

## Irish Section Postgraduate Symposium

# Insights into the role of macrophage migration inhibitory factor in obesity and insulin resistance

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High-fat diet (HFD)-induced obesity has emerged as a state of chronic low-grade inflammation characterised by a progressive infiltration of immune cells, particularly macrophages, into obese adipose tissue. Adipose tissue macrophages (ATM) present immense plasticity. In early obesity, M2 anti-inflammatory macrophages acquire an M1 pro-inflammatory phenotype. Pro-inflammatory cytokines including TNF- $\alpha$ , IL-6 and IL-1 $\beta$  produced by M1 ATM exacerbate local inflammation promoting insulin resistance (IR), which consequently, can lead to type-2 diabetes mellitus (T2DM). However, the triggers responsible for ATM recruitment and activation are not fully understood. Adipose tissue-derived chemokines are significant players in driving ATM recruitment during obesity. Macrophage migration inhibitory factor (MIF), a chemokine-like inflammatory regulator, is enhanced during obesity and is directly associated with the degree of peripheral IR. This review focuses on the functional role of macrophages in obesity-induced IR and highlights the importance of the unique inflammatory cytokine MIF in propagating obesity-induced inflammation and IR. Given MIF chemotactic properties, MIF may be a primary candidate promoting ATM recruitment during obesity. Manipulating MIF inflammatory activities in obesity, using pharmacological agents or functional foods, may be therapeutically beneficial for the treatment and prevention of obesity-related metabolic diseases.

### **Obesity: Inflammation: Insulin resistance: Adipose tissue macrophages: Macrophage migration inhibitory factor**

The incidence of obesity has escalated to epidemic proportions. In 2008, an estimated 1.45 billion adults were overweight worldwide (BMI > 25 kg/m<sup>2</sup>), 500 million of whom were obese (BMI > 30 kg/m<sup>2</sup>)<sup>(1)</sup>. Alarming, in 2010 approximately 43 million children under 5 years of age were classed as overweight<sup>(2)</sup>. This global surge in obesity can be largely attributed to the increased availability and consumption of high-fat foods in combination with sedentary lifestyle<sup>(3,4)</sup>. Consequently, there has been a substantial rise in obesity-related co-morbidities; dyslipidemia,

hypertriglyceremia, insulin resistance (IR), type-2 diabetes mellitus (T2DM), CVD and non-alcoholic fatty liver disease, collectively known as the metabolic syndrome<sup>(1,5)</sup>. As such, obesity presents an astronomical financial cost and burden on health care systems<sup>(6)</sup>. Accumulating evidence indicates that chronic low-grade inflammation is the critical link in the pathogenesis of obesity-related disorders. Disproportionate production of pro- and anti-inflammatory adipose tissue-derived cytokines from hypertrophied adipocytes is a critical determinant of

**Abbreviations:** ATM, adipose tissue macrophage; CCL, CC-chemokine ligand; CCR, CC-chemokine receptor; DIO, diet-induced obese; HFD, High-fat diet; IR, insulin resistance; JNK, c-Jun N-terminal kinases; MCP-1, monocyte chemoattractant protein-1; MIF, migration inhibitory factor; T2DM, type-2 diabetes mellitus; TLR, toll-like receptor.

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obesity-induced IR<sup>(7,8)</sup>. The increased presence of macrophages within obese adipose tissue has emerged as the predominant source of inflammatory mediators, which contribute to adipose tissue dysfunction and systemic IR<sup>(8,9)</sup>. Unravelling the mechanisms responsible for adipose tissue macrophage (ATM) infiltration, activation and ensuing adipocyte dysregulation is imperative to our understanding of the pathophysiological progression to IR and T2DM. Macrophage migration inhibitory factor (MIF) is a principal regulator of innate immune responses and has been implicated in numerous inflammatory and autoimmune diseases such as septic shock and rheumatoid arthritis<sup>(10)</sup>. Several studies have documented an association between MIF and metabolic deregulation and a relationship between MIF and obesity has transpired<sup>(11,12)</sup>. This review summarises the importance of macrophages in high-fat diet (HFD)-induced adipose tissue inflammation and IR and outlines the known role of MIF in inflammation and metabolic complications.

### Obesity and insulin resistance

IR is the hallmark of obesity-induced metabolic complications and is defined by the inability of metabolic tissues including adipose tissue, skeletal muscle and liver to appropriately respond to insulin action<sup>(13)</sup>. Insulin is a pleiotropic hormone secreted by pancreatic  $\beta$ -cells. It signals via the tyrosine phosphorylation of the insulin receptor substrate molecule, which subsequently activates a cascade of intracellular signalling events. Insulin activates two main signalling pathways one of which is phosphatidylinositol-3-kinase-AKT pathway primarily responsible for mediating glucose uptake<sup>(14,15)</sup>. Upon insulin stimulation, the GLUT-4 promptly translocates from intracellular storage sites to the plasma membrane, facilitating glucose uptake<sup>(16,17)</sup>. Insulin also exhibits anti-lipolytic activities in adipose tissue; inhibiting hormone-sensitive lipase activity and reducing NEFA release<sup>(18)</sup>. Adipose tissue IR materialises as impaired insulin-mediated glucose uptake and elevated lipolysis inducing hyperlipidaemia, hyperglycaemia and hyperinsulinaemia. Insulin-resistant skeletal muscle displays reduced glucose disposal<sup>(19)</sup>. On the other hand, the liver exhibits selective IR, whereby the inhibitory effect of insulin on hepatic gluconeogenesis is interrupted while its action on lipogenesis is enhanced, resulting in chronic hyperglycaemia and hypertriglyceridemia<sup>(20,21)</sup>. Considerable evidence places emphasis on the importance of inflammation in pathogenesis of obesity-induced IR. Macrophage-derived inflammatory mediators including TNF- $\alpha$  and IL-6, impede insulin signalling<sup>(9)</sup>. Thus, we focus on the role of macrophage-mediated inflammation in the development of IR.

