Altered signalling and gene expression associated with the immune system and the inflammatory response in obesity

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White adipose tissue functions not only as an energy store but also as an important endocrine organ and is involved in the regulation of many pathological processes. The obese state is characterised by a low-grade systemic inflammation, mainly a result of increased adipocyte as well as fat resident- and recruited-macrophage activity. In the past few years, various products of adipose tissue including adipokines and cytokines have been characterised and a number of pathways linking adipose tissue metabolism with the immune system have been identified. In obesity, the pro- and anti-inflammatory effects of adipokines and cytokines through intracellular signalling pathways mainly involve the nuclear factor kappa B (NF-κB) and the Jun N-terminal kinase (JNK) systems as well as the I kappa B kinase beta (IKK-β). Mitogen-activated protein kinase (MAPK) and extracellular-signal-regulated kinase (ERK) pathways, which lead to signal transducer and activator of transcription 3 (STAT3) activation, are also important in the production of pro-inflammatory cytokines. Obesity increases the expression of leptin and other cytokines, as well as some macrophage and inflammatory markers, and decreases adiponectin expression in adipose tissue. A number of cytokines, e.g. tumour necrosis factor alpha (TNF-α) and monocyte chemotactic protein 1 (MCP-1), and some pro-inflammatory interleukins, leuckocyte antigens, chemochines, surface adhesion molecules and metalloproteases are up-regulated whereas other factors are down-regulated. The present paper will focus on the molecular mechanisms linking obesity and inflammation with emphasis on the alteration of signalling and gene expression in adipose cell components.

Adipokines: Cytokines: Gene expression: Inflammation: Obesity

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Background

Obesity represents one of the most important public health issues for the coming years and excess body weight is currently the sixth most important risk factor contributing to the overall burden of disease worldwide[1,2]. Obesity is associated with a low-grade inflammation of white adipose tissue (WAT) resulting from chronic activation of the innate immune system, which can subsequently lead to insulin resistance, impaired glucose tolerance and even diabetes[3,4]. There is much evidence that the adipose tissue secretes many factors, collectively termed "adipokines", that include leptin, adiponectin, resistin, retinol binding protein 4 (RBP4) and numerous classical cytokines and immune-associated factors including tumour necrosis factor alpha (TNF-α), interleukin (IL) 1β, IL-6, and monocyte chemotactic protein 1 (MCP-1)[5,6]. Mounting evidence highlights the role of adipose tissue in the development of a systemic inflammatory state that contributes to obesity-associated vasculopathy and cardiovascular risk. Circulating mediators of inflammation secreted by adipocytes and adipose tissue-derived macrophages participate in the mechanisms of vascular insult and atheromatous change[5].

The degree of macrophage infiltration of WAT has been shown to be associated with obesity. Higher expression of the CD68 gene marker has been reported in obese human WAT, which confirms a positive association between BMI and the adipose tissue content of macrophages[7,8]. The number of macrophages present in WAT is directly correlated with adiposity and with adipocyte size in both human subjects and mice. There seems to be a differential expression of pro- and anti-inflammatory factors with increasing adipocyte size resulting in a shift toward dominance of pro-inflammatory adipokines largely as a result of a dysregulation of hypertrophic, very large cells[9].

Although lymphocytes are not a constituent of WAT, there is often a close physical proximity between lymphocytes and WAT, particularly in lymph nodes, which are generally surrounded by pericapsular adipose tissue. Data indicate the presence of intriguing two-way paracrine interactions between lymphocytes and adjacent adipocytes[10]. Additionally, it is well established that leptin is an important modulator of T-cell function[11] and a connection between obesity and T cells may exit. Recently, higher numbers of T cells have been found in WAT of diet-induced obese insulin-resistant mice than in lean[12].

