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The autocrine and paracrine roles of adipokines

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Abstract.

Obesity, defined by an excess of adipose tissue, is often associated with the development of various metabolic diseases. The increased and inappropriate deposition of this tissue contributes to hyperglycemia, hyperlipidemia, insulin resistance, endothelial dysfunction and chronic inflammation. Recent evidence suggests that factors expressed and secreted by the adipose tissue, adipokines, may contribute to the development of these abnormalities by mechanisms including inhibition of adipogenesis, adipocyte hypertrophy and death, immune cell infiltration and disruption of tissue metabolism. The presence of adipokine receptors in adipocytes renders these cells available to autocrine and paracrine effects of adipokines. In this review the reported local effects of adipokines on adipose tissue structure, inflammation and regulation of metabolic functions, in the face of over-nutrition and consequent obesity, are outlined. Elucidating the local regulation of white adipocyte development and function could help in the design of effective, tissue-specific therapies for obesity-associated diseases.

Introduction. Obesity, affected by the expanding adipose tissue mass, has necessitated the study and understanding of this organ. It is now accepted that the adipose tissue is an important, dynamic participant in regulating whole-body metabolism and is crucial for glucose and energy homeostasis (Rosen and Spiegelman, 2006). Various diseases are closely associated with increased adipose tissue mass, including type 2 diabetes, atherosclerosis, hypertension, osteoarthritis and certain cancers (Greenberg and Obin, 2006). Obesity-associated changes in the structure and function of adipose tissue and its distribution are affected by and effect changes in the adipokine secretory repertoire and profoundly influence the susceptibility of an individual to the associated pathologies (Björntorp, 1991).

Obesity has been described as a low grade inflammatory condition, with both the cellularity and the secretions of adipose tissue reflecting these changes. Current evidence suggests that over-nutrition leads to adipocyte hypertrophy, followed by cell death, which may act as a stimulus for immune cell infiltration into the tissue (Strissel et al., 2007; Murano et al., 2008). Monocyte infiltration and differentiation in particular has been shown to correlate with adipocyte hypertrophy, as well as body mass (Weisberg et al 2003). These macrophages, in response to the endocrine and metabolic milieu prevalent in the obese adipose tissue, switch their phenotype from one of a non-inflammatory resident macrophage to that of a lipid engorged foam cell, expressing dendritic cell markers, such as CD11c (Lumeng et al., 2007). The secretions of these macrophages, such as IL-6 and TNF α , along with those from the hypertrophied adipocyte, such as MCP-1 and leptin, regulate the pathological changes of obesity, like insulin resistance and endothelial dysfunction (Figure 1). These autocrine/paracrine effects augment the endocrine changes mediated by the molecules in other organs such as liver, heart and skeletal muscle. All the signals released from or expressed by the adipose tissue are referred to as adipokines here, even though these include cytokines, chemokines and products of tissue enzyme activity (Table 1).

i) Adipokines, adipose tissue structure and regional distribution.

Two histologically and functionally distinct types of adipose tissue have been identified: white (WAT) and brown adipose tissue (BAT). In mice, BAT exists throughout adulthood, while in humans it was thought to exist mainly in the neonatal period, having largely disappeared within the first years after birth. However, recent evidence suggests that adults retain metabolically active BAT depots that can be cold-induced and respond to sympathetic nervous system activation (Frühbeck et al., 2009).

While BAT is specialized in the production of heat and lipid oxidation, WAT functions as insulation, an exemplary store of excess energy as triacylglycerols, mediator of glucose homeostasis and an important endocrine/immune organ that secretes adipokines, that include inflammatory cytokines, chemokines, acute phase proteins and complement-like factors (Table 1 lists some of these). These adipokines may act as endocrine factors that regulate many central and peripheral processes, including appetite, energy metabolism, glucose and lipid metabolism, inflammatory responses, angiogenesis, blood pressure and reproductive function. The endocrine effects of adipokines have been extensively and expertly reviewed elsewhere (Halberg et al., 2008).

WAT is composed of many cell types, with about 50% of it being adipocytes, 10% resident macrophages and the rest comprising preadipocytes, endothelial and epithelial cells. Both the adipocytes and the non-adipocyte cells secrete adipokines. Adiponectin, and to a lesser extent, leptin, are largely adipocyte products, with signals such as MCP-1 and IL-6 being produced by the hypertrophied fat cell and the non-fat cells producing many of the other inflammatory signals. In obesity, there is macrophage infiltration into WAT, with the number of these macrophages being directly correlated to adipocyte size (Cousin et al., 1999). In addition the phenotype of the macrophage is also altered to reflect a more inflammatory state (M1; Lumeng et al., 2007). It appears that these alterations in the cellularity of the tissue may lead to changes in local adipokines secretion and can potentially cause dysregulation of WAT-mediated disposal of dietary glucose and lipids, and in turn, contribute to hyperglycemia, hyperlipidemia, insulin resistance and chronic inflammation (Trayhurn, 2005).

