

# Adipose tissue macrophages: Regulators of adipose tissue immunometabolism during obesity



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## ABSTRACT

**Background:** Adipose tissue macrophages (ATMs) are a well characterized regulator of adipose tissue inflammatory tone. Previously defined by the M1 vs M2 classification, we now have a better understanding of ATM diversity that departs from the old paradigm and reports a spectrum of ATM function and phenotypes in both brown and white adipose tissue.

**Scope of Review:** This review provides an updated overview of ATM activation and function, ATM diversity in humans and rodents, and novel ATM functions that contribute to metabolic homeostasis and disease.

**Major Conclusions:** While the paradigm that resident ATMs predominate in the lean state and obesity leads to the accumulation of lipid-associated and inflammatory ATMs still broadly remains rigorously supported, the details of this model continue to be refined and single cell data provide new insight into ATM subtypes and states.

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**Keywords** Adipose tissue; Macrophage; Obesity; Inflammation; Lipids

## 1. INTRODUCTION

It has been almost 20 years since the investigation of adipose tissue (AT) biology and metabolism collided with immunology research as studies highlighted the presence of resident adipose tissue leukocytes such as adipose tissue macrophages (ATMs) [1]. These discoveries led to a reassessment of the structure, composition, and regulation of adipose tissue function. We now know that a network of leukocytes exists in adipose tissue that includes components of both the innate and adaptive immune systems. Innate immune components such as ATMs, adipose tissue dendritic cells (ATDC), natural killer (NK) cells, and innate lymphoid cells (ILC) dominate the resident leukocyte population in adipose tissue [2–4]. Recent studies improve the delineation of resident adipose tissue leukocytes distinct from blood-derived leukocytes found in adipose tissue and support the presence of a distinct pool of leukocytes sequestered within lean and obese adipose tissue [5–7].

The next challenges in the field of AT immunometabolism center on expanding our understanding of AT inflammation in the context of how the wide range of AT leukocytes communicate with each other and separate from how each cell type stands alone. In this light, ATMs

serve as a key inflammatory node in the coordination of immune responses between innate and adaptive immune cells. The capacity of macrophages to function in antigen presentation, cytokine production, host defense, and patrolling the local microenvironment to maintain homeostasis has drawn attention to the diversity of ATM subtypes or states revealed by recent single cell transcriptomic studies [8]. ATM diversity aligns with the diversity of AT, which we now know encompasses multiple types (e.g., brown, beige/brite, white) that span a range of functions related to their anatomic location and role in metabolism [9]. This diversity provides a future challenge and opportunity to understand new aspects of macrophage biology in AT. Here we provide a review of recent insights and advances in our understanding of the function of ATMs and their role as regulators of AT immunometabolism presented in the context of their diversity. The initial model of “M1 vs M2” ATMs proposed by our group many years ago is outdated and overly simplistic in relation to our current understanding of macrophage diversity [10]. We hope to highlight novel concepts in ATM regulation that can set the stage for future discoveries and expansion. In addition, we highlight future areas for development and investigation that will move the field toward a more comprehensive understanding of ATMs in health and disease.

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**Abbreviations:** AT, Adipose tissue; ATM, Adipose tissue macrophage; MMe, Metabolically activated macrophage; CLS, Crown-like structures; LAM, Lipid-associated macrophage; HFD, High-fat diet; ND, Normal diet; VAT, Visceral adipose tissue; BAT, Brown adipose tissue; WAT, White adipose tissue; eWAT, Epididymal white adipose tissue; iWAT, Inguinal white adipose tissue; T2D, Type 2 Diabetes

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for many types of tissue macrophages including in adipose tissue, liver, and the brain [21,25,26].

Surprisingly, the capacity of weight loss or calorie restriction to reverse the accumulation of LAMs in adipose tissue appears to be limited [26–28]. Weight loss interventions in mice do not restore AT immune cells to the lean state which may have implications for health and metabolic risk for individuals who were formerly obese.

### 3. ATM ACTIVATION AND FUNCTION

The range of functions of ATMs is vast and includes their capacity to proliferate in response to adipose tissue remodeling, control of fibrotic responses and ECM production, activate adaptive immune cells such as T cells by their function as antigen presenting cells, production of metabolic enzymes and factors that control intercellular communication in adipose tissue, modulation of adipocyte insulin sensitivity, and regulation of adipose tissue progenitor activation. (Figure 2) We will highlight some of the more recent advances in identifying novel ATM functions and mechanisms of action.

