THE EXPANDED ADIPOSE TISSUE plays a key role for the metabolic abnormalities associated with obesity. One mechanism for this is through the induction of insulin resistance commonly seen in obesity. The adipose tissue can influence whole body insulin sensitivity in different ways. Both the increased body fat mass and the associated cellular insulin resistance lead to elevated circulating FFA levels, which, by itself, augments insulin resistance. In addition, the adipose tissue secretes many cytokines and hormones (adipokines) that cross-talk with the liver, skeletal muscle, and also the pancreas. Important molecules released by human adipose tissue include adiponectin, leptin, IL-6, IL-8, and monocyte chemoattractant protein-1 (MCP-1). The profile of secreted adipokines becomes altered in obesity-favoring proinflammatory factors, which promote insulin resistance, whereas adiponectin, a molecule with several beneficial actions, is reduced.

The increased storage of surplus triglycerides in the adipose cells can be accomplished in two different ways, by expanding the available adipose cells (hypertrophy) or by recruiting new fat cells (hyperplasia). In adult man, hypertrophy of the fat cells is the most common form of accommodating the lipids, whereas hyperplasia predominates in the prepubertal age. Hypertrophic obesity is also more strongly associated with whole body insulin sensitivity. Here, we review recent findings linking hypertrophic obesity with inflammation and a dysregulated adipose tissue, including local cellular insulin resistance with reduced IRS-1 and GLUT4 protein content. In addition, the number of preadipocytes in the abdominal subcutaneous adipose tissue capable of undergoing differentiation to adipose cells is reduced in hypertrophic obesity. This is likely to promote ectopic lipid accumulation, a well-known finding in these individuals and one that promotes insulin resistance and cardiometabolic risk. We also review recent results showing that TNFα, but not MCP-1, resistin, or IL-6, completely prevents normal adipogenesis in preadipocytes, activates Wnt signaling, and induces a macrophage-like phenotype in the preadipocytes. In fact, activated preadipocytes, rather than macrophages, may completely account for the increased release of chemokines and cytokines by the adipose tissue in obesity. Understanding the molecular mechanisms for the impaired preadipocyte differentiation in the subcutaneous adipose tissue in hypertrophic obesity is a priority since it may lead to new ways of treating obesity and its associated metabolic complications.

Wnt signaling; tumor necrosis factor-α; adipose cells

Obesity. Recruitment of new fat cells is less common in adults, but when it occurs it is usually the type of obesity seen in individuals characterized as “obese but metabolically normal.” Around 20–30% of obese individuals can be characterized in this way (23).

A recent study has shown that there is also a continuous turnover of fat cells in adult man, ~10%/yr (22). It is reasonable to assume that the new adipocytes are formed from mesenchymal stem cells or other precursor cells that become committed to preadipocytes. Here, we will briefly review this process (adipogenesis) and its known regulation. The importance of the master regulator of cell fate and differentiation, the Wnt signaling pathway, will also be discussed as well as our recent findings linking inflammation with Wnt activation.

Adipocyte Differentiation and the Role of Wnt Activation

Commitment of mesenchymal stem cells to a defined lineage is a complex process and, so far, largely unknown. For the adipose lineage, it can be divided into two phases: commitment into preadipocytes and terminal differentiation to adipocytes. Preadipocytes are morphologically indistinguishable from fibroblasts. Before differentiation to adipocytes, the preadipocytes enter the cell cycle and undergo a finite number of cell divisions (mitotic clonal expansion), and this seems to be necessary before the cells can enter terminal differentiation. The transcriptional regulation of adipogenesis is well characterized and determined by a sequential activation of transcription factors, where induction of peroxisome proliferator-acti-
Differentiation and induction of PPARγ/H9253 inhibition of Wnt signaling is a prerequisite for preadipocyte differentiation. Nevertheless, the fully differentiated adipose cell phenotype, including proteins related to insulin signaling and action, requires the activation of C/EBPα as well (20, 29).

The canonical Wnt signaling pathway is an extracellular pathway that controls cell proliferation, cell survival, and cell fate. Activation of the Wnt signaling cascade has been shown to regulate the differentiation of mesenchymal progenitor cells toward osteogenesis, myogenesis, or adipogenesis (Fig. 1). Wnt ligands are secreted glycoproteins that function in a paracrine and autocrine manner. They bind and cross-link the low-density lipoprotein-related receptors (LRP) and frizzled receptors, thereby initiating signaling cascades and where the canonical Wnt/β-catenin pathway has been characterized most extensively (17). Following binding of the canonical Wnt ligands, β-catenin becomes hypophosphorylated and thereby stabilized and translocated to the nucleus, where it acts as a coactivator for the transcription factor T cell factor/lymphoid enhancer factor (TCF/LEF). So far, 19 Wnt ligands have been identified, and among these, Wnt10b is a likely candidate for the endogenous Wnt inhibiting adipogenesis. Wnt10b is highly expressed in adipose tissue stromal cells, and its expression declines rapidly following initiation of adipogenesis (21). Inhibition of Wnt signaling is a prerequisite for preadipocyte differentiation and induction of PPARγ and C/EBPα in both human and mouse 3T3-L1 preadipocytes (see Fig. 1) (10, 16). Overexpression of Wnt10b in mice caused a 50% decrease in the amount of adipose tissue, and the mice were resistant to diet-induced obesity (28). Disruption of the Wnt signal results in spontaneous differentiation of the preadipocytes, and Wnt10b-null mice exhibited an increased adipogenic potential, i.e., lipid formation, in myoblasts (26).

