

# The role of adipose tissue dysfunction in the pathogenesis of obesity-related insulin resistance

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## Abstract

Research of the past decade has increased our understanding of the role adipose tissue plays in health and disease. Adipose tissue is now recognized as a highly active metabolic and endocrine organ. Adipocytes are of importance in buffering the daily influx of dietary fat and exert autocrine, paracrine and/or endocrine effects by secreting a variety of adipokines. The normal function of adipose tissue is disturbed in obesity, and there is accumulating evidence to suggest that adipose tissue dysfunction plays a prominent role in the development and/or progression of insulin resistance. Obese individuals often have enlarged adipocytes with a reduced buffering capacity for lipid storage, thereby exposing other tissues to an excessive influx of lipids, leading to ectopic fat deposition and insulin resistance in situations where energy intake exceeds energy expenditure. In addition, adipose tissue blood flow is decreased in obesity. This impairment may affect lipid handling in adipose tissue and, thereby, further contribute to excessive fat storage in non-adipose tissues. On the other hand, adipose tissue hypoperfusion may induce hypoxia in this tissue. Adipose tissue hypoxia may result in disturbances in adipokine secretion and increased macrophage infiltration in adipose tissue, events that are frequently observed in obesity. In this review, it is discussed how enlarged adipocytes, an impaired blood flow through adipose tissue, adipose tissue hypoxia, adipose tissue inflammation and macrophage infiltration are interrelated and may induce insulin resistance.

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## 1. Introduction

The prevalence of obesity has reached epidemic proportions globally, with more than 1 billion adults being overweight, of whom at least 300 million are obese [1]. This poses a major public health issue, since obesity is a major contributor to the global burden of chronic diseases. Abdominal obesity plays a central role in the metabolic syndrome and is a major risk factor for chronic diseases, such as type 2 diabetes mellitus and cardiovascular disease [2]. Not surprisingly, the prevalence of obesity-related disorders is also increasing at an alarming rate. In fact, obesity is the most important risk factor for the development of type 2 diabetes [3], which is further stressed by

the fact that obesity, body fat distribution and weight gain throughout adulthood are important predictors of diabetes [3,4]. Furthermore, adiposity is associated with insulin resistance even over relatively normal ranges of body fatness. Although the relationship between obesity, insulin resistance and cardiovascular disease is well-recognized [5], the mechanisms involved remain relatively poorly understood.

Adipose tissue dysfunction plays a crucial role in the pathogenesis of obesity-related insulin resistance and type 2 diabetes, as has recently been reviewed [6–8]. The aim of this review is to discuss the evidence that enlarged adipocytes, an impaired adipose tissue blood flow (ATBF), adipose tissue hypoxia, local inflammation in adipose tissue and adipose tissue macrophage infiltration seem to be interrelated and may lead to disturbances in adipokine secretion, lipid overflow, and excessive fat storage in non-adipose tissues, which together

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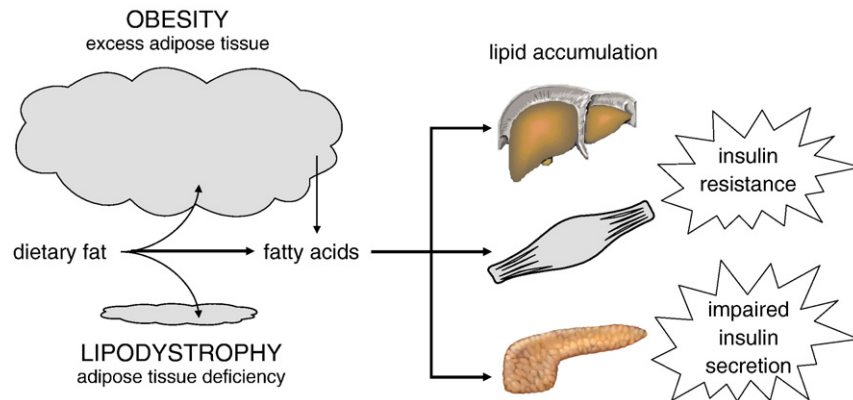


Fig. 1. The normal function of adipose tissue is to buffer the daily influx of dietary fat. When the buffering capacity for lipid storage in adipose tissue is decreased, as in obesity (when the fat cells are overloaded) and lipodystrophy (when the adipose tissue necessary to perform such a function is lacking), other tissues are exposed to an excessive influx of fatty acids and TAG, which in turn may result in TAG storage that interferes with insulin sensitivity (skeletal muscle and liver) and insulin secretion (pancreas).

may result in the development and/or progression of insulin resistance.

## 2. Adipose tissue as lipid storage depot

Obesity is the result of an imbalance between energy intake and energy expenditure. When energy intake exceeds energy expenditure the energy surplus is stored in various organs. Adipose tissue is the main lipid storage depot in our body, and is of crucial importance in buffering the daily influx of dietary fat entering the circulation. Adipose tissue exerts its buffering action by suppressing the release of non-esterified fatty acids into the circulation and by increasing the clearance of triacylglycerol (TAG). In obesity, adipose tissue is overloaded with TAG and the buffering capacity for lipid storage in adipocytes is decreased, especially in the postprandial state [9]. It could be argued that TAG storage in adipocytes of obese subjects has reached a near-maximum level and that these adipocytes are therefore not able to effectively store even more lipids.