Although not yet conclusively proven, the current hypothesis is that adipokines, cytokines, and other factors produced and released by WAT are responsible for the chronic

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inflammatory state of visceral obesity. This outcome is associated with increased levels of inflammatory markers, such as C-reactive protein (CRP) and IL-6 in the circulation of obese subjects[6,13,14] and an increased production and secretion of a wide range of inflammatory molecules by WAT, including TNF-α and IL-6, which may have local effects on WAT physiology but also systemic effects on other organs[3]. Human WAT is a potent source of pro-inflammatory cytokines although the majority of this release is due to the non-fat cells in the adipose tissue, except for leptin and adiponectin that are primarily secreted by adipocytes[4,6]. Human adipocytes secrete at least as much plasminogen activator inhibitor-1 (PAI-1), MCP-1, IL-6 and IL-8 *in vitro* as they do leptin, but the non-fat cells of adipose tissue secrete even more of these proteins. The secretion of leptin, on the other hand, by the non-fat cells is negligible. The release of IL-8, MCP-1, vascular endothelial growth factor (VEGF), transforming growth factor beta 1 (TGF-β1), IL-6, prostaglandin E$_2$ (PGE$_2$), TNF-α, cathepsin S, hepatocyte growth factor (HGF), IL-1β, IL-10, resistin, CRP and interleukin-1 receptor antagonist (IL-1Ra) by adipocytes is less than 12 % of that by the non-fat cells present in human adipose tissue[15]. Fig. 1 summarises the secretion of adipokines, cytokines and other factors by adipocytes and macrophages in WAT.

Cell signalling underlying inflammatory changes in obesity

In obesity, the pro-inflammatory effects of cytokines through intracellular signalling pathways mainly involve the nuclear factor kappa B (NF-κB) and the Jun N-terminal kinase (JNK) systems[3,16], as well as the I kappa B kinase beta (IKK-β), which is a central coordinator of inflammatory responses through activation of NF-κB. Signalling pathways leading to IKK-β and NF-κB are activated in insulin-responsive tissues of obese and high-fat-fed animals[16] (Fig. 2).

Adiponectin may be a local regulator of inflammation in the adipocyte and adipose tissue *via* its regulation of the NF-κB and peroxisome proliferator-activated receptor gamma 2 (PPARγ2) transcription factors. Whereas lipopolysaccharide (LPS) induces an increase in NF-κB activation, adiponectin suppresses both NF-κB activation and the induction of IL-6 expression by LPS in both pig adipocytes and cultured

3T3-L1 adipocytes. In addition, adiponectin antagonises an LPS-induced increase in TNF-α mRNA expression. Adiponectin also induces an upregulation of PPARγ2 mRNA[17]. Adiponectin interacts with at least two known cellular receptors (ADIPOR1 and ADIPOR2), which in turn leads to the activation of peroxisome proliferator-activated receptor (PPAR), AMP-activated protein kinase (AMPK) and p38 mitogen-activated protein kinase[18]. Adiponectin also regulates the expression of several pro- and anti-inflammatory cytokines; its main anti-inflammatory function might be related to its capacity to suppress the synthesis of TNF-α and interferon-γ (IFNγ) and to induce the production of anti-inflammatory cytokines such as IL-10 and IL-1Ra. Activation of PPARγ2 exerts anti-inflammatory effects through the inhibition of the transcriptional activation of pro-inflammatory response genes (Fig. 2).

Leptin signals through its receptor OBRb to induce activation of the mitogen-activated protein kinases (MAPKs) p38 and extracellular-signal-regulated kinase (ERK) and of signal transducer and activator of transcription 3 (STAT3) (Fig. 2). This results in production of the pro-inflammatory cytokines TNF-α, IL-6 and IL-12[6]. Leptin also induces the production of nitric-oxide synthase 2 (NOS2) and, thereby, reactive oxygen species (ROS), enhances macrophage phagocytosis, and induces the activation, proliferation and migration of monocytes. Signal transduction pathways activated by leptin in immune cells include not only the JNK signal transducer and activator of transcription system (particularly STAT3) but also phosphatidylinositol 3-kinase (PI3K) and MAPK. In endothelial cells, leptin induces oxidative stress and upregulation of adhesion molecules[19].

