue favoring proinflammatory molecules, chemo-attractants, and growth factors and diminishes the expression of anti-inflammatory adipokines. Apart from effects on adipocyte metabolism, secretion of these factors can also influence tissue angiogenesis and macrophage recruitment (90, 296, 302).

Angiogenesis can also be induced in the absence of tissue hypoxia by other mechanisms. A recent report suggests that IL-19 can promote angiogenesis in the absence of hypoxia via a direct effect on vascular cells and an indirect effect by activating macrophages (141). Furthermore, it was recently reported that a potent HIF-1 inhibitor, echinomycin, completely inhibited adipogenesis in 3T3-L1 cells, as well as in high-fat-diet mice. This inhibitory effect was independent of HIF-1 pathways (318).

C. Activation of Angiogenesis by VEGF

Balanced endogenous pro- and anti-angiogenic factors normally regulate angiogenesis and endothelial cell growth. However, in pathological angiogenesis, as, for instance, in cancer, a shift occurs in the balance of regulators, where the expression and/or activation of proangiogenic factors such as VEGFs and its receptors are significantly upregulated (46).

The VEGF family of growth factors is crucial for endothelial cell proliferation, migration, and survival and thus angiogenesis (54, 110, 176, 207). Most of VEGF actions in adipose tissue are mediated by VEGF-A through the VEGF receptor-2 (VEGFR2), although other VEGFs can also play a role in the angiogenic process (54).

A study of angiogenesis and adipogenesis in *db/db* mice demonstrated that the administration of anti-VEGF antibodies inhibits angiogenesis and also the formation of adipogenic/angiogenic cell clusters. These and other data support the close cross talk between adipogenesis and angiogenesis, and that VEGF is a key mediator of that process (213). There is evidence of similar effects with anti-VEGF antibodies also in human tissue (171).

Adipose tissue-specific VEGF knockout mice have reduced vessel density, severe inflammation, and apoptosis within adipose tissue and develop severe insulin resistance and glucose intolerance when fed a high-fat diet (279). In contrast, transgenic mice overexpressing VEGF-A showed elevated vascularization in both BAT and WAT, were protected from high-fat-diet-induced hypoxia and inflammation, and also had enhanced BAT function and improved insulin sensitivity (71).

Local overexpression of VEGF-A, specifically in adipose tissue of obese mice, was also reported to improve vascularization, mitigate inflammation, and improve systemic glucose metabolism (277, 278). The WAT contained less hypoxic areas, and HIF-1 α protein levels were significantly reduced compared with that in wild-type animals (71, 277).

Although not all data have been consistent, the current concept is that induction of VEGF is important during adipose tissue expansion, allowing sustained angiogenesis. On the other hand, VEGF inhibition may be useful in the context of preexisting adipose tissue dysfunction and obesity, inhibiting inflammation and fibrosis and improving insulin sensitivity.

However, human studies have also shown that markers of enhanced angiogenesis in SAT are associated with the development of insulin resistance by enhancing lipid storage (148). A positive correlation between circulating VEGF-A levels and BMI has also been reported in a relatively small group of healthy subjects (184). In addition, VEGF-A165b is an inhibitory isoform of VEGF-A that has been described as a novel antiangiogenic factor in human obesity (212).

A recent publication from the same group reported that obesity-induced upregulation of WNT5A controls adipose tissue angiogenesis via modulation of VEGF-A165b expression. Their data suggest that WNT5A negatively regulates adipose tissue angiogenesis via VEGF-A165b in human obesity (144).

The divergent results of the effects and associations with VEGF may be due to its additional effects on metabolism rather than its effects in vascularization. Another VEGF family protein, VEGF-B, has been shown to regulate endothelial cell uptake and transport of fatty acids (102), and, later, the same group demonstrated that the endothelium

can function as an effective barrier for lipid transport, even in the context of obesity and dyslipidemia (103). Inhibition of VEGF-B/VEGFR1 signaling limited ectopic lipid accumulation in muscular tissues, restored peripheral insulin sensitivity and muscle glucose uptake, and preserved islet function.

Different conclusions have been reported in a recent publication showing that VEGF-B induces adipose tissue angiogenesis, leading to beneficial metabolic effects in obese mice (237). The authors describe that the VEGFB/VEGFR1 pathway can be used to recruit the VEGF-A/VEGFR2 proangiogenic pathway in the adipose tissue. VEGF-B increases the bioavailability of VEGF-A for VEGFR2, displacing it from VEGFR1, and, thereby, increasing adipose tissue vascularization, reducing inflammation, and reversing metabolic complications (237).

VEGFR2 can also be activated by non-VEGF ligands with an affinity comparable to that of VEGFA. It has also been reported that VEGFR2 can induce endothelial sprouting, migration, and invasion through the binding of the BMP-4 antagonist GREM1 (206, 274).

D. Angiogenesis and Adipose Precursor Cells

If adipogenesis and angiogenesis are not well synchronized, recruitment and differentiation of adipocyte precursor cells are also impaired, contributing to adipose cell hypertrophy (14, 17, 289).

In agreement with this concept, it has been reported that the adipose niche is located close to the growing vasculature, and that the adipocyte precursor cells may have endothelial origin. Lineage-tracing studies using the preadipocyte marker ZFP423 or the endothelial marker VE-cadherin suggest that white, and also brown, adipocytes originate from endothelial precursor cells (93, 295). However, this view has been challenged when using other endothelial markers (25, 26). Nevertheless, human adipocyte progenitor cells proliferate in response to angiogenic stimuli in conjunction with adipose tissue angiogenesis (204).

