Articles in PresS. Am J Physiol Endocrinol Metab (March 3, 2009). doi:10.1152/ajpendo.00015.2009

Getting the Message Across – Mechanisms of Physiological Cross-Talk by Adipose Tissue

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Abstract

Obesity is associated with resistance of skeletal muscle to insulin-mediated glucose uptake, as well as resistance of different organs and tissues to other metabolic and vascular actions of insulin. In addition, the body is exquisitely sensitive to nutrient imbalance, with energy excess or a high fat diet rapidly increasing insulin resistance even before noticeable changes occur in fat mass. There is a growing acceptance of the fact that, as well as acting as a storage site for surplus energy, adipose tissue is an important source of signals relevant to, *inter alia*, energy homeostasis, fertility and bone turnover. It has also been widely recognised that obesity is a state of low-grade inflammation, with adipose tissue generating substantial quantities of pro-inflammatory molecules. At a cellular level, the understanding of the signaling pathways responsible for such alterations has been intensively investigated. What is less clear, however, is how alterations of physiology, and of signaling, within one cell or one tissue are communicated to other parts of the body. The concepts of cell signals being disseminated systemically through a circulating 'endocrine' signal have been complemented by the view that local signaling may similarly occur through 'autocrine' or 'paracrine' mechanisms. Yet while much elegant work has focussed on the alterations in signaling which are found in obesity or energy excess, there has been less attention paid to ways in which such signals may propagate to remote organs. This review of the integrative physiology of obesity critically appraises the data and outlines a series of hypotheses as to how inter-organ cross-talk takes place. The hypotheses presented include the 'fatty acid hypothesis', the 'portal hypothesis', the 'endocrine hypothesis', the 'inflammatory hypothesis', the 'overflow hypothesis', a novel 'vasocrine' hypothesis, and a 'neural hypothesis' - and the strengths and weaknesses of each hypothesis are discussed.

(297 words)

Key Words: Adipocyte; inflammation; insulin resistance; adipocytokines; endothelium

Introduction

The global epidemic of obesity is bringing in its wake a catastrophic increase in the prevalence of metabolic diseases (1). Obesity is a major cause of insulin resistance, which has been implicated in the rising prevalence of diabetes, metabolic syndrome and cardiovascular disease (2). But independent of increased fat mass, a positive energy balance rapidly induces systemic insulin resistance because of systemic signaling of nutrient balance by adipose tissue and perhaps liver (3). Thus during the last 15 years, adipose tissue has been widely recognized to play a more important role than simply that of a fuel depot. It is also a vital source of information to other insulin sensitive organs and tissues of its own mass and of the organism's nutritional state.

The other area of knowledge which has grown exponentially over the same time frame is the recognition of obesity representing a state of low-grade inflammation. Adipose tissue synthesises and secretes circulating hormones and 'adipokines' which act as systemic inflammatory mediators and signals of the organism's nutritional status. Furthermore, fat within organs and around blood vessels appears to have important metabolic consequences. These mechanisms involve both a nutrient sensing mechanism within adipocytes and other cells (autocrine effects), and intercellular (paracrine) or inter-organ (endocrine) 'cross-talk,' representing a propagation of signals, particularly from fat, to entrain the metabolic cooperation of other target organs such as liver and skeletal muscle. While there has been an explosion in the understanding of these signaling mechanisms at the molecular and cellular level, the elegance of understanding at this level is rarely accompanied by a growth in sophistication at the level of integrative physiology. Thus adipose tissue signals which are expressed or secreted in greater quantities by adipose tissue from obese subjects, or by visceral compared to subcutaneous fat, may or may not satisfy criteria to support their role as an endocrine (systemic or portal) 'adiposity signal.'

We hypothesize that adipose tissue is connected by a myriad of different forms of communication with other organs and tissues, including neural and vascular routes, and so literally sits at the "crossroads" of multiple interorgan exchanges. Additionally, interactions among different cell types within adipose tissue contribute to the overall impact of this organ. Clearly, any unifying hypotheses imputed to account for these signaling effects need to explain various complex attributes of fat and insulin resistance,

including the particularly adverse effects of truncal fat on the risk of both diabetes and cardiovascular disease. Therefore, this article will examine the major putative mechanisms whereby adipose tissue is a key mediator of obesity-related organ cross-talk. Seven hypotheses will be presented – 1. a ‘fatty acid hypothesis’, 2. a ‘portal hypothesis’, 3. an ‘endocrine hypothesis’, 4. an ‘inflammatory hypothesis’, 5. an ‘overflow hypothesis’, 6. a novel ‘vasocrine’ hypothesis, and 7. a ‘neural hypothesis’ - along with the strengths and pitfalls of the evidence for each hypothesis.

Insulin Resistance in various target organs

To consider the systemic effects of fat on insulin action, the concept of insulin resistance in individual target organs needs to be defined. The term “insulin resistance” generally refers to resistance to the metabolic effects of insulin, including the suppressive effects of insulin on endogenous glucose production (primarily in **Liver**), and the stimulatory effects of insulin on peripheral glucose uptake and glycogen synthesis (predominantly in **Skeletal Muscle**). In short, a reduction of insulin-mediated peripheral glucose uptake produces an elevation of glucose concentrations predominately in the postprandial state, while insensitivity of the suppression of hepatic glucose output to insulin is responsible for fasting hyperglycemia. Of note, mitochondrial density and activity in skeletal muscle and liver may contribute importantly to insulin sensitivity in different tissues (4,5). Insulin resistance in **adipose tissue** is characterized by a decreased suppression of adipose tissue lipolysis by insulin, resulting in elevated circulating non-esterified fatty acid (NEFA) levels. Indeed, the suppressive effect of insulin on NEFA levels is impaired in obese insulin-resistant individuals (6,7) and in type 2 diabetes mellitus (T2DM) (8). In addition to this, it has been shown that impaired glucose uptake into fat resulted in both hepatic and skeletal muscle insulin resistance (9). Furthermore, selective over-expression of the insulin-glucose transporter GLUT4 in adipose tissue both increased adipose tissue mass and improved whole-body insulin sensitivity (10,11). These findings suggest that adipose tissue provides a ‘sink’ to protect other tissues from toxic effects of excessive NEFA, and/or that the increased fat mass generated in this model produced factor(s) promoting insulin sensitivity. *This evidence of ‘cross-talk’ between adipose tissue and other organs indicates that adipose tissue serves not only as a target organ of insulin but can modulate insulin’s effects on other target tissues, which will be the focus of this article.*

More recently, it has become clear that insulin activates intracellular signaling pathways in a variety of other tissues, including **Vascular Endothelium** and **Nervous Tissue**. Since both tissues may coordinate the body’s response to insulin in obesity and other insulin resistant states, the relevance of insulin resistance in these tissues will be presented briefly.

Normally, the **vascular endothelial cell** is sensitive to various actions of insulin, which include stimulating the expression and activation of endothelial nitric oxide synthase (12), with consequent vasodilatation (13,14). In obesity, impaired insulin-mediated vasodilatation is a recognized precursor of atherothrombotic vascular disease (15,16). Whether insulin-mediated vasodilatation in vessels supplying skeletal muscle plays any role in increasing insulin-dependent glucose uptake remains disputed (17,18,19). However, insulin clearly has a substantially greater impact on capillary recruitment to increase nutritive tissue perfusion than it has on total limb blood flow (19,20). Such recruitment, probably through diversion of flow from non-nutritive circuits (21), has a rapid timescale and a dose-response relationship in the physiological concentration range, making it likely to represent an important physiological mechanism (19,22). Bergman and his group have shown how important interstitial space accessibility is to insulin's mechanism of action, not only in muscle and adipose tissue (23,24), but curiously, also for its liver effect (25). Furthermore, this group has recently shown that a high-fat diet reduces the access of insulin to target tissues, potentially by inhibiting capillary recruitment (26).

A growing body of evidence indicates that **brain** insulin action is required for physiological glucose homeostasis (27,28,29). This counters the longstanding perception of the brain as an insulin insensitive organ (30). While peripheral insulin at basal concentrations has been shown to stimulate global brain glucose uptake (31), this effect is markedly reduced in insulin resistant subjects (32). Indeed, while glucose disposal occurs in an insulin-independent manner in the majority of neurons, neurons in the hypothalamus and other discrete brain areas express the insulin-responsive glucose transporter GLUT4 (33). Targeted impairments in insulin receptors either in all neurons (34) or specifically in the hypothalamus (29) rapidly cause hyperphagia and diet-dependent obesity. Central inhibition of insulin action also dramatically reduces the ability of exogenously infused insulin to blunt hepatic glucose output, suggesting an important role for hypothalamic insulin receptors in the regulation of hepatic glucose metabolism (28). Therefore, resistance to the central appetite-suppressing and metabolic effects of insulin may play a seminal role in the development of insulin resistance.

The individual hypotheses concerning mechanisms whereby adipose tissue signals its mass to insulin target tissues will now be outlined and discussed. Before exploring

other possible adipose signals, a brief outline of the actions of NEFA on insulin signaling is presented. Of note, while a discussion of genetic factors is beyond the scope of this review, it is likely that genetic factors would influence an individual's metabolic responses to a given degree of fat accumulation.

