Adipose Tissue Macrophages, Low Grade Inflammation and Insulin Resistance in Human Obesity

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Abstract: Obesity was first described as a low-grade inflammatory condition more than a decade ago. However, it is only relatively recently that obese individuals have been described with increased macrophage infiltration of adipose tissue, as well as an increase in the number of "M1" or "classically activated" macrophages. Furthermore, macrophages have been identified as the primary source of many of the circulating inflammatory molecules that are detected in the obese state and are postulated to be causal both in the development of insulin resistance and in the progression to type 2 diabetes. There is also novel evidence to suggest that macrophages inhibit adipocyte differentiation, potentially leading to adipocyte hypertrophy, altered secretion of adipokines and ectopic storage of lipid within liver, muscle and other non-adipose tissues. Currently, it is not clear what causes increased macrophage infiltration of adipose tissue in obese individuals. Theories include altered signalling by adipocytes, nutritional induction of metabolic endotoxemia or reduced angiogenesis and local adipose cell hypoxia. Importantly, PPAR-gamma agonists have been shown to alter macrophage phenotype to "M2" or an "alternatively activated" anti-inflammatory phenotype and may induce macrophage specific cell death. Consequently, excitement surrounds the potential for specific inhibition of macrophage infiltration of adipose tissue *via* pharmacotherapy for obese patients and more particularly as adjunct therapy to improve insulin sensitivity in obese individuals with insulin resistance and overt type 2 diabetes.

Key Words: Obesity, insulin resistance, inflammation, adipose tissue macrophages, type 2 diabetes.

CHRONIC LOW GRADE SYSTEMIC INFLAMMATION IN OBESITY

Adipose tissue was once thought to be an inert mass whose sole function was the storage of fat. However, it is now recognized that adipose tissue is an active endocrine organ that secretes numerous adipokines, cytokines and chemokines. The rapidly increasing prevalence of obesity in developed countries is now well described as are the strong associations between obesity and increased risk of many diseases including cardiovascular disease, metabolic syndrome, type 2 diabetes and some types of cancers.

Obesity was first recognised as a chronic low grade inflammatory condition approximately a decade ago, when Hotamisligil and colleagues described elevations in the cytokine tumor necrosis factor-alpha (TNFα) following diet induced obesity in rodents and its effect on insulin sensitivity [1]. This study was part of a paradigm shift in the way we view adipose tissue. Since then, increased adipose mass has been linked with increases in numerous other inflammatory molecules including; C-reactive protein (CRP), plasminogen activated inhibitor (PAI-1), serum amyloid A, migration inhibitory factor (MIF), resistin, inducible nitric oxide synthase (iNOS), interleukin-6 (IL6), colony stimulating factor-1 (CSF1) and monocyte chemoattractant protein-1 (MCP1) [2-5]. Importantly, many *in-vitro* and *in-vivo* studies have shown that inflammatory cytokines directly induce insulin resistance (Fig. **1**). For example, TNFα infusion significantly reduced insulin stimulated glucose uptake in lean men by inhibitory serine phosphorylation of insulin receptor substrate-1 (IRS-1) [6]. MCP-1 and CRP also led to inhibitory serine phosphorylation of IRS-1 and impaired insulin stimulated glucose uptake in human skeletal muscle cells and in L6-myotubes [7, 8].

MACROPHAGES INFILTRATION OF ADIPOSE TISSUE

Adipose tissue is a heterogeneous tissue that is composed of mature adipocytes and smaller cells that constitute the stromal-vascular fraction. This fraction includes pre-adipocytes, fibroblasts, endothelial cells, histiocytes, and macrophages. Macrophages are mononuclear phagocytes involved in immunological and inflammatory processes, whose function is to provide an immediate defence against foreign organisms and to clear resultant cellular debris. Flow cytometry studies of the stromavascular fraction have shown that 3-10% is made up of resident macrophages [9]. Relatively recently, adipose tissue from obese humans and rodents was described as infiltrated by an increased number of macrophages [3, 10]. Interestingly, macrophage infiltration in obesity appears to be

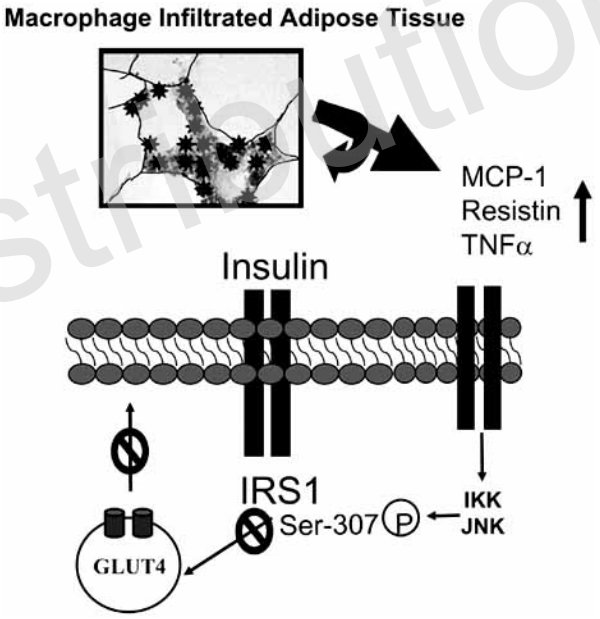

Fig. (1). Cytokines Induction of Insulin Resistance.