Noncanonical Wnt signaling has been less characterized, but at least two noncanonical Wnt signaling pathways have been proposed, the planar cell polarity pathway and the Wnt/Ca²⁺ pathway (8). The noncanonical Wnt signals are transduced through receptors such as RYK and ROR2 that cross-link with the frizzled receptor and activate a number of small G proteins, where some are dishevelled dependent and others are Ca²⁺-dependent effector molecules (18). Activation of the Ca²⁺-dependent Nemo-like kinase is implicated in activation of the transcription factor TCF/LEF (14, 15). However, the role of the noncanonical Wnt signaling pathways for (pre)adipocyte differentiation has not been studied much.

Although inhibition of the canonical Wnt signal is an absolute prerequisite for induction of adipogenesis, maintained Wnt signal promotes myo- and osteogenesis (Fig. 1). An important molecule for the termination of Wnt activation is Dickkopf (Dkk)1. Dkk1 is a secreted protein that binds to the Kremen receptor and forms a ternary complex with LRP and frizzled receptors. It is induced by PPARγ ligands (Gustafson B, Eliasson B, and Smith U, unpublished data), and as a consequence β-catenin levels are reduced, thus favoring adipogenesis. However, increasing Dkk1 is negative for osteogenesis. TNFα has been shown to increase Dkk1 in osteoblasts, thus reducing bone formation. Increased Dkk1 has also been seen in rheumatoid arthritis (5), where the inflammation both increases bone destruction and, by increasing Dkk1, reduces new bone formation. The effect of PPARγ ligands to increase Dkk1 expression in (pre)adipocytes (Gustafson B, Eliasson B, and Smith U, unpublished data) is intriguing since it could also provide an explanation for the reduced bone formation seen in diabetic patients treated with these drugs (13).

Inflammation in the Adipose Tissue Can Activate the Wnt Signal

Hypertrophic obesity is associated with an infiltration of macrophages into the adipose tissue (27). This promotes inflammation and introduces TNFα into the tissue. Although preadipocytes also express TNFα at the gene and protein level, they appear unable to cleave the prohormone and thus actively secrete TNFα (12).

TNFα is a powerful inhibitor of preadipocyte differentiation. Interestingly, we (10) and others (2) have found that TNFα can activate the Wnt signal during the early stages of adipogenesis. This effect is not seen by other proinflammatory molecules like MCP-1 or resistin (11). The signaling pathway for this cross-

**Fig. 1.** An overview of preadipocyte and adipocyte differentiation and associated changes in Wnt10b expression. C/EBPβ/δ and -α, CCAAT/enhancer-binding protein-β/δ and -α, respectively; PPARγ2, peroxisome proliferator-activated receptor-γ2; GM-CSF, granulocyte-macrophage colony-stimulating factor; MIP-1α, macrophage inflammatory protein-1α; MCP-1, monocyte chemotactic protein-1.
talk by TNFα with Wnt is currently unclear but important to elucidate. Like Wnt ligands, TNFα suppresses the induction of PPARγ and C/EBPα (10). Furthermore, expression of a dominant-negative TCF7L2 blocks the inhibition of adipogenesis by TNFα even in the presence of elevated β-catenin levels (2). These data clearly document that the early inhibitory effect of TNFα on adipogenesis is exerted through the Wnt/β-catenin pathway.

The Adipose Tissue in Hypertrophic Obesity is Dysfunctional

Enlargement of the adipose cells alters the phenotype such that lipolysis is increased and the adipose cells become insulin resistant via reduced insulin receptor substrate (IRS-1) and glucose transporter 4 (GLUT4) protein expression (reviewed in Ref. 9). One important mechanism for this is the associated proinflammatory activation in the tissue (Fig. 2). Several cytokines and chemokines like IL-6, IL-8, and MCP-1 are increased, and the infiltration of macrophages leads to IL-1β and TNFα secretion. Several of these cytokines have been shown to reduce both IRS-1 and GLUT4 expression and induce insulin resistance (9). The increased MCP-1 and serum amyloid A secretion also seems important for attracting monocytes into the tissue by increasing adhesion molecules and binding to the endothelial cells. Following macrophage conversion, they promote inflammation in the adipose tissue.

Taken together, adipose cell enlargement leads to a dysregulated adipose tissue characterized by a proinflammatory profile, increased lipolysis, reduced adiponectin release, and insulin resistance. In addition, the increased inflammation has consequences for the preadipocytes that cannot undergo normal differentiation with lipid storage, as discussed above.