Resistin increases the production of TNF-α, IL-1β, IL-6 and IL-12. However its effect on monocyte and macrophage functions are unknown. The receptor for resistin is also unknown, but this adipocytokine induces the activation of p38, ERK and PI3K[6].

Insulin resistance is a pathophysiological component of type 2 diabetes and obesity, and also occurs in states of stress, infection, and inflammation associated with an upregulation of cytokines. Pro-inflammatory cytokines, such as TNF-α, have been shown to inhibit insulin signaling by suppressing phosphorylation of insulin receptor and insulin receptor substrate (IRS) proteins. Although serine phosphorylation of IRS-1 by JNK appears to be one of the inhibitory mechanisms, other data suggest that suppression of cytokine signaling (SOCS) protein-mediated inhibition of IRS phosphorylation is also involved in TNF-α-mediated inhibition of insulin signaling. In both obesity and LPS-induced endotoxaemia there is an increase in SOCS proteins, SOCS-1 and SOCS-3, in liver, muscle, and, to a lesser extent, in WAT. In concordance with these increases by LPS, tyrosine phosphorylation of the insulin receptor is partially impaired and phosphorylation of the IRS proteins is almost completely suppressed[20].

Recently, it has been shown that saturated fatty acids, which are released in large quantities from hypertrophied adipocytes *via* the macrophage-induced adipocyte lipolysis, serve as a naturally occurring ligand for Toll-like receptors 4 (TLR4), thereby inducing the inflammatory changes in both adipocytes and macrophages through NF-κB activation[21].

The molecular mechanisms linking obesity and inflammation with emphasis on the alteration of signalling and