Macrophage infiltrated adipose tissue secrete increased levels of inflammatory cytokines, such as tumor necrosis factor alpha (TNFα) and monocyte chemoattractant protein -1 (MCP-1). These cytokines bind receptors that ultimately lead to the activation of Inhibitor kB Kinases (IKK) and JUN N-terminal Kinase (JNK) and subsequently inhibitory serine phosphorylation of insulin receptor substrate-1 (IRS-1). This inhibits the insulin signalling pathway preventing insulin stimulated glucose transporter-4 (GLUT-4) translocation to the cell surface and reducing glucose uptake.

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limited to adipose tissue as no differences are seen in the type or number of resident macrophages in muscle and/or liver of obese mice [11]. There is also increasing evidence that adipose tissue macrophages (ATMs) are the main source of the increase in the circulating inflammatory molecules detected in obesity [12, 13].

In morbidly obese humans, macrophages form crown-like structures that completely surrounding necrotic adipocytes and fuse to form lipid containing giant multi-nucleated cells that stain strongly for MAC-2 [14]. Adipose samples from lean subjects do not stain positive for MAC-2, indicating an absence of these giant lipid filled cells. It has not been reported whether adipose samples from moderately overweight individuals stain for MAC-2. Together, these studies highlight the extent of macrophage infiltration of adipose tissue in marked obesity. Furthermore, there is a growing body of evidence to suggest that the inflammatory process within the expanded fat mass is a primary event which may be involved in the development of insulin resistance and may also contribute to progression to type 2 diabetes. However, insulin resistance begins at much lower levels of fatness than have been studied to date.

In rodents, there is conflicting evidence whether macrophage infiltration occurs early during weight gain. Some studies have reported that the expression of inflammatory genes in adipose tissue is increased within 1-3 weeks following the initiation of a high fat diet [11, 15, 16]. Furthermore, elevations in circulating levels of plasma MCP-1 were detected after 4 weeks high fat feeding [16]. However, Xu *et al.* [11] quantified macrophage number by histology and observed no detectable changes in ATM number until after 8 weeks of high fat feeding, which is well after the usual development of insulin resistance in rodent models [17].

Macrophages can be stimulated to express distinct patterns of surface markers, chemokines and cytokines and it was recently described that the phenotype of macrophage detected in adipose tissue differs between high fat fed and lean chow fed animals [18]. In this study Lumeng *et al*. [18] observed that ATMs from lean mice expressed genes that are characteristic of M2 or "alternatively activated" macrophages, including *Ym1, arginase 1, and Il-10* whereas diet-induced obese mice had increased expression of genes such as *TNF*α and *iNOS* that are characteristic of M1 or "classically activated" macrophages. This study suggests that it is not only important to quantitate the total number of macrophages, but also the phenotype of macrophage in the tissue.

The cause of macrophage infiltration of adipose tissue is discussed in more detail later, but it has been proposed by us and others [3] that macrophage recruitment is dynamic and may be affected by changes in nutritional status and adiposity (Fig. **2**). Indeed, substantial weight losses induced by bariatric surgery significantly reduce macrophage number in morbidly obese individuals [3]. Weight loss was also associated with significant reductions in the expression of *MCP1 and hypoxia inducible factor 1*α *(HIF1*α*)* and a switch in macrophage activation from adverse M1 to M2 phenotype, with increased staining for the anti-inflammatory cytokine IL10. An 18 kg weight loss by 15-week lifestyle intervention also reduced adipose tissue expression of macrophage-specific markers (CD14, CD68), IL-6, IL-8, and TNFα in morbidly obese individuals [3]. However, this study did not investigate changes in ATM phenotype or location, which is arguably of greater importance. Most importantly, these studies have only investigated a very large change in adiposity in severely obese individuals who have a range of co-morbidities. It is well known that insulin resistance occurs at much lower body weight, and may be induced in as little as 3 weeks of overfeeding and reduced by more moderate weight loss in humans. Therefore, it remains to be established whether moderate changes in fatness (either gain or loss) alter the total number of macrophages or alter the phenotype of macrophages detected. Particularly, in question is whether over-nutrition and/or weight gain will increase ATMs in all individuals, independently of prior adiposity and/or in particular, genetic predisposition to type 2 diabetes or obesity.