Consequently, non-adipose tissues are exposed to an excessive influx of TAG and fatty acids, which could lead to accumulation of these lipid fuels in the form of TAG when the capacity to oxidize fatty acids is not sufficient (Fig. 1). A large number of observations suggest that TAG accumulation in non-adipose tissues, such as skeletal muscle [10–12], pancreatic islets [13] and the liver [14,15], may play an important role in the development of insulin resistance and/or impaired insulin secretion in obese individuals. In addition, increased delivery of fatty acids to the liver leads to higher glucose production [16–18], elevated hepatic very low-density lipoprotein (VLDL)–TAG output [19], and reduced insulin clearance by the liver [20–22], resulting in conditions associated with insulin resistance, such as glucose intolerance, hyperlipidemia, and hyperinsulinemia, respectively [23] (Fig. 2).

The importance of the buffering function of adipose tissue is further emphasized by the adverse metabolic consequences in situations where adipose tissue is lacking. A deficiency of adipose tissue, as in lipodystrophy, is also associated with insulin

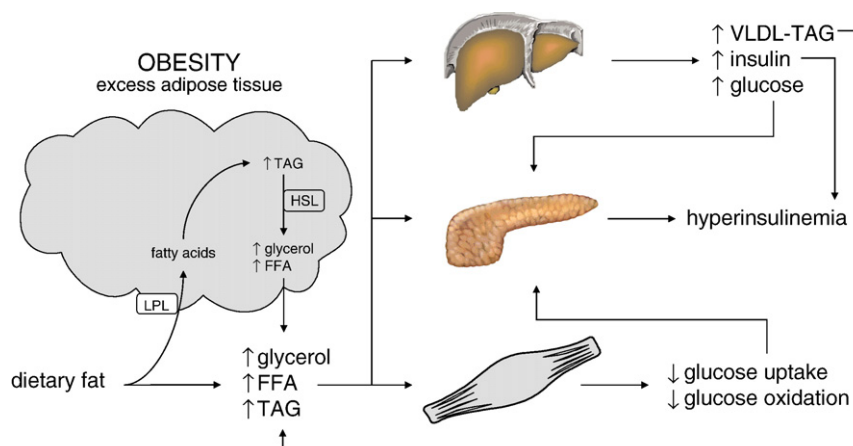


Fig. 2. LPL and HSL act on circulating TAG-rich lipoprotein particles and stored TAG, respectively, to release fatty acids. The fatty acids that are released are transported into the circulation or are re-esterified. A decreased buffering capacity for lipid storage in adipose tissue results in an increased flux of fatty acids and TAG to skeletal muscle, liver, and pancreas. In addition to lipid accumulation in these tissues, this leads to an increased liver glucose production, elevated hepatic VLDL–TAG output, and decreased insulin clearance by the liver. Together with disturbances in skeletal muscle glucose metabolism, these events result in conditions related to insulin resistance, such as glucose intolerance, hyperinsulinemia, and hyperlipidemia. LPL, lipoprotein lipase; HSL, hormone-sensitive lipase; TAG, triacylglycerol; FFA, free fatty acids; VLDL, very low-density lipoprotein.

resistance and a high incidence of type 2 diabetes [24]. Mice models that lack adipose tissue are severely insulin resistant and have an elevated lipid content in skeletal muscle and liver [25]. Surgical implantation of adipose tissue from healthy mice into these lipodystrophic animals reduced TAG content in these tissues, and reversed insulin resistance in a dose-dependent manner [26]. Thus, both excess adipose tissue and too little (or complete absence of) adipose tissue may elicit insulin resistance. In other words, an impaired capacity to store the daily influx of dietary fat in adipose tissue may result in ectopic fat deposition and insulin resistance in situations where energy intake exceeds energy expenditure.

### 3. Importance of adipose tissue blood flow in lipid metabolism

Tissue-specific regulation of blood flow is required to meet local metabolic and physiological demands under varying conditions. Blood flow may be an important regulator of metabolism in both muscle [27] and adipose tissue [28–30]. There is evidence that disturbances in adipose tissue blood flow (ATBF) may affect adipose tissue lipid handling, thereby contributing to an increased lipid supply to non-adipose tissues, which in turn may lead to ectopic fat deposition as discussed above. In lean, healthy individuals, ATBF is responsive to nutrient intake [31–36]. The ATBF response to nutrient intake may be of great importance in the regulation of metabolism by facilitating signalling between adipose tissue and other tissues [37]. ATBF controls the supply of circulating TAG-rich lipoprotein particles to adipose tissue lipoprotein lipase [30], which is responsible for hydrolysis of these particles into fatty acids and glycerol. It has been demonstrated that both fasting ATBF [38–42] and ATBF responsiveness to nutrients [40,41] are reduced in obesity. An impaired postprandial ATBF seems to be associated with insulin resistance [40,43], which may partly be explained by a decrease in TAG clearance in the postprandial period. Indeed, it has been reported that plasma TAG extraction in adipose tissue is decreased in obese compared to lean subjects both in the fasting and postprandial state [44]. Further evidence supporting a role for ATBF in lipid metabolism comes from a study where ATBF was manipulated pharmacologically to examine the effects of ATBF on adipose tissue metabolism. Intravenous adrenaline infusion, resulting in an elevation of ATBF, increased the extraction of TAG in adipose tissue [30]. Thus, disturbances in ATBF regulation may contribute to a reduced TAG clearance, lipid overflow and lipid-induced insulin resistance. On the other hand, there is evidence that insulin resistance may lead to vascular dysfunction, since obesity is associated with impaired endothelium-dependent vasodilatation in various vascular beds in response to insulin [45–47]. In other words, although cause and consequence cannot be clearly defined based on available data, it is evident that ATBF and insulin resistance are related.