Depot specific differences in adipose tissue secretory function are also dependent on the anatomical location of this tissue, which can accumulate in different compartments within the body. It is deposited peripherally in subcutaneous depots, such as mammary and gluteofemoral regions, and internally in visceral depots, as in the omental and epicardial regions. The expansion of these depots may occur both through adipocyte hypertrophy and hyperplasia. Accumulation of excess fat in the visceral compartment, even if subcutaneous fat mass is normal, carries the greater metabolic risk (Weyer et al., 2000; Wajchenberg, 2000; Smith et al., 2001). Insulin resistance, the precursor to type 2 diabetes, is closely associated to adipocyte hypertrophy in visceral, as opposed to subcutaneous, adipose tissue. It has been proposed that inhibition of adipogenesis, the process of conversion of pre-adipocytes to lipid-laden adipocytes, in subcutaneous depots, in the face of a continuing ability to differentiate visceral adipocytes normally, leads to type 2 diabetes (Danforth, 2000). This may be because the excess lipid that would have been stored in the subcutaneous depots is shunted to liver and skeletal muscle

and results in impaired insulin action within these tissues and the development of peripheral insulin resistance (Smith et al., 2001).

Visceral depots also secrete more of the proinflammatory cytokines, such as IL-6, visfatin and MCP-1 (Greenberg and Obin, 2006, Madani, et al., 2009), which may be responsible for the insulin resistance. Conversely, in comparison with visceral adipose tissue, the subcutaneous depots appear less metabolically active and secrete more leptin and less free fatty acids (Wajchenberg, 2000). Therefore, excessive accumulation of subcutaneous adipose tissue is more likely to be associated with hyperleptinemia and may lead to central leptin resistance and greater susceptibility to further weight gain (Wajchenberg, 2000; Smith et al., 2001). Thus it is apparent that differential regional deposition of adipose tissue can impact on disease outcome, perhaps mediated by their altered adipokine repertoire.

ii) Adipokine receptors and signal transduction.

The presence of several of the adipokine receptors in adipocytes from both subcutaneous and visceral depots potentially renders these cells available to autocrine/paracrine regulation by adipokines. It has been demonstrated that human adipocytes express leptin receptors, both the long form (huOb-R), as well as the short forms B219.1 to B219.3 (Kielar et al., 1998, Apran-Husmann et al., 2001). The IL-6 receptor, together with the signal-transducing gp130 unit, is also present in isolated mature human adipocytes, as well as in those differentiated *in vitro* (Päth et al., 2001). The two TNF α receptors, (TNFR1 and TNFR2) are ubiquitously expressed, and interestingly, the expression of TNFR2 in human adipose tissue is positively correlated with BMI (Hotamisligil et al., 1997). Similarly, both adiponectin receptors are expressed (mRNA) in human adipose tissue and in isolated adipocytes from both subcutaneous and omental depots. Their levels correlate negatively with indices of obesity and metabolic dysfunction and are increased by physical exercise (Rasmussen et al., 2006, Nannipieri et al., 2007, Blüher et al., 2007). However, expression of the receptors is not proof of locally acting adipokines. Most of the evidence comes from *in vitro* studies that have used supra-physiological or pharmacological doses of these molecules to elicit a response. In the absence of tissue specific receptor knockout models or pharmacological inhibitors of the receptors local effects remains speculative.

In addition to the specific adipokine receptors, all toll-like receptors (TLRs 1-10, apart from 5 and 7) are expressed both in the stromavascular fraction and in mature adipocytes, from human subcutaneous and visceral adipose tissue (Kopp, 2009). These TLRs are essential innate immune receptors that classically alert the immune system to the presence of pathogens (Zhang and Schluesener, 2006). They recognize a large number of ligands, which includes LPS, zymosan and glycosylphosphatidylinositol lipid, and are capable of inducing the expression of a variety of inflammatory genes, like cytokines and chemokines. They can thus attract and activate T-cells in response to chemokines, such as RANTES. Their role in obesity or in response to excess energy intake is currently being investigated.

The signalling pathways that are apparent in the adipocytes and can be activated by the adipokines include those most commonly implicated in inflammation such as the NF κ B and JAK/STAT pathways (Table 2).

iii) Adipokine regulation of adipose mass and blood flow.

During adipogenesis, fibroblast-like preadipocytes differentiate into lipid-laden and insulin-responsive adipocytes. This process occurs in several stages and involves a cascade of transcription factors, among which peroxisome proliferator-activated receptor gamma (PPAR γ) and CCAAT/enhancer-binding proteins (C/EBPs) are considered the crucial determinants of adipocyte fate. The involvement of additional factors, including Krupel-like factors (KLFs), Wingless and INT-1 proteins (Wnts), and cell-cycle proteins has also been reported (Lefterova and Lazar, 2009). Differentiation of fibroblast-like preadipocytes to mature adipocytes is a continuous process in adipose tissue. Although the number of total adipocytes in an individual is determined during childhood and adolescence, there is constant turnover and about 10% of all adipocytes are renewed per year at all adult ages and throughout the spectrum of BMI (Spalding et al., 2008). Several of the proinflammatory adipokines, such as TNF α , act as potent inhibitors of adipogenesis, possibly via inhibition of PPAR γ -dependent phenomena, as shown by the fact that thiazolidinediones prevent TNF α -induced anti-adipogenesis (Cawthorn and Sethi, 2008). Furthermore, both in 3T3L1 (Cawthorn et al., 2007) and in human pre-adipocytes (Isakson et al., 2009), this TNF α -effect is dependent on Wnt/ β -catenin/T-cell factor 4 pathway.