MACROPHAGES INFILTRATE VISCERAL AND SUBCUTANEOUS ADIPOSE TISSUE

Visceral adipose tissue depots are more lipolytic, with higher fat turnover and fatty acid release and hence are more metabolically adverse as compared to subcutaneous depots [20]. In fact, visceral depots are now recognised to be developmentally different [19] and we predict that visceral depots will behave differently in respect to macrophage infiltration and cytokine secretion although this is yet to be clearly established. Visceral adipose depots are known to express more IL6, MCP-1 and CSF1 and other macrophage or inflammatory related genes [21]. Recently, visceral adipose tissue was described as infiltrated by an increased number of macrophages as compared to subcutaneous depots in both lean and obese individuals, potentially contributing to the well described adverse effects of visceral adiposity [21, 22]. In addition, visceral macrophage numbers were positively related with obesity in a wide cross sectional investigation and importantly, intra-abdominal fatness was associated with increased macrophage infiltration. It is postulated that macrophage deposition within visceral adipose tissue is more likely to be responsible for enhanced systemic cytokine levels [23], although visceral adipose tissue constitutes just 5-20% of total adipose mass. However, the portal drainage of visceral fat may increase the inflammatory response through a hepatic first pass effect. Although the apparent increase in MCP-1 secretion that is reported from visceral explants of adipose tissue was lost once adjusted for the increased number of macrophages detected in visceral adipose tissue [24]. Together, this data suggest visceral fat is infiltrated with increased macrophage number and that this contributes significantly to circulating cytokines and hence inflammation in individuals with abdominal obesity.

WHAT CAUSES MACROPHAGE INFILTRATION IN OBESITY?

Numerous theories into the underlying cause of macrophage recruitment into adipose tissue have been proposed. These include macrophage recruitment in response to altered adipokine signalling and cell size, local adipose hypoxia and nutritional endotoxemia. There is also a striking overlap between the biology of adipocytes and macrophages and some evidence to suggest that preadipocytes may be converted to macrophages under appropriate conditions (Fig. **2**) [25].

a. Altered Adipocyte Signalling and Cell Size?

Obesity results in an altered secretion pattern of adipokines, chemokines and cytokines which may be actively involved in macrophage recruitment (Fig. **2**). Indeed, it is well known that obesity results in increased leptin and lower adiponectin secretion from adipose tissue. Leptin exerts mildly pro-inflammatory effects, activating macrophage phagocytosis and cytokine production and adiponectin manifests anti-inflammatory activity as shown by inhibiting macrophage production of TNFα and IL6 and other actions, including binding bacterial lipopolysaccharide [26]. Other secreted factors that are dysregulated in obesity and may be involved in macrophage recruitment include MCP-1, MIF1 and CSF1.

Obesity is associated with increased adipocyte size (hypertrophy). Larger fat cells secrete more chemoattractant and immune-related genes, including *serum amyloid A, transmembrane 4 L six family member 1 and chemokine CXC Motif ligand 2* [27] which may be involved in macrophage recruitment. Interestingly, increased adipocyte size has clearly been associated with insulin resistance and progression to type 2 diabetes [28, 29] and in both rodents and humans, adipocyte size is a strong direct predictor of macrophage infiltration of adipose tissue [10, 30]. There is increasing evidence however that this association may be due to, rather