1. Obesity and Insulin Resistance – the Fatty Acid Hypothesis

The original mechanism proposed for the effect of NEFA on glucose uptake was that of Randle, who some forty years ago postulated that fatty acids inhibit glucose oxidation in the tricarboxylic acid cycle by direct substrate competition in heart and diaphragm muscle (35,36). As initially proposed by Randle, an increase in fatty acid availability results in an elevation of the intramitochondrial acetyl coenzyme A/coenzyme A and NADH/NAD+ ratios, with subsequent inactivation of pyruvate dehydrogenase. This, in turn, causes citrate concentrations to increase, leading to inhibition of phosphofructokinase. Subsequent increases in intracellular glucose-6-phosphate concentration were predicted to inhibit hexokinase II activity, resulting in an increase in intracellular glucose concentrations and a decrease in muscle glucose uptake. Recent observations by Shulman's group have used 13C-NMR to measure intracellular concentrations of glucose-6-phosphate, and have shown substantial decreases in obese patients and those with type 2 diabetes, rather than the increase predicted by Randle (37). These findings have led to the proposal of an alternative mechanism whereby increased NEFA leads to down-regulation of insulin signaling in human skeletal muscle (37). Increased NEFA concentrations have been shown to lead to serine/threonine phosphorylation of insulin receptor substrates (IRS-1 and IRS-2), subsequently reducing the ability of the IRS to activate phosphatidylinositol 3- kinase (PI3-kinase) and glucose transport (38). This may occur through the mediation of the serine kinase protein kinase C- θ (PKC-θ), since an acute elevation of plasma fatty acids for 5 hours resulted in the activation of PKC-θ in skeletal muscle and was associated with decreased tyrosine phosphorylation of IRS-1 (39). Indeed, inactivation of PKC-θ was protective against fat-induced insulin resistance in skeletal muscle (40) and against fatty acid-induced insulin resistance in endothelial cells (41). Additionally, overexpression of uncoupling protein 3 (UCP3) in skeletal muscle was recently shown to protect against fat-induced insulin resistance and reduce PKC-θ activity in muscle, presumably by dissipating the mitochondrial proton gradient and promoting conversion of intramyocellular fat into thermal energy (42). These findings are all in line with elegant studies using NMR-spectroscopy, showing that NEFA-induced insulin resistance occurs through the impairment of trans-membrane glucose transport (43), and that this in turn is the consequence of reduced substrate activation downstream from the insulin receptor, IRS-1 and PI3-kinase.

Elevated NEFA levels also impair the suppressive effects of insulin on hepatic glucose production. Various mechanisms appear to contribute to this phenomenon. NEFAs have been shown to stimulate gluconeogenesis in vitro (44), probably due to increased ATP and NADH production (45) and gluconeogenic gene expression (46). Additionally, chronic high-fat feeding in rats is associated with a higher activity ratio of hepatic glucose-6-phosphatase (G-6-Pase)/glucokinase (GK), thus favouring increased hepatic glucose output (47). Increased NEFA levels acutely induce the expression of hepatic G-6-Pase in normal rats (48). Transcriptional regulation of hepatic genes by lipids could potentially be mediated via the peroxisome proliferator-activated receptors (PPARs) (49).

In addition to total NEFA levels, certain species of NEFA and their products appear to be of considerable physiological relevance. In particular, increasing evidence appears to link tissue ceramide accumulation to insulin resistance, especially that induced by saturated fat and obesity (50). Not only did cell-permeable ceramide analogues induce insulin resistance in muscle cells, but palmitate-induced insulin resistance was prevented by inhibiting *de novo* ceramide synthesis (51). Recent observations have associated increased liver fat and insulin resistance with increased adipose ceramide content, further suggesting a connection between ceramide production and insulin resistance in humans (52).

Elevated concentrations of NEFA not only produce hepatic and peripheral insulin resistance (53), but impair endothelial-dependent vasodilatation (54), perhaps through direct effects on the nitric oxide synthase enzyme (and thus nutritive blood flow), as well as inhibition of insulin action. The impact of tissue fat accumulation will be discussed under ‘Overflow Hypothesis’.

The Fatty Acid Hypothesis proposes, then, a central role for these molecules as the systemic signal for obesity-associated insulin resistance. Other observations on the powerful influence of visceral fat on insulin resistance, and on risk of diabetes and cardiovascular disease, have led to this model being further developed as a ‘Portal Hypothesis,’ as will now be outlined.

2. Obesity and Insulin Resistance – the Portal Hypothesis

While insulin resistance can occur in the absence of obesity, increased fat mass (55), particularly when distributed centrally (56,57,58), is a powerful contributor to this state. Obesity is also a major predictor of diabetes and cardiovascular disease, with truncal or upper body obesity being the major contributory phenotype for both conditions (59,60). The widely accepted explanation for the deleterious effect of truncal fat is the so-called "Portal Hypothesis" (61), which suggests that the higher rates of lipolysis in visceral fat (62) expose the liver directly to high concentrations of non-esterified fatty acids (NEFA). The consequence is hepatic insulin resistance, followed by peripheral hyperinsulinemia and insulin resistance in skeletal muscle. Bergman and colleagues have shown that a six to twelve week high-fat diet in dogs, associated with a more than two-fold increase in trunk adiposity, results in increased hepatic fat infiltration and insulin resistance (63,64) There are, however, several observations that challenge the tenets of the 'Portal Hypothesis'. First, an elegantly balanced study has shown that the large majority of NEFA to which the liver is exposed is generated by peripheral rather than by visceral fat, even in obese subjects, despite their proportionate increase in visceral fat (65). This observation questions the link between visceral obesity and hepatic insulin resistance, and implies that NEFA concentrations in the systemic circulation should correlate more closely with insulin resistance than generally seen (66). A second observation, is that in aging rats the removal of 'visceral fat' leads to improved insulin sensitivity and prevents the age-related deterioration of insulin action (67). Nevertheless, the 'visceral' fat removed in this study was epididymal and perirenal fat, both of which drain into the systemic circulation, and neither procedure changed systemic concentrations of NEFA.

There is furthermore evidence that adipose signaling of insulin resistance is not mediated through elevated concentrations of NEFA. Kahn and colleagues created a mouse in which insulin resistance was limited to adipose tissue by knocking out the GLUT4 glucose transporter specifically in adipocytes (68). *In vitro*, liver and muscle insulin action in these mice were completely normal, but in the whole animal, insulin resistance was found in both organs, implying that there is cross-talk between insulin-resistant fat and other organs. The fact that systemic NEFA concentrations were unaffected by the adipose tissue GLUT4 knockout shows that these were not likely to have been the signaling molecules (68). It is necessary, then, to explore alternative mechanisms by which adipose tissue can signal its own mass or its metabolic state to remote tissues and organs.

3. Obesity and Insulin Resistance – the Endocrine Hypothesis

Adipose tissue, in addition to its role as an energy storage depot, elaborates endocrine hormones, including adiponectin, leptin and other metabolic mediators (69,70) (Table 1). The so-called 'Endocrine Hypothesis' postulates that obesity may contribute to insulin resistance by altering the levels of these key adipose-derived circulating hormones (Figure 1). A number of these candidate molecules are pro-inflammatory cytokines, and will be further considered below under the 'Inflammatory Hypothesis.' Of the other widely recognised adipocytokines, perhaps the most interesting candidate for systemic signaling is **adiponectin**. Adiponectin is produced in substantial amounts by adipose tissue. Unlike most adipose tissue products, however, it is negatively related to fat mass (71), possibly as a consequence of inhibition by TNF-α or cortisol (72,73). In population studies, low adiponectin concentrations are associated cross-sectionally with insulin resistance and longitudinally with increased risks of diabetes and cardiovascular disease (74,71,75,76). There are, nevertheless, inconsistencies among adiponectin studies which partially result from the existence of different molecular species with different biological activities; and the high-molecular-weight to low-molecular-weight adiponectin ratio, rather than the absolute adiponectin concentration, has been strongly associated with insulin sensitivity (77,78). In mouse models, overexpression or infusion of adiponectin enhances insulin action and protects against endothelial damage (79) and, indeed, modestly increasing levels of circulating adiponectin in ob/ob mice actually improved insulin action despite inducing morbid obesity (80). Conversely, adiponectin gene knockout produces a reduction in mitochondrial content and function (81), with consequent insulin resistance. Protection from endothelial damage and the anti-inflammatory action of adiponectin appear to be mediated through a potent suppression of IKK beta activated by TNF-α or hyperglycemia (82), or reduction of oxidative stress (83). Thus the actions of this molecule may represent, reciprocally, a mediator of the systemic effects of obesity.

In addition to adiponectin, another potentially insulin-sensitizing fat-derived protein has recently been identified. Visceral adipose tissue-derived serpin (**vaspin**) is highly expressed in visceral adipocytes, and reduced tissue expression and serum levels are associated with worsening diabetes in an obese rat model (84). The expression of this adipose-derived protein is induced by adipogenesis (85). Administration of vaspin to obese high-fat-fed mice improved glucose tolerance and insulin sensitivity, and

reversed the altered expression of genes relevant to insulin resistance (84). Intriguingly, however, vaspin expression was detectable only in a minority of human adipose samples, and its expression in visceral adipose was positively associated with obesity and impaired glucose tolerance, although its expression in subcutaneous adipose was correlated with insulin sensitivity (86). The fat-derived hormone **leptin** also has a complex relationship to insulin sensitivity. While the appetite centres in the brain are its main target, leptin also appears to have effects on insulin action in peripheral tissues, as well as on blood vessels and pancreatic ß-cells (70,87). There are some indications that, as with insulin, obesity is associated with a state of peripheral leptin resistance (88). Moreover, hyperinsulinemia promotes both insulin resistance and stimulation of leptin production and secretion from adipose tissue. This may in turn enhance leptin resistance by further desensitizing the leptin signal transduction pathway, constituting a vicious cycle (87).