IL-6 (20 ng/ml) also inhibits differentiation and lipid accumulation of 3T3L1 fibroblasts. The main adipogenic transcription factor affected by IL-6 is C/EBP α , whereas earlier activation of C/EBP δ , C/EBP β and PPAR γ remain intact (Gustafson et al., 2006). Chemerin, acting via the relevant receptor in 3T3L1 and human preadipocytes also impairs PPAR γ expression and cellular differentiation (Gorlaski et al., 2007, Roh et al., 2007). Interestingly, with all these adipokines, not only is adipogenesis inhibited, but in addition, the cells acquire a more macrophage-like phenotype (Isakson et al., 2009) and express a number of inflammatory genes, like IL-6, MCP1 and PAI-1 (Gustafson et al., 2006, Gorlaski et al., 2007). Resistin had no clear effect on the *in vitro* differentiation of 3T3-L1 adipocytes (Kim et al., 2001, Gong et al., 2004), however, *in vivo* data from a transgenic mouse model support an inhibitory role of resistin in adipogenesis (Kim et al., 2004). The same appears to apply to PAI-1, as primary adipocytes from PAI-1 deficient mice show accelerated differentiation and overexpression of PAI-1 in 3T3L1 adipocytes suppressed CEBP α and PPAR γ expression, and, subsequently differentiation (Liang et al., 2006).

Data on adipokines that may act as promoters of differentiation are scarce. In this context the effects of adiponectin on adipogenesis appear to be diverse. In brown pre-adipocytes derived from human bone marrow, adiponectin suppressed differentiation, via activation of COX-2 and increased PGE2 synthesis (Yokota et al., 2002). On the other hand, in 3T3L1 adipocytes adiponectin overexpression promoted preadipocyte proliferation and accelerated expression of adipogenic transcription factors and adipogenesis (Fu et al., 2005). There are also some data that macrophage migration inhibitory factor (MIF),

which is induced and released by adipocytes after treatment with TNF α (Hirokawa et al., 1997), might facilitate adipogenesis. Knocking down of MIF in 3T3L1 preadipocytes restricted their proliferation and suppressed expression of C/EBP δ ; as a result expression and activation of C/EBP α and PPAR γ and cell differentiation were also inhibited (Ikeda et al., 2008).

The renin – angiotensin system (RAS) and the relevant receptors, AT $_1$ and AT $_2$, are expressed in adipose tissue (Engeli et al., 2000). The effects of the locally produced angiotensin II appear to be dependent on the balance between the expression of the two receptors. Thus, initial *in vitro* experiments suggested that angiotensin II promotes pre-adipocyte differentiation via the AT $_2$ receptor (Engeli et al., 2000). Subsequently, however, it was shown that the *in vivo* effects of angiotensin II are mediated primarily via AT $_1$ and in this case the end result is expansion of adipose tissue, primarily because of adipocyte hypertrophy, and thus metabolic dysfunction (Massiéra et al., 2001, Tomono et al., 2008).

While not truly adipokines, products of enzyme activity within the adipose tissue such as NEFA and the prostaglandins, also have documented, potent autocrine/paracrine effects on adipose tissue differentiation and metabolism (Frayn, 1998, Richelsen, 1992, Négrel, 1999). Prostacyclin especially has been shown to be an inducer of adipogenesis by activating both C/EBP and PPAR γ (Vassaux et al., 1992).

The nitric oxide (NO) pathway has also come under some scrutiny as endothelial function, determined largely by bioavailability of NO, and insulin sensitivity, are both modulated by adiposity (Caballero, 2003, Ritchie et al., 2004). Although it has been postulated that adipokines, such as leptin, TNF α and IL-6, act on the endothelium to produce detrimental effects (Clarke and Mohamed-Ali, 2004), to date none has clearly been identified. NO is generated in adipose tissue and adipocytes (Ryden et al., 2001). While both eNOS, and to a lesser extent iNOS, are expressed in adipose tissue and adipocytes, only eNOS expression increases with obesity (Ryden et al., 2001). Asymmetric dimethylarginine (ADMA) is a novel risk factor for cardiovascular disease that inhibits nitric oxide (NO) bioavailability and causes endothelial dysfunction (Vallance and Leiper, 2002). ADMA is cleared by catabolism through the activity of dimethylarginine dimethylaminohydrolase (DDAH1 and 2; Vallance and Leiper, 2002). In obesity the ADMA generated may act as an autocrine and/or paracrine inhibitor of eNOS, thus potentially affecting several NO-mediated functions of the adipose tissue, such as local blood flow (Ardilouze et al., 2004), lipolysis (Gaudiot, 1998), glucose metabolism (Tanaka et al., 2003) and mitochondrial biogenesis (Nisoli et al., 2004).

