

Recent advances in the relationship between obesity, inflammation, and insulin resistance

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ABSTRACT. It now appears that, in most obese patients, obesity is associated with a low-grade inflammation of white adipose tissue (WAT) resulting from chronic activation of the innate immune system and which can subsequently lead to insulin resistance, impaired glucose tolerance and even diabetes. WAT is the physiological site of energy storage as lipids. In addition, it has been more recently recognized as an active participant in numerous physiological and pathophysiological processes. In obesity, WAT is characterized by an increased production and secretion of a wide range of inflammatory molecules including TNF- α and interleukin-6 (IL-6), which may have local effects on WAT physiology but also systemic effects on other organs. Recent data indicate that obese WAT is infiltrated by macrophages, which may be a major source of locally-produced pro-inflammatory cytokines. Interestingly, weight loss is associated with a reduction in the macrophage infiltration of WAT and an improvement of the inflammatory profile of gene expression. Several factors derived not only from adipocytes but also from infiltrated macrophages probably contribute to the pathogenesis of insulin resistance. Most of them are overproduced during obesity, including leptin, TNF- α , IL-6 and resistin. Conversely, expression and plasma levels of adiponectin, an insulin-sensitising effector, are down-regulated during obesity. Leptin could modulate TNF- α production and macrophage activation. TNF- α is overproduced in adipose tissue of several rodent models of obesity and has an important role in the pathogenesis of insulin resistance in these species. However, its actual involvement in glucose metabolism disorders in humans remains controversial. IL-6 production by human adipose tissue increases during obesity. It may induce hepatic CRP synthesis and may promote the onset of cardiovascular complications. Both TNF- α and IL-6 can alter insulin sensitivity by triggering different key steps in the insulin signalling pathway. In rodents, resistin can induce insulin resistance, while its implication in the control of insulin sensitivity is still a matter of debate in humans. Adiponectin is highly expressed in WAT, and circulating adiponectin levels are decreased in subjects with obesity-related insulin resistance, type 2 diabetes and coronary heart disease. Adiponectin inhibits liver neoglucogenesis and promotes fatty acid oxidation in skeletal muscle. In addition, adiponectin counteracts the pro-inflammatory effects of TNF- α on the arterial wall and probably protects against the development of arteriosclerosis. In obesity, the pro-inflammatory effects of cytokines through intracellular signalling pathways involve the NF- κ B and JNK systems. Genetic or pharmacological manipulations of these effectors of the inflammatory response have been shown to modulate insulin sensitivity in different animal models. In humans, it has been suggested that the improved glucose tolerance observed in the presence of thiazolidinediones or statins is likely related to their anti-inflammatory properties. Thus, it can be considered that obesity corresponds to a sub-clinical inflammatory condition that promotes the production of pro-inflammatory factors involved in the pathogenesis of insulin resistance.

Keywords: adipocyte, macrophage, obesity, diabetes, visceral adipose tissue, adipokines

It is now well established that obesity is an independent risk factor for type 2 diabetes, dyslipidemia, and cardiovascular diseases (CVD). There is also strong evidence that, for a given adiposity, there is a large heterogeneity in the metabolic and cardiovascular risk mainly linked to the location of excessive adipose tissue. Visceral adipose tissue accumulation is an important predictive

factor of lipid, glucose or atherogenic disturbances, while location of adipose tissue in the lower part of the body is not associated with increased alterations at the metabolic level. Since the description of the metabolic syndrome in the eighties by Reaven [1], the awareness of its deleterious consequences and the dramatic rise in the prevalence of obesity led physicians and public health

services to consider it the major health problem linked to related morbidities.

This review will mainly focus on the mechanisms by which excess adipose tissue can lead to insulin resistance and type 2 diabetes, and promote the onset of CVD.

A recent and striking discovery is that obesity is associated with a low-grade inflammation process in adipose tissue, the pathophysiological mechanisms of which remained poorly understood, underlining the relationship between fat cells and the immune system. Another physiological and pathological aspect that has generated a considerable sum of experimental and clinical work during the last decade is that adipocytes have the capacity to synthesize and secrete several factors collectively called adipokines. Some of them appear to play an important role in obesity-associated insulin resistance and cardiovascular complications [2, 3].

