Review

Adipose Tissue, Inflammation and Atherosclerosis

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Metabolic syndrome is associated with dysfunctional adipose tissue that is most likely a consequence of the enlargement of adipocytes and infiltration of macrophages into adipose tissue. Obesity and ectopic lipid deposition are major risk factors for diseases ranging from insulin resistance to type 2 diabetes and atherosclerosis. Enlargement of adipocytes, due to impaired adipocyte differentiation, leads to a chronic state of inflammation in the adipocytes and adipose tissue with a reduction in the secretion of adiponectin and increase in the secretion of proinflammatory cytokines such as interleukin (IL)-6, IL-8 and monocyte chemoattractant protein (MCP)-1. The secretion of cytokines like tumour necrosis factor (TNF)-α, mainly from macrophages, enhances local inflammation. These proinflammatory cytokines might also substantially affect cardiovascular function and morphology. Furthermore, a proinflammatory state in adipose tissue can lead to local insulin resistance with an impaired inhibitory effect of insulin on the release of FFAs and endothelial dysfunction that clearly promotes cardiovascular diseases and type 2 diabetes. The underlying mechanisms of ectopic fat accumulation in various tissues and the impact on metabolic syndrome and its association with insulin resistance are discussed.

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Introduction

For many years, adipose tissue was regarded merely as a heat insulator and a store of excess free fatty acids (FFAs) that could be released when needed. However, following the identification of adipokines, adipose tissue is now recognized to play a central role in the pathophysiology of insulin resistance and metabolic syndrome. Waist circumference, an easy-to-measure marker of metabolic risk and used to define metabolic syndrome, has been shown to correlate with different components of the syndrome supporting the notion that visceral obesity plays an important role in the development of cardiovascular diseases as well as insulin resistance and type 2 diabetes. Several recent studies have increased the understanding of adipose tissue biology and endocrine function. Obesity, characterized by enlarged adipocytes, and insulin resistance are associated with impaired adipogenesis and a low-grade chronic inflammation that to a large extent emanates from adipose tissue. The secretion of bioactive molecules from adipose tissue might have an effect on insulin sensitivity in the liver and peripheral tissues, and have a negative impact on the cardiovascular system. In this review, current knowledge of low-grade chronic inflammation in adipose tissue with local and systemic effects will be discussed.

Adipose Tissue

Adipose tissue in mammals can be divided into white and brown adipose tissue (WAT and BAT). WAT is not homogenous, and can be divided into subcutaneous and visceral adipose tissue. Subcutaneous adipose tissue stores excess calories and can be further divided into upper and lower body obesity, while visceral adipose tissue (omentum and mesenteric) supplies the inner organs with energy. There is 3 to 4
times more subcutaneous than visceral adipose tissue\textsuperscript{3}, and it appears that these two tissue types can interact in a coordinated and compensatory manner\textsuperscript{4}.

Although WAT and BAT share many metabolic characteristics, WAT stores energy whereas BAT dissipates energy. The role of BAT in adult humans is unclear but it has been recently proposed that it can be induced to increase glucose uptake\textsuperscript{5}. Furthermore, genes characteristic of human BAT have been shown to negatively correlate with obesity and insulin sensitivity\textsuperscript{6, 7}.

Adipose Tissue as an Endocrine Organ

Adipose tissue is a very complex cell. Under normal conditions, it is involved in lipid synthesis, uptake and storage. Secreted adipokines function as either endocrine, paracrine or autocrine mediators. Increases in adipocyte size can lead to deleterious alterations in insulin sensitivity caused by a decrease in adiponectin secretion, an increase in the release of FFAs and the induction of inflammatory mediators.

The Adipocyte

The adipocyte is a very complex cell. Under normal conditions, it is involved in lipid synthesis, storage and secretion of anti-inflammatory molecules, but it can also be induced to secrete a number of inflammatory factors such as MCP-1 and IL-6. By acting as transmitters of endocrine or paracrine signals, the secreted adipokines can promote either inflammation or altered insulin sensitivity of the adipocyte (Fig. 2).