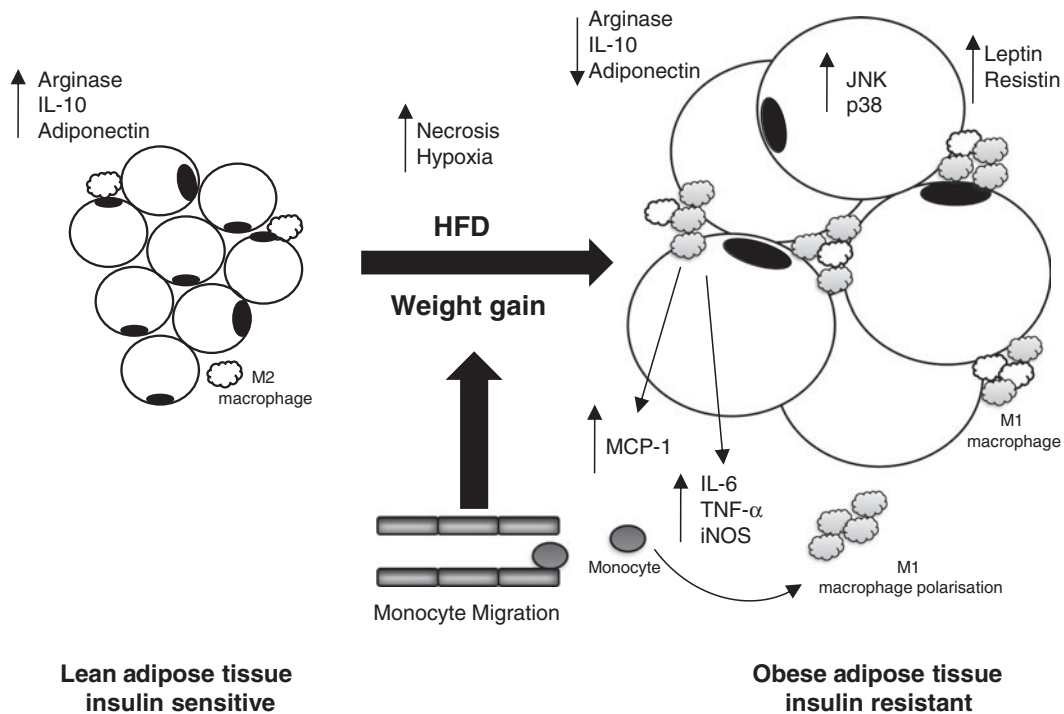
### Obese adipose tissue and inflammation

Obesity instigates a typical host inflammatory response<sup>(22)</sup>. Circulating levels of inflammatory mediators including acute phase proteins: plasminogen activator inhibitor and C-reactive protein and inflammatory cytokines TNF- $\alpha$ , IL-6 and IL-8 are systemically elevated<sup>(23–25)</sup>. Adipose

tissue is considered a critical interface in the pathogenesis of obesity-induced IR. Once perceived as a mere inert storage depot, adipose tissue is now known to be a vital endocrine organ paramount in maintaining systemic glucose and lipid homeostasis<sup>(26)</sup>. This metabolically active organ secretes copious amounts of adipokines, most notably leptin and adiponectin<sup>(27,28)</sup> and regulates NEFA flux to and from adipocytes maintaining energy homeostasis<sup>(18,29)</sup>. HFD-induced adipose tissue expansion leads to an increase in pro-inflammatory cytokine secretion and a decrease in anti-inflammatory cytokine production<sup>(26,30)</sup>. Indeed Hotamisligil and co-workers were the first to demonstrate that TNF- $\alpha$  expression was significantly increased in the adipose tissue of obese mice<sup>(7)</sup>. Moreover, chronic treatment with recombinant TNF- $\alpha$  was shown to disrupt insulin signalling in 3T3-L1 adipocytes. TNF- $\alpha$  activated intracellular inhibitor of NF- $\kappa$ B kinase- $\beta$ , which in turn, leads to reduced insulin receptor substrate-1 and GLUT-4 mRNA expression in 3T3-L1 adipocytes<sup>(7,31)</sup>. Conversely, whole body deletion of TNF- $\alpha$  or its corresponding receptor TNF receptor 1 (TNFR1) gene partially protected mice from HFD-induced IR<sup>(32)</sup>. Subsequently, a multitude of adipose tissue derived cytokines including IL-6, IL-1 $\beta$  and CC-chemokine ligand 2 (CCL2)/monocyte chemoattractant protein (MCP)-1 were identified to be overproduced during obesity in human subjects and rodents<sup>(22,33)</sup>.

### Adipose tissue macrophages in obesity

Despite compelling evidence signifying an association between chronic adipose tissue inflammation and IR, the cell types responsible for orchestrating this inflammatory response remained unexplored. In 2003, two separate studies demonstrated that expanding adipose mass is accompanied by a progressive infiltration of macrophages into adipose tissue (Fig. 1)<sup>(8,9)</sup>. Weisberg *et al.* conducted transcriptional profile analysis on adipose tissue isolated from genetic and diet-induced obese (DIO) mouse models and demonstrated 1304 transcripts correlated to body mass, 30% of which encoded proteins that were characteristic of macrophages. Immunohistochemistry analysis of adipose tissue confirmed that macrophage-specific marker F4/80 expression was positively associated with adipocyte size and body mass. Furthermore, ATM were deemed the predominant source of pro-inflammatory TNF- $\alpha$ <sup>(8)</sup>. Xu *et al.* showed macrophage-specific genes to be significantly up-regulated in adipose tissue prior to overt hyperinsulinemia, an effect that was dramatically reduced on treatment with the insulin-sensitising drug rosiglitazone<sup>(9)</sup>. In support of these studies, diet or surgery-induced weight loss coincided with reduced ATM infiltration, CCL2/MCP-1 gene expression and low-grade inflammation in morbidly obese individuals<sup>(34,35)</sup>. These findings placed macrophage biology at the forefront of investigations in an attempt to decipher how immune and metabolic responses converge in obesity and IR. *In vitro* analysis demonstrated that paracrine ATM-adipocyte interactions exacerbate local inflammation<sup>(30,36)</sup> and potentiated the development of IR and T2DM<sup>(37)</sup>. Indeed Suganami *et al.* showed that 3T3-L1



**Fig. 1.** Obesity-induced adipose tissue macrophage (ATM) recruitment. M2 ATM are characteristic of lean adipose tissue. Adipocyte-derived arginase, adiponectin and M2-derived IL-10 are enhanced. During obesity, adipose tissue expansion is accompanied by adipocyte necrosis, hypoxia and enhanced pro-inflammatory cytokine secretion which attract pro-inflammatory M1 macrophages into adipose tissue. In this obesogenic state pro-inflammatory IL-6, IL-1 $\beta$ , TNF- $\alpha$ , inducible nitric oxide synthase (iNOS), monocyte chemoattractant protein (MCP)-1 and adipokines leptin and resistin are up-regulated, exacerbating the inflammatory milieu, while arginase, adiponectin and IL-10 are decreased. Inflammatory signalling pathways c-Jun N-terminal kinases (JNK) and p38 are activated leading to impaired insulin action and systemic insulin resistance (IR).