Other molecules that may play a role in fat mass signaling include **Resistin** and **Visfatin**. Resistin in mice is produced by adipocytes and induces hepatic and systemic insulin resistance (70,89). In humans, however, its origin appears to be from macrophages (90). Despite their different cellular origins, evidence that resistin may be related to obesity-induced insulin resistance is accumulating. Increased levels of resistin are associated with insulin resistance (91) and decreased levels of resistin are associated with improved insulin sensitivity with rosiglitazone therapy (92,93). In vitro experiments have also shown that resistin downregulates the expression of insulin receptors (94). Intriguingly, intracerebroventricular administration of resistin induces hepatic insulin resistance, suggesting that resistin may impact insulin resistance via a central mechanism (95), which is supported by the observation that resistin mRNA is present in the hypothalamus and significantly depleted in the mouse pituitary gland by food restriction (96). Visfatin has been described as a molecule originating from visceral fat, but with concentrations inversely proportional to insulin sensitivity (97,98)

A newly described, and very interesting, adipocytokine is **Retinol Binding Protein 4** (RBP4) (99,100,101). This molecule has been shown by Kahn's group to be produced by insulin resistant adipose tissue, whether the insulin resistance is a consequence of glucose transporter knockout, obesity, or high fat feeding (101). The gene appears to be more highly expressed in visceral than subcutaneous adipose tissue (102), and this may be influenced by single nucleotide polymorphisms in the RBP4 gene (103). This

protein has been shown to inhibit insulin action in skeletal muscle and liver, and insulin sensitivity in mice is improved by a small molecule which lowers RBP4 levels through increased renal excretion (101). However, the role of this new protein in human insulin resistance has not reached a consensus (104,105,106), in part because of issues of assay methodology (107) and the presence of free and complexed RBP4 in the circulation (108).

Finally, the recently identified endogenous ligand of the human G protein-coupled APJ receptor, **apelin,** deserves mention here. Apelin is widely distributed in various tissues, exerting a broad range of physiological actions, including heart contractility, blood pressure, fluid homeostasis, and appetite (109,110). It is a positive regulator of angiogenesis, induced by hypoxia, and leads to NO dependent vasodilatation (109,111). Furthermore, apelin has been recognized to be an endocrine regulator of AMPK synthesized and secreted by adipocytes (112). It has been shown to have a substantial glucose-lowering effect, enhancing glucose utilization in skeletal muscle and adipose tissue (112). Acute infusion of apelin in chow-fed mice drastically reduced glucose levels, stimulating peripheral glucose uptake via AMPK-, Akt-, and endothelial nitic oxide synthase (eNOS) dependent pathways (113). In obese and hyperinsulinemic mice and humans, plasma apelin concentrations are increased and correlate with plasma insulin levels (113) and insulin has been shown to directly influence the gene expression of apelin in adipocytes (112). Interestingly, a hypocaloric diet in obese women associated with weight loss reduced increased plasma and adipose tissue apelin expression (114). It has been hypothesized that apelin homeostasis may also be impaired in and contribute to obesity-associated disorders, potentially by developing adipose tissue mass via angiogenesis and/or preadipocyte proliferation (112). It remains, however, that the exact role of apelin in glucose homeostasis has yet to be elucidated.

While this section has documented single candidates responsible for adipose tissue cross-talk, insights into the mechanisms of homeostasis in human physiology would make it more likely that multiple, rather than single, cross-talk mechanisms exist. As such, it is likely that different metabolic perturbations in adipose tissue are associated with a variety of alterations of adipocytokine expression and secretion, with a plethora of different signals necessary to overcome physiological homeostasis. This general understanding may also apply to the ‘Inflammatory Hypothesis,’ where a role is

proposed for pro-inflammatory molecules in inducing insulin resistance. And these inflammatory signals may operate either via systemic (endocrine) or local (paracrine, portal, or 'vasocrine') routes.

4. Obesity and Insulin Resistance – the Inflammatory Hypothesis

4a. Inflammatory signaling – the role of molecular signals

The inflammatory hypothesis proposes that pro-inflammatory molecules generated from fat, or in the obese state, may induce insulin resistance (Figure 1). This hypothesis shares characteristics with the Endocrine Hypothesis, in that the molecules responsible may act via an endocrine route. However we are categorizing this as a separate entity, considering these molecules, which are principally inflammatory in nature, are mainly produced by adipose tissue macrophages, as opposed to an adipocyte source for the molecular candidates for the Endocrine Hypothesis (Table 1). The concept that inflammation may play a role in causing insulin resistance stems from the observation that high doses of salicylates are able to lower blood glucose concentrations (115,116). Furthermore, it is now clear that obesity is a chronic low grade inflammatory state. This concept was initially proposed by the pioneering work of Hotamisligil et al. (117), who first demonstrated that TNF-α, one of the primary inflammatory cytokines, was expressed in adipose tissue, that its expression was induced in obese states, and that its increased expression could contribute to systemic insulin resistance (117). Subsequent studies, which included an evaluation of TNF-α-null and TNF-α receptor–null mice, have largely supported this hypothesis (118). Moreover, recent studies using gene microarray techniques found increased levels of multiple pro-inflammatory gene expression in adipose tissue from obese rodents compared to lean counterparts (119,120). The fact that these effects appear to precede the development of insulin resistance during high-fat feeding further supports the notion that adipose-derived inflammatory factors may have a causative role in the development of obesity- and high-fat diet-induced insulin resistance. Perhaps the most compelling evidence comes from studies where the IKK–β and nuclear factor κB (NF-κB) pathways are activated in the liver by high-fat feeding in mice and obese Zucker rats, in parallel to the development of insulin insensitivity in both the liver and skeletal muscle (121). More directly, the animals were protected from high-fat diet-induced insulin resistance when IKK-β was lacking in the myeloid cell line (122). These observations raise the intriguing

possibility that adipose-derived pro-inflammatory cytokines, perhaps in combination with circulating NEFA, may play a major role in fat mass signaling – a so-called Inflammatory Hypothesis. The inflammatory signals are produced by adipose tissue and either systemically induce insulin resistance or are first propagated from fat to liver, and then systemically, to skeletal muscle and other target tissues. The latter hypothesis is an extension of the Portal and Endocrine Hypotheses; however, instead of predominantly NEFA-derived signals, these signals are a combination of various adipocytokines.

Increased NEFA levels also contribute to systemic inflammation and affect insulin action. Recent studies suggest that NEFA effects on inflammatory pathways are mediated via the Toll-Like Receptor-4 (TLR-4) (123), which has an important role in inflammation and immunity. Structural similarities between saturated fatty acids and lipopolysaccharide (LPS), the canonical bacterial antigen, have been postulated to explain the stimulatory effects of NEFA on TLR-4. Indeed, mice with a loss-of-function mutation in TLR4 are protected against the development of diet-induced obesity (124). This mechanism also appears to be relevant to vascular inflammation and insulin resistance, since disruption of TLR-4 blocks the ability of palmitate to activate IKK-β and to inhibit both insulin signal transduction and insulin-stimulated NO production (125). Additionally, NEFA induces a direct pro-inflammatory response in skeletal muscle which can be prevented by one session of endurance exercise in humans (126).

Which, then, are the inflammatory adipocytokine candidates responsible for organ cross-talk in obesity-induced insulin resistance? Of the adipocytokines, **TNF-α** has been best characterised for its action in inducing insulin resistance through inflammatory pathways, with consequent effects on IRS-1 and Akt phosphorylation (127,128,129) and on AMP-kinase signaling (130). Rodent studies suggest that shedding of the membrane-bound form of TNF-α via the activity of a converting enzyme is needed for paracrine activity (131) and that cell signaling occurs via the TNFR1 receptor (132). An increase in TNF-α messenger RNA expression has been observed in adipose tissue from four different rodent models of obesity and diabetes, with elevated local and systemic levels of TNF-α protein (133). Interestingly, the actions of TNF-α on insulin signaling are very similar to those of NEFA. TNF-α and NEFA have both been shown to activate the c-Jun amino-terminal kinase (JNK) and the inhibitor of

nuclear factor κB kinase (IKK-β) inflammatory pathways (134), the most likely pathways for mediating the inhibition of insulin signaling (128), as seen in both a skeletal muscle (135) and an adipocyte cell line (136). Additionally, TNF-α induces endothelial cell activation, expression of adhesion molecules, and inhibition of endothelial function (137). In parallel, TNF-α induces endothelial cell insulin resistance through a MAP-kinase pathway (138). Recent interesting evidence is that insulin sensitivity is improved by treatment through neutralizing TNF-α with the monoclonal antibody, infliximab, in patients with rheumatoid arthritis or ankylosing spondylitis (139,140), indicating that TNF-α is indeed an important adipocytokine that may be at least partially responsible for an insulin resistant state. Other studies, however, showed no effect on improving insulin sensitivity after treatment with a TNF-α monoclonal antibody or receptor (117,141,142). These conflicting results could be due to differences in duration of treatment (shorter in the latter experiments) or selection of patient groups, insufficient power of the studies or the potential paracrine action of the molecule. Although TNF-α may not be a good candidate as a *systemic* fat-derived signal, due to its low circulating concentration other than in conditions such as septic shock (127), it is still possible that it has important paracrine or autocrine effects within adipose tissue, or that its portal delivery is more important than its systemic effect. In fact, *in vitro* studies demonstrated that the macrophage activation state is influenced by contact with adipocytes and that factors derived from the macrophage, in turn, induce insulin resistance in adipocytes, an action which is partially reversed by a TNF-α neutralizing antibody (143).