BMP-4 plays a key role in regulating adipogenic precursor cell commitment and differentiation. We found BMP-4 to be induced and secreted by differentiated (pre)adipocytes, and BMP-4 secretion is increased in large adipose cells (95). This provides an additional link between expansion of the adipose tissue and angiogenesis, since both BMP-2/4 have been described to mediate proangiogenic effects *in vitro* and *in vivo* (163, 244).

BMP-4 also promotes the proliferation and migration of endothelial cells via stimulation of VEGF-A and Ang-1 and their respective receptors VEGFR2 and TIE2. These effects

increase the proangiogenic activity of endothelial cells (280).

A recent publication reported that BMP-4 overexpression enhances the number of blood vessels in mouse adipose tissue. It also demonstrated an important interplay between angio- and adipogenesis by BMP-4 and that BMP-4 promotes precursor cells to produce proangiogenic factors during differentiation (285). Furthermore, BMP-4 regulates the expression of several micro-RNAs in endothelial cells associated with the modulation of angiogenesis (73).

BMP-2/4 have particularly been studied in relation to angiogenesis, and little is known about potential effects of other BMPs in regulating adipose tissue angiogenesis. However, BMP-4 is a secreted protein by adipose cells and of major importance as a regulator of human SAT precursor cell commitment and differentiation (98). Thus BMP-4 seems well placed as an important coregulator of both adipogenesis and the needed enhancement of angiogenesis.

V. ADIPOSE TISSUE INFLAMMATION

A. Chronic Inflammation Links Hypertrophic Obesity to Insulin Resistance

The last two decades of studies have resulted in numerous solid data identifying chronic low-grade inflammation as an important link between obesity, systemic insulin resistance, and T2D. Serum concentrations of several proinflammatory markers [e.g., C-reactive protein, TNF- α , IL-1 β , IL-6, and monocyte chemoattractant protein-1 (MCP-1)] have been shown to correlate with, or predict, incidents of T2D in humans (174, 272). Furthermore, C-reactive protein has been found to be positively correlated with abdominal adipose tissue accumulation in men (174). Notably, IL-6 (245), IL-1 β (130), and TNF- α can directly inhibit insulin signaling, and targeting these cytokines by neutralizing antibodies has shown some beneficial anti-diabetic effects in clinical studies (49, 159, 205, 215).

At the molecular level, obesity activates the master mediators of inflammation, I κ B-kinase/nuclear factor- κ -light chain enhancer of activated b-cell (IKK- β /NF- κ B) and c-Jun NH₂-terminal kinase (JNK), to induce expression and release of inflammatory markers and mediators. Both IKK and JNK are also known to inhibit insulin action through subsequent serine phosphorylation and inhibition of the insulin signaling adapter proteins, IRS-1/2. This, then, interferes with IRS-insulin receptor coupling, promotes IRS degradation, and reduces downstream insulin signaling. Genetic deletion or pharmacological antagonism of these inflammatory pathways (e.g., high dose of salicylates) have been reported to attenuate obesity-induced inflammation and insulin resistance (160). In fact, antibody therapy tar-

getting IL-1 β was recently shown to reduce the incidence of recurrent cardiovascular events in patients with a history of CVD (236).

Activation of inflammation, specifically in the adipose tissue involving both the innate and adaptive immune systems, is the hallmark of obesity-induced chronic inflammation. Nevertheless, not all obese subjects develop chronic low-grade inflammation. Indeed, dysfunctional SAT with hypertrophic adipose cells, rather than the increased fat mass itself, plays a key role for the induction of chronic low-grade inflammation and insulin resistance in metabolically unhealthy subjects. Extensive studies have revealed essential roles for adipose tissue resident immune cells, particularly macrophages but also other immune cells, and their cross talk with adipocytes in mediating obesity-associated chronic low-grade inflammation (FIGURE 6).

B. Hypertrophic Adipocytes Cross Talk With Immune Cells Through Cytokines, FFA, Leukotrienes, micro-RNA, and FABPs to Enhance Inflammation

As discussed previously, SAT in insulin-resistant obesity is characterized by adipocyte hypertrophy and impaired adipogenesis. Interestingly, the secretory profile of hypertrophic adipocytes has also been shown to shift toward a more

proinflammatory signature. In fact, the levels of the secreted proinflammatory factors IL-6, IL-8, MCP-1, and TNF- α are elevated, positively correlated with adipose cell size (264, 268) and also increased in adipose cells from insulin-resistant subjects (245). MCP-1 is a chemoattractant promoting recruitment and infiltration of macrophages, which enhance the inflamed status of the adipose tissue. Adipose tissue macrophages, rather than adipose cells per se, are the main source of elevated inflammatory cytokines in the tissue. Macrophage-secreted cytokines can, in turn, further amplify the inflammatory phenotype of the adipose cells. For instance, TNF- α inhibits adipocyte differentiation, increases lipolysis, and induces a macrophage-like phenotype in preadipocytes (94, 168). Nevertheless, most of these findings are based on animal studies, and it is still unclear whether the resident/infiltrated macrophages are the dominating cells responsible for the secreted local cytokines in inflamed human adipose tissue.

It is well established that macrophage-specific markers are elevated in SAT in obesity, and their expression levels correlate with markers of insulin resistance and the metabolic syndrome in humans (151, 190, 311). This association with obesity is reversible following weight reduction, as shown, for instance, with bypass surgery-induced weight loss, where macrophage infiltration and markers of inflammation are reduced (41).