Fat mass is dependent on both adipocyte cell number and size. The number of adipocytes is determined during early adulthood and changes in fat mass are attributed to changes in adipocyte cell size\textsuperscript{10}. The adipocyte turnover rate in humans was recently established to be ~10% per year\textsuperscript{10}. Fat cell mass increases with increasing body fat to a maximum of ~0.7 −0.8 \textmu g lipids per cell, and thereafter there is a more rapid increase in fat cell number\textsuperscript{11}. In subjects with obesity, adipose tissue has a lower capacity for the recruitment of new adipocytes\textsuperscript{12}. Tschoukalova et al. found that the number of committed preadipocytes was decreased in patients with obesity independent of fat location\textsuperscript{13}. In a recently published study from our laboratory, we found that the number of mesenchymal precursor cells was increased in obese individuals but differentiation of preadipocytes into adipocytes was decreased, suggesting an impaired differentiation of preadipocytes in obesity\textsuperscript{14}. Therefore, it appears that most adult-onset obesity is related to the hypertrophy of adipocytes, and enlarged adipocytes is the best obesity-fac-
tor in correlation with insulin resistance compared with other factors related to obesity\(^1, 14\).

Large adipocytes are more insulin-resistant and lipolytic, and release more inflammatory cytokines and less adiponectin (Fig. 2\(^15\)). They are also more frequently found in individuals with obesity-related metabolic disorders\(^11\). Thus, the relative number of large adipocytes might be the most important determinant of metabolic activity and the response to environmental changes.

**Features of Metabolic Syndrome**

The term “metabolic syndrome” includes hypertension, dyslipidemia, glucose intolerance and insulin resistance, variables that all are associated with obesity\(^16\). Dysfunctional adipose tissue with low-grade, chronic and systemic inflammation links the metabolic and vascular pathogenesis including dyslipidemia, low-grade inflammation and insulin resistance and is a hallmark of disorders such as type 2 diabetes and cardiovascular disease (Fig. 3). However, lifestyle factors and, to a lesser degree, genetic factors, are also involved\(^17\).

**Visceral and Subcutaneous Adipose Tissue**

A high level of visceral fat is an independent risk factor for glucose intolerance, insulin resistance, and cardiovascular disease. Visceral fat refers to the intra-peritoneal fat composed of the greater and lesser omentum and mesenteric adipose tissue. Visceral fat accounts for -20% of total body fat in men compared with only -6% in pre-menopausal women. A male risk profile has been characterized in women with abdominal obesity\(^11\). Enlarged adipocytes in the viscera are characterized by an increased lipolytic state and are resistant to the anti-lipolytic effects of insulin\(^18\).

Waist circumference is an index of the body’s periartrial and periarteriolar fat. It is an easy-to-measure marker of metabolic risk and is used to identify individuals with metabolic syndrome. However, the adverse and contributing effects of abdominal subcutaneous fat should not be overlooked as up to 80% of total adipose tissue can be composed of subcutaneous fat\(^19\).

Visceral and subcutaneous adipose tissue deposits have constitutive biological differences that are characteristic of their physiological roles. Even though both deposits serve as energy reservoirs to maintain systemic equilibrium, there are differences in levels of lipid mobilization, adipokine production and adipocyte differentiation that might be of importance in responses to diet and exercise\(^20\). Visceral fat secretes higher levels of complement factors, adiponectin, and inflammatory markers such as IL-6, IL-8, angiotensinogen and plasminogen activator inhibitor-1\(^1, 4, 21, 22\).

Rates of FFA lipolysis are approximately 50% greater in obese individuals than in healthy subjects, and higher in subjects with upper body obesity than those with lower body obesity. However, abdominal subcutaneous fat is probably the main source of increased levels of circulating FFA\(^20\). The altered FFA metabolism and endocrine function in visceral obese individuals imply that visceral adipose tissue is involved in the pathophysiology of metabolic syndrome but this does not exclude a contribution from subcutaneous adipose tissue.