Interleukin-6 (IL-6) is perhaps the cytokine which most clearly plays a systemic role as the main driver of hepatic production of the acute-phase reactant C-reactive protein (CRP) (144). It is generated in sufficient quantities by adipose tissue to represent up to 30% of circulating levels in healthy subjects (69). The role of IL-6 in insulin resistance has been the topic of substantial debate (145,146). This cytokine may mediate hepatic insulin resistance (147). In fact, in mice with constitutive overexpression of inflammatory pathways in hepatocytes, circulating levels of IL-6 were increased 2- to 3-fold, while neutralisation of IL-6 using monoclonal antibodies improved insulin resistance (148). A recent study related portal vein concentrations of cytokines to systemic levels of inflammation and showed a substantial secretion of IL-6 from visceral fat as well as a close correlation between portal levels of this cytokine and systemic levels of CRP (149). In contrast, IL-6 generation by exercising muscle has been postulated as a mechanism whereby glucose uptake is increased during physical

activity (150); and infusion of IL-6 in doses insufficient to produce systemic symptoms enhances muscle insulin action through activation of AMP-activated protein kinase (151). It is interesting to note that CRP itself may have a direct effect on insulin receptor substrate-1 serine phosphorylation in L6 myocytes (152). These effects occur at physiological concentrations of CRP (152) and are consequent upon the activation of extracellular signal-regulated protein kinase 1/2 (ERK1/2) and c-Jun N-terminal kinase (JNK). This may represent the indirect effect of IL-6 on muscle insulin resistance via increased levels of CRP.

Finally, there is growing evidence linking plasminogen activator inhibitor-1 (**PAI-1)** with obesity and insulin resistance; PAI-1 is a key regulatory protein of tissue fibrinolysis, cell migration, angiogenesis and tissue remodelling (153). It has been shown that adipose tissue is a significant source of PAI-1, with the bulk of production derived specifically from the stromal vascular fraction in visceral fat (154,155,156). Fascinatingly, PAI-1 deficiency reduces adiposity, improves the metabolic profile in genetically obese mice (157), and attenuates diet-induced obesity and insulin resistance in C57BL/6 mice (158,159). Furthermore, in mouse models, the absence or inhibition of PAI-1 through genetic alteration in adipocytes protects against insulin resistance by promoting glucose uptake and adipocyte differentiation via increased peroxisome proliferator activated receptor-γ (PPAR-γ) expression (160).

There are, however, differences between liver and skeletal muscle in the responses to activation of inflammatory signaling pathways. In mice, the liver nuclear factor kappa B (NF-κB) pathway is activated by high-fat feeding and is integral to producing hepatic insulin resistance (122,121). Activation of the NF-κB pathway, through muscle-specific transgenic expression of activated IKK-β, however, results in muscle wasting, but without increasing expression of cytokines or effects on skeletal muscle insulin action (161,162). In parallel, inactivation of the NF-κB pathway in mouse skeletal muscle is without effect on glucose homeostasis, even after induction of obesity (163). Interestingly, while skeletal muscle NF-κB appears to be unconnected to obesity-related insulin resistance, the peripheral insulin resistance induced by high-fat feeding in mice is almost completely prevented in animals lacking a myeloid cell line NF-κB pathway (122). Taken together, these data suggest that the peripheral insulin resistance which follows hepatic insulin resistance in high fat feeding, as described in ‘Portal Hypothesis,’

may depend more significantly on activation of inflammatory pathways in circulating mononuclear cells than of such signaling mechanisms in local target tissue.

Again, the multitude and complexity of inflammatory cytokines produced by adipose tissue would seem to confirm the concerted effect of these products at points upstream in the insulin signaling pathway on subsequent insulin resistance. The question then becomes, why does adipose tissue from obese subjects secrete more inflammatory cytokines than that from lean subjects? What happens in the adipose tissue bed to make it more inflammatory?

4b. Inflammatory signaling - the role of adipose tissue macrophages

Not all adipose tissue beds are alike in the amount of adipocytokines they produce. Please refer to Table 1 which outlines the cellular origins and depot specificity of the various adipocytokines discussed here. Surgical fat removal in animals, an established method for studying the influence of fat accumulation on systemic metabolism, could provide valuable information regarding the role of visceral fat, as subcutaneous lipectomy results in a compensatory increase in the amount of visceral fat (164). A study in which mice underwent partial subcutaneous lipectomy showed the development of insulin resistance in association with a significantly greater fat accumulation and higher TNF-α expression in visceral adipose tissue (VAT) (165). The transplantation of VAT back into the subcutaneous compartment after lipectomy reversed the systemic and tissue features of insulin resistance. This indicates that the functional difference between SAT and VAT is, at least in part, consequent upon the interaction of adipocytes with their milieu, a hypothesis which is supported by *in vitro* studies (166). The expression and secretion of interleukin-6 are substantially greater in visceral and truncal fat than in subcutaneous fat (167), and its production is also increased by lipid accumulation in the adipocyte (168,169).

One major difference between different adipose tissue beds, and between states of over- and under-nutrition, is the degree of infiltration by macrophages. Observations demonstrating the existence of adipose tissue macrophages, their increase in number with obesity, a high-fat diet, and increasing adipocyte size (170,171,172), and reduction in number with weight loss (173) or increased intake of n-3 fatty acids (174), have raised the possibility that these cells, rather than the adipocytes themselves, are the

main source of adipocytokine production. Cell separation studies have shown that adipose tissue macrophages are responsible for the expression of most of the TNF-α and a substantial amount of other cytokines (172). Moreover, if macrophage inflammatory pathways are inactivated, such as by creating a mouse lacking the IKK-β enzyme in myeloid cells, the animal is protected from the effect of a high-fat diet on both hepatic and peripheral insulin resistance (122). Recent elegant studies have shown that the phenotypic shift of adipose tissue macrophages to a proinflammatory state in obesity results from recruitment of inflammatory macrophages from the circulation, rather than from a switch of resident macrophage phenotype (175, 176). This suggests that the consequences of adipose tissue excess are mediated through recruitment of macrophages and their secretion of inflammatory mediators, which may also play a part in further stimulating cytokine release from adjacent adipocytes. The systemic effects arising from such a process will thereby depend on endocrine signals generated in fat by both adipocytes and macrophages, with other inflammatory molecules, such as TNF-α, exerting effects in paracrine, rather than endocrine, fashion. It is also possible that the circulating inflammatory signal in obesity is not an individual cytokine, but a systemic state of blood monocyte activation (177), which may respond to local chemoattractant molecules and trigger inflammatory cascades in remote tissues. In support of this paradigm, a recent study showed that in obesity the circulating mononuclear cell population is indeed characterized by increased levels of inflammatory mediators such as IKKβ and suppressor of cytokine signaling-3 (SOCS3), suggesting that the circulating mononuclear cell in obesity is ‘inflamed’ (178).

Thus, if obesity is a low grade inflammatory state, the question arises how macrophages are recruited into the adipose tissue. As mentioned before, it has been shown that macrophage content increases with adipocyte size (170). Interestingly, adipose tissue macrophages in obesity form crown-like structures around necrotic adipocytes, indicating the possibility that adipocytes may achieve a certain maximum size before cell death which then attracts macrophages to clear the necrotic debris (179). This finding is consistent with the previously mentioned study (172), which showed higher numbers of macrophages in adipose tissue consisting mainly of larger adipocytes.

Therefore, it may be hypothesized, then, that hypertrophied adipocytes, as a result of obesity, have abnormal secretory properties, such as increased expression of

monocyte chemoattractant protein 1 (MCP-1), colony stimulating factor-1 (CSF-1) and transforming growth factor β (TGF-β) (180,181,182,183), thereby recruiting more monocytes into adipose tissue than normal adipocytes (184,185,186) (Figure 1). Previous studies have further supported this concept of an interaction between adipocytes and macrophages (143,187). It is notable that the inflammatory properties of macrophages in fat seem to require the presence of adipocytes. FAT-ATTAC mice, whose adipocytes undergo apoptosis, showed significantly lower concentrations of circulating pro-inflammatory cytokines compared to wild type mice, despite the significant number of macrophages in the adipocyte-free adipose tissue pads (187). Furthermore, as referred to above, a study by Lumeng et al. (143,188,189) suggests two way cellular cross-talk between adipocytes and macrophages, mediated in part by TNF-α.