Therefore, it must also be kept in mind that at the tissue level, obesity is not an exclusively adipocyte disease, but also involves other cell types that reside in WAT. This concept helps us to understand the pathophysiological mechanisms at the root of insulin resistance and type 2 diabetes.

ADIPOSE TISSUE INFLAMMATION DURING OBESITY: A LINK WITH COMPONENTS OF THE METABOLIC SYNDROME

Obesity is associated with a chronic inflammatory response, characterized by abnormal adipokine production, and the activation of some pro-inflammatory signalling pathways, resulting in the induction of several biological markers of inflammation [4-9]. Conversely, a reduction in body weight is accompanied by a decrease or even a normalization of these biological parameters [10-13]. This association is meaningful, and several animal models suggest that these inflammatory processes have a causal relationship with obesity and its co-morbidities such as insulin resistance, type 2 diabetes and CVD.

The role of fat cells in metabolic dysfunctions has long been considered, but their potential role in an inflammatory process is a new concept. However, recently, several findings have converged to indicate that adipocytes share with immune cells certain properties such as complement activation [14] and pro-inflammatory cytokine production [4]. Fat cell precursors also share features with macrophages. Preadipocytes have the capacity for phagocytosis in response to several stimuli [15, 16]. Moreover, numerous genes that code for transcription factors, cytokines, inflammatory signalling molecules, and fatty acid transporters are essential for adipocyte biology, and are also expressed and functional in macrophages [17-19].

A body of evidence suggests the presence of an overall, low-grade inflammation in obesity, with altered levels of several circulating factors such as an increase in the plasma levels of C-reactive protein (CRP), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), and other biological markers of inflammation [2, 3, 20-25]. In addition, there is a correlation in healthy individuals between body mass index (BMI) and CRP levels [20]. IL-6 has been reported to increase liver CRP production [20, 26]. Interestingly, adipose tissue IL-6 content is higher in obese patients displaying an increased CRP level. Otherwise, there is at least a

two-fold higher risk of type 2 diabetes within 3-4 years in obese individuals with higher CRP levels [27].

At the adipose tissue level, TNF- α has been shown to be over-expressed in WAT from different animal models of obesity, and is considered to be a molecule that makes a link between inflammation and obesity. Recombinant TNF- α decreases insulin sensitivity, while TNF- α - or TNF- α receptor-null mice have an increased sensitivity in response to this hormone [4, 28]. Thus it is likely that overproduction of TNF- α by WAT from obese animal models contributes to insulin resistance.

Other adipose-specific molecules that are involved in the control of energy metabolism, also regulate immune responses. For example leptin, in addition to its key role in food intake and energy expenditure also regulates immune processes. Leptin-deficient mice or humans display an altered immune status [29-31]. The reduction in leptin levels could be responsible for fast-associated immunosuppression [32].

Adipose tissue macrophage infiltration during obesity

Large-scale studies of gene expression using micro-array approaches have already highlighted that in WAT from rodent genetic models of obesity the expression of genes coding for proteins involved in inflammatory processes was markedly altered [33]. Unexpectedly, it was observed that these variations in gene expression in WAT were essentially related to a macrophage infiltration in WAT of these obese mice [34, 35]. These locally present macrophages are responsible for the major part of the locally-produced TNF- α , and for an important part of the production of IL-6 and inducible nitric oxide synthase (iNOS) [34]. This macrophage infiltration has also been reported in WAT of obese patients [34, 36-40].

It is noteworthy that a reduction in body weight is accompanied, not only by an improvement in the inflammatory process and the co-morbidities, but also by a decrease in the expression of genes coding for inflammation proteins [37]. This phenomenon is already detectable with a small decrease in body weight. Three months following by-pass surgery, there is a significant decrease in both macrophage infiltration and in the steady state levels for mRNA involved in the inflammatory response [38]. Thiazolidinediones are able to reproduce this kind of effect on WAT gene expression [35].