### 4. Link between adipocyte size and insulin resistance

Enlargement of adipocytes is frequently observed in obesity and has also been demonstrated in pre-diabetic individuals and

in type 2 diabetics [48–50]. The increased adipocyte size may represent a failure in the recruitment of new adipocytes due to impaired differentiation, which may have a genetic origin [51]. An impaired adipocyte differentiation appears to be a precipitating factor in the development of type 2 diabetes [49,50]. In accordance with this, it has recently been shown that fat cell enlargement is an independent marker of insulin resistance [52]. In fact, enlarged abdominal subcutaneous adipocyte size and insulin resistance appear to be independent and additive predictors of the development of type 2 diabetes [50].

One potential link between adipocyte size and the development and/or progression of insulin resistance could be the release of fatty acids from adipose tissue. Mobilization of fatty acids from stored adipocyte TAG stores is mediated by hormone-sensitive lipase [53] and the recently characterized adipose triglyceride lipase [54]. Fat mobilization is strongly inhibited by insulin. It has been suggested that the enlarged adipocytes of obese subjects may be resistant to the antilipolytic effect of insulin, which would result in an increased release of fatty acids into the circulation. However, adipocytes from obese and type 2 diabetic subjects appear to be equally responsive to the antilipolytic effects of insulin compared to control subjects despite the presence of systemic insulin resistance [55,56]. There are data to suggest that non-esterified fatty acids may not provide the link between adipocyte size and insulin resistance [50,52], but it is important to note that these studies measured fasting rather than postprandial fatty acid concentrations. The latter might play a more important role in the development of insulin resistance. A placebo-controlled, double-blind cross-over study using a thiazolidinedione (TZD) insulin-sensitizer provided evidence to suggest that systemic insulin concentration is an important determinant of fatty acid release from adipose tissue [57]. Since adipose tissue appears to be normally insulin-responsive with respect to inhibition of lipolysis in obese and type 2 diabetic subjects, hyperinsulinemia as often present in insulin resistant conditions will decrease fasting lipolysis. On one hand, this might be a mechanism to protect subjects with excess adipose tissue mass against the detrimental effects of a high circulating fatty acid concentration [58]. On the other hand, decreased adipocyte lipolysis in hyperinsulinemic conditions may contribute to a continuous increase in adipocyte size, which may further impair the dynamic function of adipose tissue to store lipids in order to accommodate an increased energy supply. Thus, it may be that an impaired buffering capacity for lipid storage in the postprandial state and the consequent prolonged elevation of non-esterified fatty acid concentration [34], rather than an increased fasting adipocyte lipolysis, provides the link between adipocyte size and insulin resistance. Although adipocytes of insulin resistant subjects seem to respond normally to insulin-induced inhibition of lipolysis, both rodent and human *in vitro* studies have demonstrated that enlarged adipocytes are insulin resistant with respect to glucose uptake [52,59–63]. It has been shown that changes in adipose tissue glucose uptake can have secondary effects on whole-body glucose metabolism in rodents [64], but it is unlikely that a reduced glucose uptake in enlarged adipocytes in response to insulin directly causes systemic

insulin resistance, since skeletal muscle is responsible for more than 80% of insulin-stimulated glucose disposal [65].

Further evidence supporting the importance of adipocyte size in the development of insulin resistance and type 2 diabetes comes from pharmacological studies. It has been demonstrated that insulin-sensitizing TZDs, acting via the peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ), stimulate adipocyte differentiation resulting in an increase in the number of small adipocytes and a decrease in the number of large adipocytes [66–68]. In addition to the formation of new adipocytes from resident adipose tissue pre-adipocytes and mesenchymal progenitor cells, it has recently been reported that TZDs promote the trafficking of bone marrow-derived circulating progenitor cells to adipose tissue and stimulate their differentiation into adipocytes [69]. Finally, a novel non-TZD PPAR $\gamma$  agonist has been shown to induce adipocyte differentiation in Zucker fatty rats by stimulating PPAR $\gamma$ , thereby increasing the number of small adipocytes, resulting in improved insulin sensitivity [70].

In conclusion, one reason why enlarged adipocytes predispose to the development of type 2 diabetes could be that enlarged adipocytes may not be able to effectively store dietary fatty acids due to defects in the ability of adipose tissue to respond rapidly to the dynamic situation after meal intake by switching between fatty acid uptake and release. As a consequence, lipid overflow, ectopic fat deposition and insulin resistance may develop. Another explanation for the observation that adipocyte hypertrophy is associated with insulin resistance and the development of type 2 diabetes relates to the secretory function of adipocytes, as will be discussed below.