Among the chemo-attractants responsible for monocyte recruitment, monocyte chemoattractant protein-1 (**MCP-1)**, which is also known as CC-motif chemokine ligand-2 (CCL-2), is considered one of the most important candidates. Mice deficient in the receptor for this ligand are resistant to the adverse effects of high-fat feeding on insulin action and show decreased adipose tissue macrophage infiltration, reduced production of adipocytokines and less hepatic steatosis (186). Another recent study has shown that over-expressing or knocking-out MCP-1 in mouse adipocytes produces increased or decreased hepatic steatosis and insulin resistance, respectively (184). Interestingly, Saltiel's group has recently shown that macrophages in mice recruited during a high-fat diet have a different inflammatory profile from resident macrophages or those found in lean mice (188,189) that such macrophages are recruited from the circulation, and that such recruitment is in part dependent upon the CCL-2 receptor (CCR) (175). This suggests that macrophages, recruited during high-fat feeding and dependent on MCP-1 signaling, may be responsible for the inflammation and consequent insulin resistance. MCP-1 exerts its effects primarily on the more pro-inflammatory 'classically activated' subtype of macrophages, recruiting them to sites of tissue damage (190). MCP-1 also circulates in plasma and may itself play an endocrine role in inhibiting insulin action in skeletal muscle (191). Surprisingly, in a recent study by Inouye et al., MCP-1 deficient mice on a high-fat diet did not show any reductions in adipose tissue macrophages, yet demonstrated worsening hyperinsulinemia and hyperglycemia (192). This study therefore suggests an independent metabolic role of MCP-1.

MCP-1 may also have an auxiliary function in establishing greater adipose tissue accumulation in obesity (193,194,195,196,197,198). White adipose tissue (WAT) has the capability to expand, and histological evaluation reveals that fat is highly vascularized, suggesting that the presence of blood vessels may be necessary to maintain its mass. Indeed, studies have demonstrated that either general or adipose-specific inhibition of angiogenesis can prevent the development of obesity in mice in association with an improved metabolic profile (195,197). MCP-1, in addition to acting as an important adipocyte-generated chemotactic factor for monocytes and macrophages as described above, is also substantially produced by several tumors and contributes directly to tumor angiogenesis by mechanisms involving TGF-β-mediated vascular smooth muscle cell migration (193) independent of monocyte recruitment (198). It is possible, then, that in situations of obesity or positive energy balance, recruited macrophages may significantly contribute to the expansion of adipose tissue vasculature, thereby activating a vicious cycle (194).

In light of the importance of the adipose macrophage in insulin resistance, a series of somewhat counter-intuitive observations relate to the role of adipose tissue cortisol metabolism. Many previous animal studies have shown that the administration of an inhibitor of 11-β hydroxysteroid dehydrogenase (**11-β HSD**) type I, or genetic deficiency of 11-β HSD type I, ameliorated insulin resistance (199,200,201) while adipose specific over-expression of 11-βHSD type I produced a phenotype remarkably similar to human metabolic syndrome (200,202), including visceral obesity, insulin-resistant diabetes, hyperlipidemia, hyperphagia and hypertension. 11-βHSD type 1 is an enzyme that regenerates active cortisol from inactive cortisone in a tissue-specific manner, leading to local cortisol excess within key metabolic tissues as a consequence of heightened enzyme activity in obesity (203). Although the anti-inflammatory properties of cortisol might suggest that adipose macrophages may be further activated by lowering local cortisol levels through inhibition of 11-βHSD type I, a recent study opened up the possibility that inhibiting 11-β HSD type I renders the macrophage less rather than more inflammatory (204), suggesting discordant effects of local endogenous cortisol production vs. high-dose exogenous glucocorticoid therapy.

The proposed paradigm, then, is that in response to any change in milieu which induces insulin resistance in the adipocyte - whether it be positive energy balance, high glucose concentrations, high NEFA levels, or locally- or systemically-produced

cytokines – the adipocyte or stromal vascular fraction in adipose tissue will generate MCP-1. This would result in macrophage recruitment and/or activation with subsequent hepatic and systemic insulin resistance, potentially in association with further fat mass growth. Any of the mechanisms which have been proposed as responsible for insulin resistance within target tissues, such as reactive oxygen species (ROS) (205,206,207), endoplasmatic reticulum (ER) stress (208), and AMP kinase/malonyl coenzyme A (169,209), or mitochondrial dysfunction (210), may act as the mechanism within the adipocyte whereby MCP-1 is induced and macrophages are recruited (211). The reader is referred to a recent review for a detailed discussion of the potential mechanisms whereby insulin-sensitive tissues sense nutrient excess, thereby generating insulin resistance in adipose tissue and skeletal muscle (3).

To hypothesize which mechanisms are operative in adipose tissue, it is necessary to delineate changes which occur in response to metabolic challenges in other target tissues. Elegant work from Hotamisligil's group has shown that a high-fat diet and obesity cause ER stress in liver cells, perhaps in consequence of ROS generation (208). The chronic metabolic milieu of hyperglycemia, increased levels of NEFA, and proinflammotory cytokines also increase the generation of ROS in endothelial cells and adipocytes (206,207,205). Systemic markers of oxidative stress increase with obesity, consistent with a role for ROS in the development of obesity-induced insulin resistance (212). These alterations could be the downstream effect of activation of NADPH oxidase by lipid accumulation in the adipocyte, with consequent increases in ROS production (213). Ruderman and colleagues have defined an AMP-kinase/malonyl-coenzyme A nutrient sensing mechanism in muscle, liver and endothelial cells that plays an important role in activating or inhibiting insulin signaling (169). Some data suggest that these mechanisms may also occur in adipocytes where they may regulate inflammatory cascades (191). Indeed, this might explain how positive or negative energy balance rapidly influences insulin resistance and several associated characteristics much more dramatically than might be anticipated with small changes in fat mass. Additionally, mitochondrial dysfunction is found in skeletal muscle of elderly insulin resistant subjects (210), although whether this is a consequence of the AMP-kinase/malonyl coenzyme A mechanism, oxidant stress, and/or adiponectin action is unclear (3).

Recently, a family of proteins collectively known as sirtuins are gaining increasing attention as possible regulators of insulin action, mitochondrial function, and systemic and adipose inflammation (214). Sirt1 reproduces some of the beneficial effects of caloric restriction, promoting fat mobilization through suppression of peroxisome proliferator-activated receptor-gamma (PPAR-*γ*), resulting in decreased triglyceride storage and increased secretion of free fatty acids (FFA) (215). Sirt1 may also modulate inflammation, by inhibiting the transcription of nuclear factor-kappa B (NF-kappa B), one of the key regulators of inflammation (216). Finally, a role has been proposed for adipose tissue hypoxia, consequent upon tissue hypoperfusion in obesity, causing dysregulated adipocytokine production in obesity (217). In particular, local adipose tissue hypoxia in obese mice has been shown to decrease adiponectin mRNA expression and increase gene expression of plasminogen activator inhibitor type-1 (PAI-1), leptin, and C/EBP homologous protein (CHOP), an endoplasmic reticulum (ER) stress–mediated protein, thus contributing to the metabolic syndrome in obesity (217).

5. and 6. Obesity and Insulin Resistance – the Overflow and Vasocrine Hypotheses

When adipocytes exceed their storage capacity and the process of adipocyte proliferation fails, fat begins to accumulate in tissues not suited for lipid storage such as liver, muscle or blood vessel, which then may form specific metabolites that inhibit insulin signal transduction in these tissues (Figure 2). High-fat feeding results in triglyceride accumulation in hepatocytes (218,186), termed hepatosteatosis (219,220), and consequent hepatic insulin resistance. In a similar fashion, the accumulation of intramyocellular lipids may contribute to the muscle insulin resistance of physical inactivity, obesity or a high-fat diet (221). The deposition of intracellular triglycerides in these insulin target organs may contribute to insulin resistance by the intracellular presence of a constant NEFA source, acting either as a substrate or a signal modulator for these cells (222), or by activation of inflammatory pathways (223,224). The triggers for ectopic fat deposition are unclear, with possible roles for elevated concentrations of NEFA (fasting or throughout the day) or for circulating inflammatory cytokines (225). A recent patient study has shown a close correlation between the degree of macrophage infiltration in omental fat and the degree of hepatic inflammation and fibrosis (226). In mice, modulation of macrophage infiltration in adipose tissue by manipulating the expression of MCP-1 (184) or its receptor (186) produced corresponding changes in

hepatic steatosis and hepatic/muscle insulin sensitivity. It is possible, then, that the 'Overflow' and 'Inflammatory Hypotheses' of insulin resistance are complementary, with the deposition of ectopic fat both consequent upon and contributing to (121,225,227) circulating inflammatory signals.