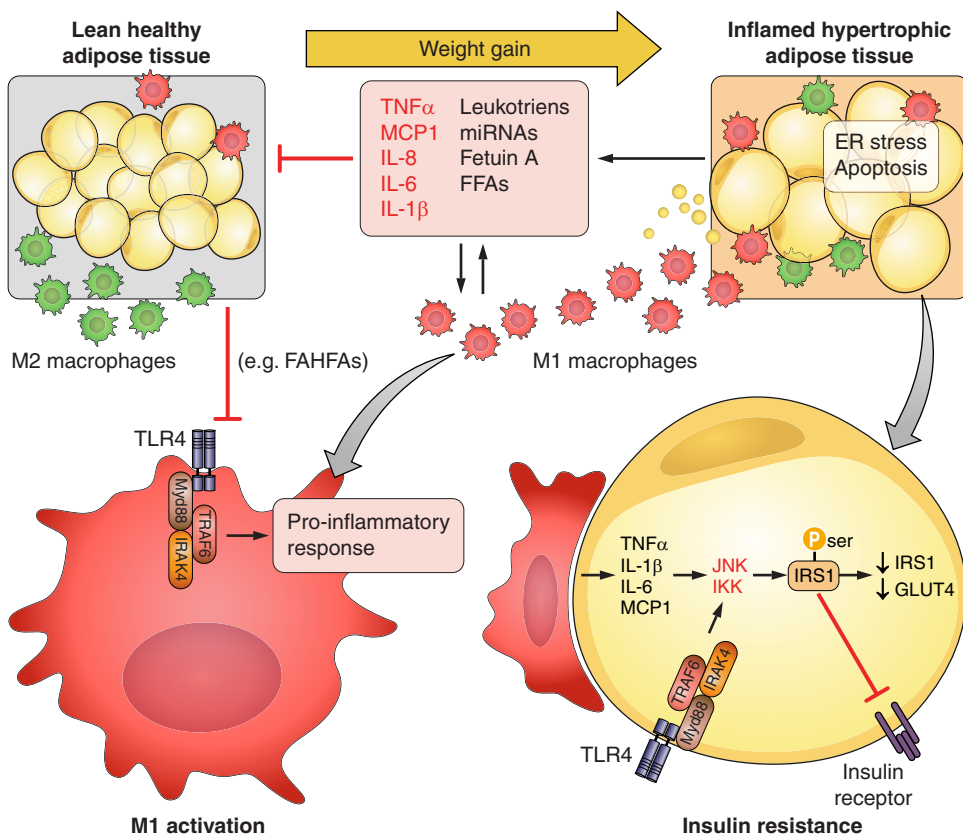


FIGURE 6. Hypertrophic adipocytes and macrophages cross talk to induce inflammation and insulin resistance. Inability to store excess fat, together with increased lipolysis in hypertrophic adipocytes, lead to increased systemic levels of free fatty acids (FFAs). Hypertrophic adipose cells have a more proinflammatory secretory profile compared with nonhypertrophic and healthy adipose cells. Enlarged adipocytes secrete inflammatory molecules [e.g., monocyte chemoattractant protein-1 (MCP-1), IL-6 and IL-8, miRNA, leukotriene, and fetuin A]. These factors, together with elevated FFA, promote recruitment of the proinflammatory M1 macrophages by activating key cellular pathways [e.g., TLR-4 signaling pathway]. The activated M1-macrophages will further induce a prominent inflammatory milieu in the adipose tissue and trigger the master mediators of inflammation IKK- β /NF- κ B (IKB-kinase/nuclear factor- κ -light chain enhancer of activated b-cell) and JNK (c-Jun NH₂-terminal kinase) in the adipocytes and attenuate cellular insulin sensitivity. ER, endoplasmic reticulum; FAHFAs, fatty acids of hydroxy-fatty acids; GLUT-4, glucose transporter-4; IRAK4, IL-1 receptor-associated kinase; IRS-1, insulin receptor substrate-1; TRAF6, TNF receptor associated factor-6.

Based on their function and *in vitro* stimulation, macrophages were first characterized in two main classes. Proinflammatory M1 macrophages are the dominating population in obese and inflamed adipose tissue, whereas the anti-inflammatory M2 macrophages, primarily important for phagocytosis, tissue repair, and angiogenesis, are the resident macrophage population in lean and metabolically healthy states. Angiogenesis in dysfunctional adipose tissue has been discussed previously. Macrophages may have a regulatory role for this as M1 macrophages express and secrete factors at the initiation steps of angiogenesis, whereas M2 macrophages secrete factors involved in later stages (271).

In vitro studies have demonstrated that M2 macrophages secrete anti-inflammatory cytokines (e.g., IL-10) that can increase adipogenesis and enhance insulin signaling (189). At the molecular level, macrophage-specific PPAR- γ and JNK have key regulatory functions for M2 and M1 activation, respectively (36, 108). Although experimental studies have shown a shift of resident M2 macrophages to M1 proinflammatory macrophages in obesity (37, 154) (FIGURE 6), increased levels of proliferating M2 macrophages have been detected in adipose tissue of both obese mice and humans, for example, around the dead/dying adipocytes (crown-like structures) (101). M2 macrophages have also been implicated in progression of other pathogenic events, such as fibrosis and tumor growth (85).