### 5. Adipose tissue as an endocrine organ

Until recently, adipose tissue was seen as a passive organ for energy storage. Research of the past decade has shown the complex nature of adipose tissue and clearly demonstrated that the traditional view of adipose tissue is no longer valid. Adipocytes are now known to express and secrete a variety of adipokines, which may act at both the local (autocrine and/or paracrine) and systemic (endocrine) level. These factors among others include cytokines, growth factors, adiponectin, resistin, adipsin, leptin, acylation stimulating protein (ASP), plasminogen activator inhibitor-1 (PAI-1), lipoprotein lipase (LPL), and components of the renin–angiotensin system (Fig. 3) [37,71,72]. Thus, in addition to the ability of adipose tissue to modulate its own metabolic activities, adipocytes signal to other tissues to regulate their metabolism. On the other hand, adipose tissue expresses numerous receptors that allow it to respond to afferent signals, such as hormones and signals from the central nervous system. Since the recognition of adipose tissue as an endocrine organ, there has been great interest in the possibility that adipose tissue-derived factors may contribute to the metabolic and hemodynamic disturbances seen in obesity and insulin resistance.

It is well-established that chronic low-grade inflammation is a hallmark of obesity, insulin resistance and type 2 diabetes [73–76]. An interesting feature of the inflammatory response

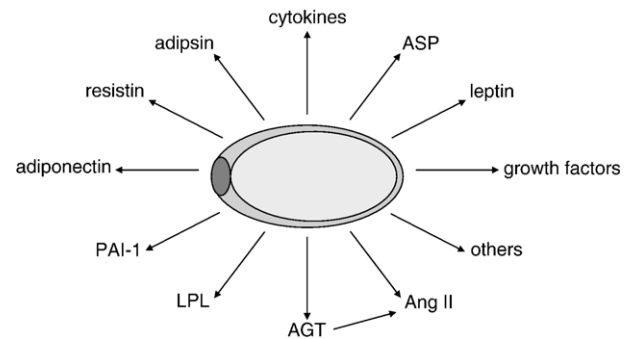


Fig. 3. Adipocytes express and secrete a variety of substances, which may exert autocrine, paracrine and/or endocrine functions. Several of these factors may play an important role in the metabolic and hemodynamic disturbances observed in obesity and insulin resistance. AGT, angiotensinogen; Ang II, angiotensin II; ASP, acylation stimulating protein; LPL, lipoprotein lipase; PAI-1, plasminogen activator inhibitor-1.

that often emerges in the presence of obesity is that it appears to be triggered and to reside predominantly in the expanded adipose tissue [77–81]. Adipose tissue of obese insulin resistant subjects is characterized by increased expression and/or secretion of inflammatory molecules, including tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) [79,82–84], interleukin (IL)-6 [83–86], PAI-1 [87,88] and leptin [84,89–91]. Conversely, the insulin-sensitizing factor adiponectin is downregulated in obese and insulin resistant humans [92–94]. It has been demonstrated that adipocyte size is an important determinant of adipokine secretion. A variety of adipokines that may link obesity to insulin resistance appear to be overexpressed in enlarged human and rodent adipocytes [95–97]. Furthermore, it has very recently been shown using cultured human adipocytes that large adipocytes seem to differentially express pro-inflammatory and anti-inflammatory factors compared to smaller adipocytes, with a shift toward dominance of pro-inflammatory adipokines [98]. In agreement with these observations, weight-loss resulted in a reduced adipocyte size and beneficial alterations in the secretory pattern [99]. A similar change in adipokine expression and secretion was observed when adipocyte differentiation was stimulated using TZD treatment. The increased number of small adipocytes and the decreased number of large adipocytes in white adipose tissue of troglitazone-treated obese rats resulted in normalization of the increased expression level of TNF- $\alpha$  [68]. Interestingly, there are clear interactions between adipokines. Downregulation of adiponectin expression may at least partly be due to the action of adipokines that are overexpressed in obesity, including TNF- $\alpha$  and IL-6 [78,100,101]. This in turn will affect the inflammatory response, since adiponectin attenuates inflammatory responses to multiple stimuli [102]. Furthermore, TNF- $\alpha$  has been demonstrated to increase IL-6 production in 3T3-L1 adipocytes [103,104]. Thus, TNF- $\alpha$  appears to play a pivotal role with respect to the production of several other adipokines [105]. In addition to adipose tissue, other metabolically active organs may exert secretory effects. Human skeletal muscle seems to produce and secrete IL-6 [106–109] and angiotensinogen [39]. Furthermore, it has been suggested that IL-6 may stimulate



hepatic production of C-reactive protein [76,110,111]. Therefore, non-adipose tissues may also contribute to the inflammatory response in obesity.