This increase in macrophage infiltration could represent the cause and/or the consequence of the low-grade inflammation state associated with obesity [41, 42]. The cellular and molecular mechanisms responsible for this macrophage infiltration remain largely unknown. Although it has been suggested that macrophages present within WAT could derive from preadipocytes [16, 43], some experiments have shown that macrophages probably originate mostly from bone marrow precursors [34]. Leptin could promote macrophage diapedesis from blood flow to WAT [39]. The fat cell is also able to synthesize and secrete a chemokine, MCP-1 (monocyte chemoattractant protein-1), a recruiting factor for circulating monocytes that is over-expressed in obesity [44]. It has been proposed that factors secreted by the human, hypertrophied, mature adipocyte can activate endothelial cells present in WAT.

In turn, endothelial cells can favour monocyte adhesion and transmigration leading to macrophage infiltration [39, 45].

It is now generally considered that both over-production of pro-inflammatory cytokines by WAT from obese animals or humans (especially by macrophages that reside in WAT), and the deficiency in anti-inflammatory adipokines could be involved in the pathophysiology of insulin resistance.

ADIPOKINES, INFLAMMATION AND INSULIN RESISTANCE

The name of adipokine is nowadays generally given to any protein that can be synthesized and secreted by adipocytes (*figures 1 and 2*) [46]. Several studies have shown that adipokine production is altered in obesity, type 2 diabetes and metabolic syndrome. This is observed for leptin, TNF- α , IL-6, adiponectin and resistin and will be more extensively discussed in this review. Other adipokines such as angiotensinogen, PAI-1 or the recently discovered visfatin are also important players in vessel and metabolism regulation but will not be discussed here.

Leptin

Leptin, is the product of the *ob* gene. It is involved in the regulation of energy homeostasis [47] and is almost exclusively expressed and produced by WAT and more particularly by differentiated mature adipocytes [48]. Circulating levels [49] and adipose tissue mRNA expression of leptin [50] are strongly associated with BMI and fat mass in obesity. Thus, leptin appears as a real marker of adipose tissue mass in lean humans where the subcutaneous fraction represents about 80 % of total fat. Indeed, leptin

mRNA expression is higher in subcutaneous adipose tissue (SAT) than in visceral adipose tissue (VAT) in human [51]. Although leptin acts mainly at the level of the central nervous system to regulate food intake and energy expenditure, there is a relationship between leptin and the low-grade inflammatory state in obesity, suggesting that leptin could exert peripheral biological effects as a function of its cytokine-like structure [48]. Indeed, leptin receptors belong to the cytokine class I receptor family, and several published works have reported that there is an increased inflammatory response associated with the presence of hyperleptinemia without obesity [52, 53], and that leptin is able to control TNF- α production and activation by macrophages [52]. However, the underlying mechanisms have not been clearly identified.

TNF- α

TNF- α is a pro-inflammatory cytokine produced by a variety of cell-types, but mainly by macrophages and lymphocytes. It can be produced by adipose tissue although this production is weak in humans. Nonetheless, TNF- α is thought to play a major role in the pathophysiology of insulin resistance in rodents [4] through the phosphorylation of the insulin receptor substrate-1 (IRS-1) protein on serine residues. This could prevent its interaction with the insulin receptor beta subunit, and stop the insulin signaling pathway. Although clinical studies have shown that VAT is closely linked to insulin resistance, TNF- α mRNA expression was similar in SAT and VAT [54, 55]. Moreover, TNF- α is weakly expressed either in subcutaneous or in deep human adipose tissue depots and this expression is not always modified in obesity [56]. This corresponds with the evaluation of *in vivo* secretion, which showed that TNF- α production by subcutaneous abdomi-