In response to different environmental factors, both M1 and the alternatively activated M2 macrophages can, however, express different markers and exhibit distinct functions (91). Notably, coexpression of both M1 and M2 markers have been identified in some macrophages, including alveolar macrophages in patients with chronic obstructive pulmonary disease and foamy macrophages of human atherosclerosis (23, 276). Nevertheless, the simplified M1/M2 classification has been over-interpreted as evidence has revealed a more complex spectrum of macrophage populations beyond the classic M1/M2 binary states (317). Thus, *in vivo*, an imbalanced multidemonstrational macrophage polarity rather than a simple bipolar M1/M2 subset infiltration is probably more likely and important for progression of dysfunctional adipose tissue.

In addition to macrophages, the presence of different types of T-lymphocytes in the adipose tissue has been confirmed by several investigations. The proinflammatory T-helper cell type 1 (Th1) cells are accumulated in obese, dysfunctional adipose tissue and augment M1-like macrophage polarization by enhancing the release of interferon- γ , IL-1 β , IL-6, and TNF- α , whereas the anti-inflammatory T-helper cell type 2 (Th2) and T-regulatory cells are the dominating T cells in lean adipose tissue and promote M2-like macrophage polarization (53). Interestingly, a shift from regula-

tory T cells to activating Th1 cells has been observed in individuals with BMI >30 (307).

Adipocytes, like many other cell types, release microvesicles that can contain RNA, protein, and lipids. In fact, a novel study identified adipose tissue as an important source of circulating exosomal micro-RNAs (291) and revealed new ways for the adipose cells to cross talk with other cells and tissues. For instance, following a high-fat diet, adipose cells secrete microvesicles targeting and activating macrophages. This occurs through a specific micro-RNA, miR-155, which promotes M1-like polarization (333) and inhibits adipose cell insulin signaling. miR-155 is also increased in obese adipose tissue (216), upregulated by TNF- α , and can inhibit adipogenesis (143). The impact of miR-155 on macrophage activation has also been studied previously (129) and shown to promote inflammation and fibrosis in the liver (19). Together, these findings identify a novel way for adipose and immune cells to cross talk and to synergistically augment local and systemic chronic inflammation in obesity.

Leukotrienes (LTs) are proinflammatory factors, mainly secreted by leukocytes, and have important functions in the innate and adaptive immune systems. However, it is known that adipocytes also secrete LTs, which may attract and activate immune cells (e.g., macrophages) (210). LTs are increased in obese adipose tissue, and the secreted levels are positively correlated with degree of adipocyte hypertrophy. Recently, LTB₄, a member of LTs, was identified as a powerful chemoattractant and a potent inhibitor of insulin signaling in liver and muscle cells. LTB₄ binds to its receptor LTB₄R to exert its action through activation of the IKK- β /NF- κ B and JNK pathways. Adipocytes lack this receptor but secrete LTB₄ and can thus activate macrophages and their tissue infiltration. Genetic or pharmacological inhibition of LTB₄R results in attenuation of inflammation and improved insulin sensitivity in obese rodent models (175). Thus LT secretion is another way for adipocytes to cross talk with immune cells, especially macrophages, to enhance chronic inflammation and systemic insulin resistance.

The FABPs function as lipid chaperons and have been implicated in regulation of lipid homeostasis. In particular, FABP-4 was studied as an important intracellular adipogenic marker and regulator of lipogenesis/lipolysis in adipocytes. It is well-established that FABP-4 can exist and function as both intracellular and extracellular secretory forms to modulate inflammatory and metabolic pathways. The intracellular FABP-4 in macrophages has been shown to regulate lipid metabolism and inflammatory responses. It can also bind and stabilize LTs. Moreover, both genetic and chemical inhibition of FABP-4 have been demonstrated to promote anti-atherogenic effects in animal models. FABP-4 is secreted from both adipocytes and macrophages, and the plasma levels are indeed increased in obesity and are posi-

tively correlated with markers of insulin resistance and CVD in humans (123). Also, a study in mice has shown that circulating FABP-4 contributes to obesity-induced metabolic disorders (42).

Recently, adipocytes were shown to secrete a novel family of lipids, FAHFAs, to exert several beneficial effects on insulin sensitivity (326). Some of these lipids also have anti-inflammatory effects and can attenuate macrophage activation, both in vitro and in vivo (157, 208, 326). In contrast, hypertrophic expansion of the SAT adipose cells is associated with both lower tissue and serum levels of the protective FAHFA lipids (326).

These data provide evidence for a dynamic adipose tissue that exerts extensive cell-cell and intertissue regulatory cross talk through the secretion of both specific adipokine peptides and lipids and where the secretory profile is dependent on the state of the tissue, i.e., healthy non-hypertrophic or inflamed and hypertrophic.

C. FFA and Hypoxia/ER Stress

A metabolic complication of obesity is the increased adipose tissue lipolysis and associated elevated FFA and very low-density lipoprotein-triglyceride levels. Apart from the antagonistic effect of elevated serum FFA levels on whole body glucose disposal, saturated FFAs also act as ligands for macrophage activation by promoting Toll-like receptor (TLR)-4 signaling to enhance infiltration and inflammation in the adipose tissue. Excess FFA and its toxic lipid intermediates, including ceramides (CER) and palmitates, also place the obese adipose tissue in a stressful situation and induce ER stress. The additional contributions by tissue hypoxia to angiogenesis and inflammation have been discussed previously.