adipocytes exposed to RAW264 macrophage-derived conditioned media exhibited increased MCP-1, IL-6 and TNF- $\alpha$  mRNA expression, while RAW264 macrophages incubated with adipocyte-conditioned media displayed enhanced IL-6 and TNF- $\alpha$  expression. TNF- $\alpha$  emerged as the dominant inflammatory mediator, anti-TNF- $\alpha$  neutralising antibody inhibited the up-regulation of adipocyte MCP-1 expression. RAW264 macrophages treated with adipocyte-derived NEFA demonstrate increased inflammatory phenotype, suggesting NEFA are an important contributor to macrophage inflammation<sup>(30)</sup>. Permana *et al.* and Lumeng *et al.* extended these findings demonstrating that macrophage-secreted factors decreased adipocyte sensitivity by inducing a marked reduction in GLUT-4 and insulin receptor substrate-1 expression coincident with attenuated AKT phosphorylation and impaired insulin-stimulated glucose uptake<sup>(36,38)</sup>.

A dynamic interplay between multiple intracellular signalling pathways within insulin-responsive tissues is imperative in modulating both local and systemic inflammation and subsequent IR. Macrophage-derived pro-inflammatory cytokines and NEFA have been implicated in the chronic activation of c-Jun N-terminal kinases (JNK) and inhibitor of NF- $\kappa$ B kinase- $\beta$  protein kinases, critical inflammatory regulators in the progression of obesity-related complications<sup>(39)</sup>. Activated JNK impairs insulin action via serine phosphorylation of insulin receptor

substrate-1, which disrupts downstream activation of phosphatidylinositol-3-kinase-AKT<sup>(31)</sup>. In genetic and DIO rodent models, lack of JNK-1 but not JNK-2 reduced adiposity coincident with attenuated IR and enhanced insulin signalling in liver tissue<sup>(40)</sup>. Furthermore, whole body JNK-1 knockout and adipose-specific JNK-1 deficiency ameliorated obesity-induced hepatic inflammation and IR<sup>(41,42)</sup>. On the other hand, deletion of JNK-1 in haematopoietic cells did not alter body weight, but provided protection from HFD-induced IR and inflammation, while abrogation of JNK-1 in non-haematopoietic cells reduced adiposity concomitant with improved insulin sensitivity<sup>(43)</sup>. Similarly, myeloid-specific deletion of inhibitor of NF- $\kappa$ B kinase- $\beta$  alleviated HFD promoted IR<sup>(44)</sup>. Taken together, these two aforementioned studies provide convincing evidence that obesity associated IR is strongly dependent on myeloid cell activity such as ATM.

Dysregulated fatty acid flux has been implicated in the activation of inflammatory macrophages. Toll-like receptor (TLR)-4 is a member of the pattern-recognition receptor family involved in innate immunity<sup>(45)</sup>. Studies indicate that SFA can stimulate both macrophage and adipocyte inflammation by activating the NF- $\kappa$ B and JNK signalling pathway via TLR-4 *in vitro*<sup>(46)</sup>. Acute lipid infusion promoted adipose tissue inflammation via NF- $\kappa$ B and systemic IR in WT but not TLR4<sup>-/-</sup> mice. Coincident DIO TLR4<sup>-/-</sup> mice exhibited attenuated IR, reduced ATM

recruitment and improved adipose tissue inflammation<sup>(46,47)</sup>. Mice exhibiting a loss of function mutation in TLR-4 displayed reduced HFD-induced weight gain, increased energy expenditure and enhanced insulin sensitivity in peripheral tissues<sup>(47)</sup>. Furthermore, haematopoietic cell specific deletion of TLR ameliorated HFD-induced hyperinsulinemia, hepatic and adipose tissue IR, concomitant with impaired ATM content<sup>(48)</sup>. It is clear from these studies that TLR-4 plays a critical role in adipose tissue inflammation and IR; moreover, macrophages have transpired as the predominant mediators of TLR-4 responses in adipose tissue<sup>(49)</sup>.

#### Adipose tissue macrophages in mice v. men: limitations of the M1/M2 classification

Macrophages are professional phagocytic cells<sup>(50)</sup> derived from peripheral blood mononuclear cells and are present in the majority of tissue types. Macrophages present diverse functionality and are classified into two populations (1) 'classically activated' M1 macrophages or (2) 'alternatively activated' M2 macrophages<sup>(51)</sup>. Helper T-cell 1 derived interferon- $\gamma$  or lipopolysaccharide can prime macrophages towards a pro-inflammatory phenotype<sup>(52)</sup>. M1 macrophages secrete copious amounts of pro-inflammatory cytokines; TNF- $\alpha$ , IL-1 $\beta$  and IL-6, produce increased nitric oxide synthase and reactive oxygen species<sup>(53)</sup>. Conversely, M2 macrophages exhibit an anti-inflammatory potential and are divided into sub-populations associated with wound healing and regulatory immunity. IL-4 and IL-13 produced during innate or adaptive immunity promote wound-healing macrophages by increasing arginase activity contributing to extracellular matrix production and tissue repair<sup>(53)</sup>. Macrophages treated with IL-4 and/or IL-13 *in vitro* display reduced pro-inflammatory cytokine signature<sup>(54)</sup>. Regulatory macrophages are induced by a transient signal such as PG in combination with a TLR ligand and are characterised by their ability to produce anti-inflammatory IL-10 and down-regulate IL-12 production<sup>(53)</sup>.