MACROPHAGE CYTOKINES AND OTHER FACTORS
TNF-α
IL-6
MCP-1
Resistin
Adipsin

ADIPOCYTOKINES
■ Adiponectin
■ Leptin
■ Resistin
■ Visfatin
■ RBP-4

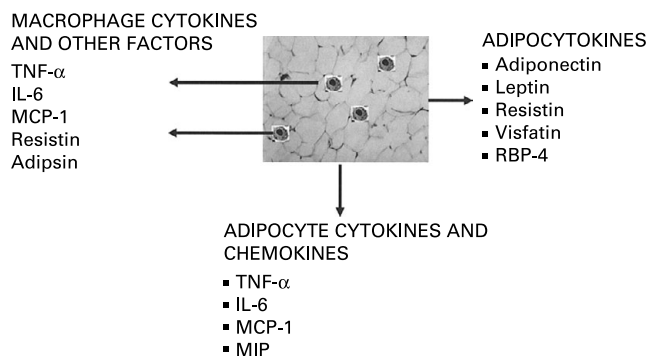**ADIPOCYTE CYTOKINES AND CHEMOKINES**
■ TNF-α
■ IL-6
■ MCP-1
■ MIP

Fig. 1. Adipocytokines, pro-inflammatory cytokines and chemokines, and other factors synthesised by adipocytes and macrophages of the white adipose tissue. IL-6, Interleukin 6; MCP-1, Monocyte chemotactic protein; MIP, Macrophage inflammatory protein; RBP4, Retinol binding protein 4; TNF-α, tumour-necrosis factor.
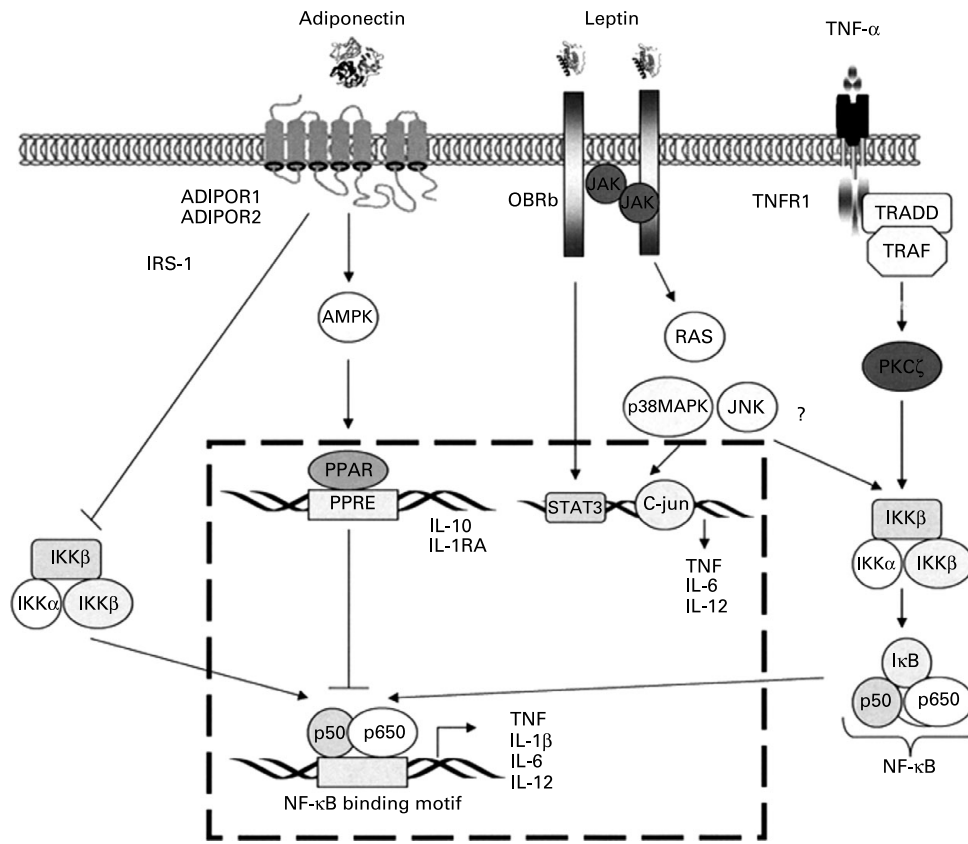
Fig. 2. Model for the pro- and anti-inflammatory effects of adiponectin, leptin and TNF-α. ADIPOR1 and ADIPOR2, adiponectin receptors; AMPK, AMP-activated protein kinase; IL, interleukin; IL-1RA: IL-1 receptor antagonist; IκB, inhibitor of NF-κB; JNK, Janus kinase; LPS, lipopolysaccharide; p38MAPK, mitogen-activated protein kinase p38; NF-κB, nuclear factor-κB; OBRb, leptin receptor PPAR, peroxisome-proliferator-activated receptor; PPRE, peroxisome-proliferator response element; STAT3, signal transducer and activator of transcription 3; TNF-α, tumour-necrosis factor; TNFR, TNF receptor; TRADD, TNFR1-associated via death domain; TRAF, TNFR-associated factor.

gene expression in adipose cell components will be presented in this review.

Altered expression of inflammation-related genes in obesity

Acquired obesity, independent of genetic influences, is able to increase the expression of macrophage and inflammatory markers and decrease adiponectin expression in adipose tissue. Table 1 summarises the genes for adipokine, cytokine and other factors whose expression is altered in inflammation associated with obesity.

Within monozygotic twin pairs, identified from the population-based FinnTwin16 cohort, acquired obesity was significantly related to increased adipocyte size and increased adipose tissue mRNA expressions of leptin, TNF-α and the macrophage marker CD68, and decreased mRNA expressions of adiponectin and PPAR-γ2. Intrapair differences in liver fat correlated directly with those in leptin and CD68 expression. CD68 expression and serum TNF-α concentrations were correlated with insulin resistance[8]. Abdominal adiposity and leptin are independent predictors of adiponectin gene expression and in human adipocytes, adiponectin gene expression is strongly related to IKKBβ mRNA[22].