ER stress is a cellular reaction that is triggered by the accumulation of unfolded proteins in the ER on environmental insults. However, chronic ER stress can also lead to increased cell apoptosis/autophagy and induction of inflammation. Cell apoptosis can by itself increase infiltration of immune cells and thus enhance the inflamed state. The unfolding protein response machinery is triggered by the ER stress sensors, inositol requiring factor inositol requiring enzyme-1, protein kinase RNA-like ER kinase, and activating transcription factor-6, to activate NF- κ B and JNK and several proinflammatory genes. Obesity has been shown to induce ER stress in the adipose tissue (33, 257, 336), and reducing ER stress has been reported to improve insulin resistance in diet-induced obesity model (145). In obese individuals, gastric bypass surgery-mediated weight loss, as well as physical exercise, have been shown to attenuate SAT ER stress, which were associated with improved insulin sensitivity (86, 146).

Mechanistically, FFAs induce ER stress by directly affecting the ordering of ER membrane and blocking the ER-specific Ca^{2+} -ATPase pump, which results in accumulation of misfolded proteins. In addition, FFAs induce ER stress through activation of the LPS responsive TLRs, TLR-2/TLR-4 (195, 231). TLR-mediated signaling plays a crucial role in the regulation of the innate immune response by activating the IKK- β /NF- κ B axis and proinflammatory cytokine secretion. These data point out that there is a mutual relationship between inflammation and ER stress, and thus it is challenging to identify the cause and consequence relationship in this context.

FFAs have also been reported to increase adipose cell reactive oxygen species production, a pathogenic event that can inhibit ER- Ca^{2+} channels and induce ER stress (145). It is also noteworthy that exposure to high glucose potentiates ER stress in vitro in human adipose cells, Müller cells (retinal glial cells), and human umbilical vein endothelial cells (9, 258, 334). Together, these data indicate that ER stress may also be a pathogenic link between chronic hyperglycemia and diabetic complications.

D. TLR-4 Links FFA to Obesity-Associated Inflammation and Fibrosis

A growing body of evidence reveals a key role for TLRs, mainly TLR-4, in enhancing and maintaining obesity-associated inflammation and insulin resistance (260). TLR-4, expressed in both adipose and immune cells, can be activated by LPS and FFA to initiate the signaling cascades that activate the JNK and IKK- β /NF- κ B pathways to trigger induction of proinflammatory cytokines (e.g., IL-6, TNF- α , and IL-1 β) and subsequent inhibition of insulin signaling (FIGURE 6).

Fetuin A, a glycoprotein secreted by liver and adipose tissue, can also be induced by FFA and is elevated in obesity in both humans and rodents. Novel findings have identified an important role for fetuin A as a necessary adapter protein for FFA to promote TLR-4 activation upon caloric excess (220). Moreover, fetuin A has recently been implicated in enhancing the infiltration of macrophages into the adipose tissue in obesity (52).

The cross talk between adipose cells and macrophages has important pathophysiological consequences at both local and systemic levels. The elevated serum levels of FFA and proinflammatory factors, the presence of macrophages in many tissues, together with the fact that TLR-4 is widely expressed in human tissues (including liver, adipose tissue, skeletal muscle, pancreas, kidney, and brain), make adipose tissue a major regulator of obesity-related systemic inflammation and insulin resistance. This, in turn, also contributes to secondary organ damage, such as diabetic vascular injury, nephropathy, and liver disease (NAFLD/NASH). In

addition, TLR-4 has recently been in focus as a link between diabetes and Alzheimer's disease. TLR-4 has been proposed to induce insulin resistance and mitochondrial dysfunction in the brain and to increase amyloid- β deposition and inflammation, eventually resulting in neuronal degeneration (124).

TLR-4 activation may also provide an important link to adipose tissue fibrosis in obesity. LPS- and high-fat-diet-induced TLR-4 activation in mice enhanced adipose tissue fibrosis, and this was mediated by upregulation of the pro-fibrotic factor TGF- β (304). However, this effect was not mediated by macrophages themselves, as genetic deletion of macrophage-specific TLR-4 had no impact on fibrotic activity (133). This indicates the involvement of other TLR-4-expressing cells, e.g., adipose cells and/or other immune cells promoting obesity-associated fibrosis.

VI. ECTOPIC LIPID ACCUMULATION—A RESULT OF ADIPOSE TISSUE DYSFUNCTION

When SAT, the largest adipose tissue depot in humans, reaches its limitation for the ability to store fat, further caloric overload leads to the accumulation of ectopic fat in other tissues, such as liver, skeletal muscle, and heart. It is well established that excessive tissue lipid accumulation leads to the formation of different lipotoxic molecules, including CER and palmitates, which promote local inflammation and insulin resistance (59). Furthermore, recent evidence indicates the accumulation of ectopic fat in the pancreas, which may contribute to β -cell dysfunction, as well as in the kidney (reviewed by Refs. 32, 48). In addition, increased visceral/intra-abdominal fat accumulation, associated with an abdominal obesity, is a marker of ectopic fat accumulation in various organs (287) (FIGURE 7).

Although ectopic fat accumulation in today's affluent society is a consequence of limited fat storage capacity leading to negative metabolic consequences, it may have had survival advantages in times of shortage of food and thus have been preserved. Lipids released by visceral fat targets the liver and, like ectopic fat in the liver, can be a source of local energy supply, important for maintaining gluconeogenesis and whole body glucose levels.