The role of NO in energy expenditure, food intake and body weight gain has been explored recently in eNOS $^{-/-}$ mice (Nisoli et al., 2003). In the lean state these animals show no significant differences to wild-type littermates. However, on a weight-gaining diet the eNOS $^{-/-}$ mice were significantly heavier, with this weight gain attributed to greater feed efficiency (described as weight gain per gram of food intake). Adipose tissue from these eNOS $^{-/-}$ animals also had less mitochondrial DNA and mitochondrial enzymes, COXIV and Cyt c , suggesting lower mitochondrial biogenesis (Nisoli et al.,

2003).

Recent studies have looked at ADMA in human obesity and shown a close relationship between BMI and plasma levels of ADMA and the L-arg/ADMA ratio, independent of other metabolic risk factors (Eid et al., 2004). Overweight and obese subjects had significantly elevated ADMA levels and lower L-arg/ADMA ratio compared to lean or normal individuals. Enzymes responsible for the biosynthesis and degradation of ADMA are expressed in the human adipose tissue (Spoto et al., 2007). This study showed ADMA release from adipose tissue from two healthy patients and reported higher DDAH2 in visceral adipose tissue and higher DDAH1 in the subcutaneous depot (Spoto et al., 2007). Interestingly adiponectin has been shown to reduce ADMA accumulation in human cultured endothelial cells (Eid et al., 2007). Modulation of this pathway in the adipose tissue has not, as yet, been investigated, especially with reference to the adipokines.

iv) Role of adipokines in inflammation.

The secretory function of adipocytes can also be modulated by adipokines, as shown mainly in *in vitro* models. TNF α is known to suppress adiponectin production by adipocytes, while it induces a number of pro-inflammatory mediators like IL-6, MCP-1 and PAI-1 (Cawthorn and Sethi, 2008), primarily via activation of Foxo1 (Ito Y et al, 2009). Treatment of differentiated human adipocytes with TNF α also significantly decreased the expression of angiotensinogen and haptoglobin (Wang et al., 2005). With regards to leptin, TNF α is thought to stimulate release of a preformed pool of leptin by 3T3L1 adipocytes and by murine adipose tissue *in vivo* (Kirchgessner et al., 1997). On the other hand, in human adipose tissue explants, TNF α has a stimulatory effect on leptin expression and release only in the presence of dexamethasone (Trujillo et al., 2006). Finally, TNF α upregulates expression of apelin in 3T3F442A adipocytes, in murine and human adipose tissue and increases circulating levels of apelin in mice *in vivo* (Daviaud et al., 2006). Therefore, TNF α might mediate the increased apelin levels noted in obesity (Boucher et al., 2005).

IL-6 has similar effects to TNF α on adipokine regulation. Given that it suppresses expression of PPAR γ in rat adipocytes (Tanaka et al., 1999), 3T3L1 and 3T3F442A adipocytes (Lagathu et al., 2003) and in human subcutaneous adipose tissue explants (Sopasakis et al., 2004), it is not surprising that IL-6 inhibits adiponectin expression and/or release (Fasshauer et al., 2003a, Sopasakis et al., 2004, Simons et al., 2007). Furthermore, IL-6 can induce PAI-1 (Rega et al., 2005), MCP-1 (Fasshauer et al., 2004), as well as its own expression and release (Fasshauer et al., 2003b, Lagathu et al., 2003). Finally, IL-6 acts as a negative regulator of visfatin expression in 3T3L1 adipocytes (Kralisch et al., 2005) and does not alter leptin release (Bruun et al., 2002). Release of pro-inflammatory cytokines, TNF α and IL-6, is also induced by resistin in isolated human subcutaneous adipocytes, an effect which is possibly mediated by the NF κ B and JNK pathways (Kusminski et al., 2007). It has been proposed that leptin, at least in part, regulates its own release by adipose tissue, based on findings in mice deficient for the leptin receptor, which present with high circulating leptin levels (Zhang et al., 1997). Furthermore, leptin stimulates adiponectin and visfatin expression and release by

adipocytes and murine adipose tissue, both *ex vivo* and *in vivo* (Delporte et al., 2004, Tan et al., 2009).

Adiponectin exerts anti-inflammatory effects on adipocytes, as in other cellular populations, primarily via inhibition of the NF κ B pathway (Ajuwon et al., 2005). As a result, release of a number of pro-inflammatory cytokines by adipocytes is significantly suppressed (IL-6, IL-8, GRO α , MCP-1) (Dietze-Schroeder et al., 2005, Ajuwon et al., 2005, Sell et al., 2006). These changes might be of particular pathophysiological significance, given that they are adequate to inhibit adipocyte-induced skeletal muscle insulin resistance (Dietze-Schroeder et al., 2005). Similar anti-inflammatory actions might be exerted by vaspin, which was able to induce expression of adiponectin, while inhibiting expression of leptin, resistin and TNF α in murine WAT (Hida et al., 2005). Finally, apelin administration resulted in increased circulating adiponectin and reduced leptin concentrations without changes in mRNA expression of these two adipokines in adipose tissue. It seems likely however, that these effects might be secondary to whole-body changes in adiposity and energy expenditure, rather than the primary apelin effect (Higuchi et al., 2007).