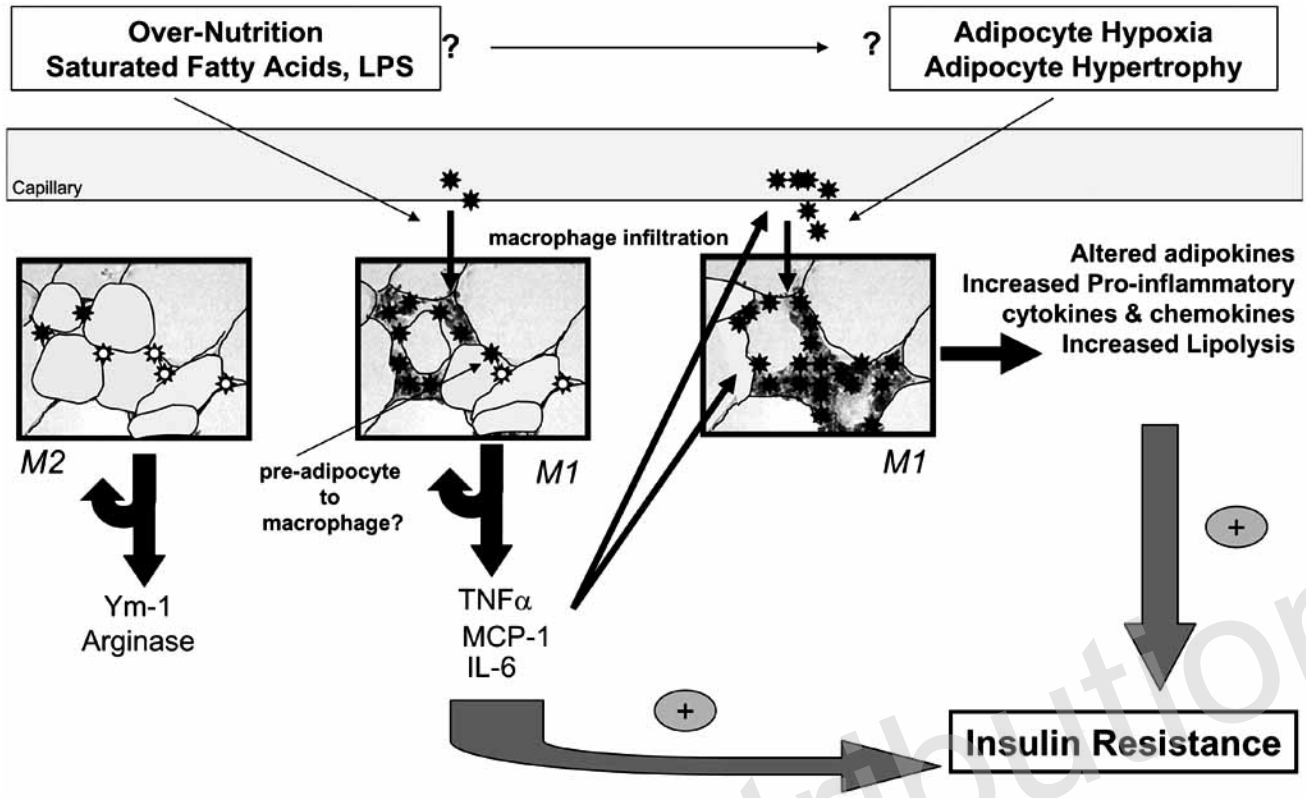

Fig. (2). Macrophage infiltration of adipose tissue is dynamic and altered by changes in nutrition and adiposity.

Postulated theory whereby over-consumption of high energy foods leads to macrophage infiltration of adipose tissue and altered secretion of chemokines and cytokines such as reductions in alternatively activated M2 macrophages that express Ym-1 and arginase and increases in clasically activated M1 macrophages that express inflammatory cytokines such as interlekin-6 (IL-6) and tumor necrosis factor alpha (TNFα) which directly impact insulin sensitivity in skeletal muscle. Macrophages also may indirectly lead to insulin resistance *via* effects on increasing adipocyte size and altered expression of adipokines and excess fatty acid release, leading to inappropriate storage of triglyceride in non-adipose tissues.

than the cause of, macrophage infiltration of adipose tissue [31]. For instance, addition of medium from human macrophages to pre-adipocytes that were isolated from either omental or subcutaneous adipose tissue completely suppressed adipogenesis as evidenced by decreased triglyceride accumulation and decreased FAS and PPAR-gamma expression [32]. Interestingly, Hammerstedt *et al.* [33] reported that TNFα, but not MCP1 or resistin inhibited adipogenesis by down regulating the wnt-signaling pathway. Recently, we and other have proposed that obesity and diabetes is associated with a reduced proportion of pre-adipocytes within adipose tissue [30] and that some individuals have a reduced ability to switch on adipogenesis in response to overfeeding. This inability for adipogenesis may promote adipocyte hypertrophy under hyperphagic conditions, ectopic lipid deposition and insulin resistance [34, 35]. It is possible that macrophage infiltration within adipose tissue affects fat expansion through a paracrine action on adipocyte differentiation, and thus may indirectly contribute to insulin resistance by increased lipid spill over to ectopic lipid depots.

b. Nutritional Endotoxemia?

Lipopolysaccheride (LPS) is known to act *via* the toll-like receptor-4 (TLR4) to activate the NFκB pathway and subsequent transcription of various inflammatory cytokines such as TNFα and IL-6. Recently, it was described that low-level endotoxemia from the gut flora is increased in fed versus fasted mice and more than doubles following 4-weeks of very high-fat feeding [36]. Furthermore, the high fat fed mouse had increased weight gain and increased expression of inflammatory factors from adipose tissue and increased glucose and insulin areas under the curve in response following oral glucose load [36]. Interestingly, the authors showed that the high fat fed response was similar to the low dose LPS infusion response for 4 weeks. Saturated fatty acids can also bind and activate TLR4-NFκB pathway and TLR4 is expressed by the 3T3L1 preadipocyte cell line as well as in human adipocytes [37, 38]. Importantly, loss of function mutations in TLR4 prevent insulin resistance and diabetes in high fat fed animals [39]. Thus, prolonged over-nutrition or increased saturated fatty acid intake may increase nutritional endotoxemia, stimulate the TLR4-NFkB pathway in adipocytes, which may be causal in macrophage infiltration of obese adipose tissue. However, this has yet to be tested in a human model although a polymorphism is known to affect innate immune function and atheroma inversely in man [40].

c. Local Adipose Tissue Hypoxia?

Hypoxia occurs when oxygen availability does not match demand by surrounding tissue and is proposed to underlie the inflammatory response as adipose tissue mass expands. There is increasing evidence showing that obese adipose tissue is hypoxic [41, 42]. Obesity is associated with increased expression of hypoxia-inducible factor-alpha (HIF1α) in humans and rodents [3, 42]. Furthermore, immunohistochemistry studies of adipose tissue have shown that pimonidazole detected hypoxic areas are co-localized with macrophages [43]. Recently, Strissel *et al.* [44] observed that hypoxia was a transient phenomenon with temporal increases in adipocyte size and hypoxia, followed by adipocyte cell death and macrophage infiltration until 16 weeks of high fat feeding. By 20

weeks however, they observed an increase in the number of small adipocytes (adipogenesis), along with reduced adipocyte death and reduced macrophage infiltration. This study suggests that macrophages infiltrate adipose tissue as a direct result of necrotic cell death. However, this study also raises conjecture that rodent high fat feeding models create artificial conditions with rapidly expanding adipose mass which leads to local adipose tissue hypoxia. Whether this process will be observed under the long steady weight gain that commonly occurs in humans is not clear. Indeed, in the classical twin overfeeding study by Bouchard and colleagues [45], weight gain was approximately 10% over 14 weeks rather than the 140% weight gain that is observed in animal studies [44]. Regardless, the hypoxic model of macrophage infiltration may have implications both for pathological conditions of excess fat and/or ectopic fat deposition and successful expansion with vascular sufficiency and warrants further investigation.