In 2007, the 'phenotypic switch' model was proposed, which suggested that in an obesogenic environment ATM are polarised from an M2 to an M1 activation state<sup>(55)</sup>. In these studies, M1 and M2 macrophages were characterised by differential expression of specific surface markers. M1 macrophages are defined by the presence of general macrophage marker F4/80 and myeloid cell markers CD11c, CD11b (F4/80<sup>+</sup>CD11c<sup>+</sup>CD11b<sup>+</sup>)<sup>(55,56)</sup>. M2 ATM are sparsely scattered in the interstitial spaces between adipocytes in lean adipose tissue<sup>(55)</sup> and express significantly elevated levels of anti-inflammatory IL-10 in response to NEFA<sup>(57)</sup>. Immunofluorescence analysis demonstrated that CD11c<sup>+</sup> M1 cells are the predominant cell population involved in crown-like structure formation surrounding necrotic adipocytes in obese mice<sup>(55,57)</sup>. Nguyen *et al.* demonstrated bone marrow-derived dendritic cells expressing F4/80<sup>+</sup>CD11b<sup>+</sup>CD11c<sup>+</sup> are activated via TLR2/4 and JNK signalling pathways in response to NEFA and exhibit enhanced inflammatory signature secreting elevated levels of IL-1 $\beta$  and IL-6. Moreover, exposure of skeletal

muscle cells to CD11c<sup>+</sup>-conditioned media impaired glucose uptake. Coincident adipose tissue derived F4/80<sup>+</sup>CD11b<sup>+</sup>CD11c<sup>+</sup> cells treated with NEFA *ex vivo* displayed heightened inflammatory activity; increased TNF- $\alpha$ , CC-chemokine receptor 2 (CCR2) and TLR-4 expression, compared with CD11c<sup>-</sup> cells<sup>(57)</sup>. Diphtheria toxin-induced ablation of CD11c<sup>+</sup> cells *in vivo* normalised insulin sensitivity in obese mice coincident with reduced local and systemic inflammatory gene expression<sup>(58)</sup>.

The M1/M2 macrophage classification system has been largely defined *in vitro* and is considered as an oversimplification of macrophage phenotype *in vivo*. It has become increasingly apparent that macrophages display a combination of M1/M2 properties. A recent DIO mouse model demonstrated mixed M1/M2 ATM profiles, wherein three distinct ATM populations were characterised in obese adipose tissue; MGL1<sup>+</sup>/CD11c<sup>-</sup>, MGL1<sup>-</sup>/CD11c<sup>+</sup> and MGL1<sup>med</sup>/CD11c<sup>+</sup><sup>(59)</sup>. Importantly, the previously defined as M2 ATM (MGL1<sup>+</sup>/CD11c<sup>-</sup>), acquired an alternative transcriptional profile on exposure to an HFD. Classical M2 transcripts including IL-1Ra and IL-10 were up-regulated in addition to the expression of a small number of M1 transcripts including inducible nitric oxide synthase. M1 MGL1<sup>-</sup>/CD11c<sup>+</sup> ATM exhibited a characteristic increase in pro-inflammatory genes and gene expression analysis revealed genes involved in matrix remodelling and angiogenesis that were up-regulated. Intriguingly 60–70% of CD11c<sup>+</sup> cells co-expressed MGL1 (MGL1<sup>med</sup>/CD11c<sup>+</sup>) and this sub-population displayed an intermediate phenotype between MGL1<sup>+</sup>/CD11c<sup>-</sup> and MGL1<sup>-</sup>/CD11c<sup>+</sup> ATM<sup>(59)</sup>. These findings corroborate with Westcott *et al.*, which unexpectedly showed that mice lacking in the traditional alternatively activated M2 MGL surface marker displayed significant reduction in CD11c<sup>+</sup> ATM infiltration, attenuated inflammation and hepatic steatosis<sup>(60)</sup>. A further study also defined three ATM populations in obese adipose. In accordance with the 'phenotypic switch' model, DIO caused a shift from MR<sup>+</sup>/CD11c<sup>-</sup> (M2) ATM population to MR<sup>-</sup>/CD11c<sup>+</sup> (M1) population. However, an increase in a third population was observed; the MR<sup>-</sup>/CD11c<sup>-</sup> double negative population, which simulated an M2-like population<sup>(61)</sup>. Li and colleagues further highlight the complexity of M1/M2 prototype *in vivo*. In their study, HFD-induced IR mice were fed a normal chow diet and CD11c<sup>+</sup> cell functionality was assessed. CD11c<sup>+</sup> ATM number remained consistent; however, inflammatory phenotype was attenuated<sup>(62)</sup>.

Further adding to the complexity of M1/M2 classification extrapolating the significance of ATM in IR from rodent models to human subjects is confounded by differences in gene expression and cell surface receptors; for instance, human subjects exhibit reduced arginase and CD11c expression in ATM<sup>(63,64)</sup>. Thus, the contribution of ATM to HFD-induced IR in human subjects is less well understood. Human ATM recently demonstrated a mixed M1/M2 phenotype. As such, CD206<sup>+</sup> cells increased in human subcutaneous adipose tissue and displayed a remodelling phenotype for instance expressing high levels of matrix metalloproteinase-9; paradoxically these macrophages also expressed elevated levels of pro-inflammatory

genes TNF- $\alpha$ , IL-8<sup>(65)</sup>. Furthermore, weight loss significantly altered the expression of M1 and M2 surface markers, promoting a less inflammatory profile<sup>(66)</sup>. Analysis of ATM in obese women demonstrated functionally distinct sub-populations. Coincident with obese rodent models CD11c expressing cells were predominantly located in a crown-like structure in adipose tissue. A subset of CD11c cells co-expressed M2 marker CD206 and exhibit features of M1 and M2 macrophages. Importantly, CD11c<sup>+</sup>CD206<sup>+</sup>/CD11c<sup>-</sup> ratio significantly correlated with levels of IR. *Ex vivo* analyses demonstrated CD11c<sup>+</sup>CD206<sup>+</sup>ATM secreted significantly higher levels of pro-inflammatory IL-6, TNF- $\alpha$  and IL-8 relative to CD11c<sup>-</sup>ATM. Furthermore, CD11c<sup>+</sup>CD206<sup>+</sup>ATM-conditioned media impaired insulin-stimulated glucose uptake into adipocytes<sup>(67)</sup>.