## 6. Importance of inflammation in insulin resistance

Studies in the past decade left little doubt that inflammatory pathways are critical in the mechanisms underlying insulin resistance and type 2 diabetes, at least in cultured cells and animal models [112–116]. Accumulating evidence suggests that this may also be the case in humans. Much progress has been made in identifying mechanisms by which the inflammatory response may cause insulin resistance. Dysregulation of adipokine production and/or secretion may both have local and systemic effects. First, it has been shown that TNF- $\alpha$  inhibits the differentiation of human adipocyte precursor cells and 3T3-L1 cells via inhibition of two master regulators of adipocyte differentiation, namely the transcription factor CCAAT/enhancer binding protein- $\alpha$  and PPAR $\gamma$ 2 [117–119]. Like TNF- $\alpha$ , IL-6 may inhibit adipocyte differentiation [101]. Furthermore, TNF- $\alpha$  has been shown to induce apoptosis in both human pre-adipocytes and mature adipocytes [120]. These TNF- $\alpha$ -induced effects together may result in enlargement of remaining fat cells and, consequently, a reduced adipose tissue lipid buffering capacity and further impairment of adipokine secretion, leading to insulin resistance as discussed earlier. Secondly, certain adipokines may affect lipid metabolism directly. TNF- $\alpha$  increases lipolysis in human and 3T3-L1 adipocytes, which appears to be mediated by activation of the extracellular signal-related kinase pathway and reduction in protein expression of perilipin [121–123], which is an adipocyte protein that coats the lipid storage droplet, thereby acting as gatekeeper to hydrolysis of the lipid droplet [124]. In addition, IL-6 has been shown to stimulate lipolysis in vivo in humans [125]. Therefore, these local effects on adipose tissue lipolysis may contribute to the development of insulin resistance by promoting the release of fatty acids from adipose tissue into the circulation, which may then result in lipid accumulation and insulin resistance in other tissues such as skeletal muscle and liver. Furthermore, decreased adiponectin concentrations may have detrimental effects on fat oxidation, since it has been demonstrated that adiponectin increases fat oxidation via activation of AMP-activated protein kinase in rat skeletal muscle and C2C12 myocytes [126–128]. Thirdly, there seems to be cross-talk between insulin receptor signalling and inflammatory pathways [113,116]. TNF- $\alpha$  can impair insulin sensitivity by triggering different key steps in the insulin signalling pathway in rodent skeletal muscle [129,130]. Furthermore, both in vitro (C2C12 myocytes) and in vivo experiments in rodent suggest that a reduction in adiponectin concentration may decrease skeletal muscle glucose uptake [127,131,132] and increase gluconeogenesis [128].

In conclusion, there is substantial evidence for the concept that local inflammation in the expanded adipose tissue mass in obese individuals is at least partly responsible for obesity-related insulin resistance. However, it is important to note that the effects of adipokines on metabolism and insulin sensitivity

are generally studied in isolation, which makes it difficult to predict the interactive effects and the net impact on insulin sensitivity in vivo in humans. The mechanisms underlying the low-grade inflammation that is often present in obesity are not yet well understood, but recent data indicate that adipose tissue of obese individuals is infiltrated by macrophages, which may be a major source of locally-produced pro-inflammatory adipokines [133,134].

## 7. Adipose tissue inflammation and macrophage infiltration

Adipose tissue is a heterogeneous tissue containing different cell types, including mature adipocytes, pre-adipocytes, endothelial cells, vascular smooth muscle cells, leukocytes, monocytes and macrophages. Due to this heterogeneity, several studies have been performed to establish the cellular origin of the adipokines that are expressed in adipose tissue. Macrophages are now recognized as important non-adipocyte cells that contribute to adipose tissue production of inflammatory factors. In fact, it has been reported that non-adipocyte cells in adipose tissue are responsible for the majority of inflammatory factors secreted by this tissue, except for leptin and adiponectin that are primarily secreted by adipocytes [135]. However, it is important to note that these findings should be interpreted with some caution, since it is not known whether quantitative comparisons between cell fractions in vitro (following collagenase digestion and cell incubation) reflect the physiological situation in vivo. Changes in adipocyte size and adipose tissue mass result in physical changes in the surrounding tissue, which may modulate adipocyte function as discussed earlier in this review. Obese adipose tissue is characterized by progressive infiltration by macrophages as obesity develops [133,134]. In line with these findings, macrophage infiltration in adipose tissue is positively associated with body mass index, adipocyte size and insulin resistance [133,134,136–138]. In turn, weight-loss induced a regression of adipocyte hypertrophy and macrophage infiltration in adipose tissue, resulting in an improvement of the inflammatory profile of gene expression [136,139]. Therefore, macrophage infiltration in adipose tissue in obesity could be integral to the inflammatory response in this tissue. It is reasonable to assume that cross-talk between adipocytes and macrophages is important in the development of insulin resistance. Indeed, the effects of macrophage-secreted factors on adipocytes may contribute significantly to the systemic inflammation and insulin resistance associated with obesity [140]. It has been shown that macrophage-secreted factors impair human fat cell differentiation [141] and induce inflammatory events in 3T3-L1 adipocytes by activating the nuclear factor kappa B (NF- $\kappa$ B) pathway [140]. Macrophages may have different phenotypes [142,143]. Although the functional consequences are not yet completely evident, it has recently been demonstrated that recruited adipose tissue macrophages have unique (inflammatory) properties compared with the resident adipose tissue macrophages [144], which may imply that recruited rather than resident adipose tissue macrophages are involved in the inflammatory response in obesity.