DOES REDUCING MACROPHAGE INFILTRATION PREVENT INSULIN RESISTANCE?

Although it is increasingly recognised that macrophage infiltration and chronic low grade inflammation is higher in the obese state, and evidence is building that macrophages may alter secreted factors that impact on impaired skeletal muscle insulin signalling *in vitro*, it has yet to be indisputably established whether increased macrophage infiltration is causal in initiating insulin resistance. In Table **1**, we have summarised intervention studies altering both macrophage infiltration and insulin sensitivity.

a. Evidence from Cells and Genetically Modified Rodents

Monocyte chemoattractant proteins and their receptors play an important role in the recruitment of monocytes into various tissues. Lumeng *et al.* [18] examined mice carrying a homozygous deletion of the gene encoding C-C motif chemokine receptor-2 (CCR2), which is the receptor for MCP1, after high fat feeding challenge. High fat-fed knockout animals had reduced inflammation, decreased macrophage infiltration in adipose tissue, and improved insulin sensitivity as compared to wild type [46]. However, a subsequent study performed by a separate group could not replicate these findings following 34 weeks high fat feeding [47].

Interaction between macrophages and adipocytes in the context of both indirect and direct co-culture studies have shown that macrophage-secreted factors activate the NFκB pathway stimulating the expression of TNFα and blocking insulin action in adipocytes (Fig. 1) [48]. Interestingly, this response was inhibited following co-culture with TLR4-mutant macrophages [49], suggesting that TLR4 is necessary for an inflammatory response. Whole body TLR4 knockouts also have reduced circulating inflammatory cytokines and macrophage infiltration of adipose tissue and have improved insulin sensitivity following high fat feeding [50, 51]. Together, these studies highlight the importance of the TLR4 pathway in activating inflammation with obesity and suggest that the increased release of fatty acids from enlarged adipocytes may feed forward to induce inflammatory and insulin signaling changes in macrophages and adipocytes.

Interestingly, co-culture of macrophages and adipocytes also reduces adiponectin secretion [32, 51] and a recent study of adiponectin transgenic ob/ob animals found that while these animals have increased fat mass as compared to ob/ob animals, they are protected from macrophage infiltration and inflammation in adipose tissue and have a healthy metabolic phenotype despite massive obesity [52]. This is seen in conjunction with reduced adipocyte size, potentially contributing to the metabolically healthy phenotype observed despite massive obesity.

b. Evidence from Drug Treatments

PPAR gamma is required for fat cell development and is the molecular target of antidiabetic thiazolidinediones (TZDs), which exert insulin-sensitizing effects in adipose tissue, skeletal muscle, and liver. Recently, a macrophage specific knockout of PPAR gamma was shown to develop insulin resistance under chow-fed conditions and this was exacerbated by high fat feeding [53]. This finding raised interest into the effects of TZDs and other drugs on macrophage infiltration of adipose tissue. TZD treatment reduced expression of inflammatory genes and decreased macrophage numbers in adipose tissue in rodents and in non-diabetic individuals with impaired glucose tolerance [11][54].

Metformin is another insulin sensitizer that acts at least partially *via* an activation of AMP-activated protein kinase (AMPK). There is conflicting evidence whether metformin suppresses ATM infiltration in adipose tissue. Dandona *et al.* [55] observed that metformin reduced circulating levels of MIF, as well as MIF expression from circulating blood monocytes, but did not examine macrophage number of phenotype per se. Isoda *et al.* [56] observed that metformin reduced NFκB translocation to the nucleus reducing expression of various pro-inflammatory cytokines in human smooth muscle cells. Other studies in humans have not been able to show an effect of metformin after 10 weeks on macrophage phenotype or number [57].

Together, these studies suggest that limiting macrophage infiltration of adipose tissue by pharmacological intervention will be metabolically beneficial to obese patients, at least in terms of reducing insulin resistance. However, it must be remembered that a low

Table 1. Does Macrophage Infiltration of Adipose Tissue Causes Insulin Resistance?