A. Hepatic Lipid Accumulation

The liver is particularly prone to the accumulation of ectopic lipids in obesity associated with a dysfunctional or reduced SAT as in lipodystrophy/lipoatrophy. The global prevalence of NAFLD, which is the most common chronic liver disease worldwide, is estimated to be 24% (328). The prevalence for NAFLD is increasing in parallel with the prevalence of obesity and is as high as 80% in obese patients

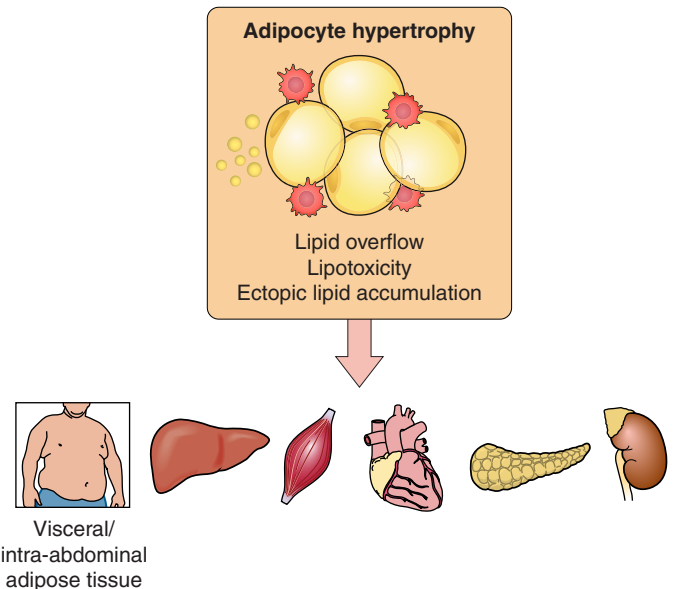


FIGURE 7. A dysfunctional subcutaneous adipose tissue (SAT) with caloric overload leads to the accumulation of ectopic lipids in different organs/tissues. When the capacity of SAT reaches its limitation to store excess fat, and hypertrophic adipocytes become inflated, ectopic fat accumulation is promoted. The increased uptake of FFAs into these organs/tissues, without a corresponding increase in lipid utilization, promotes accumulation of toxic lipid intermediates, which lead to cellular dysfunction, referred to as lipotoxicity. [The figure was partly generated using Servier Medical Art, provided by Servier, licensed under a Creative Commons Attribution 3.0 unported license.]

compared with 16% in individuals with normal BMI and without metabolic risk factors (203). Even if the prevalence for NAFLD increases with age in adults, it has also more frequently been reported in children and adolescents, due to the increasing rates of obesity and T2D in these groups (109). NAFLD can progress from simple, reversible steatosis into NASH at a prevalence of 5–10% (40). NASH is a considerably more severe disease characterized by inflammation and fibrosis, which causes the liver cells to swell and become damaged (12). Ultimately, this condition can lead to cirrhosis and hepatocellular carcinoma.

Lipid overflow from increased intake of dietary fat, FFAs released from lipolysis of the expanded adipose tissue, and DNL in the liver increase the hepatic FFA pool and contribute to triacylglycerol (TAG) accumulation with ~15, 60, and 25%, respectively (68). However, not only is dietary fat a major risk factor for development of NAFLD, but also increased intake of carbohydrates, especially fructose (267). Fructose-enriched diets promote DNL, which eventually could contribute to increased levels of intrahepatic lipids (290).

Lipids accumulate in the cytoplasm as lipid droplets and give rise to lipid metabolites, but it is probably not the TAG per se that are harmful to the cells (198). Instead, the imbalance between fatty acid oxidation, lipid disposal, and

storage will lead to the synthesis of toxic lipid intermediates, such as diacylglycerol (DAG) and CER (169). DAG is associated with impaired insulin signaling and insulin resistance via the activation of hepatic protein kinase C- ϵ (158, 252), which leads to a reduced insulin-stimulated phosphorylation of IRS-2 and Akt2 and thus the ability to activate glycogen synthesis and reduce gluconeogenesis (reviewed by Ref. 229). The role of CER has been less studied, but it is also emerging as a potentially important pathway for hepatic insulin resistance, and a diet rich in saturated fatty acids has been shown to increase *de novo* CER synthesis (76) in the liver (reviewed by Ref. 217) and induce insulin resistance (153).

Adiponectin, primarily released by adipocytes, is an anti-inflammatory adipokine targeting the hepatocytes and preventing lipid accumulation by increasing β -oxidation and suppressing DNL. Serum levels of adiponectin are also decreased in obesity and in patients with NAFLD (reviewed by Ref. 292). Indeed, an important insulin-sensitizing effect of adiponectin seems to be mediated via increased cellular ceramidase activity, thereby depleting the cells of CER (121).

A consequence of the increased hepatic lipid pool is the development of mitochondrial dysfunction, increased oxidative stress, production of reactive oxygen species, and ER stress. Ultimately, hepatic inflammation with increased release of proinflammatory cytokines, produced by hepatocytes and macrophage-like Kupffer cells (18), will be induced and contribute to the progression from simple steatosis to NASH (63). The chronic low-grade inflammation can also enhance hepatic lipid accumulation through increased lipid uptake due to increased cellular fatty acid transporters, enhanced TAG synthesis, and decreased fatty acid oxidation (reviewed by Ref. 182).

Although the different experimental studies discussed above, performed mainly in cells or animal models, have shown that increased hepatic lipid accumulation can induce insulin resistance and inflammation, this concept is not equally straightforward in humans. For instance, genetic predisposition for increased liver fat is seen in individuals carrying specific palatin-like phospholipase domain containing 3 variants (297), but most studies have shown that this is not associated with insulin resistance or other markers of the metabolic syndrome (324). The reason for this is unclear, but emphasizes that the signals are not directly the TAGs accumulated, but, more likely, activation of specific metabolites and pathways. There is no single pathway universally responsible for the development of NAFLD, and it is still a debate whether NAFLD is a consequence or a cause of insulin resistance.