Visfatin (VF) is a recently described adipokine preferentially secreted by visceral adipose tissue with insulin mimicing properties[23]. Subcutaneous abdominal adipose tissue (SCAAT) visfatin is highly expressed in lean, more insulin-sensitive subjects and is attenuated in subjects with high levels of inflammatory markers, which suggests a role for this adipokine in inflammation associated with obesity[24].

An alteration of inflammation-related genes has been described in the obese subject. An up-regulation (rather than down-regulation) in adipocytes of obese individuals implies an active role for these cells in WAT inflammation. Increased levels of transcription for these inflammation related genes will presumably result in increased secretion of these factors from adipocytes. Weight loss improves the inflammatory profile of obese subjects through a decrease of pro-inflammatory factors and an increase of anti-inflammatory molecules; the genes are expressed mostly in the stromavascular fraction of adipose tissue, which is shown to contain numerous macrophages[25].

TNF-α mRNA expression has been reported to be up-regulated in adipose tissue from several rodent models of obesity and diabetes and from obese humans and this elevated expression has been assumed to be associated with the development of insulin resistance[4,26]. However, the biological signal of TNF-α may be influenced by the expression of the two TNF-α receptors: the p60 TNF receptor, TNFR60, and the p80 TNF receptor, TNFR80. TNFR60 expression level is positively correlated with BMI and fat cell size, whereas

Table 1. Altered gene expression of adipokines, cytokines and other factors involved in inflammation associated to obesity

Up-regulated genes	
CCL11	Eotaxin-1 (chemokine, CC motif, ligand 11)
CCL2	Chemokine, CC motif, ligand 2, also termed as monocyte chemotactic protein (MCP-1)
CCR1	Macrophage inflammatory protein 1 alpha (MIP-1α)
CCR2	C-C chemokine receptor 2 (MCP-1 receptor)
CD53	Leukocyte surface antigen CD53
CD68	Macrophage antigen CD68 or Macrosialin
CKLFSF3	Chemokine-like factor super family 3
CTSS	Cathepsin S
HLA-DRA	Major histocompatibility complex, class II, DR alpha
ICAM1	intercellular adhesion molecule-1 (ICAM-1)
IL-8	Interleukin 8
ITGB2	Integrin β2
LEP	Leptin
MMP9	Metalloprotease 9
PLA2G7	Phospholipase A2, group VII
TNFA	Tumour-necrosis factor alpha
TNFR60	P60 tumour-necrosis factor receptor
VCAM1	Precursor of vascular cell adhesion molecule 1 isoform
Down-regulated genes	
ADIPOQ	Adiponectin
PBEF1	Pre-B-cell colony-enhancing factor 1 or Visfatin
IL1F5	Interleukin family member 5
ADAMTS1	Disintegrin and metalloprotease with thrombospondin motifs-1 preproprotein
FN1	Fibronectin 1 isoform 1 preprotein
MMP15	Matrix metalloproteinase 15 preprotein
PPARG	Peroxisome-proliferator activated receptor gamma

References: 8, 24–36.

TNFR80 and TNF mRNA levels show positive associations with serum insulin and triglyceride concentrations[27].

Major quantitative changes in adipokine gene expression have been demonstrated to occur during differentiation of human adipocytes and that TNF-α has a pleiotropic effect on inflammation-related adipokine production, the synthesis of MCP-1 and nerve growth factor (NGF) being highly induced by the cytokine[28]. Likewise, eleven genes have been found to be up- or down-regulated in TNF-α-treated human adipocytes. Among six up-regulated genes revealed by array analysis, only eotaxin-1, MCP-1, and a precursor of vascular cell adhesion molecule 1 isoform (VCAM1) were confirmed by real-time polymerase chain reaction (PCR). Similarly, among five down-regulated genes identified by array, IL-1 family member 5 (IL1F5), a disintegrin and metal-loprotease with thrombospondin motifs-1 preproprotein (ADAMTS*1*), fibronectin 1 isoform 1 preprotein (FN1), and matrix metalloproteinase 15 preprotein (MMP15) were confirmed by real-time PCR. There was a substantial increase (50-fold) in eotaxin-1 in response to TNF-α[29].