Model	Intervention	Outcomes	Ref
MCP1 -/- (whole body)	HFD	⇓ ATM, ⇓ Circulating inflammatory cytokines, ⇑ Insulin sensitivity in knockout	[18]
TLR4 -/- (whole body)	HFD	⇓ ATM infiltration, ⇓ Circulating inflammatory cytokines, ⇑ Insulin sensitivity in knockout	[[48, 49]
Peritoneal macrophages from TLR4 -/-	SFA	⇓NFkB activation, ⇓ Pro-inflammatory cytokines in knockout.	[49]
3T3-L1 fibroblasts, J774A.1 and RAW264 macrophages	Co-Culture	⇑ NFkB signalling, ⇑ Pro-inflammatory cytokines, ⇓ Adiponectin, ⇓ Insulin stimulated glucose transport	[46]
Ob/Ob Adiponectin transgenic	HFD	⇑ Fat mass, ⇓ ATM infiltration ⇑ Insulin sensitivity in transgene	[50]
Macrophage specific PPARgamma -/-	HFD	⇑ ATM, ⇓ Insulin sensitivity in knockout	[51]
Glucose Tolerant Human	Pioglitazone10wk	⇓CD68 mRNA, ⇓ MCP1 mRNA, ⇑ Insulin sensitivity after treatment	[55]
Glucose Tolerant Human	Metformin 10wk	⇔ CD68 mRNA, ⇔ MCP1 mRNA, ⇔ Insulin sensitivity after treatment	[55]
Obese Human	Metformin 6wk	⇓ MIF expression, ⇔ glucose, ⇔ insulin after treatment	[53]
Human smooth muscle cells	Metformin	⇓ NFkB activation, ⇓ pro-inflammatory cytokines, ⇑ Insulin signalling after treatment	[54]

MCP1 = monocyte chemoattractant protein-1; ATM = adipose tissue macrophages, NfKB = nuclear factor-kappa B, HFD = High fat diet, SFA = saturated fatty acid stimulation, MIF, monocyte inhibitory factor.

level of resident macrophages is necessary to clear necrotic debris and that complete suppression of function is likely to interfere with normal biological processes.

CONCLUSION

With the preliminary data currently available inhibition of macrophage infiltration of adipose tissue of obese or pre-diabetic individuals seems a rational therapeutic target for the pharmacotherapy of type 2 diabetes and/ or the Metabolic Syndrome. However, a number of questions regarding the role of macrophage infiltration in human obesity remain to be answered. For example, what is the cause/s of macrophage infiltration? Does moderate fat gain alter macrophage number and/or macrophage phenotype in humans? Are some individuals predisposed to this? Is macrophage infiltration causal in development of insulin resistance and/or progression to type 2 diabetes or an accessory after the initiation? Over the past 3 years, preliminary data suggest that preventing M1 macrophage infiltration of adipose tissue may diminish insulin resistance. Consequently, great excitement surrounds the potential for specific inhibition of macrophage infiltration of adipose tissue as pharmacotherapy for obese patients but more particularly as adjunct therapy to improve insulin sensitivity in obese individuals with insulin resistance.

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ABBREVIATIONS

ATM	=	Adipose Tissue Macrophages
TNFα	=	Tumor Necrosis Factor-alpha
MCP-1	=	Monocyte chemoattracant protein-1
CRP	=	C-Reactive protein
IL6	=	Interleukin-6
IL10	=	Interleukin-10
PPAR	=	Peroxisome proliferator activated receptor
PAI-1	=	plasminogen activated inhibitor-1
iNOS	=	inducible nitric oxide synthase
HIF1α	=	hypoxia inducible factor 1α (HIF1α)
TLR4	=	toll like receptor-4

REFERENCES

[1] Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. Science 1993; 259: 87-91.

[2] Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006; 444: 860-7.

[3] Cancello R, Henegar C, Viguerie N, Taleb S, Poitou C, Rouault C, *et al.* Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. Diabetes 2005; 54: 2277-86.

[4] Heilbronn LK, Rood J, Janderova L, Albu JB, Kelley DE, Ravussin E, *et al.* Relationship between serum resistin concentrations and insulin resistance in nonobese, obese, and obese diabetic subjects. J Clin Endocrinol Metab 2004; 89: 1844-8.

[5] Heilbronn LK, Noakes M, Clifton PM. Energy restriction and weight loss on very-low-fat diets reduce C-reactive protein concentrations in obese, healthy women. Arterioscler Thromb Vasc Biol 2001; 21: 968-70.

[6] Krogh-Madsen R, Plomgaard P, Moller K, Mittendorfer B, Pedersen BK. Influence of TNF-{alpha} and IL-6 infusions on insulin sensitivity and expression of IL-18 in humans. Am J Physiol Endocrinol Metab 2006; 291: E108-14.

[7] Sell H, Dietze-Schroeder D, Kaiser U, Eckel J. Monocyte chemotactic protein-1 is a potential player in the negative cross-talk between adipose tissue and skeletal muscle. Endocrinology 2006; 147: 2458-67.

[8] D'Alessandris C, Lauro R, Presta I, Sesti G. C-reactive protein induces phosphorylation of insulin receptor substrate-1 on Ser307 and Ser612 in L6 myocytes, thereby impairing the insulin signalling pathway that promotes glucose transport. Diabetologia 2007; 50: 840-9.

[9] Curat CA, Miranville A, Sengenes C, Diehl M, Tonus C, Busse R, *et al.* From blood monocytes to adipose tissue-resident macrophages: induction of diapedesis by human mature adipocytes. Diabetes 2004; 53: 1285-92.

[10] Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, Jr. Obesity is associated with macrophage accumulation in adipose tissue. J Clin Invest 2003; 112: 1796-1808.