Since there is currently no pharmacological treatment approved for NAFLD/NASH, weight loss following diet and lifestyle intervention is the most efficient therapy to reduce

liver fat and insulin resistance (reviewed by Ref. 223). Interestingly, it was recently shown that acute dietary intake of saturated fat rapidly increases liver fat accumulation and insulin resistance (115). Overall, it is the energy content of the diet that mostly influences liver fat accumulation, and even a small weight loss will reduce it (325). Furthermore, around 10–15% body weight reduction in overweight and obese individuals with NAFLD was found to lower the insulin resistance (223).

Due to its association with systemic dyslipidemia and insulin resistance, NAFLD is also a risk factor in humans for the development of incident CVD and T2D (178). There are also studies suggesting that early lipid accumulation in the liver and hepatic insulin resistance precede skeletal muscle insulin resistance and actively contribute to the development of the metabolic syndrome and T2D (reviewed by Ref. 39).

B. Ectopic Fat in Other Organs/Tissues

Ectopic fat deposition in other organs and/or tissues, such as skeletal muscle, pancreas, epi-/pericardial adipose tissue, myocardium, and perivascular adipose tissue, can also lead to lipotoxicity with impact on insulin resistance and obesity-related comorbidities in humans.

Skeletal muscle, which makes up ~40% of the body mass, is an important regulator for whole body glucose metabolism and lipid utilization (131), and excessive accumulation of intramyocellular lipids (IMCLs) is associated with the development of insulin resistance and T2D (222). Increased uptake of FFAs by myocytes without a corresponding increase in lipid oxidation promotes accumulation of IMCLs. Intramuscular TAG is essential for lipid homeostasis in skeletal muscle, and the composition is important since unsaturated, compared with saturated, fatty acids have a protective effect through the promotion of TAG accumulation, which leads to decreased accumulation of the toxic lipid metabolites, DAG and CER (181, 232). Both of these lipid intermediates are associated with impaired insulin signaling and insulin resistance (3, 128), and they have also been shown to trigger inflammatory signaling pathways, leading to increased secretion of proinflammatory cytokines (including TNF- α , IL-1 β , and IL-6) and mitochondrial dysfunction (reviewed by Ref. 196).

Although increased lipid accumulation in the skeletal muscles has long been considered an important pathway for the induction of whole body insulin resistance, a confusing observation is that highly trained and insulin-sensitive individuals performing endurance exercise, such as marathon runners, also have increased IMCLs (70). These individuals also have high lipid turnover, due to a high oxidative capacity (70). Thus it is probably not the accumulation of intramuscular lipids per se that causes the detrimental ef-

fects on insulin sensitivity, but rather the accumulation of the toxic lipid intermediates, such as DAG and CER. In support of this concept, DAG and CER not only influence insulin signaling directly, but also enhance proinflammatory pathways and impair mitochondrial functions.

Ectopic fat, accumulated in the skeletal muscle and liver, induces the secretion of cytokines, which, in an autocrine/paracrine and endocrine fashion, enhances local and systemic insulin resistance, inflammation, as well as the progression of vascular disease (reviewed by Ref. 177). In contrast, ectopic fat, which accumulates in the epi-/pericardial areas, in and around the blood vessels and in the myocardium itself, seems to have mostly local effects and is associated with cardiac insulin resistance, myocyte apoptosis, and contractile dysfunction. Epicardial fat, which also seems to contain brown adipose cells (250), is an energy source for the heart and is considered to protect the myocardium from high levels of FFAs and unlikely to increase cardio-metabolic risk (209). Despite that, several human studies have shown an association between increased amount of epicardial fat, either due to, or independent of, obesity for the onset and development of coronary artery disease (316). However, this association can be confounded by the presence of other markers of ectopic fat and/or visceral obesity. The epicardial fat depot is also able to synthesize and secrete cytokines, adipokines, and vasoactive factors, which can be released and transported into the adjacent myocardium and coronary arteries (125). Ultimately, these factors could contribute to the development of CVD (99).

The myocardium extracts FFAs from the blood to meet the energy demand of the heart since it has a limited ability to synthesize FFAs itself. If the supply of FFAs exceeds the capacity for lipid oxidation, fat accumulates in the cardiomyocytes as intramyocardial TAG, and a high rate of conversion of FFA into TAG is associated with increased production of intermediates, such as CERs, DAGs, long-chain acyl-CoAs, and acylcarnitines, which leads to mitochondrial dysfunction and impaired cardiac function (335). Despite the strong link between accumulation of CER and cardiomyocyte lipotoxicity in animal models of obesity, the relationship in the human heart remains unclear (reviewed by Ref. 65).

Excess fatty acid uptake by perivascular adipose tissue, a diet-regulated depot around the vasculature shown to share important features with BAT, also enhances the secretion of various cytokines and chemokines that provoke inflammation (113). Although only a small adipose depot, accounting for ~3% of total body fat (263), it has important local cross talk with the vasculature. Insulin resistance in the vasculature, at least in part a result of the ambient inflammatory milieu, affects endothelial cells and vascular smooth-muscle cells, which are implemented in the development of CVD (166).