From these *in vitro* data it is reasonable to speculate that in obesity the inflammatory signals are amplified in the adipose tissue and perpetuate both the cellular and the secretory changes reported. However no concrete evidence is currently available to support this hypothesis.

In view of adipose tissue expansion in obesity, the ability to form new vessels to prevent adipocyte hypoxia, or the failure to do so, might be of particular importance (Trayhurn et al., 2008). Among the adipokines, leptin is known to promote angiogenesis in several experimental models (Sierra-Honigmann et al., 1998, Bouloumié et al., 1998). In adipose tissue it also induces angiostatic signals (angiopoietin-2) and tissue de-vascularization (Cohen et al., 2001). Adiponectin, by activating AMP kinase in endothelial cells, is also able to induce angiogenesis (Ouchi et al., 2004), as shown in adiponectin-deficient mice, which have impaired responses to ischemia (Shibata et al., 2004). IL-6 also has the potential to act as an angiogenic factor, by inducing release of VEGF, as has been reported in both human subcutaneous and visceral adipose tissue explants and in isolated adipocytes and preadipocytes (Rega et al., 2007). Adipose derived VEGF may also directly induce angiogenesis in the tissue (Vona-Davis and Rose, 2009). Apelin and HGF have also been shown to induce angiogenesis, both in human umbilical vein endothelial cells *in vitro*, and in adipose tissue in transplantation model *in vivo*. Interestingly, given that hypoxia induces its expression in 3T3F442A adipocytes, they may represent a compensatory mechanism of adipocytes against oxygen deprivation in obesity and other disease states (Kunduzova et al., 2008; Bell et al., 2008).

v) Autocrine/paracrine regulation of glucose and lipid metabolism.

Deposition of energy in adipose tissue occurs primarily via hydrolysis of circulating triglycerides by lipoprotein lipase (LPL), uptake of fatty acids and re-esterification to glycerol within the cell or via de novo lipogenesis. Interestingly, ASP is a potent stimulator of triglyceride synthesis in adipocytes *in vitro* (Baldo et al., 1993) and is

released by adipose tissue *in vivo* in the postprandial period, when net fatty acid uptake by the tissue occurs (Saleh et al., 1998). TNF α has opposing effects on free fatty acid uptake by adipocytes, probably by interfering with the expression and activity of LPL and fatty acid binding and transport proteins (Cawthorn and Sethi, 2008). It has also been shown that IL-6 inhibits LPL activity in 3T3L1 adipocytes and in murine adipose tissue *in vivo* (Greenberg et al., 1992), whereas resistin increases LPL activity (Kim et al., 2007). In view of some of the opposing effects of these adipokines on fatty acid uptake and the lack of *in vivo* data it is difficult to speculate on their precise role in obesity.

In the face of negative energy balance, adipose tissue releases free fatty acids after intracellular hydrolysis of triglycerides by adipose triacylglycerol lipase (ATGL) and hormone-sensitive lipase (HSL). TNF α is a potent stimulator of lipolysis. At least two distinct mechanisms have been implicated: downregulation of perilipin, leading to exposure of the lipid droplet to the lipases, and suppression of the antilipolytic GTP-binding membrane proteins G α_i (Rydén and Arner, 2007). Leptin also induces lipolysis in mouse or rat adipocytes (Frühbeck et al., 1997). In human adipocytes, however, from either lean or obese healthy subjects, leptin treatment failed to modulate lipolysis (basal or isoproterenol-stimulated or insulin-mediated anti-lipolysis), (Apran-Husmann et al., 2001, Elimam et al., 2002).

It has been reported that IL-6 increases basal, as well as isoprenaline-stimulated, lipolysis in 3T3L1 (Petersen et al., 2005) and in human mammary adipocytes (Päth et al., 2001); however, very high IL-6 concentrations (≥ 100 ng/ml) were used in these experiments. Under physiological conditions, adipocytes are chronically exposed to IL-6 at the lower ng/ml range, which however exceed circulating concentrations by orders of magnitude (Sopasakis et al., 2004). Finally, recent data indicate that chemerin acts as an inhibitor of both basal and stimulated lipolysis in 3T3L1 adipocytes, possibly via a reduction in intracellular cAMP levels (Gorlaski et al., 2007).