### Fatty acids and adipose tissue macrophage polarisation

At present, there is a plethora of research attempting to decipher the molecular mechanisms controlling this 'phenotypic switch'. One hypothesis is that lipids may regulate macrophage polarisation. The identification that the fatty acid sensor PPAR $\gamma$  displayed anti-inflammatory properties was the first unifying link between macrophage lipid metabolism and inflammation<sup>(68,69)</sup>. PPAR $\gamma$  is a ligand-activated transcription factor central to lipid homeostasis. It is highly expressed in adipocytes and macrophages and is essential for adipocyte differentiation and lipid storage<sup>(70,71)</sup>. PPAR $\gamma$  activity is markedly enhanced by IL-4<sup>(72)</sup> and in DIO mice, myeloid cell-specific disruption of PPAR $\gamma$  impaired M2 activation predisposing mice to adipose tissue inflammation and systemic IR<sup>(73)</sup>. Furthermore, lack of PPAR $\gamma$  suppressed numerous genes involved in nutrient uptake, fatty acid synthesis and  $\beta$ -oxidation<sup>(73)</sup>. Short-term treatment with PPAR $\gamma$  agonist rosiglitazone enhanced M2 ATM number and reduced adipose tissue pro-inflammatory IL-18 gene expression in obese mice<sup>(74)</sup>. Congruent with this, human monocytes treated with PPAR $\gamma$  agonist GW1929 or rosiglitazone, were primed towards an M2 activation state expressing enhanced surface expression of M2 MR and reduced M1 surface marker CD163 *in vitro*. Intriguingly, in atherosclerotic lesions treatment with PPAR $\gamma$  agonists GW1929 or rosiglitazone did not induce M2 marker expression in resting or M1 macrophages implying PPAR $\gamma$  solely primes native monocytes to an M2 polarisation state<sup>(68)</sup>. More recently, two distinct ATM subsets were characterised: CCR2<sup>+</sup>F4/80<sup>Hi</sup> and F4/80<sup>Low</sup> in *db/db* obese mice, mice deficient in leptin receptor activity, based on responsiveness to PPAR $\gamma$  agonist. In the absence of an agonist CCR2<sup>+</sup>F4/80<sup>Hi</sup> ATM were abundantly expressed; however, in response to PPAR $\gamma$  agonist CCR2<sup>+</sup>F4/80<sup>Hi</sup> ATM sub-population were reduced coincident with improved insulin sensitivity and inflammation<sup>(75)</sup>. Bassaganya-Riera *et al.*<sup>(76)</sup> extended this work demonstrating F4/80<sup>Hi</sup> and F4/80<sup>Low</sup> ATM subsets differentially express PPAR $\gamma$  and lack of PPAR $\gamma$  favours an M1 ATM polarisation state.

The adipose tissue 'expandability hypothesis' postulates that adipose tissue loses its ability to adequately store

excess fat resulting in lipid spill over which promotes ectopic lipid accumulation and inflammation in macrophages<sup>(69,77)</sup>. Transcriptional profile analysis of adipocytes and ATM from leptin-deficient (*ob/ob*) mice, demonstrated that increasing adiposity was associated with a reduction in lipogenic, lipid uptake and lipid storage gene expression in adipocytes but up-regulation in ATM. Correspondingly, a large portion of CD11c<sup>+</sup> M1 macrophages were present within adipose tissue. Lipidomic analysis revealed ATM selectively accumulated SCFA which coincided with M2 to M1 phenotypic switch. Finally, rosiglitazone treatment improved adipocyte lipid storage capacity, improved insulin sensitivity coincident with increased M2 markers. Correspondingly, mice over-expressing PPAR $\gamma$  target gene diacylglycerol acyltransferase 1 in macrophages and adipocytes exhibited enhanced weight gain but were protected from typical obesity-induced macrophage infiltration and inflammatory activation coincident with attenuated IR. Furthermore in this context, NEFA failed to directly activate macrophages which over-expressed diacylglycerol acyltransferase 1<sup>(78)</sup>. Taken together, these data suggest that obesity reduces adipocyte storage capacity and metabolic capabilities, which induces lipid partitioning from adipocytes into macrophages favouring M1 ATM polarisation<sup>(77)</sup>.

### Obesity-induced adipose tissue macrophage recruitment

A vital question that must be addressed regarding ATM is what triggers ATM infiltration during obesity. The precise mechanisms underpinning the initiation of ATM recruitment are unclear and are currently under intense investigation<sup>(64)</sup>. Adipocyte death and/or hypoxia are possibly both responsible for recruitment. Adipocyte necrosis increases with obesity due to adipocyte hypertrophy, and thus a plausible explanation for macrophage recruitment during obesity is for the removal of dead and dying cells<sup>(79)</sup>. Indeed, in obese mice and human subjects greater than 90% of ATM were shown to be specifically localised in a crown-like structure surrounding necrotic adipocytes<sup>(79)</sup>. These activated macrophages exhibit increased IL-6 and TNF- $\alpha$  expression and were shown to ingest residual lipid droplets forming large lipid-laden multinucleate giant cells, a characteristic feature of chronic inflammation<sup>(79)</sup>. Similarly, adipocyte apoptosis is markedly increased in obese mice and human subjects<sup>(80)</sup>. Hypoxia is a pathogenic state whereby the surrounding tissue is devoid of adequate oxygen supply resulting in decreased oxygen tension. Local hypoxia is observed in obese adipose tissue in mice and human subjects<sup>(81,82)</sup>. In DIO mice, adipose tissue hypoxia is correlated with enhanced pro-inflammatory gene expression and reduced adiponectin expression. Conversely, weight loss improved adipose oxygenation and increased adiponectin expression<sup>(83)</sup>. Moreover, hypoxic conditions up-regulate additional adipokines and adipose-tissue-derived cytokine expression, including leptin and IL-6<sup>(84)</sup>. Hypoxia-inducible factor-1 $\alpha$  is a critical regulator in cellular hypoxic response, which is rapidly degraded under

normoxic conditions and is increased early in obesity. Over-expression of hypoxia-inducible factor-1 $\alpha$  in a transgenic mouse model initiated adipose tissue fibrosis, with an accompanying increase in local inflammation<sup>(85)</sup>. In human subjects, phosphorylated levels of p38 are significantly up-regulated in visceral adipose tissue. Disrupting p38 activity reduced hypoxia-induced stromal vascular fraction inflammatory responses, implicating a role for p38 activity in the regulation of hypoxia<sup>(86)</sup>. These studies strongly suggest that adipocyte cell death and hypoxia-induced inflammation may be potential underlying causes of ATM infiltration during obesity.