[11] Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, *et al.* Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. J Clin Invest 2003; 112: 1821-30.

[12] Fain JN, Madan AK, Hiler ML, Cheema P, Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology 2004; 145: 2273-82.

[13] Maury E, Ehala-Aleksejev K, Guiot Y, Detry R, Vandenhooft A, Brichard SM. Adipokines oversecreted by omental adipose tissue in human obesity. Am J Physiol Endocrinol Metab 2007; 293: E656-65.

[14] Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, *et al.* Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J. Lipid Res 2005; 46: 2347-55.

[15] Brake DK, Smith EOB, Mersmann H, Smith CW, Robker RL. ICAM-1 expression in adipose tissue: effects of diet-induced obesity in mice. Am J Physiol Cell Physiol 2006; 291: C1232-39.

[16] Chen A, Mumick S, Zhang C, Lamb J, Dai H, Weingarth D, *et al.* diet induction of monocyte chemoattractant protein-1 and its impact on obesity. Obesity Res 2005; 13: 1311-20.

[17] Storlien LH, James DE, Burleigh KM, Chisholm DJ, Kraegen EW. Fat feeding causes widespread *in vivo* insulin resistance, decreased energy expenditure, and obesity in rats. Am J Physiol Endocrinol Metab 1986; 251: E576-83.

[18] Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest 2007; 117: 175-84.

[19] Frayn KN. Visceral fat and insulin resistance--causative or correlative? Br J Nutr 2000; 83(Suppl 1): S71-7.

[20] Gesta S, Tseng YH, Kahn CR. Developmental origin of fat: tracking obesity to its source. Cell 2007; 131: 242-56.

[21] Harman-Boehm I, Bluher M, Redel H, Sion-Vardy N, Ovadia S, Avinoach E, *et al.* Macrophage infiltration into omental versus subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity. J Clin Endocrinol Metab 2007; 92: 2240-7.

[22] Cancello R, Tordjman J, Poitou C, Guilhem G, Bouillot JL, Hugol D, *et al.* Increased infiltration of macrophages in omental adipose tissue is associated with marked hepatic lesions in morbid human obesity. Diabetes 2006; 55: 1554-61.

[23] Curat CA, Wegner V, SengenÃ¨s C, Miranville A, Tonus C, Busse R, *et al.* Macrophages in human visceral adipose tissue: increased accumulation in obesity and a source of resistin and visfatin. Diabetologia 2006; V49: 744-7.

[24] Bruun JM, Lihn AS, Pedersen SB, Richelsen B. Monocyte chemoattractant protein-1 release is higher in visceral than subcutaneous human adipose tissue (AT): implication of macrophages resident in the AT. J Clin Endocrinol Metab 2005; 90: 2282-9.

[25] Charriere G, Cousin B, Arnaud E, Andre M, Bacou F, Penicaud L, *et al.* Preadipocyte conversion to macrophage. Evidence of plasticity. J Biol Chem 2003; 278: 9850-5.

[26] Peake PW, Shen Y, Campbell LV, Charlesworth JA. Human adiponectin binds to bacterial lipopolysaccharide. Biochem Biophys Res Commun 2006; 341: 108-15.

[27] Jernas M, Palming J, Sjoholm K, Jennische E, Svensson P-A, Gabrielsson BG, *et al*. Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression. FASEB J 2006; 20: 1540-2.

[28] Weyer C, Foley JE, Bogardus C, Tataranni PA, Pratley RE. Enlarged subcutaneous abdominal adipocyte size, but not obesity itself, predicts Type II diabetes independent of insulin resistance. Diabetologia 2000; 43: 1498-1506.

[29] Dubois SG, Heilbronn LK, Smith SR, Albu JB, Kelley DE, Ravussin E, *et al.* Decreased expression of adipogenic genes in obese subjects with type 2 diabetes. Obesity 2006; 14: 1543-1552.

[30] Tchoukalova Y, Koutsari C, Jensen M. Committed subcutaneous preadipocytes are reduced in human obesity. Diabetologia 2007; 50: 151-7.

[31] Lacasa D, Taleb S, Keophiphath M, Miranville A, Clement K. Macrophage-secreted factors impair human adipogenesis: involvement of proinflammatory state in preadipocytes. Endocrinology 2007; 148: 868-77.

[32] Constant VA, Gagnon A, Landry A, Sorisky A. Macrophage-conditioned medium inhibits the differentiation of 3T3-L1 and human abdominal preadipocytes. Diabetologia 2006; 49: 1402-11.

[33] Hammarstedt A, Isakson P, Gustafson B, Smith U. Wnt-signaling is maintained and adipogenesis inhibited by TNF[alpha] but not MCP-1 and resistin. Biochem Biophys Res Commun 2007; 357: 700-706.

[34] Danforth E. Failure of adipocyte differentiation causes type II diabetes mellitus? Nat Genet 2000; 26: 13-13.

[35] Heilbronn L, Smith SR, Ravussin E. Failure of fat cell proliferation, mitochondrial function and fat oxidation results in ectopic fat storage, insulin resistance and type II diabetes mellitus. Int J Obes Relat Metab Disord 2004; 28(Suppl 4): S12-21.