**TNF\(\alpha\) and IL-6**

Several studies suggest that tumour necrosis factor (TNF\(\alpha\)) and IL-6 are both involved in obesity-related insulin resistance and that TNF\(\alpha\) is one of the most important mediators of inflammation\(^23\). In contrast to IL-6, TNF\(\alpha\) is secreted not by adipocytes but instead by infiltrating macrophages in adipose tissue, and functions as a paracrine and/or autocrine factor\(^24, 25\). It has been shown that adipose tissue is a significant source of circulating IL-6 and also related to BMI and adipocyte size\(^15\). TNF\(\alpha\) and IL-6 are known to promote lipolysis and the secretion of FFA, which contribute to an increase in hepatic glucose production and insulin resistance\(^26\). Both cytokines impair adipocyte differentiation and instead promote inflam-
mation\textsuperscript{27}. Furthermore, IL-6 promotes inflammation not only in adipose tissue but in endothelial cells and liver cells\textsuperscript{28}. IL-6 also promotes insulin resistance by interfering with the insulin signaling in adipose tissue\textsuperscript{29}.

In a recent study in obese individuals, adipokine levels in the radial artery were compared with those in the portal circulation to evaluate the possibility that visceral fat supports systemic inflammation by secreting inflammatory cytokines into the portal circulation that drains visceral fat\textsuperscript{29}. The authors found a 50\% increase in the secretion of IL-6 into the portal vein, but no other differences among the inflammatory adipokines tested. They also found a direct correlation between the concentrations of IL-6 and systemic C-reactive protein (CRP) in the portal vein. These findings indicate that visceral fat is an important site for IL-6 secretion and provide a potential link between visceral fat and systemic inflammation in individuals with abdominal obesity\textsuperscript{22}.

A further study in overweight men showed that circulating levels of IL-6 were associated with visceral adiposity, whereas TNF\(\alpha\) showed an association with overall obesity\textsuperscript{30}. These results support the hypothesis that IL-6 mediates the hyperinsulinemic state related to excess visceral fat while TNF\(\alpha\) seems to contribute to the insulin resistance of overall obesity\textsuperscript{30}.

**Ectopic Fat Accumulation**

Ectopic fat is defined as the deposition of triglycerides within cells of non-adipose tissue that normally contain only small amounts of fat. A positive energy balance produces a pattern similar to lipodystrophy with the accumulation of excess lipids in the liver, skeletal muscle and pancreas indicating that adipose tissue is not capable of sequestering nutritional lipids away from these organs\textsuperscript{31}. The accumulation of fat in skeletal muscle and viscera is associated with insulin resistance and cardiovascular disease.

Lipid accumulation in liver and muscle is an early hallmark of type 2 diabetes. In the pancreas, lipid accumulation has been shown to precede suppressed glucose-mediated insulin production\textsuperscript{22}. In the lipid-overloaded heart, metabolic dysregulation may induce insulin resistance resulting in impaired glucose oxidation and, ultimately, heart failure\textsuperscript{33}. The accumulation of ectopic fat is now considered part of metabolic syndrome. However, although the evidence for its deleterious effects is strong, whether ectopic lipid accumulation precedes or succeeds insulin resistance is not clear.

**Epicardial Adipose Tissue**

Epicardial adipose tissue is metabolically active and a source of FFA and several bioactive adipokines such as adiponectin, TNF\(\alpha\), IL-1, IL-6, neutral growth factor and resistin\textsuperscript{34, 35}. Some of these factors might substantially affect cardiovascular function and morphology and, thereby, directly contribute to the development of the cardiovascular complications of increased adiposity as well as insulin resistance\textsuperscript{34, 35}. Epicardial fat reflects cardiac and visceral adiposity and is related to intima-media thickness, an increase in vascular stiffness, and inflammation which plays a major part in disease progression\textsuperscript{36, 37}. Individuals with coronary artery disease show increased macrophage infiltration into epicardial fat, suggesting a state of chronic inflammation\textsuperscript{36}.

Epicardial adipose tissue may also affect the coronary arteries and myocardium through paracrine and/or direct secretion of pro-inflammatory adipokines. Epicardial fat has a higher rate of FFA and triacylglycerol uptake than subcutaneous fat, but also a higher rate of fatty acid breakdown\textsuperscript{39}.

**Perivascular Fat**

Perivascular adipose tissue (PAT) surrounds blood vessels in changing amounts and is produced from the vascular lamina adventitia in response to circulating factors and local stimuli\textsuperscript{40}. PAT has been considered largely a passive structural support for arteries. However, it can play an active role in regulating vascular tone and releases adipocyte-derived vascular relaxation factors into blood vessels\textsuperscript{41}. Excess calories and inactivity enlarge PAT depots with potentially unfavorable consequences and an increase in PAT is suggested to mediate morphologic changes associated with an increase in vascular stiffness seen in obesity\textsuperscript{42}.