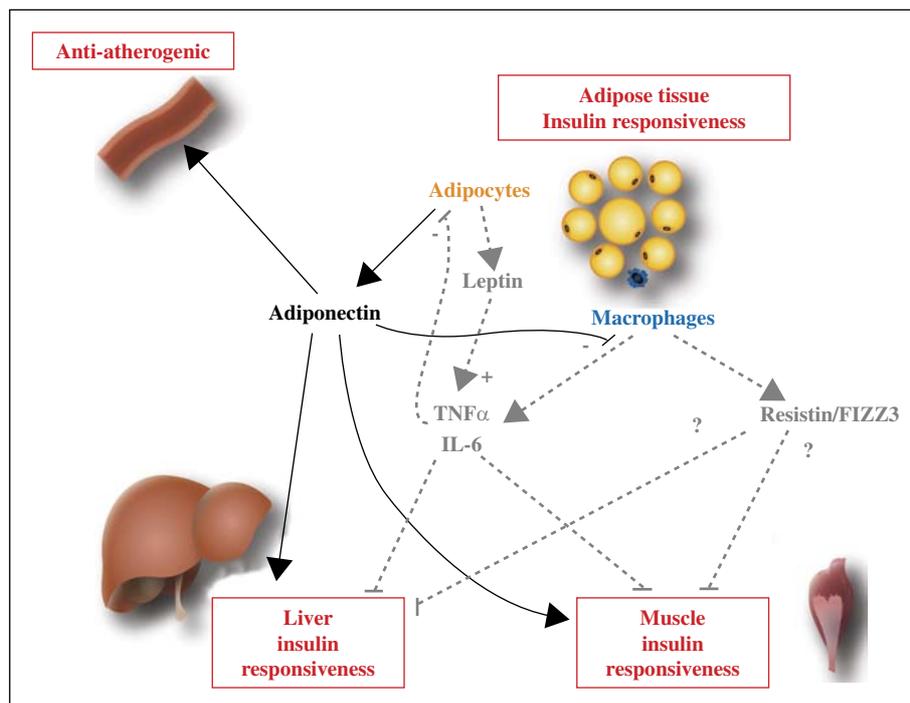


Figure 1

Adipokine expression and secretion by adipose tissue in lean subjects.

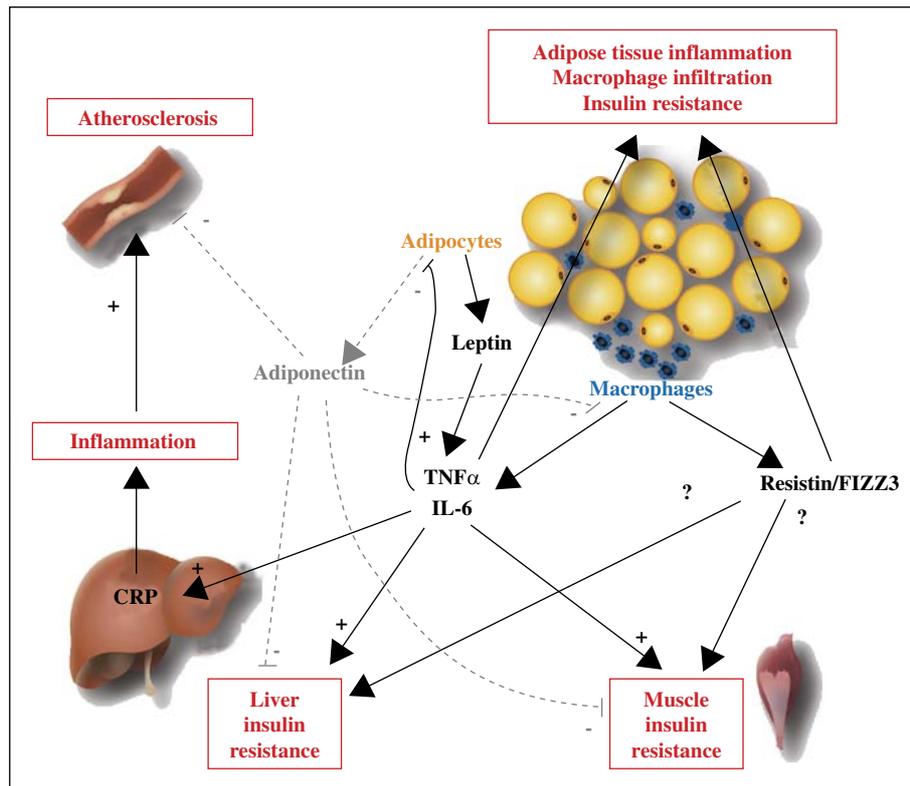


Figure 2

Adipokine expression and secretion by adipose tissue in insulin-resistant, obese subjects.