Do macrophages infiltrate adipose tissue and initiate the inflammatory response or does the initial inflammatory response emerge from the adipocyte and further propagate with the recruitment of macrophages? Several studies have been performed to elucidate the triggers for macrophage infiltration in adipose tissue, which are probably multifactorial. It has recently been demonstrated that monocyte chemoattractant protein-1 (MCP-1) may play an important role in macrophage infiltration in adipose tissue. MCP-1 is a chemokine and member of the small inducible cytokine family, which plays a role in monocyte and lymphocyte recruitment to sites of injury and infection [145]. MCP-1 is produced by macrophages, endothelial cells and adipocytes [146,147], and its expression is closely related to the number of residing macrophages [148]. It has been reported that MCP-1 expression in adipose tissue is increased in obese rodents [149,150]. In addition, circulating concentrations of MCP-1 are elevated in obese [146,151] and diabetic individuals [152,153], and its concentration have been found to decrease after weight-loss [154]. In vitro studies in adipocytes and myocytes have shown that MCP-1 may induce insulin resistance [149,155]. However, circulating concentrations do not always correlate with obesity and diabetes [156]. Therefore, MCP-1 may predominantly exert local (autocrine/paracrine) effects in adipose tissue rather than having a direct systemic pathogenic role. The early timing of MCP-1 expression prior to that of other macrophage markers during the development of obesity supports the idea that MCP-1 is produced initially by cells other than macrophages [134]. Interestingly, in obesity, MCP-1 expression and/or secretion can be stimulated by TNF- $\alpha$ , insulin, IL-6 and growth hormone in pre-adipocytes and 3T3-L1 adipocytes [134,149,157,158], whereas its secretion from human adipocytes is decreased by stimuli that increase insulin sensitivity, including adiponectin and TZD treatment [159,160]. Thus, alterations in adipokine

secretion may change MCP-1 expression and secretion, which in turn could influence macrophage infiltration in adipose tissue. Knockout studies have shown that C–C chemokine receptor 2 (CCR2) and its ligand MCP-1 are required for accumulation of macrophages in adipose tissue [161,162]. CCR2 and MCP-1 knockout mice both are characterized by decreased adipose tissue macrophage infiltration, reduced pro-inflammatory gene expression in adipose tissue, decreased hepatic triacylglycerol content and improved insulin sensitivity on a high-fat diet compared with wild-type animals [161,162]. In accordance with these observations, adipose tissue-specific overexpression of MCP-1 increases adipose tissue macrophage content and decreases insulin sensitivity [161,163]. Thus, MCP-1 seems to play an important role in macrophage infiltration in adipose tissue and may contribute to the development of insulin resistance. Whatever the initial stimuli to recruit macrophages into adipose tissue are, once these cells are present in adipose tissue they, along with adipocytes and other cell types, could perpetuate a vicious cycle of macrophage recruitment and production of pro-inflammatory adipokines, which may result in progressive loss of adipocyte function and development of obesity-related insulin resistance (Fig. 4).

In conclusion, it seems unlikely that macrophages initiate inflammation in adipose tissue. Rather, macrophages, and likely other types of immune cells, are thought to amplify an inflammatory response that has already been established. Although the precise mechanisms underlying the disturbances in adipokine secretion and subsequent macrophage infiltration in adipose tissue that are often present in obesity remain to be established, it is highly likely that this relates to events within adipose tissue itself. Interestingly, there is accumulating evidence to support the view that adipose tissue hypoxia may play a major role in obesity-related impairments in adipokine expression/secretion and subsequent insulin resistance.

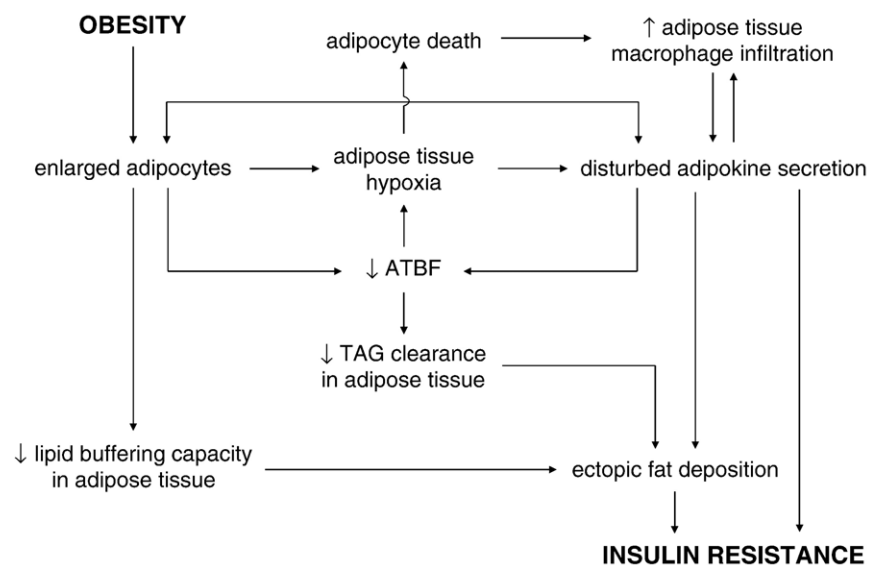


Fig. 4. Proposed mechanisms how adipose tissue dysfunction may play a crucial role in the pathogenesis of obesity-related insulin resistance and type 2 diabetes. Enlarged adipocytes, an impaired ATBF, adipose tissue hypoxia, local inflammation and macrophage infiltration in adipose tissue seem to be interrelated, and may lead to disturbances in adipokine secretion and lipid accumulation in non-adipose tissues, which together may result in the development and/or progression of insulin resistance. ATBF, adipose tissue blood flow.