Insulin plays a central role in the regulation of adipocyte metabolism, by acting as a potent inhibitor of lipolysis, while stimulating glucose uptake and fatty acid re-esterification. TNF α antagonises the effects of insulin on adipocytes in multiple levels (Cawthorn and Sethi, 2008, Rydén M and Arner P, 2007). Firstly, it reduces protein levels of GLUT-4, insulin receptor (IR) and insulin receptor substrate-1 (IRS-1) in adipocytes, possibly by suppressing PPAR γ expression and activity. Secondly, it disrupts downstream insulin signaling by inhibiting tyrosine phosphorylation of IR and IRS-1. Finally, it increases the intracellular concentrations of diacylglycerols and ceramides, which then in turn, compromise insulin signaling further. Leptin also interferes with insulin signaling (Pérez et al., 2004, Kraus et al., 2002) and insulin-mediated glucose uptake (Kraus et al., 2002) in mouse or rat adipocytes. As was noted for lipolysis, these findings are not reflected in human adipocytes: in this case, leptin does not appear to affect basal glucose uptake, and there is controversy as to whether or not it inhibits insulin-induced glucose uptake (Zhang et al., 1999, Apran-Husmann et al., 2001) and insulin-induced lipogenesis (Elimam et al., 2002). The initial reports regarding IL-6 effects on glucose uptake by 3T3L1 adipocytes were contradictory (Stouthard et al., 1996, Huppertz et al., 1996). Subsequently, high concentrations of IL-6 (≥ 20 ng/ml) were

shown to suppress expression, both at mRNA and protein levels, of GLUT-4 and IRS-1 and thus to reduce glucose uptake. In contrast to TNF α , IL-6 does not interfere with the phosphorylation of IR and IRS-1 (Rotter et al., 2003, Lagathu et al., 2003). Resistin seems to act as another inhibitor of glucose uptake in differentiated human subcutaneous preadipocytes and adipocytes, although its effects appear modest (McTernan et al., 2003). Insulin-sensitizing effects of other adipokines have also been reported. Overexpression of adiponectin in 3T3L1 adipocytes resulted in increased expression of GLUT4 and enhanced insulin-mediated glucose uptake (Fu et al., 2005); on the other hand, treatment of pig adipocytes with adiponectin (full-length and globular at 30 μ g/ml) inhibited insulin-mediated lipogenesis (Jacobi et al., 2004). Vaspin, a product of murine visceral adipose tissue, induced expression of GLUT-4 in WAT after *in vivo* administration and improved whole-body insulin sensitivity and glucose tolerance (Hida et al., 2005). Omentin, which is also preferentially expressed and release by visceral adipose tissue, appears to increase adipocyte insulin sensitivity and promote insulin-stimulated glucose uptake, via stimulation of Akt phosphorylation (Yang et al., 2006). Finally, chemerin promotes insulin-mediated IRS-1 phosphorylation and concomitantly enhances glucose uptake in 3T3L1 adipocytes (Takahashi et al., 2008).

In addition to studies on adipocytes, whole-body animal models can offer further insight in the function of adipokines. Thus, it has been suggested that leptin *in vivo* inhibits fat accumulation in adipose tissue. It is known that high circulating leptin levels in obesity are not effective in body weight regulation due to the accompanying leptin resistance. However, induction of hyperleptinemia in leptin-sensitive lean rats results in rapid depletion of fat stores. In addition, constitutive overexpression of the leptin receptor in adipose tissue, which overcomes leptin resistance, prevents high fat diet-induced obesity. At a molecular level, this is accompanied by induction of leptin signaling pathways (STAT-3), activation of AMPK and PCG-1 α , and upregulation of UCP-1 and UCP-2 in WAT (Wang et al., 2005). Because of the failure of adipose tissue as an energy store, these mice show ectopic fat deposition and metabolic dysregulation (Wang et al., 2008). Data from a transgenic model suggest that resistin impairs fatty acid re-esterification in the adipose tissue and thus leads to increased free fatty acid release and systemic insulin resistance (Pravenec et al., 2006).

Conclusion. There is accumulating evidence that in obesity, adipose tissue expands, through adipocyte hypertrophy that is facilitated by inhibition of adipogenesis and adipocyte death. These areas of cell death form focal points for macrophage infiltration into the tissue. The infiltrating macrophage switch from a non-inflammatory phenotype to form foam cells. Continued accumulation of body fat is accompanied by adipocyte hyperplasia and hypertrophy, along with further increases in macrophage numbers. All stages of this process are accompanied by changes in, and are mediated by, autocrine and paracrine effects of adipokines on adipogenesis, angiogenesis, inflammation and adipocyte metabolism. Many of these effects may impact on the development of endothelial dysfunction and insulin resistance, important precursors of various metabolic diseases, such as atherosclerosis and type 2 diabetes. Understanding the putative role of these molecules in shifting the adipocytes from the large, insulin-resistant to the small, insulin-responsive phenotypes, may constitute an attractive strategy for treating metabolic

disease. Targeted induction of specific adipokines may facilitate such a shift in adipose tissue cellularity as well as in adipocyte morphology and function. Thus, elucidating the mechanisms underlying WAT secretory function and the ability to modulate this may, in future, be important in treating the pathological consequences of obesity.