nal adipose tissue was quantitatively negligible in lean and obese subjects [57]. This suggests that adipose tissue is not directly implicated in the increased circulating TNF- α levels observed in obesity in human. It can be hypothesized that other mechanisms involving a systemic effect of leptin or of other adipokines may induce TNF- α secretion by other cell types such as macrophages. Nevertheless, the precise role of TNF- α in human obesity requires further investigation.

Interleukin-6

Interleukin-6 is produced by many cell types (fibroblasts, endothelial cells, monocytes), and many tissues including adipose tissue. It is now well known that IL-6 production by adipose tissue is enhanced in obesity [5, 6]. It is thought that 15 to 30 % of circulating IL-6 levels derives from adipose tissue production in the absence of an acute inflammation [57]. Secretion is higher in VAT than in SAT [5, 58]. Accordingly, IL-6 mRNA expression is higher in VAT than in SAT [5]. However, in adipose tissue, the greater proportion of IL-6 is not produced by mature adipocytes but rather by cells of the stroma vascular fraction including preadipocytes, endothelial cells and monocytes-macrophages [5, 58]. Interleukin-6 is a multi-functional cytokine acting on many cells and tissues. One of the main effects of IL-6 is the induction of hepatic CRP production, which is now known to be an independent, major risk marker of cardiovascular complications [59]. Interestingly, there is a strong relationship between IL-6 protein content in adipose tissue and circulating levels of both IL-6 and CRP [60]. In addition, IL-6 has been recently proposed to play a central role in the link between

obesity, inflammation and coronary heart diseases [61]. As VAT can produce higher IL-6 amounts than SAT [5], this could partly explain the relationship between central fat depots and cardiovascular risk complications in human. Moreover, IL-6 production by adipose tissue could directly affect liver metabolism by inducing VLDL secretion and hypertriglyceridaemia, since the VAT is closely connected to the liver by the venous portal system [62]. Recent studies have suggested that IL-6 could be involved in insulin resistance and its complications [6, 63]. The IL-6 receptor belongs to the cytokine class I receptor family involving JAK/STATs (Janus kinases/signal transducers and activators of transcription) signal transduction pathway [64]. Janus kinase activation induces STAT phosphorylation, dimerisation and translocation to the nucleus to regulate target gene transcription [64]. It is now clearly established that a strong interaction occurs between cytokine and insulin signalling pathways, and generally leads to an impaired biological effect of insulin. Although the exact mechanisms have not yet been clearly elucidated, it could involve tyrosine phosphatase activation [65] or an interaction between suppressor of cytokine signalling (SOCS) proteins and the insulin receptor [66-68]. Whatever the mechanisms involved, it has now been clearly demonstrated that cytokines such as TNF- α and IL-6 are able to decrease insulin action [65-70]. Therefore, in addition to the aggravation of the cardiovascular risk linked to inflammation, the chronic increase in circulating cytokine levels could contribute to insulin resistance.

Adiponectin

Adiponectin, having been discovered by several groups, has been attributed several different names: ACRP30 (adipocyte complement-related protein of 30 kDa) or adipoQ in mouse and GBP28 (gelatin-binding protein 28) or APM1 (adipose most abundant gene transcript 1) in human [71]. It is highly expressed in adipose tissue. Plasma levels of adiponectin, which constitutes 0.01% of circulating proteins, are between 5 to 30 mg/L in lean control subjects while those of leptin are between 2 to 8 µg/L. The expression of adiponectin mRNA is dependent on the adipose tissue localisation. It is lower in VAT than in SAT [72].