## 8. Role for adipose tissue hypoxia in insulin resistance?

Epidemiological and clinical studies have shown that obstructive sleep apnea (OSA) may contribute to the development of insulin resistance [164–166], possibly via cycles of intermittent hypoxia resulting from periodic collapse of the upper airway during sleep. Epidemiological studies have revealed an association between the degree of hypoxia and insulin resistance [164–166]. Interestingly, intermittent hypoxia causes acute insulin resistance in mice due to decreased skeletal muscle glucose utilization [167]. Based on these findings it can be speculated that hypoxia in certain tissues may induce insulin resistance. Is it possible that adipose tissue hypoxia contributes to obesity-related insulin resistance?

Adipocyte size increases up to 140–180  $\mu\text{m}$  in diameter during the development of obesity [168]. However, the capacity for adipocyte hypertrophy is limited. The reason for this could be that enlarged adipocytes endure less than adequate oxygen supply, since the diffusion distance for oxygen is at most 100  $\mu\text{m}$  [169]. In situations where oxygen availability does not meet the demand of the surrounding tissue, hypoxia occurs. Is there evidence that adipose tissue hypoxia is present in obesity? Indeed, it has very recently been reported that white adipose tissue of obese mice is hypoxic, as demonstrated both by pimonidazole staining and a markedly increased lactate concentration in adipose tissue [170]. These observations were strengthened by measurements of hypoxia-inducible gene expression, which appeared to be significantly elevated in the obese animals [170]. In line with these findings, it has been shown that weight-loss decreased the expression of hypoxia-responsive genes [136].

What could be the cause of adipose tissue hypoxia in obesity? It could be that oxygen pressure and/or oxygen content of the blood is reduced in obese subjects. However, measurements in arterial blood of obese and control mice showed no differences in oxygen pressure, haemoglobin concentration or oxygen saturation between both groups [170]. A more likely explanation for adipose tissue hypoxia relates to blood flow through this tissue. Observations that adipose tissue mass is sensitive to angiogenesis inhibitors [171,172] and that adipocytes secrete multiple angiogenic factors, including PAI-1, leptin, matrix metalloproteinases and vascular endothelial growth factor [172–175], suggest that adipose tissue development and vascularization in this tissue are closely associated. However, it has recently been suggested by Trayhurn and Wood [176] that the expansion of adipose tissue mass during the progressive development of obesity may lead to hypoxia in certain parts of adipose tissue, because angiogenesis is insufficient to maintain normoxia in the entire adipose tissue depot. As will be discussed in more detail later in this section, this may lead to increased production of inflammatory factors, acute phase proteins, and angiogenic factors by adipose tissue in obesity, the function of which is to increase blood flow and vascularization, and these events may involve the key controller of the cellular response to hypoxia, the transcription factor hypoxia-inducible factor-1 (HIF-1) [176–178]. Thus, decreased blood supply to adipose tissue may underlie adipose tissue

hypoxia in obesity (Fig. 4). It is well-established that ATBF per unit tissue mass is reduced in obese humans [38–42] and rodents [170,179]. It has been shown that both small and large adipocytes of obese rodents are hypoxic, suggesting that local hypoperfusion rather than adipocyte size is the main culprit for adipose tissue hypoxia [170]. However, adipocyte size may be related to local blood flow. An inverse relationship between adipocyte size and ATBF has been found in dogs [180] and rabbits [181], although data are less evident when blood flow is expressed per adipocyte rather than per unit adipose tissue weight [180,181]. In addition to disturbances in fasting ATBF [38–42], the postprandial enhancement of ATBF is reduced in obese subjects [40]. Therefore, it is tempting to speculate that an impaired ATBF responsiveness to nutrient intake may worsen adipose tissue hypoxia in the postprandial period.

The crux of the matter is whether adipose tissue hypoxia may provide an important link between obesity and insulin resistance? Hypoxic cells respond by altering gene expression to ensure adaptation. Hypoxia leads to the expression of HIF-1 $\alpha$ , which when combined with HIF-1 $\beta$  forms the transcription factor HIF-1 [182–184]. HIF-1 is a key regulator in the response to alterations in oxygen tension and modulates the expression of genes that are involved in angiogenesis, erythropoiesis, inflammation and glucose metabolism [175,182,184,185]. It has recently been shown that hypoxia increased the expression of certain glucose transporters and glucose transport in human adipocytes, which may lead to disturbances in cellular glucose homeostasis [186]. Interestingly, recent studies have demonstrated that hypoxia also dysregulates the expression of several key adipokines. Adiponectin and PPAR $\gamma$  mRNA expression were reduced, whereas PAI-1 and visfatin mRNA expression were increased in hypoxic 3T3-L1 adipocytes compared with normoxic control cells [170,187]. In accordance with these findings, hypoxia has been reported to induce PAI-1 production and inhibit adiponectin synthesis in 3T3-L1 adipocytes [188]. More recently, it has been demonstrated that hypoxia, which induced HIF-1 $\alpha$  protein synthesis, evoked marked alterations in the expression and secretion of inflammation-related adipokines in human adipocytes [189]. Furthermore, adipose tissue hypoxia was observed in dietary obese mice and was associated with increased expression of inflammatory genes and decreased expression of adiponectin. Weight-loss improved oxygenation and reduced inflammation in these animals [190]. Although these data do not provide evidence that HIF-1 $\alpha$  is causally involved in the modulation of adipokine expression, it is interesting to note that chemically-induced hypoxia using  $\text{CoCl}_2$ , a known inducer of HIF-1 $\alpha$ , evoked qualitatively similar changes in adipokine gene expression in human adipocytes as observed during hypoxia [189]. In addition, both HIF-1 $\alpha$  expression and macrophage infiltration in human adipose tissue were shown to be increased in obesity and reduced after weight-loss [136]. Furthermore, distinct changes in gene expression occur in macrophages when they experience hypoxia *in vitro* [191]. These include upregulation of molecules required for macrophage survival, tissue revascularization and recruitment and activation of more macrophages and other