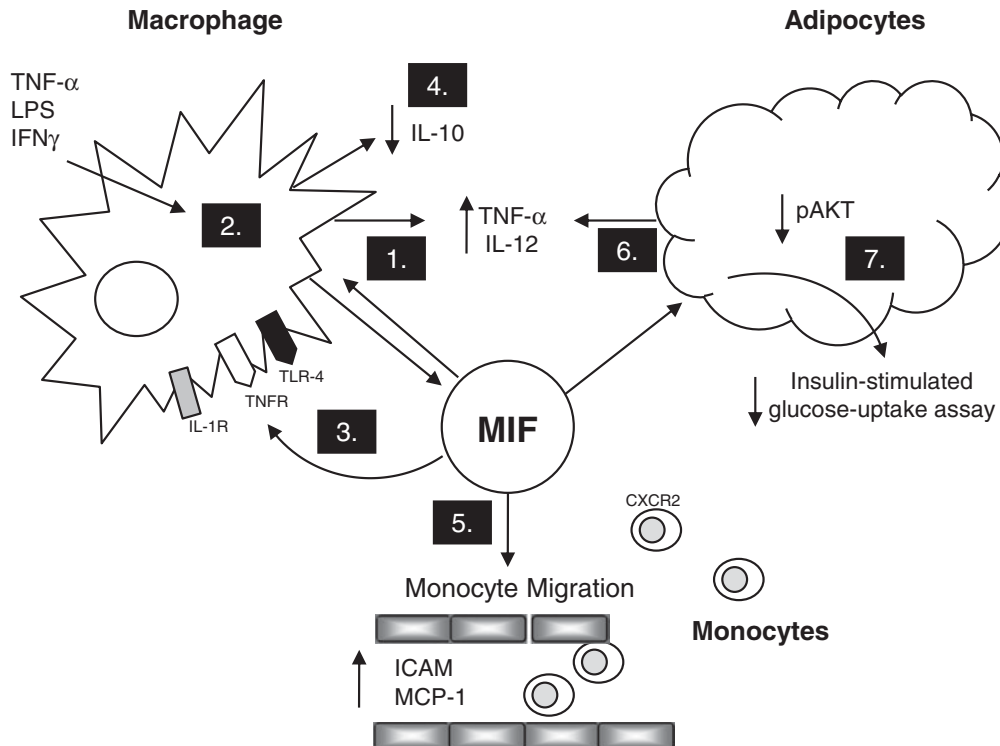
Chemokines have emerged as crucial mediators in obesity-associated ATM recruitment into adipose tissue. They are small pro-inflammatory cytokines which instigate monocyte recruitment from the bone marrow to the site of inflammation<sup>(87,88)</sup>. More than 50 cytokines/chemokines have been described and categorised into four distinct groups in accordance with the location of the conserved cysteine residue; CCL, CXCL, CL and CX3CL<sup>(89,90)</sup>. The pathophysiological role of CCL2/MCP-1 and its receptor CCR2 has been most thoroughly investigated. CCL2 is ubiquitously expressed and found in monocytes and adipocytes and thus has been implicated in several biological processes and disease states<sup>(91)</sup>. Circulating concentrations and adipose tissue expression levels of CCL2 positively correlate to adiposity<sup>(9)</sup>. In addition, 3T3-L1 adipocytes treated with CCL2 exhibited a marked reduction in insulin-simulated glucose uptake *in vitro*<sup>(92,93)</sup>. Absence of CCR2 in DIO mouse model partially ameliorated HFD-induced obesity, hepatic steatosis coincident with attenuated systemic IR. Immunohistochemistry analysis demonstrated reduced ATM recruitment, concomitant with reduced adipose tissue TNF- $\alpha$  expression. Further expression analysis revealed that CCR2 directly altered genes involved in lipid metabolism and adipocyte differentiation, with CCR2<sup>-/-</sup> mice exhibiting increased expression of fatty acid binding protein-4 and PPAR $\gamma$ . Correspondingly, short-term treatment with a CCR2 antagonist decreased macrophage infiltration and enhanced systemic insulin sensitivity in DIO mice<sup>(51)</sup>. In support of these findings, a subsequent study demonstrated lack of CCL2-attenuated adipose tissue inflammation and IR, while over-expression of CCL2 in adipose tissue caused increased ATM number coincident with exaggerated systemic IR<sup>(94,95)</sup>. However, CCR2 and CCL2 knockout mice models retain significant number of ATM<sup>(51,94)</sup>. The role of CCL2 in obesity maybe further confounded by the effect of CCL2 on metabolism. Lack of CCR2 reduced HFD-induced obesity, while plasma CCL2 levels decreased exponentially with pharmacological-induced weight reduction in DIO mice<sup>(51,93)</sup>. A number of studies have disputed the involvement of the CCR2/MCP-1 axis in obesity and IR<sup>(93,96)</sup>. Abrogating CCR2 in DIO mice did not alter body weight, glucose homeostasis or ATM infiltration<sup>(93)</sup>. Coincidentally, Inouye and co-workers showed that CCL2-deficient mice exhibited increased HFD-induced weight gain and comparable macrophage recruitment capabilities to control mice. CCL2-deficient mice also displayed impaired glucose tolerance, and in fact presented increased plasma glucose and

reduced adiponectin levels compared with control mice<sup>(96)</sup>. These studies suggest that infiltrating macrophages are not solely pro-inflammatory and indicate that factors independent of CCR2/CCL2 are involved in macrophage recruitment.

Emerging data have proposed a pathogenic role for alternative chemokines in obesity. CX3CL1, a human chemokine is expressed in adipocytes and increased with obesity<sup>(97,98)</sup>. Furthermore, genetic variations within CX3CR1 gene correlate to increased weight circumference, IR and reduced adiponectin<sup>(98)</sup>. A causal role for CX3CL1 in adipose tissue inflammation has been displayed whereby CX3CL1 induced monocyte adhesion to human adipocytes *ex vivo*<sup>(98)</sup>. A role for keratinocyte-derived chemokine, a mouse orthologue for human IL-8, in obesity-induced ATM recruitment has been proposed. Keratinocyte-derived chemokine and its receptor CXCR2 have been implicated in co-ordinating macrophage infiltration into atherosclerotic lesions<sup>(99,100)</sup>. Circulating concentrations and adipose tissue expression levels are heightened during obesity. Furthermore, keratinocyte-derived chemokine induced an inflammatory response in 3T3-L1 adipocytes *in vitro*, up-regulating IL-6, TNF- $\alpha$  and MCP-1 expression. Another CXCR2 ligand is CXCL5. CXCL5 is abundantly expressed in adipose tissue, predominantly within the stromal vascular fraction. Circulating CXCL5 levels increased with obesity concomitant with enhanced secretion from obese adipose tissue explants compared with lean *ex vivo*. Furthermore, exogenous CXCL5 treatment reduced muscle insulin sensitivity via activation of suppressor of cytokine signalling pathway<sup>(90)</sup>. Confirming the importance of both keratinocyte-derived chemokine and CXCL5 in obesity-induced inflammation and IR mice deficient in CXCR2 exhibit reduced ATM recruitment and attenuated IR in HFD-induced obese mice models<sup>(90,101,102)</sup>. Another potential chemokine receptor that could mediate ATM recruitment and alter IR is CCR5<sup>(103)</sup>. CCR5 and its ligands are up-regulated in both obese human and rodent models<sup>(103,104)</sup>. DIO mice lacking CCR5 are protected from the development of hepatic steatosis and exhibit improved insulin sensitivity. Furthermore, deletion of CCR5 alters both ATM number and the M1/M2 polarisation state with a corresponding reduction in adipose tissue inflammation and improved insulin sensitivity<sup>(103)</sup>. These studies illustrate that chemokines are central to ATM recruitment; however, it is yet to be fully established what enhances their secretion.