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Table 1: Signals derived from adipose tissue

Immune characteristics	Adipokine	References
Cytokine-like molecules	Leptin	Klein et al., 1996; Zhang et al., 1994
	IL-6/IL-6sR	Mohamed-Ali et al., 1997
	TNF α /TNFsR1/TNFsR2	Hotamisligil et al., 1993; Mohamed-Ali et al., 1999
	IL-8	Bruun et al., 2001
	IL-10	Juge-Aubry et al., 2005
	MIF	Hirokawa et al., 1997
Complement-like factors	Adipsin	Cook et al., 1987
	ASP	Cianflone et al., 1989
	Adiponectin	Hu et al., 1996; Maeda et al., 1996; Scherer et al., 1995
Chemokines	MCP-1	Gerhardt et al., 2001
	RANTES	Madani et al., 2009; Schaffler et al., 2006
	MIP-1 α	Gerhardt et al., 2001
	Chemerin	Goralski et al., 2007
Acute phase proteins	Serum Amyloid A	Benditt & Meek, 1989; Lin et al., 2001
	C-Reactive Protein	Ouchi et al., 2003
	Haptoglobin	Friedrichs et al., 1995
	PAI-1	Alessi et al., 1997
Growth factors	HGF	Fain et al., 2004
	NGF	Peeraully et al., 2004
	TGF β	Samad et al., 1997
	VEGF	Claffey et al., 1992; Mick et al., 2002
	Visfatin	Fukuhara et al., 2002
Adhesion molecules	VCAM-1	Fain et al., 2007
	ICAM-1	Brake et al., 2006
Enzymatic pathways	Components of the COX pathway: PGI ₂ , PGE ₂	Ogston et al., 2008; Shaw & Ramwell, 1968
	Components of the NO pathway: NO, ADMA	Spoto et al., 2007
	RAS	Frederich et al., 1992
	MMPs/TIMPs	Maquoi et al., 2002
Others	Apelin	Boucher et al., 2005
	RBP4	Zovish et al., 1992
	Resistin	Steppan et al., 2001
	Omentin	Schaffler et al., 2005
	Vaspin	Hida et al., 2005

IL: interleukin; TNF: tumour necrosis factor; MIF: macrophage migration inhibitory factor; MCP: macrophage chemotactic protein; RANTES: regulated upon activation, normally T cell expressed and secreted; MIP: macrophage inhibitory protein; PAI: plasminogen activator inhibitor; HGF: hepatocyte growth factor; NGF: nerve growth factor; VEGF: vascular endothelial growth factor; VCAM: vascular cell adhesion molecule; ICAM-1: intracellular adhesion molecule; COX: cyclo-oxygenase; NO: nitric oxide; ADMA: asymmetric dimethylarginine; RAS: renin angiotensin system; MMP: matrix metalloproteinase; TIMP: tissue inhibitor of metalloproteinases; RBP: retinol binding protein.

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Table 2. Signaling pathways activated by adipokines in adipocytes

Adipokine / Function	Signaling pathway	Cell type	Effect
Adiponectin: Adipogenesis	• COX2/PGE2	• Human bone marrow brown preadipocytes (Yokota et al., 2002)	• (↓) CEBP α / PPAR γ
	Secretory Functions • PPAR γ /NF- κ B(↓)	• 3T3L1/Porcine adipocytes (Ajuwon et al., 2005)	• (↓) IL-6/ TNF α
IL-6:	Adipogenesis • Dvl/ β -catenin	• 3T3L1 (Gustafson et al., 2006)	• (↓) CEBP α
	Metabolic Functions • ERK	• Porcine adipocytes (Yang et al., 2008)	• (↓) perilipin
	Secretory Functions • MAPK • MAPK-JAK2	• 3T3L1 (Fasshauer et al., 2003, Kralisch et al., 2005) • 3T3L1 (Fasshauer et al., 2004)	• (↓) adiponectin/ visfatin • (↑) MCP-1
Leptin:	Adipogenesis • ERK-STAT1	• Murine preadipocytes (Rhee et al., 2008)	• (↓) PPAR γ
	Metabolic Functions • MAPK-STAT3	• Murine brown adipocytes (Kraus et al., 2002)	• (↓) IR kinase activity
	Secretory Functions • MAPK-PKA	• Murine adipocytes (Mehebig et al., 2005)	• Activation of NOS
Resistin:	Metabolic Functions • SOCS3 • PKB/AMPK	• 3T3-L1 (Steppan et al., 2005) • 3T3-L1 (Kim et al., 2007)	• (↓) tyrosine phosphorylation of IR • (↑) LPL activity
TGFβ:	Adipogenesis • Smad3/4	• 3T3L1 (Choy et al., 2003)	• (↓) CEBP β / δ activity