Due to limitations in detection techniques, nonalcoholic fatty pancreas disease (NAFPD) has been less studied, and the prevalence of NAFPD in the general population is unknown (reviewed by Ref. 89). Despite that, human studies have shown that pancreatic lipid accumulation interferes with insulin secretion (309). However, if NAFPD directly contributes to β -cell dysfunction in T2D, which has been shown *in vitro* as well as in animal studies, in humans it remains to be elucidated (reviewed by Ref. 89).

Even if caloric overload is still the main predictor of ectopic fat accumulation, other factors, such as genetic predisposition, aging, disease conditions, lifestyle, and environmental conditions, are also involved.

VII. CONCLUSIONS AND PERSPECTIVES

This review is mainly focused on human adipose tissue function, growth, and expansion and their relation to the development of obesity-associated complications, including inflammation, ectopic fat accumulation, insulin resistance, and T2D.

The amount of adipose tissue, both high as in obesity and low as in lipodystrophy/lipoatrophy, is associated with insulin resistance and its different metabolic consequences. In both cases, the major problem is the limited storage capacity of excess lipids in the adipose tissue, in particular SAT as the largest and best storage site for lipids.

There is a remarkable individual difference in the ability of SAT to expand, and this greatly affects the metabolic consequences for an individual facing an energy surplus. When the SAT expansion has exceeded its limits, lipids are stored in ectopic depots, including the liver, heart, and skeletal muscles, enhancing local and systemic insulin resistance and inflammation.

Several studies have also shown that the obesity-associated metabolic complications are not primarily dependent on the amount of SAT, but rather different measures of accumulated non-SAT adipose tissue and ectopic lipids. Abdominal obesity in humans is a well-known risk marker of future risk of T2D and CVD, and it is also a marker of increased accumulation of ectopic fat and expansion of visceral/intra-abdominal non-SAT. Together, these data show that obesity-associated health risks are not simply based on an individual's BMI, and that additional clinical markers, including adipose tissue distribution (waist circumference in particular), are important for risk evaluation. Hopefully, ongoing large "omics" studies will also identify sensitive markers of future health risk in both nonobese and obese individuals, allowing focused intervention strategies. One such example is our laboratory's recent finding of increased plasma mannose levels as a novel marker of future risk of both CVD and T2D (193).

It is now also becoming increasingly clear that SAT expansion in humans is under genetic, and probably also epigenetic, regulation, which provides novel insight into the mechanisms for insulin resistance and T2D. We have shown that individuals with a close family history of T2D (FDR) are characterized by markers of an impaired ability to commit and differentiate new adipose cells, leading to inappropriate SAT adipose cell hypertrophy for a given BMI and associated insulin resistance and risk of T2D. Interestingly, this apparent defect was not seen in individuals with a family history of obesity. Similarly, it was also recently demonstrated that nonobese individuals with a genetic predisposition for insulin resistance are characterized by partial “lipodystrophy,” or, more likely, impaired SAT adipogenesis, as in FDR.

In this review, we have also emphasized the importance of angiogenesis for functional adipose tissue growth and the role of inflammation and hypoxia in regulating angiogenesis. This must also be a carefully regulated process, since adipose tissue will not grow unless adequately oxygenated. It also implies a close cross talk between the adipose tissue cells and the endothelial cells, but additional work is required to define the molecular regulation. An interesting, but not yet sufficiently explored, link between adipogenesis and angiogenesis is BMP-4, which is highly secreted by expanded adipose cells and a key regulator of adipose precursor cell commitment and differentiation. BMP-4 is also an activator of angiogenesis, and the possibility that adipose tissue BMP-4 secretion is an important feedback regulator of both adipose tissue expansion and angiogenesis needs to be further addressed.

The adipose tissue is also a large and important endocrine organ, releasing many important adipokines, such as leptin, adiponectin, TNF- α , retinol binding protein 4, IL-1, IL-6, BMP-4, and the novel FAHFA lipids. The secretion of these adipokines and the anti-inflammatory and insulin-sensitizing FAHFA lipids is much dependent on the functional state of the adipose tissue and is altered in hypertrophic obesity.

BMP-4 has recently attracted much attention as a regulator of differentiation of adipose precursor cells into white or an oxidative beige/brown phenotype. SAT is a plastic tissue, where the adipose cells can remain white and primarily lipid storing or become beige/brown and oxidative. The latter possibility provides an interesting opportunity for weight reduction.

We have found increased BMP-4 signaling, either through BMP-4 itself or through inhibition of the endogenously secreted BMP antagonists, to induce human adipose precursor cells to become beige/brown and oxidative. In addition, we found increased serum levels of BMP-4 in fully mature mice to induce SAT cells to assume a beige/brown and oxidative phenotype, while, instead, reducing BAT activation

and promoting a lipid-laden, beige, and less functional BAT phenotype. Since the adipose tissue secretes BMP-4 and serum levels are increased in human obesity, like in murine models, we postulate that the predominantly beige BAT phenotype in human obesity is due to the increased BMP-4 levels coming from WAT rather than being a cause of the obesity. The reduced expression of UCP1 and other markers of oxidative capacity in SAT in obesity seem at odds with this concept. However, SAT precursor and adipose cells become BMP-4 resistant in obesity which prevents their beige/brown response to the endogenously secreted BMP-4. In humans, this BMP-4 resistance is due to increased secretion of the BMP-4 antagonist GREM1, while, in mice, Noggin is upregulated. Antagonizing GREM1 is an interesting possibility to promote beige/browning of human SAT, which may reduce body weight, enhance insulin sensitivity, and prevent T2D.

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