### Macrophage migration inhibitory factor

MIF is a multifunctional pro-inflammatory cytokine whose actions are responsible for an array of inflammatory processes. Macrophages have been identified as a primary source and target of MIF *in vitro* and *in vivo*<sup>(105)</sup>. Uniquely, MIF is rapidly released from preformed intracellular pools in response to inflammatory stimuli: Lipopolysaccharide, TNF- $\alpha$  and interferon- $\gamma$  and can elicit its effects in a paracrine and autocrine manner<sup>(105–107)</sup>. In this regard, MIF can inhibit immunosuppressive



**Fig. 2.** Role of macrophage migration inhibitory factor (MIF) in inflammation and adipose tissue dysfunction. 1. MIF activates macrophages inducing inflammatory cytokine secretion; TNF- $\alpha$  and IL-12. 2. Inflammatory stimuli lipopolysaccharide (LPS), TNF- $\alpha$  and interferon- $\gamma$  can induce MIF secretion from macrophages. 3. Endogenous MIF can regulate toll-like receptor-4 (TLR-4), IL-1 receptor (IL-1R)1, TNF receptor (TNFR) expression. 4. MIF inhibits macrophage-derived anti-inflammatory IL-10 secretion. 5. MIF is involved in monocyte recruitment via transmigration and recruitment of monocytes by up-regulating intracellular adhesion molecule-1 (ICAM) and monocyte chemoattractant protein-1 (MCP)-1 and can also be by binding to CXC-chemokine receptor (CXCR)2. 6. MIF can promote TNF- $\alpha$  secretion from adipocytes. 7. MIF can impair insulin-stimulated glucose uptake into adipose tissue and down-regulate phosphorylated AKT expression.

glucocorticoids, promote secretion of a variety of cytokines including TNF- $\alpha$ , IL-2, IL-6, IL-8, interferon- $\gamma$ , IL-1 $\beta$  and inhibit IL-10 propagating a pro-inflammatory response<sup>(105–108)</sup>. Endogenous MIF regulates innate immunity via up-regulation of TLR-4, IL-1R1 and TNFR expression<sup>(109,110)</sup>. Roger and co-workers demonstrated that MIF<sup>-/-</sup> macrophages were hyporesponsive to lipopolysaccharide and failed to secrete TNF- $\alpha$  and IL-6 due to profound suppression of inhibitor of NF- $\kappa$ B kinase- $\beta$ /NF- $\kappa$ B activity<sup>(109)</sup>. In addition, MIF deficiency suppressed IL-1 and TNF- $\alpha$ -induced mitogen-activated protein kinases activity and cell proliferation in mouse fibroblasts. Reconstitution of an upstream mitogen-activated protein kinase or MIF reversed these affects<sup>(110)</sup>. Furthermore, MIF impairs p53-dependent apoptosis sustaining activated macrophage lifespan, thus further amplifying the inflammatory response<sup>(111)</sup>. Another mechanism identified by which MIF promotes inflammation is through amplification of transmigration, recruitment and activation of leukocytes at the site of inflammation through up-regulation of adhesion molecules such as intracellular adhesion molecule-1 and chemokine MCP-1<sup>(112–114)</sup>. MIF can exert its chemotactic properties via CXCR2 and CXCR4 in macrophages and T-cells, respectively<sup>(114)</sup>. Binding of

MIF to CXCR4 on the surface of fibroblasts and T-cells induced JNK propagating CXCL8 secretion<sup>(115)</sup>. Intriguingly, an alternative MIF receptor CD74, traditionally involved in the activation of mitogen-activated protein kinases pathway, has recently been demonstrated to also mediate macrophage chemotactic responses<sup>(116)</sup>. Given its diverse functionality and prominent role in macrophage biology MIF has been implicated in a multitude of inflammatory diseases<sup>(107)</sup>. More recently, a pathogenic role has been ascribed to MIF in obesity-associated inflammation and related metabolic complications (Fig. 2).

#### The role of macrophage migration inhibitory factor in obesity and type-2 diabetes mellitus

Epidemiological studies implicate MIF in obesity and T2DM<sup>(117–120)</sup>. Obese individuals with a BMI of 37.5 (SD 4.9) kg/m<sup>2</sup> have significantly elevated plasma MIF concentrations (2.8 (SD 2.0) ng/ml) compared with lean individuals with a BMI of 22.6 (SD 3.4) kg/m<sup>2</sup> (1.2 (SD 0.6) ng/ml). During obesity MIF mRNA expression is up-regulated by 60% in circulating peripheral blood mononuclear cells<sup>(117,118)</sup>. Furthermore, a positive

association between elevated plasma MIF concentrations and peripheral blood mononuclear cells MIF mRNA with BMI, NEFA concentration and impaired glucose tolerance has been shown<sup>(120,121)</sup>. A polymorphism within the MIF gene promoter (MIF\_794[CATT]<sub>5-8</sub>) was associated with obesity in a Japanese population<sup>(122)</sup>. Treatment with the anti-diabetic drug metformin decreased plasma MIF concentration from 2.3 (SD 1.4) to 1.6 (SD 1.2) ng/ml in obese individuals following a 6-week intervention. Cessation of drug treatment reversed these effects<sup>(118)</sup>. Furthermore, a weight loss programme in morbidly obese individuals that achieved a 14.4 kg reduction in body weight was associated with lower serum MIF concentrations and improved pancreatic  $\beta$ -cell function<sup>(119)</sup>.