Ectopic fat deposition also occurs around blood vessels. While there may be parallel roles for this ectopic fat in propagating signals to remote organs and tissues, this fat differs in microanatomy and physiology in that it is intracellular in its deposition, and hence signals to insulin sensitive cells in a 'paracrine' fashion. We have demonstrated that there is a cuff of adipose tissue around the origin of nutrient arterioles, isolated from cremaster muscles from obese Zucker rats (228). Using a variety of insulin signaling pathway inhibitors, we have shown that in these animals, the PI3-kinase insulin signaling pathway is impaired, and nitric oxide production is suppressed (229). This has led us to propose that in states of obesity, perivascular fat may signal to the vessel wall, both locally ('paracrine') and downstream ('vasocrine'), through outside-to-inside signaling, with TNF-α being a likely mediator (228) (Figure 2). Perivascular fat around nutrient arterioles may inhibit the effects of systemic insulin on local vasodilatation, with consequent inhibition of postprandial increases in nutritive blood flow and insulin action. Crucially, it is possible that such downstream delivery of inflammatory mediators through the nutritive capillary bed may impact on the insulin signaling pathways in the skeletal myocytes, producing a state of muscle insulin resistance independent of constraints on nutritive blood flow and substrate delivery. Such a hypothetical mechanism might explain the observations that inducing over-activity of the NF-κB pathway in skeletal muscle itself is without effect on muscle insulin sensitivity (161,162). It is also postulated that the increased intravascular concentration of TNF-α will be limited to the muscle nutritive capillary bed, with TNF-α binding proteins likely to inhibit any activity of the molecule in the systemic circulation. The predominant source of these adipocytokines in perivascular fat, again, is likely to be either macrophages recruited from the circulation or through activation of local resident macrophages (120), although this hypothesis needs further experimental testing.

We have proposed that the relationship between insulin resistance and vascular disease could represent the co-existent downstream consequences of inflammatory cytokines inducing endothelial dysfunction in parallel with its effects on other insulin responsive organs (230). The production of these cytokines from perivascular fat may

imply that the effects on both nutritive blood flow and insulin action are mediated via local, rather than systemic, signaling, although as indicated above there is a possible systemic contribution to the process through recruitment of activated circulating mononuclear cells to perivascular fat depots (Figure 2). Perivascular adipocytes show reduced adipocytic differentiation compared to those from subcutaneous and visceral depots, secrete less adiponectin and more proinflammatory cytokines, particularly after high fat feeding (231). Intriguingly, the role of perivascular fat may also be important in arterial disease, as has been proposed by Mazurek and colleagues (229,232). This group described substantial macrophage infiltration in epicardial fat corresponding anatomically to the location of atheromatous plaques in underlying coronary vessels. The observation that epicardial fat volume closely correlates with that of visceral fat (232) suggests that there may be a homology between perivascular fat around large arteries (with resultant outside-to-inside signaling and atherosclerosis), and visceral fat around the heart and in the peritoneum (228). If there is a corresponding homology between perivascular fat and visceral fat, the relationships of visceral obesity, insulin resistance and atherothrombotic vascular disease become clear.

Considering the aforementioned organ cross-talk, the question then follows, how fat mass might signal to endothelium, initiating endothelial inflammation and damage. Potential signals as circulating molecules have been considered earlier. Non-esterified fatty acids do not seem to be a likely candidate, because fasting concentrations are generally not substantially elevated in insulin resistant states (233). Of the cytokines, TNF-α can certainly produce endothelial activation and inhibition of nitric oxide production (234) but, as pointed out earlier, is not present in the systemic circulation of healthy obese subjects in sufficient concentrations for this effect to be likely (127). IL-6 on its own does not have a major impact on endothelial function (235). Adiponectin may function as an endothelial protector by increasing nitric oxide synthesis and reducing inflammation (71,236,237). It is also possible that endothelial cells themselves, like adipocytes, respond to over-nutrition or a high-fat diet by changes in cell redox state or through activation of AMP-kinase/malonyl coenzyme A pathways (238). A recent study, investigating the time course of high fat diet on generating insulin resistance in adult mice showed effects on aortic inflammation, endothelial dysfunction and insulin resistance within one week, whereas skeletal muscle and liver insulin resistance was measurable only after 4-14 weeks (239) These observations support a

major contributing role of vascular insulin resistance to the systemic effects of high fat feeding, although the role of peri-aortic fat was not explored in this study.

The above hypothesis now leaves one further question: If perivascular and truncal fat act as an integrated organ responsible for generating local and systemic inflammatory signals, what is it that determines this pattern of fat distribution? Central adiposity is a feature of low birth weight (240,241), aging (242) and physical inactivity (243). The Whitehall Study showed the association of low social class and work-related stress with central obesity (244). Possible mechanisms for this include the activation of the hypothalamo-pituitary-adrenal axis (245), local production of glucocorticoids (246), or effects of a dysfunctional autonomic nervous system (247). Moreover, in a rat hind limb model, increases in non-nutritive blood flow (as would occur with inactivity) produce deposition of septal fat rather than uptake of NEFA by muscle as a substrate (248). Therefore, it is likely that the individual pattern of fat distribution is of multifactorial origin, and future studies are likely to uncover additional contributing factors.

7. Obesity and Insulin Resistance – A Neural Hypothesis

As elaborated earlier in this article, it has become clear that the brain is an insulin-sensitive organ and glucose metabolism in insulin-resistant individuals is disrupted in the brain as in other target organs. What, then, could be the cause of insulin insensitivity of the brain in the state of obesity? First of all, insulin transport may become rate-limiting under certain circumstances, as has been outlined above regarding insulin's access to the interstitial space and its action in muscle and fat (213,219,209). It is notable that the capillary endothelium which comprises the blood-brain barrier is surrounded not only by pericytes but by macrophages, which are stimulated by cytokines and chemokines in inflammatory brain diseases (249) (Figure 3). It could then be proposed that in the low grade inflammatory state found in obesity, these macrophages are also involved in the generalised activation of the NFκB pathway (250,251,70). While the insulin receptor-mediated transporter in brain capillary endothelium has not been characterised at the cellular level, it is possible that serine phosphorylation of the insulin receptor substrate-1 might adversely affect insulin transport as it does in other insulin-sensitive cells (122). In such a way, the etiology of insulin resistance may be similar in the brain, blood vessels and, through vasocrine effects, in muscle.

It is likely that the brain's involvement in the regulation of glucose homeostasis serves more as a master regulator rather than as a mere target organ (Figure 3). Phrased differently, the brain's insensitivity to insulin and/or other nutrients such as glucose or NEFAs seems to influence endogenous glucose production, adipose tissue lipolysis and adipocyte proliferation. In insulin-sensitive subjects, the brain processes information from adiposity signals, such as insulin and leptin, and integrates this input with nutrient signals such as NEFA and glucose (252,253), thereby favouring the return of food intake and glucose production by the liver to their original levels. On the contrary, the brain in insulin-resistant individuals will result in elevated levels of both body fat content and hepatic glucose production due to defects in brain nutrient sensing. Rossetti and colleagues provided supporting evidence in a study where restoring hypothalamic levels of long-chain fatty acyl-CoAs in overfed rats normalized food intake and glucose homeostasis (254). This occured in association with markedly improved liver insulin sensitivity and selective activation of brainstem neurons within the nucleus of the solitary tract and the dorsal motor nucleus of the vagus (255). In short, the brain appears to sense nutrient or hormonal signals and subsequently impacts hepatic glucose production through the vagus nerve.

The hypothesis that the nervous system may affect lipid mobilization comes from the observed inability of adrenal demedullation to fully block lipid mobilization (256,257,258). Moreover, the possibility of sympathetic nervous system (SNS) innervation of white adipose tissue (WAT), has now been demonstrated neuroanatomically and functionally, with the SNS appearing to play an important role in lipolysis and the regulation of fat cell number (259) (Figure 3). Bergman's group has demonstrated that the cyclical changes in circulating NEFA concentrations are the consequence of coordinated SNS activity in a variety of adipose beds, again suggesting an integrating role for the central nervous system (260,261,262). The presence of parasympathetic nervous system (PSNS) innervation in WAT was first described by Kreier et al. who demonstrated that intra-abdominal adipose tissue receives vagal input (263), augmenting insulin's anabolic actions on glucose and NEFA metabolism (263), thereby opposing the effect of the SNS in fat. This finding is in line with data by Tracey et al. demonstrating that PSNS activation may suppress inflammatory states (264), possibly in association with NEFA action on TLR4 and the NF-κB inflammatory pathway (265). Kreier and colleagues have also proposed that the differences in body fat

distribution may reflect differential activities of the somatotopically organized sets of autonomic neurons in the central nervous system (263). Body fat mass is tightly regulated, as partial surgical lipectomy in several species triggers compensatory increases in non-excised white adipose tissue mass, independent of changes in food intake (266). In Siberian hamsters, surgical denervation of inguinal WAT produces pronounced increases in fat cell number with little change in fat cell size *in vivo* (267,268), suggesting that SNS innervation of WAT plays a role in fat pad mass. It is notable, that the magnitude of the denervation-induced increase in fat cell number varies between fat pads. This finding is in accordance with the observation that obesity typically is associated with decreases in SNS activity and WAT hypercellularity (269). However, it remains to be studied whether the regulation of body fat mass could be the result of a fine balance between the sympathetic and parasympathetic nervous systems.