Adiponectin has several particularities which distinguished it from others adipokines: 1) circulating adiponectin levels are decreased in obese and/or type 2 diabetic patients and in patients with coronary heart diseases, 2) there is a strong positive correlation between adiponectinemia and insulin sensitivity, 3) there is an inverse correlation between adiponectinemia and obesity and more particularly with abdominal obesity and 4) adiponectin may play a protective role against atherosclerosis and insulin resistance. The insulin-sensitive action of adiponectin may involve the activation of AMP activated protein kinase (AMPK), which is known to regulate cellular malonyl CoA concentrations by inhibiting acetyl CoA carboxylase [73]. This inhibition results in a decreased level of intracellular malonyl CoA and a subsequent decreased lipogenesis associated with increased mitochondrial fatty acid beta-oxidation. Adiponectin is also able to regulate liver glucose production by lowering mRNA expression of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase, two key enzymes of neoglucogenesis [71]. In addition to its insulin-sensitising effects, adiponectin may have a protective effect on the vascular wall by acting early at several steps of the atherogenesis process: 1) modulation of endothelial adhesion molecules [74], 2) transformation of macrophages into foam cells [75] and 3) modulation of vascular smooth muscle cells proliferation [76]. Moreover, adiponectin may modulate the TNF- α -induced inflammatory response, since it has been shown that adiponectin reduces TNF- α secretion of macrophages [77]. This anti-TNF- α effect may partly explain the anti-inflammatory and anti-atherogenic effect of adiponectin. By contrast both TNF- α and IL-6 reduce human adipocyte mRNA expression of adiponectin [78], which is an additional mechanism by which these two cytokines induce insulin resistance.

Two adiponectin receptors, adipoR1 and adipoR2, localized on chromosomes 1q32 and 12p13 respectively, have been recently cloned [79]. AdipoR1 is predominantly expressed in skeletal muscle while adipoR2 is mainly expressed in the liver. However, the physiological relevance and the transduction signal pathways of these two receptors remain to be determined.

Resistin

Steppan *et al.* have recently discovered resistin, also called FIZZ3 (found in inflammatory zones) or adipocyte secreted factor (ADSF) while looking for new molecular targets of thiazolidinediones in adipocytes [80]. They showed that circulating and adipose tissue resistin levels

were increased in obese rodents but decreased under treatment with thiazolidinediones. Moreover, infusion of recombinant resistin into lean control animals induced insulin resistance, while its immuno-neutralisation improved insulin sensitivity in insulin-resistant obese animals.

In cultured adipocytes, resistin reduced insulin-stimulated glucose transport, an effect which was reversed using an anti-resistin antibody. In addition, resistin inhibited adipocyte differentiation [81]. These studies suggest that resistin could be a link between adipose tissue, obesity and insulin resistance. However, additional, contradictory studies have indicated a decreased mRNA gene expression in adipose tissue from various insulin-resistant rodent models. Nevertheless, recombinant resistin caused major liver insulin resistance [82].

It was recently shown that resistin-knockout mice have lower fasting glycaemia and increased glucose tolerance and insulin sensitivity associated with a reduced liver glucose production [82]. The lack of resistin could lead to the activation of AMPK and consequently to a decreased expression of genes involved in liver neoglucogenesis, suggesting that resistin could exert effects opposite to those of adiponectin. Finally, resistin-knockout mice under a high fat diet regimen became as obese and insulin-resistant as their wild type counterparts. However, fasting glycaemia was lower in resistin-knockout mice, suggesting the implication of resistin in the hyperglycaemia and insulin resistance observed in obesity.

With regards to resistin in humans, several discrepancies have been observed since some studies have shown that adipose tissue expresses resistin while others did not find its presence or detected only very low mRNA expression in this tissue. It is believed that the adipocyte is not the major cell type producing resistin in humans, which rather is produced by circulating monocytes and macrophages [82]. Finally, most of the studies found no correlation between plasma resistin levels, BMI and insulin resistance in human. Nonetheless, the macrophage localization of resistin and its inter-relationship with adipocyte metabolism and function are currently under investigation.