inflammatory cells [192]. These data suggest that adipose tissue hypoxia modulates, either directly or indirectly via recruitment of macrophages, adipokine expression and secretion, and may therefore provide an important link between obesity and insulin resistance (Fig. 4). Secondly, cell death may occur in response to hypoxia. The severity of hypoxia determines whether cells become apoptotic or adapt to hypoxia and survive. A hypoxic environment devoid of nutrients prevents the cell undergoing energy dependent apoptosis and cells become necrotic [193]. Interestingly, it has been shown that adipose tissue macrophages are predominantly (>90%) localized to dead adipocytes that exhibit features of necrosis. These macrophages fuse and form syncytia that sequester and scavenge adipocyte debris. Furthermore, the frequency of adipocyte death was positively associated with increased adipocyte size in obese mice and humans and in hormone-sensitive lipase-deficient (HSL<sup>-/-</sup>) mice, a mouse model of adipocyte hypertrophy without obesity [194]. Thus, there is indirect evidence that hypoxia-induced adipocyte death may evoke macrophage infiltration in adipose tissue of obese individuals (Fig. 4). Thirdly, it has been shown that hypoxia inhibited adipocyte differentiation [195,196]. Hypoxia-induced inhibition of adipocyte differentiation appears to be mediated by the production of mitochondrial reactive oxygen species [195], which is in line with previous work demonstrating that mitochondrial reactive oxygen species influence pre-adipocyte size [197]. Hypoxia-mediated inhibition of adipocyte differentiation was only partly dependent on the presence of HIF-1 $\alpha$  [195]. As discussed earlier, an impaired adipocyte differentiation appears to be a precipitating factor in the development of type 2 diabetes [49,50]. Finally, HIF-1-independent adaptive responses that may relate to insulin resistance occur under hypoxic conditions. It has been shown that unfolded protein response (UPR), a HIF-1-independent signalling pathway, contributes to cellular adaptation to hypoxia [198]. Newly synthesized proteins are folded and assembled by chaperones in the endoplasmic reticulum (ER) [199]. Many disturbances, including hypoxia, cause accumulation of unfolded proteins in the ER [198,200], leading to ER stress [195,201]. It has been demonstrated that ER stress is elevated in adipose tissue and liver of obese mice, which activates the inflammatory response, thereby contributing to insulin resistance [202,203]. In line with these observations, hypoxia induced ER stress in human adipocytes, which in turn suppressed adiponectin mRNA expression [170].

In conclusion, adipose tissue hypoxia, possibly due to an impaired ATBF as a consequence of adipose tissue expansion, may be an important trigger for the induction of insulin resistance in obesity via effects on adipokine expression and/or adipocyte differentiation (Fig. 4).

## 9. Summary and perspectives

Abdominal obesity is strongly associated with insulin resistance and the development of type 2 diabetes and cardiovascular disease. Enormous progress has been made over the past few years in the attempt to understand the mechanisms underlying obesity-related insulin resistance. It is now well-recognized that

adipose tissue is a highly active metabolic and endocrine organ. Adipocytes are of crucial importance in buffering the daily influx of dietary fat and exert autocrine, paracrine and/or endocrine effects by secreting a variety of factors. In this review, different aspects of adipose tissue dysfunction have been discussed, and it has been postulated how these aspects may be interrelated and could play a crucial role in the pathogenesis of obesity-related insulin resistance and type 2 diabetes, as summarized in Fig. 4. A rapidly emerging body of evidence suggests that enlargement of adipocytes, an impaired ATBF, adipose tissue hypoxia, local inflammation and macrophage infiltration in adipose tissue are interrelated and may lead to disturbances in adipokine secretion and excessive fat storage in non-adipose tissues, which together may result in insulin resistance and ultimately type 2 diabetes. A key question is which mechanism(s) may underlie the sensing of adipocyte enlargement. Local hypoxia due to an inadequate blood flow through adipose tissue may be an important sensing mechanism of adipocyte hypertrophy. Alternatively, it may well be that certain signalling pathways within the adipocyte are triggered (e.g. by lipotoxicity) when the lipid droplets have reached a certain size, thereby preventing further enlargement of the adipocyte. Future research should be aimed at elucidating the sequelae of events that occur during the development and progression of adipose tissue dysfunction and how exactly these disturbances are linked. Unravelling the underlying mechanisms of obesity-related insulin resistance may increase the rationale for strategies to prevent and/or treat type 2 diabetes and may yield new pharmaceutical targets.

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