TNFα:	Adipogenesis	<ul style="list-style-type: none"> • TAK1/TAB1/NIK/NF-κB • Wnt/β-catenin/T-cell factor 4 	<ul style="list-style-type: none"> • Mouse bone marrow stromal cells (Suzawa et al., 2003) • 3T3-L1 (Cawthorn et al., 2007), human preadipocytes (Isakson et al., 2009) 	<ul style="list-style-type: none"> • (\downarrow) PPARγ activity • (\downarrow) PPARγ/CEBPα
	Metabolic Functions	<ul style="list-style-type: none"> • NF-κB/PPARγ(\downarrow) • Multiple kinases • Ceramide generation • Caspase-3/-6 • MEK/ERK • iNOS/NO/cGMP 	<ul style="list-style-type: none"> • 3T3-L1 (Iwata et al., 2001, Ruan et al., 2002) • 3T3-L1 (Engelman et al., 2000, Gao et al., 2003, Zhang et al., 2008), human visceral adipocytes (Fernandez-Veledo et al., 2009) • Rat brown adipocytes (Teruel et al., 2001) • 3T3-L1 (Guilherme et al., 2009) • Human adipocytes (Zhang et al., 2002) • 3T3-L1 (Lien et al., 2009) 	<ul style="list-style-type: none"> • (\downarrow) GLUT-4/IR/IRS-1/Akt protein • Serine phosphorylation of IRS-1 • (\downarrow) GLUT-4 translocation • (\downarrow) PPARγ protein • (\uparrow) intracellular cAMP and perilipin phosphorylation • HSL phosphorylation
	Secretory Functions	<ul style="list-style-type: none"> • PPARγ/CEBPβ • PKC/JNK • Foxo1/CEBPβ • MAPK-PI3 kinase-PKC-NF-κB • MAPK-PI3 kinase-JNK 	<ul style="list-style-type: none"> • 3T3-L1 (Ruan et al., 2002, Kita et al., 2005) • 3T3-L1 (Kim et al., 2005, Lim et al., 2008) • 3T3-L1 (Ito et al., 2009) • 3T3-L1 (Pandey et al., 2005) • 3T3-F442A (Daviaud et al., 2006) 	<ul style="list-style-type: none"> • (\downarrow) adiponectin • (\downarrow) adiponectin • (\uparrow) MCP-1/IL-6 • (\uparrow) PAI-1 • (\downarrow) apelin

3T3-L1 and 3T3-F442A refers only to preadipocytes or adipocytes in this Table. AMPK: AMP-activated kinase; CEBP: CCAAT enhancer-binding protein; COX: cyclooxygenase; Dvl: dishevelled; ERK: extracellular signal related kinase; GLUT: glucose transporter; HSL: hormone sensitive lipase; IL: interleukin; IR: insulin receptor; IRS: insulin receptor substrate; JAK: Janus tyrosine kinase; JNK: c-Jun-NH₂-terminal kinase; LPL: lipoprotein lipase; MAPK: mitogen activated protein kinase; MCP: monocyte chemoattractant protein; MEK: mitogen-activated protein kinase kinase; NF- κ B: nuclear factor- κ B; NIK: NF- κ B-inducing kinase; NOS: nitric oxide synthase; PAI: plasminogen activator inhibitor; PK: protein kinase; PG: prostaglandin; PI: phosphatidylinositol;

PPAR: peroxisome proliferator activated receptor; STAT: signal transducer and activator of transcription; TGF: transforming growth factor; TNF: tumour necrosis factor.

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Figure legends**Figure 1.**

The figure shows a schematic representation of the alterations in adipose tissue that accompany body weight gain. In the lean state the tissue secretes elevated levels of adiponectin and other anti-inflammatory adipokines and is insulin responsive. Increased energy intake is followed by adipocyte hypertrophy/death and chemotactic adipokine release (eg MCP-1). This facilitates macrophage infiltration into the tissue and exacerbates the inflammatory response. These secretory changes are accompanied by local insulin resistance and hypoxia. Many of the adipokines released by this inflamed adipose tissue cause insulin resistance and endothelial dysfunction, precursors to many metabolic diseases.

- Dead adipocyte
- ☀ Infiltrating macrophages

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Figure



Lean / healthy state

Adipose tissue expansion / inflammation

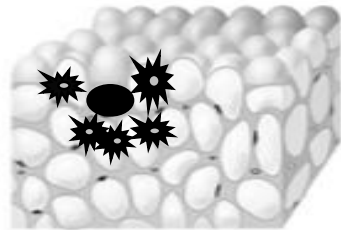
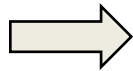
Obesity-associated pathologies

↑ Insulin sensitivity
Normal endothelial function

↑ Anti-inflammatory adipokines
↓ Pro-inflammatory adipokines

↓ Insulin sensitivity
Endothelial dysfunction

↓ Anti-inflammatory adipokines
↑↑ Pro-inflammatory adipokines



↓ Adiponectin, omentin
↑ TNF α , IL-6, IL-8, MIF, MCP-1, RANTES

↑ leptin, adipisin, chemerin, visfatin, apelin, vaspin

↑ SAA, haptoglobin, PAI-1

↑ HGF, NGF, TGF β , VEGF

Activation of COX, NOS, RAS and MMPs