INFLAMMATORY PATHWAYS IN INSULIN RESISTANCE AND TYPE 2 DIABETES

A century ago, it was suggested that inflammation could be involved in the pathophysiology of type 2 diabetes [83]. Although the molecular mechanisms involved are not clearly understood, it has been suggested that not only the pro-inflammatory effects of cytokines, but also of reactive oxygen species and free fatty acids in obesity are mediated through specific intracellular signalling pathways, involving the nuclear factor (NF)- κ B, I κ B kinase, (IKK), Activating Protein-1 (AP-1) and c-Jun NH₂-terminal kinase (JNK) signalling molecules. All these pathways could interact with insulin signalling *via* serine/threonine inhibitory phosphorylation of IRS.

Genetic or pharmacological manipulations of these different effectors of the inflammatory response modulate insulin sensitivity in different animal models. Indeed, invalidation of these genes, which mediate inflammatory responses, modulates insulin sensitivity. Heterozygous IKK- β ^{+/-} mice under high fat diet regimen or mated with

ob/ob obese mice have lower glycaemia, an improvement in insulin sensitivity and insulin signalling as compared to *IKK-β^{+/-}* mice [84, 85].

By contrast, tissue-specific, over-expression of *IKK-β* in liver and adipose tissue but not in skeletal muscle leads to systemic insulin resistance. Accordingly, selective inhibition of the *NF-κB* function in liver and adipose tissue protects against insulin resistance in nutritional and genetic animal models of obesity [86].

JNK activity, which is mainly related to the *JNK1* isoform, is increased in obese mice. In response to a high fat diet or in the context of genetically obese rodents, *JNK1*-null animals gain less body weight and are less prone to altered insulin sensitivity [87]. Liver-specific down-regulation of *JNK* signalling improves insulin responsiveness in animal models of type 2 diabetes [88].

The implication of inflammation pathways is also suggested by the protective effect of some anti-inflammatory compounds against obesity-associated insulin resistance. Aspirin not only inhibits *IKK* and *JNK* pathways [89, 90], but also other serine/threonine kinases involved in *TNF-α*-induced insulin resistance. In addition, through its antioxidant properties, aspirin decreases *NF-κB* and *AP-1* activation in response to oxidative stress [90]. Salicylate reduces the severe insulin resistance observed in genetically obese rodents [84]. In human species, high doses of salicylate improve insulin sensitivity of type 2 diabetic patients [91]. Nevertheless, the exact mechanisms by which aspirin modulates carbohydrate metabolism and insulin sensitivity remain to be investigated [92].

Other drugs with well characterized anti-inflammatory effects, such as thiazolidinediones and statins, also possess anti-diabetic properties. Thiazolidinediones have an insulin-sensitizing action that is possibly related, at least in part, to their ability to decrease adipocyte *TNF-α* production, or *TNF-α* effects on several target tissues, and by contrast to induce adiponectin expression [93]. Statins modulate endothelial functions and trans-endothelial leukocyte migration, inhibit pro-inflammatory cytokine secretion and interfere in the *NF-κB* pathway [94]. Accordingly, pravastatin is known to reduce the risk of type 2 diabetes.

CONCLUSION

During the last decade, understanding of the biology of adipose tissue and, in particular, its secretory functions have dramatically improved, and this has completely modified our understanding of the pathophysiological link between the increase of fat mass, namely obesity, insulin resistance and cardiovascular complications. The adipokines produced by adipocytes or by adipose tissue-infiltrating macrophages, are able to induce a low-grade inflammation state that could play a central role in obesity and type 2 diabetes-related insulin resistance and cardiovascular complications. New therapeutic approaches can thus be considered. However, further studies are necessary to better understand the regulation and biological functions of adipokines. New adipokines will be certainly discovered in the next few years, which will lead us to greater appreciation of the complexity of the cross-talk between metabolic tissues, and their alteration in human diseases.

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