[36] Cani PD, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, *et al*. Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes 2007; 56: 1761-72.

[37] Bes-Houtmann S, Roche R, Hoareau L, Gonthier MP, Festy F, Caillens H, *et al*. Presence of functional TLR2 and TLR4 on human adipocytes. Histochem Cell Biol 2007; 127: 131-7.

[38] Song MJ, Kim KH, Yoon JM, Kim JB. Activation of Toll-like receptor 4 is associated with insulin resistance in adipocytes. Biochem Biophys Res Commun 2006; 346: 739-45.

[39] Tsukumo DM, Carvalho-Filho MA, Carvalheira JB, Prada PO, Hirabara SM, Schenka AA, *et al*. Loss-of-function mutation in Toll-like receptor 4 prevents diet-induced obesity and insulin resistance. Diabetes 2007; 56: 1986-98.

[40] Kiechl S, Lorenz E, Reindl M, Wiedermann CJ, Oberhollenzer F, Bonora E, *et al.* Toll-like receptor 4 polymorphisms and atherogenesis. N Engl J Med 2002; 347: 185-92.

[41] Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, *et al.* Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. Diabetes 2007; 56: 901-11.

[42] Ye J, Gao Z, Yin J, He Q. Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. Am J Physiol Endocrinol Metab 2007; 293: E1118-28.

[43] Rausch ME, Weisberg S, Vardhana P, Tortoriello DV. Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. Int J Obes 2007; doi: 10.1038/sj.ijo.0803744.

[44] Strissel KJ, Stancheva Z, Miyoshi H, Perfield JW, 2nd, Defuria J, Jick Z, *et al.* Adipocyte death, adipose tissue remodeling and obesity complications. Diabetes 2007; 56: 2910-8

[45] Bouchard C, Tremblay A, Despres JP, Nadeau A, Lupien PJ, Theriault G, *et al.* The response to long-term overfeeding in identical twins. N Engl J Med 1990; 322: 1477-82.

[46] Lumeng CN, Deyoung SM, Bodzin JL, Saltiel AR. Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. Diabetes 2007; 56: 16-23.

[47] Inouye KE, Shi H, Howard JK, Daly CH, Lord GM, Rollins BJ, *et al.* Absence of CC chemokine ligand 2 does not limit obesity-associated infiltration of macrophages into adipose tissue. Diabetes 2007; 56: 2242-50.

[48] Lumeng CN, Deyoung SM, Saltiel AR. Macrophages block insulin action in adipocytes by altering expression of signaling and glucose transport proteins. Am J Physiol Endocrinol Metab 2007; 292: E166-74.

[49] Suganami T, Mieda T, Itoh M, Shimoda Y, Kamei Y, Ogawa Y. Attenuation of obesity-induced adipose tissue inflammation in C3H/HeJ mice carrying a Toll-like receptor 4 mutation. Biochem Biophys Res Commun 2007; 354: 45-9.

[50] Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. J Clin Invest 2006; 116: 3015-25.

[51] Suganami T, Tanimoto-Koyama K, Nishida J, Itoh M, Yuan X, Mizuarai S, *et al.* Role of the toll-like receptor 4/NF-{kappa}B pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. Arterioscler Thromb Vasc Biol 2007; 27: 84-91.

[52] Kim JY, van de Wall E, Laplante M, Azzara A, Trujillo ME, Hofmann SM, *et al*. Obesity-associated improvements in metabolic profile through expansion of adipose tissue. J Clin Invest 2007; 117: 2621-37.

[53] Hevener AL, Olefsky JM, Reichart D, Nguyen MT, Bandyopadyhay G, Leung HY, *et al.* Macrophage PPAR gamma is required for normal skeletal muscle and hepatic insulin sensitivity and full antidiabetic effects of thiazolidinediones. J Clin Invest 2007; 117: 1658-69.

[54] Bodles AM, Banga A, Rasouli N, Ono F, Kern PA, Owens RJ. Pioglitazone increases secretion of high-molecular-weight adiponectin from adipocytes. Am J Physiol Endocrinol Metab 2006; 291: E1100-5.

[55] Dandona P, Aljada A, Ghanim H, Mohanty P, Tripathy C, Hofmeyer D, *et al.* Increased plasma concentration of macrophage migration inhibitory factor (MIF) and MIF mRNA in mononuclear cells in the obese and the suppressive action of metformin. J Clin Endocrinol Metab 2004; 89: 5043-7.

[56] Isoda K, Young JL, Zirlik A, MacFarlane LA, Tsuboi N, Gerdes N, *et al.* Metformin inhibits proinflammatory responses and nuclear factor-kappaB in human vascular wall cells. Arterioscler Thromb Vasc Biol 2006; 26: 611-7.

[57] Di Gregorio GB, Yao-Borengasser A, Rasouli N, Varma V, Lu T, Miles LM, *et al.* Expression of CD68 and macrophage chemoattractant protein-1 genes in human adipose and muscle tissues: association with cytokine expression, insulin resistance, and reduction by pioglitazone. Diabetes 2005; 54: 2305-2313.