TNFα Induces a Macrophage-Like Phenotype in Preadipocytes

It is well established that the gene expression profile of preadipocytes is in many regards similar to that of monocytes/macrophages. In fact, 3T3-L1 preadipocytes can assume a macrophage-like phenotype when they are injected intraperitoneally (3). TNFα is capable of completely preventing the normal differentiation of both 3T3-L1 and human preadipocytes, as discussed above. We have recently examined the phenotype of human undifferentiated preadipocytes cultured for 10 days in the presence of a low concentration (5 ng/ml) of TNFα (12). The results were quite stunning. The preadipocytes assumed a clear macrophage-like phenotype with expression of markers like CD68 and macrophage inflammatory protein-1α and secretion of granulocyte-macrophage colony-stimulating factor and IL-1β, i.e., molecules considered specific for macrophages. The molecules were not expressed or secreted, or they were expressed and secreted only at very low levels, in the absence of TNFα. A similar effect was seen in the presence of LPS, suggesting a role of Toll-like receptor-4 (12). However, although human preadipocytes assumed a macrophage-like phenotype, they did not completely transdifferentiate since they did not secrete TNFα, become phagocytic, or express the scavenger receptor (12).

It is well established that most cyto- and chemokines secreted by the adipose tissue emanate from the stromal cells rather than the adipose cells per se (6, 7). Recent findings of macrophage infiltration in the adipose tissue in obesity have led to the conclusion that macrophages account for most of this secretion. We calculated to what extent the partially transdifferentiated preadipocytes could account for the secretion of MCP-1 and IL-6 using data reported in the literature. The results were almost identical, suggesting that activated proinflammatory preadipocytes can account for most, if not all, of the secretion (12). Thus, it may well be that the macrophages in the adipose tissue in obesity are recruited to “clean” the tissue from apoptotic/necrotic large fat cells, as suggested by Cinti et al. (4). In contrast, the activated preadipocytes in the adipose tissue account for the secretion of most cytokines and chemokines. It is, of course, also possible that both preadipocytes and macrophages play important roles and/or that the preadipocytes activate the macrophages in the tissue to a proinflammatory M1 phenotype. However, a key question is to understand whether it is only the macrophages that secrete TNFα or whether certain preadipocytes also are capable of cleaving and secreting TNFα. In the first scenario, macrophage infiltration would still be important as initiators of the proinflammatory and macrophage-like phenotype of the stromal preadipocytes.

Macrophage infiltration into the adipose tissue and activation of preadipocytes and adipocytes

Fig. 2. Induction of proinflammatory factors and insulin resistance in hypertrophic obesity. FFA, free fatty acids; IRS-1, insulin receptor substrate-1; GLUT4, glucose transporter 4.
Impaired Preadipocyte Differentiation in Hypertrophic Obesity in Man

We recently concluded a large study where we examined the ability of preadipocytes from the stromal fraction of the subcutaneous abdominal adipose tissue in man to undergo differentiation (12). To prevent an inhibitory effect of infiltrated monocytes/macrophages, we removed CD14- and CD45-positive cells. A striking finding was that the number of preadipocytes capable of undergoing differentiation was reduced in obesity and negatively correlated with both body mass index and adipose cell size of the donor (12). This finding is intriguing and suggests that excess lipids cannot be stored adequately in the subcutaneous adipose tissue in hypertrophic obesity and, consequently, are directed to other sites, i.e., ectopic lipid deposition in the liver and skeletal muscle as well as other depots with preadipocytes such as visceral fat, epicardial fat, etc. Many recent studies have shown that there is a correlation between amounts of lipids in these ectopic sites. For instance, liver fat correlates with amount of visceral fat and epicardial fat (1). In this scenario, ectopic fat accumulation, which clearly promotes insulin resistance in the liver and skeletal muscle (19), is a consequence of an inability to store the excess lipids in the subcutaneous adipose tissue.

The question, then, is why is there an impaired differentiation of the preadipocytes? We examined the expression of several genes in the preadipocytes and found that MAP4K4 was increased in obesity. The reason for this is unclear, but TNFα activates MAP4K4, and this in turn inhibits PPARγ activation (24, 25). Thus, it is possible that the inflammation in the adipose tissue, in particular via TNFα, inhibits preadipocyte differentiation through MAP4K4 activation and that there is cross-talk with the Wnt signaling cascade. Nevertheless, it is surprising that such an effect is long lasting and remains in the cultured cells even after several generations in vitro. Current work is focused on knocking down MAP4K4 and other genes we have found to be increased in preadipocytes from obese individuals. Understanding the mechanisms for the impaired adipogenesis may offer novel ways to prevent the metabolic consequences of obesity.

Conclusions

Adipose cell enlargement is associated with local and whole body insulin resistance and an increased inflammation in the adipose tissue. This, in turn, inhibits normal preadipocyte differentiation through activation of the Wnt signaling cascade and also induces a dysregulated adipose tissue.

Inability of the preadipocytes in the abdominal subcutaneous adipose tissue to store excess lipids is a likely cause of peripheral “spillover” and ectopic lipid accumulation. This will further augment the insulin resistance and the metabolic abnormalities associated with hypertrophic obesity.

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