**Insulin Resistance**

Large adipocytes are more frequently found in subjects with impaired glucose tolerance and type 2 diabetes than those with a similar degree of adiposity but with normal glucose tolerance, and impaired adipocyte differentiation appears to be one of the most important factors in the progression of type 2 diabetes\textsuperscript{1}. Insulin has several functions, including the transport of nutrients into cells, the regulation of gene expression and energy homeostasis. It acts on a number of target tissues and through many different intracellular signaling cascades. Elevated levels of intracellular FFAs can blunt the response to insulin and sub-
Insulin receptor substrate (IRS)-1 is a key molecule in the insulin signaling pathway, and failure to activate IRS-1 leads to systemic insulin resistance. Inhibitory phosphorylation of IRS-1 can be induced through inflammatory agents such as TNFα and IL-6, but also through activation of receptors such as the Toll-like receptors (TLR), or intracellular molecules such as lipids and reactive oxygen species (ROS). Activation of the TNFα and IL-6 receptors induces activation of important activators of inflammation i.e. IκB kinase (IKKβ) and Janus kinase (JNK). JNK is also activated by FFAs and endoplasmatic reticulum stress, factors that are associated with obesity-induced activity. IKKβ does not phosphorylate IRS-1 but causes insulin resistance through activation of NFκB. Suppressor of cytokine signalling (SOCS) inhibits insulin actions on IRS-1 either by interfering with the tyrosine phosphorylation or by targeting IRS-1 for proteosomal degradation.

The consequences of decreased insulin production as a result of ectopic lipid accumulation in the pancreas combined with a diminished activation of the insulin receptor in adipocytes results in an impairment of insulin-stimulated glucose transport, a reduced anti-lipolytic effect, an increase in the amount of FFA released, impaired preadipocyte differentiation and a decrease in lipoprotein lipase production and activity. These effects will lead to the development of insulin resistance, type 2 diabetes and cardiovascular diseases.

Macrophage Infiltration into Adipose Tissue

There are two kinds of macrophages in adipose tissue: resident/tissue (or alternatively activated) macrophages and inflammatory macrophages. In severely obese individuals, the number of macrophages is higher in visceral fat than subcutaneous fat, consistent with a more prominent role for visceral fat in insulin resistance. The infiltration of monocytes/macrophages into adipocyte tissue is probably initiated by an increase in adipocyte size. Enlargement of adipocytes is associated with an increase in physical stress and ROS production, and increased secretion of FFA and inflammatory cytokines. Of these cytokines, MCP-1 secreted from macrophages seems to be the most important. MCP-1 is expressed before inflammatory macrophage markers, providing evidence that cells other than macrophages produce it. In obesity, circulating mononuclear cells are in a proinflammatory state and are key players in endothelial dysfunction. The transmigration of blood monocytes into adipose tissue is a complex mechanism that requires the expression of adherent molecules both on the monocytes and on the endothelial cells to which they attach. After transmigration into adipose tissue, the monocytes differentiate into inflammatory macrophages. Infiltrating inflammatory macrophages constitute a major source of inflammatory mediators, especially TNFα, within adipose tissue, and it is likely that they
act synergistically with adipocytes to amplify local inflammation \(^53\). Neutralizing antibodies to TNF\(\alpha\) have been shown to inhibit inflammation in a coculture of 3T3-L1 adipocytes and a macrophage cell line, suggesting that TNF\(\alpha\) is a major macrophage-derived mediator of inflammation in adipocytes \(^54\). Resident macrophages have low levels of proinflammatory cytokine production but can be activated with an enhanced recruitment and activation of blood monocytes \(^52\). The infiltration of macrophages into adipose tissue contributes to the local and systemic metabolic effects of obesity; however, the majority of macrophages are distributed around necrotic adipocytes whose numbers increase dramatically in obese individuals. For example, Cinti et al. showed that >90% of all macrophages in the adipose tissue of obese individuals were located around dead adipocytes \(^55\). They also showed a positive correlation between adipocyte death and adipocyte size and suggested that adipocyte death helps to mediate local macrophage infiltration \(^55\). It is proposed that the inflammation in adipocytes entails a paracrine loop involving FFAs released from adipocytes and TNF\(\alpha\) released from macrophages, which, together with other factors, leads to a further increase in recruitment of monocytes \(^54\).