Some of these inflammation-related genes encode chemokines, such as MCP-1, macrophage inflammatory proteins (MIPs), such as MIP-1α, -2α, 1β and 2β,?and IL-8, that can attract monocytes/macrophages to infiltrate adipose tissue. In addition, macrophage migration inhibitory factor (MIF), a lymphokine that regulates macrophage function in inflammation, and chemokine-like factor super family 3 (CKLFSF3), a potential chemokine, has also been found upregulated in adipocytes of obese subjects[30].

Recently, it has been suggested that in human obesity, SCAAT MCP-1 gene over-expression is a biomarker of an "inflamed" adipose organ and impaired glucose metabolism[31].

In fact, in obese subjects at baseline, SCAAT gene expression profile revealed a "co-up-regulation" of MCP-1, MCP-2, MIP-1α, and CD68, and whole-body glucose disposal inversely correlated with the MCP-1 gene expression. Likewise, following hyperinsulinaemia, MCP-1 and MCP-2 mRNA levels significantly increased in lean subjects but not in obese. Accumulation of fat in the liver has also been reported to be associated with increased adipose tissue inflammation[32].

Recruited AT macrophages in obese animals represent a subclass of macrophages with unique pro-inflammatory properties. Comparisons of gene expression in recruited and resident macrophages using cDNA microarrays and real-time PCR showed that recruited macrophages overexpress genes important in macrophage migration and phagocytosis, including IL-6, inducible nitric oxide synthase (iNOS), and C-C chemokine receptor 2 (CCR2). Many of these genes were not induced in macrophages from high-fat diet-fed CCR2 knockout mice, supporting the importance of CCR2 in regulating the recruitment of inflammatory macrophages in WAT during obesity[33].

The up-regulation of inflammation-related genes in pre-adipocytes/stromal vascular cells of obese subjects may increase the recruitment of immune cells into WAT and may also result in changes in the extracellular matrix (tissue remodelling) to accommodate adipose tissue expansion in obesity. In fact, microarray expression profiling has revealed that the expression of inflammation-related genes is significantly upregulated in pre-adipocytes/stromal vascular cells of obese individuals. Quantitative PCR confirmed the upregulation of IL-8, cathepsin S (CTSS), integrin β2 (ITGB2), major histocompatibility complex, class II, DR alpha (HLA-DRA), leukocyte surface antigen CD53 (CD53), phospholipase A2, group VII

(PLA2G7) and metaloprotease 9 (MMP9) in pre-adipocytes of obese subjects[34].

The cardiovascular and metabolic hormone atrial natriuretic peptide (ANP) is able to modulate adipose tissue secretion of several adipokines and cytokines by direct action on adipocytes and macrophages or through activation of adipocyte hormone-sensitive lipase. ANP decreases the secretion of the pro-inflammatory cytokines IL-6 and TNF-α, of several chemokines, and of the adipokines leptin and RBP4 but the secretion of the anti-inflammatory molecules IL-10 and adiponectin remains unaffected; ANP directly inhibits the secretion of IL-6 and MCP-1 by macrophages[35].

Other upregulated genes encoding adhesion molecules have been found in WAT from obese subjects that may help to retain any infiltrating monocytes/macrophages in the tissue. In human obesity, elevated expression of the intercellular adhesion molecule-1 (ICAM-1) has been described in WAT from diet-induced obese subjects[36]. Likewise, increased Thy-1 cell surface antigen (THY1) expression has been found, which is a major cell surface glycoprotein that facilitates the adhesion and transendothelial migration of monocytes in activated endothelial cells[30]. In addition, CTSS is an elastolytic cysteine protease that has also been implicated in the development of atherosclerotic lesions in both animal models and humans[37].

Conflict of interest statement

The authors have no conflicts of interest to report. Concepción M Aguilera, Mercedes Gil-Campos and Ramón Cañete co-wrote the manuscript.

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