It has been demonstrated that patients with T2DM have higher serum MIF (20.7 (13.3) ng/ml) levels compared with non-diabetic controls (5.2 (SD 3.0) ng/ml)<sup>(123)</sup>. The Pima Indians display increased susceptibility to T2DM. Healthy lean Pima Indians exhibit elevated serum MIF concentrations relative to healthy lean Caucasians. This elevated MIF concentration was directly associated with impaired insulin action; supporting a link between MIF and increased susceptibility to IR and T2DM<sup>(120)</sup>. A subsequent case-control study based on the Cooperative Health Research in the region of Augsburg Survey 4 (KORA4: 1999–2000) serum MIF concentration increased exponential from normoglycemia to impaired glucose tolerance and overt T2DM. This study indicated that MIF was a more proficient discriminatory factor between healthy individuals and patients with impaired glucose tolerance or T2DM compared with IL-6 or C-reactive protein 2<sup>(124)</sup>. Results from the population-based study monitoring of trends and determinants in cardiovascular disease/KORA presented evidence of a triangular relationship between MIF levels, SNP within the MIF gene and the incidence of T2DM in women. The C allele of SNP rs1007888 was associated with increased circulating MIF, while rs1007888CC was correlated to T2DM in female subjects. Moreover, elevated MIF concentrations correlated to the incidence of T2DM to a greater extent in obese women compared with lean<sup>(125)</sup>. More recently, rs1007888 also showed association with enhanced susceptibility to gestational diabetes mellitus and postpartum metabolic syndrome<sup>(126)</sup>.

### The role of macrophage migration inhibitory factor in glucose metabolism and adipose tissue dysfunction

Given that epidemiological data suggest an association between MIF, obesity and glucose homeostasis, molecular studies have focused on whether MIF is a causal factor in relation to dysregulated glucose metabolism and adipose tissue dysfunction. Waeber *et al.*<sup>(127)</sup>, first implicated a role for MIF within glucose metabolism when they highlighted the presence of MIF within the secretory granules in rodent pancreatic  $\beta$ -cells. The production of MIF from these cells was demonstrated to be glucose dependent and on release regulate insulin secretion in an autocrine manner. Immunoneutralisation of MIF-impaired glucose-induced insulin secretion during exposure to exogenous recombinant MIF potentiated insulin release<sup>(127)</sup>.

More recently, MIF inhibition provided significant protection from palmitic-acid induced  $\beta$ -cells apoptosis<sup>(128)</sup>. Together, these studies suggested a potential pathogenic role for MIF in fatty acids induced  $\beta$ -cell dysfunction and hyperinsulinemia.

MIF has been shown to be secreted from murine and human adipocytes<sup>(12,121,129,130)</sup>. Moreover, glucose and insulin reportedly regulate adipocyte MIF expression *in vitro*<sup>(131)</sup>. Treatment of 3T3-L1 adipocytes with TNF- $\alpha$  induced MIF secretion, suggesting that TNF- $\alpha$  may regulate MIF production during obesity<sup>(129,130)</sup>. *In vivo* MIF-deficient mice were hyporesponsive to TNF- $\alpha$  administration and exhibited normoglycemia compared with control mice, indicating MIF is required for successful TNF- $\alpha$  action. Furthermore, 3T3-L1 adipocytes exposed to exogenous MIF demonstrated impaired insulin-stimulated glucose uptake and insulin receptor signal transduction. Similarly, in response to inflammatory stress, MIF<sup>-/-</sup> mice exhibit a marked improvement in adipose tissue glucose uptake compared with control mice<sup>(132)</sup>. This study suggested that MIF affects glucose homeostasis both directly and indirectly via TNF- $\alpha$  regulation. In obese patients subcutaneous adipose tissue explants were shown to release significant quantities of MIF over 24 h (10 000 pg/ml in 24 h) and these concentrations presented a direct correlation to individual donor BMI<sup>(121)</sup>. More recently, Koska *et al.* demonstrated that MIF mRNA expression in subcutaneous adipose tissue increased with adipocyte size and was negatively correlated to peripheral and hepatic IR<sup>(12)</sup>. Correspondingly, intracellular levels of MIF were found to be heightened during adipogenesis *in vitro*. Conversely, genetic deletion of MIF in rodent models and siRNA knockdown of MIF in 3T3-L1 adipocytes reduced intracellular lipid accumulation and impaired adipogenic gene PPAR $\gamma$  expression<sup>(132,133)</sup>. Verschuren and co-workers have shown that lack of MIF in an atherosclerotic mouse model (LDLR<sup>-/-</sup>MIF<sup>-/-</sup>) maintained on a standard chow diet exhibit attenuated adipose tissue inflammation and enhanced adipose tissue insulin sensitivity coincident with improved glucose homeostasis and systemic inflammation<sup>(134)</sup>. In response to an acute inflammatory challenge, IL-1 $\beta$  administration, LDLR<sup>-/-</sup>MIF<sup>-/-</sup> mice exhibited reduced circulating serum amyloid A, a marker of systemic inflammation. Subsequent histological analysis revealed that lack of MIF reduced adipocyte size and MAC3<sup>+</sup> macrophage population. Gene expression analysis indicated that the ratio of M1/M2 was reduced in LDLR<sup>-/-</sup>MIF<sup>-/-</sup> mice. Furthermore, thioglycollate-elicited macrophages isolated from MIF<sup>-/-</sup> mice exhibited reduced IL-6 secretion when stimulated with lipopolysaccharide compared with control mice. Consequently, PI3-kinase activity, a functional marker of insulin signalling, was greater in LDLR<sup>-/-</sup>MIF<sup>-/-</sup> adipose tissue. In addition, deletion of MIF reduced monocyte adhesion, macrophage lesion content and atherosclerotic lesion size<sup>(134)</sup>. On the contrary, mature MIF<sup>-/-</sup> mice maintained on a standard chow diet for 12 months demonstrated impaired glucose tolerance<sup>(11)</sup>. These contradictory findings underscore the complexity of MIF inflammatory signals within glucose metabolism depending on age.



## Conclusion

Extensive evidence implicates inflammation as a critical factor in the progression of obesity-related metabolic abnormalities. ATM accumulation significantly contributes to adipose tissue inflammation resulting in impaired insulin action, thus contributing to systemic IR and the development of T2DM. However, the triggers responsible for ATM recruitment and activation are not fully understood. Adipose tissue-derived chemokines have emerged as principal mediators in propagating inflammation and IR. Given MIF chemotactic properties, MIF may be a primary candidate promoting ATM recruitment during obesity. Pro-inflammatory MIF activity is enhanced during obesity and is directly associated with the degree of peripheral IR. Current anti-inflammatory therapeutics exhibit a broad range of actions which consequently may induce an immunocompromised phenotype<sup>(49)</sup>. Targeting the functionality of specific tissues, for example targeting ATM inflammatory responses, may prove to be more beneficial. Indeed, several population studies have identified a number of SNP within the MIF gene promoter region associated with obesity and T2DM. Thus, manipulating MIF inflammatory activities in obesity, using pharmacological agents or functional foods, may be therapeutically beneficial for the treatment and prevention of obesity-related metabolic diseases.

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