**Vasocrine Signaling**

Excess intake of calories results in increased deposition of perivascular fat around the heart and its major branches. Conditions where there is an increased amount of adipose tissue surrounding the blood vessels, overproduction of adipokines, and signaling from perivascular fat deposits to the arteries, “outside-to-inside signalling”, could then lead to inflammation and atherosclerosis (Fig. 5). Perivascular fat is also considered to be ectopic within adipose tissue, acting as an integrated organ responsible for both paracrine signaling and vessel-to-vessel signaling “vasocrine signaling”. Insulin has the rapid effect of increasing blood flow to skeletal muscle and an ability to recruit capillaries that depends on its actions to dilate precapillary arterioles \(^56\). The most potent inhibitor of insulin’s actions and endothelial nitrogen oxide (NO)-dependent vasodilation among cytokines is TNF\(\alpha\), and it is suggested that a high local concentration of cytokines leads to a blunted activation by insulin of endothelial NO synthase and increased release of endothelin-1, resulting in a constriction of the arteriole \(^40\).
Effects of Adiponectin

In 1995 and 1996, four different groups independently identified adiponectin\(^57,59\). Belonging to the complement 1q family, adiponectin forms characteristic multimers of which the high molecular weight (HMW) multimer seems to be the most important\(^60,61\). The ratio of the HMW multimer to other forms is an independent risk factor for metabolic disorders\(^62\). Adiponectin is abundantly expressed in adipose tissue and circulating levels are high, 5 to 20 \(\mu\)g/mL. Adiponectin levels negatively correlate with visceral adiposity to a greater extent than with subcutaneous adiposity.

Kim \textit{et al.} showed that although overexpression of adiponectin in ob/ob mice results in normalized glucose and insulin levels together with improvements in triglyceride and FFA levels, the mice became extremely obese due to adipocyte hyperplasia\(^63\). Despite the obesity, very few macrophages were present in adipose tissue, consistent with increased differentiation of new insulin-sensitive adipocytes\(^63\).

Adiponectin and the Vascular System

Adiponectin has effects in a number of different tissues. For example, in muscle, it counteracts insulin resistance\(^64\). In arteries, it reduces atherosclerosis and intima media thickness\(^65\). It also has effects on capillaries and the heart\(^61\). The actions of adiponectin are summarized in \textbf{Fig. 6}.

Adiponectin attenuates inflammatory actions at several levels. It reduces TNF\(\alpha\)-stimulated expression of E-selectin, vascular cell adhesion molecule-1 (VCAM-1) and IL-8 in human aortic endothelial cells\(^66-68\). It also inhibits TNF\(\alpha\)-induced activation of nuclear factor-\(\kappa\)B (NF\(\kappa\)B) and prevents the proliferation and migration of smooth muscle cells. Adiponectin inhibits foam cell formation (lipid accumulation in macrophages) as well as platelet aggregation and T-cell recruitment and accumulation\(^69\). It has been shown to inhibit Toll-like receptor-mediated activation of NF\(\kappa\)B in mouse macrophages\(^70\). Adiponectin is also an important regulator of endothelial NO synthase, a major determinant of endothelial function and angiogenesis\(^71\).

Conclusion

Adipose tissue is a central factor in the development of insulin resistance and regulation of whole-body insulin sensitivity. It is also plays an important role in vascular complications. Adipose tissue has the ability to modulate both local and systemic processes in other tissues such as the liver, pancreas, skeletal muscle, heart and brain. An increase in adipose tissue
mass results in the infiltration of macrophages and enhanced inflammation. Adipokines produced by the adipose tissue impair insulin signalling and key proteins for glucose uptake. In addition, secreted adipokines promote endothelial dysfunction, adhesion of monocytes, vascular remodelling and foam cell formation in the arterial wall, which contribute to cardiovascular complications. The reduction in adiponectin levels with increasing adiposity contributes to obesity-associated vascular complications.

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