Conclusions

In conclusion, the associations between obesity and insulin resistance, most importantly, may represent the consequences of various metabolically active substances produced by excess adipose tissue mass. Firstly, recent studies suggest that the cellular origin of adipose-generated inflammatory signals is likely, to a considerable degree, to be macrophages. These macrophages are recruited to 'inflamed fat' mostly by MCP-1 production and are responsible for both the generation of inflammatory signals themselves and modulating production of cytokines from adipocytes. Secondly, the effect of these fat-generated inflammatory molecules on liver, muscle and adipose tissue itself is local insulin resistance. Organ cross-talk is possibly mediated by elevated NEFA levels, one or more circulating cytokines, or even the circulating monocytes themselves. Additionally, these inflammatory signals, as well as the over-supply of NEFA, may contribute to ectopic fat production. The downstream effect of ectopic fat deposition is local insulin resistance, perhaps partially mediated by local accretion of triglycerides in hepatocytes or myocytes and the metabolic effects of adipocytokines. Moreover, at least in the liver and in blood vessels, ectopic fat is associated with the activation of inflammatory cascades and overproduction of cytokines, including TNF-α. Thirdly, the association of insulin resistance and central obesity with structural vascular disease and abnormal vascular function contributes to insulin insensitivity by limiting nutritive blood flow, and thus, insulin and substrate delivery to target tissues. This vascular insulin resistance may represent the consequences of outside-to-inside signaling from perivascular fat. Finally, the brain seems to be a key mediator in this multiple organ cross-talk mechanism, responding to signals from adipose tissue and to the general nutritional state, with its output determining fat mass and distribution, hepatic glucose production, and, through the effects of NEFA on TLR4, the inflammatory milieu.

Acknowledgements

The authors would very much like to thank Dr. Etto Eringa for his helpful and constructive comments on a draft of the article. We also sincerely thank Tatyana Harris for her kind and creative help with the figures.

Figure Legends

Figure 1. The Endocrine and Inflammatory Hypotheses: Endocrine and inflammatory effects of obesity on systemic insulin resistance.
CCR-2= CC-motif chemokine ligand-2 receptor
MCP-1= monocyte chemoattractant protein-1
TGF-ß= transforming growth factor beta
TNF-α= tumor necrosis factor alpha
PAI-1= plasminogen activator inhibitor-1
ATM= adipose tissue mass
NEFA= nonesterified fatty acids
RBP4= retinol binding protein 4
IL-6= interleukin-6

Figure 2. The Overflow and Vasocrine Hypotheses: Effects of excess perivascular, intrahepatic, and intramyocellular adipose tissue on systemic insulin resistance.
EC= endothelial cell
VSMC= vascular smooth muscle cell
Ms= muscle
PI3K= phosphoinositide 3-kinase
ERK= extracellular signal-regulated kinase
eNOS= endothelial nitric oxide synthase
NO= nitiric oxide

Figure 3. The Neural Hypothesis: Effects of the brain and central nervous system on glucose metabolism, peripheral insulin action, and adipose tissue mass and metabolism.
BBB= blood brain barrier
EC= endothelial cell
Mac= macrophage
Peri= pericyte
As= astrocyte
EGP= endogenous glucose production
VAT= visceral adipose tissue
SAT= subcutaneous adipose tissue

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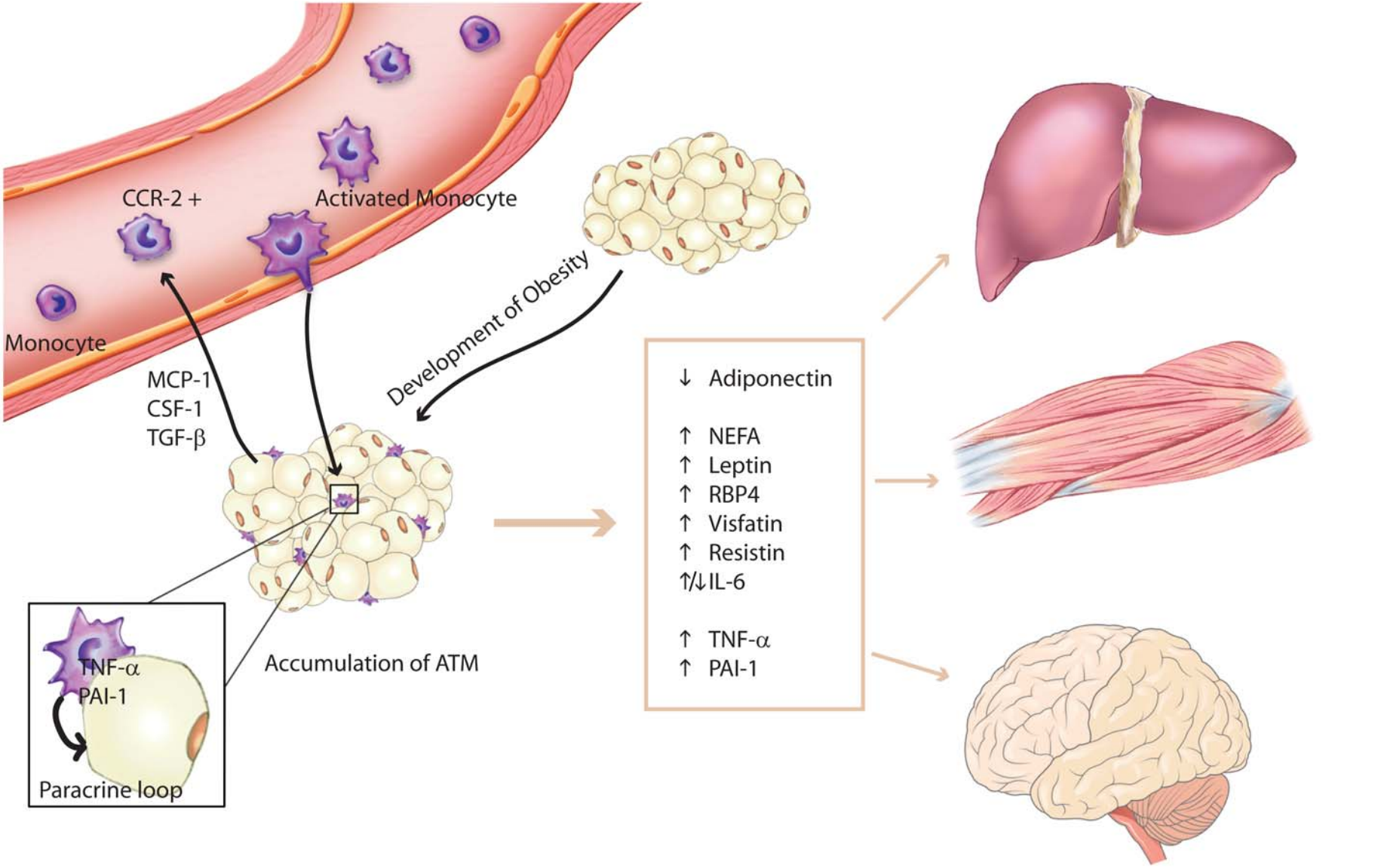
	Cellular origin	Depot specificity	Plasma levels in obesity or insulin resistant states	Effect on insulin action	Effect on inflammation	Effect on vascular endothelium	Comments
NEFA (7,8,35)	A	None	Increased during fasting and in insulin resistant states	-	Pro-inflammatory	Impairs endothelial-dependent vasodilatation	Probable role as a nutrient responsible for hypothalamic sensing in glucose homeostasis (302)
Adiponectin (71,79,82)	A	SAT > VAT (Expression)	Reduced	+	Anti-inflammatory	Protects against endothelial damage	
Leptin (270-273)	A	None	Increased	+	Suggested pro-inflammatory action in specific circumstances (273)	Not well established	Established adiposity signal to the brain
TNF-α (130,142,274-276)	M	Not well defined	Increased	-	Pro-inflammatory	Inhibits endothelial nitric oxide production, induces adhesion molecule expression	Conflicting reports of improvement of insulin action with monoclonal antibody or receptor blocker in humans and rodents
IL-6 (277-282)	A	VAT>SAT (portal vein concentration exceeds that of radial artery)	Increased	- and + (different effects on different tissues:- refer to text)	Pro-inflammatory	Potential inhibition of vasodilatation and capillary recruitment	IL-6-STAT3 signaling in the liver appears to contribute to insulin action. in the brain (282)
PAI-1 (283-287)	M	Not well defined	Increased	-	Pro-inflammatory	Prothrombotic	May play a role in adipocyte differentiation (160)
Resistin (92,95,288)	A (rodents) and M (humans)	Not defined	Increased	-	Pro-inflammatory?	Not well defined	Intracerebroventricular administration of resistin induces hepatic insulin resistance
RBP4 (99,101,102)	A	Not defined	Increased	-	Pro-inflammatory	Not defined	Issues with assay methods resulting in contradictory findings especially in human studies

MCP-1 (289-291)	A	Not defined	Increased	-	Pro-inflammatory	Not defined	Role in recruiting macrophages to adipose tissue during positive energy balance; angiogenesis
Visfatin (292,293)	A	Produced from both depots; however, different role depending on the depot	Increased (Visceral Visfatin) Decreased (Subcutaneous Visfatin)	- (Visceral Visfatin) + (Subcutaneous Visfatin)	Pro-inflammatory (Visceral Visfatin)	Not defined	Depot specificity exists in human adipose tissue: visceral origin-visfatin is associated with insulin resistance whereas subcuteneous origin-visfatin is associated with insulin sensitivity
Vaspin (84,86,294)	A	VAT>> SAT	Increased; however, this relationship seem abrogated in the presence of T2DM	+	Not well defined	Not defined	Administration of recombinant human vaspin in mouse model of diet induced obesity restored insulin sensitivity (303)
Thrombospondin-1 (295)	A	Unknown	Up (gene expression in adipose tissue)		Unknown	Unknown	
Apelin (112-114)	A	Unknown	Increased	+	Unknown	Endothelium dependent vasodilatation by triggering NO release	
Osteopontin (296-298)	M	Unknown	Increased in obesity, independently of insulin resistance	-	Unknown	Unknown	PPAR agonist reduces Osteopontin expression adipose tissue

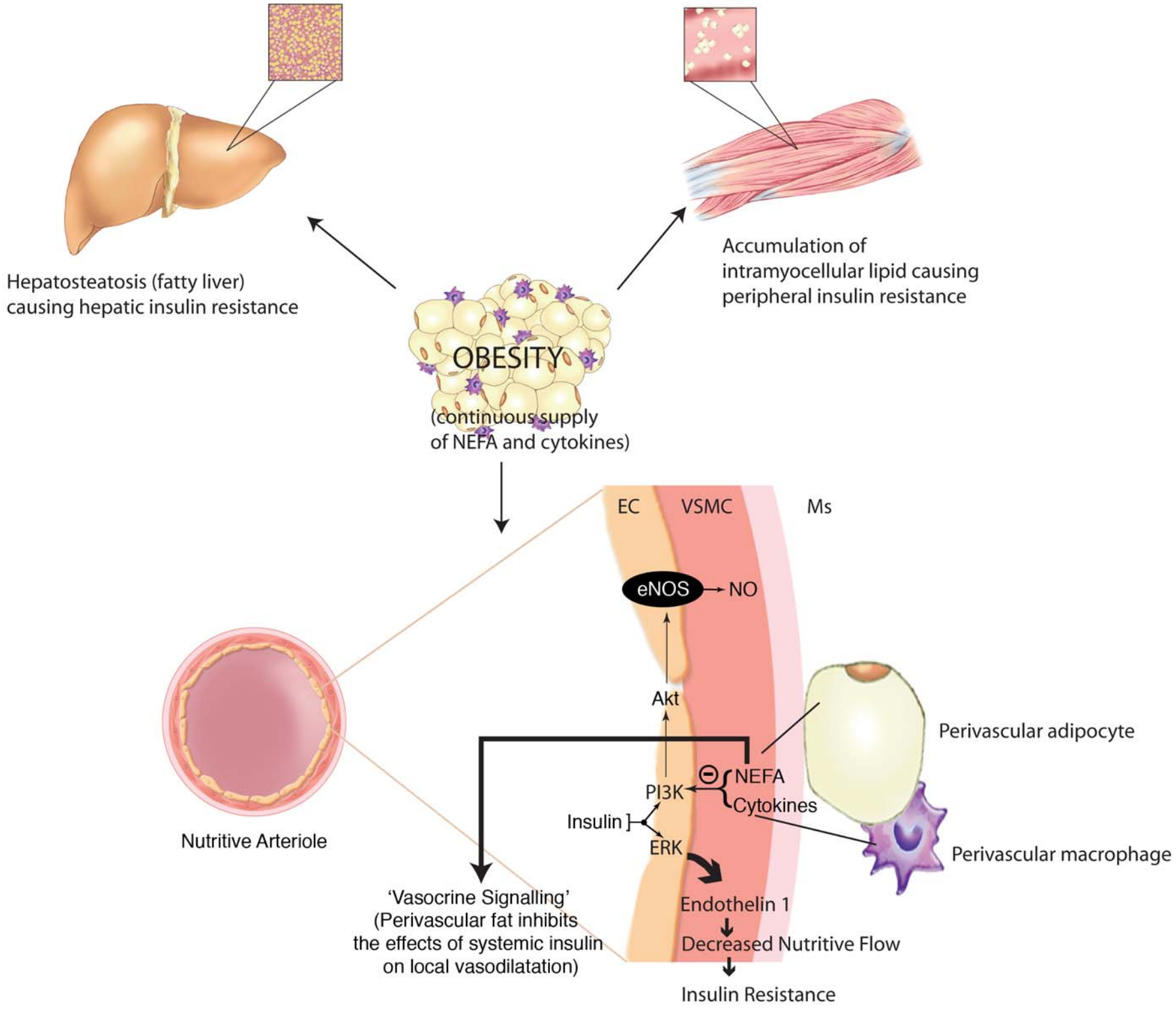
Lipocalin-2 (299-301)	A, M	Unknown	Increased	-	Unknown	Unknown	

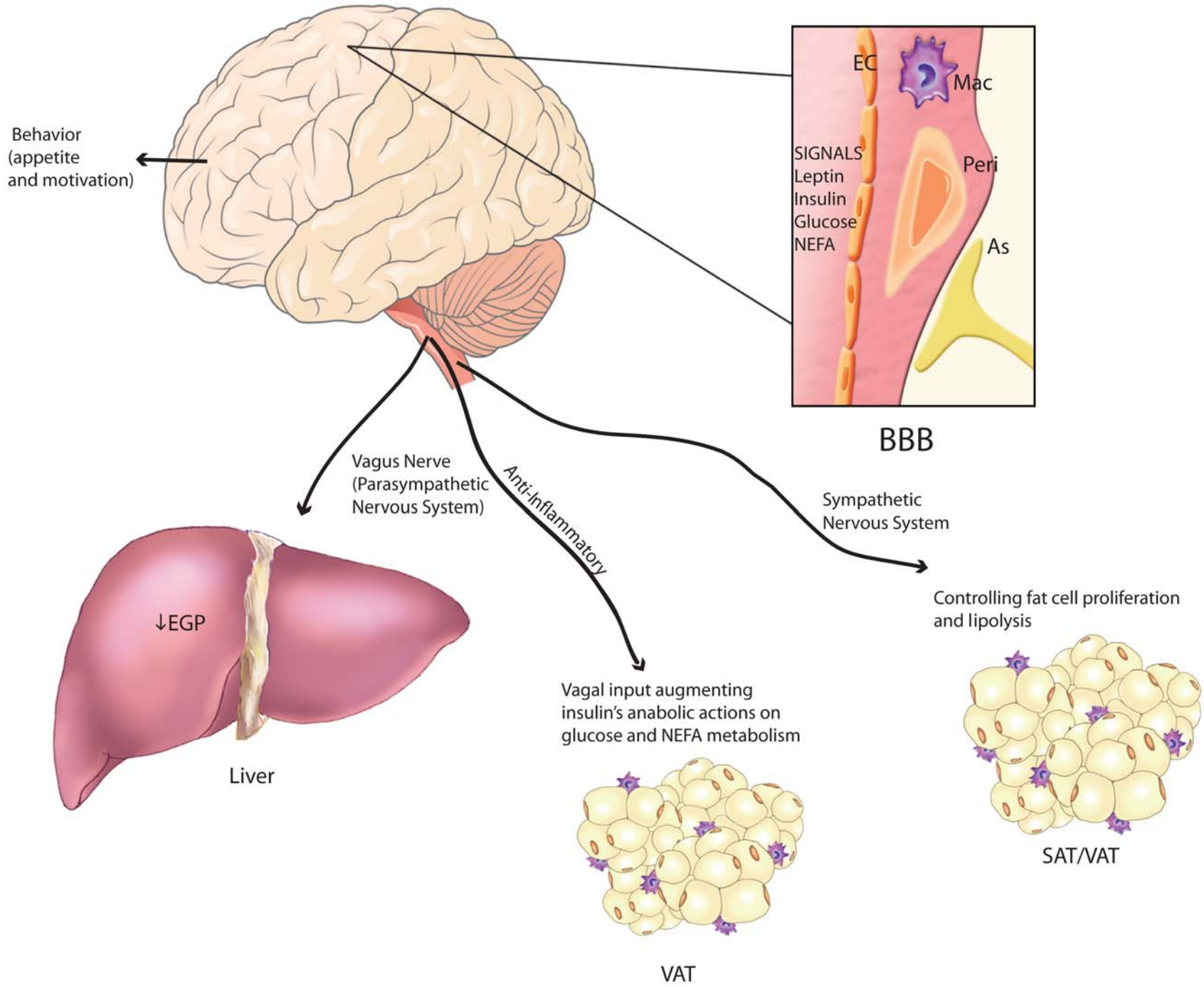
Table 1

A: adipocyte
M: adipose tissue macrophage or stromal vascular fraction
SAT: subcutaneous adipose tissue
VAT: visceral adipose tissue
+: associated with improving insulin sensitivity
- : associated with worsening insulin sensitivity



Systemic Insulin Resistance


Hepatosteatosis (fatty liver)
causing hepatic insulin resistance
Accumulation of
intramyocellular lipid causing
peripheral insulin resistance
OBESITY
(continuous supply
of NEFA and cytokines)
EC
VSMC
Ms
eNOS
NO
Akt
PI3K
Insulin
ERK
NEFA
Cytokines
Perivascular adipocyte
Perivascular macrophage
Nutritive Arteriole
'Vasocrine Signalling'
(Perivascular fat inhibits
the effects of systemic insulin
on local vasodilatation)
Endothelin 1
Decreased Nutritive Flow
Insulin Resistance


Behavior
(appetite
and motivation)
EC
Mac
SIGNALS
Leptin
Insulin
Glucose
NEFA
Peri
As
BBB
Vagus Nerve
(Parasympathetic
Nervous System)
Anti-Inflammatory
Sympathetic
Nervous System
↓EGP
Liver
Vagal input augmenting
insulin's anabolic actions on
glucose and NEFA metabolism
VAT
Controlling fat cell proliferation
and lipolysis
SAT/VAT