#### 3.1. ATM cytokine production and TLR4 signaling

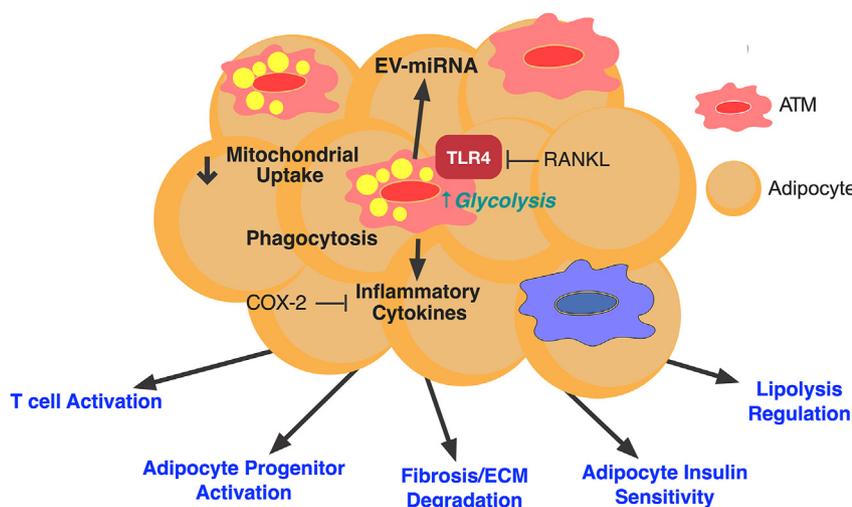
The capacity of ATMs to generate pro-inflammatory cytokines such as TNF $\alpha$  and IL-6 has been reproducibly shown to be a key feature of ATM function. TLR4 activation and its downstream signaling pathways have been shown to be essential for adipose tissue immune cell crosstalk and ATM activation and cytokine secretion [12,29,30]. Recent studies suggest inhibitory pathways in ATMs may play an important role in attempting to restrain inflammatory signals. The receptor activator of NF- $\kappa$ B ligand (RANKL) inhibits TLR4 activation in ATMs, subsequently crippling ATM cytokine production [31]. RANKL promotes the interaction of its receptor RANK with TRAF6, preventing TLR4 activation, and inhibiting iNOS, TNF $\alpha$ , and IL1 $\beta$  production in ATMs [31]. This data suggests that RANKL may be a future target to limit the inflammatory response in ATMs. TLR4 signaling also regulates COX-2 expression [32], which is induced in ATMs during obesity and has been shown to limit AT inflammation [33]. Myeloid-specific deletion of COX-2 in obese mice resulted in worsened inflammation compared to WT obese mice. COX-2

deficient mice had increased ATM proliferation, pro-inflammatory cytokine production, and crown like structures (CLS) in adipose tissue.

#### 3.2. ATM phagocytic capacity as a regulator of the adipose tissue environment

One of the earliest observations is the clustering of CD11c<sup>+</sup> LAM ATMs around dead and dying adipocytes presumable to clear debris and lipid droplets. Analysis of the major subset of resident ATMs from murine epididymal white adipose tissue (eWAT) defined by TIM4 and CD163 showed that all subsets have phagocytic capacity with the TIM4<sup>+</sup> populations having the greatest endocytic potential *in vitro* and *in vivo* [8]. The phagocytic capacity of TIM4<sup>+</sup> ATMs decreases with age but does not change with HFD. In obesity, ATMs contribute to the formation of multinucleated giant cells with high phagocytic function and the capacity to digest large particles in obese adipose tissue [34]. However, the true nature of the type of phagocytosis ATMs participate in within CLS is unclear. Studies suggest that a unique type of phagocytosis termed exophagy is the primary mechanism of CLS formation, where lysosomal enzymes are delivered to the extracellular space to facilitate the digestion of adipocyte fragments [3]. Weight loss by calorie restriction appears to activate ATM phagocytic capacity suggesting a distinct remodeling function of ATMs in settings of adipose tissue contraction [26].

Beyond the digestion of adipocytes, ATMs may participate in the uptake of other byproducts of adipocyte remodeling. Transfer of mitochondria from stressed or activated adipocytes has been shown to occur between adipocytes and macrophages in WAT and brown adipose tissue (BAT). In BAT, ATMs are responsible for removing extracellular vesicles carrying damaged mitochondria released from brown adipocytes during thermogenic activation [35]. Adipocyte to ATM mitochondrial transfer was observed to be decreased in eWAT with obesity [36]. Blockade of ATM mitochondrial transfer by inhibition of heparan sulfate biosynthesis led to increased adipose tissue mass and insulin resistance, suggesting mitochondrial clearance as a novel homeostatic function of ATMs in maintaining metabolic homeostasis. Dietary factors appear to regulate the amount of mitochondrial transfer between adipocytes and ATMs. Long-chain fatty acids suppress ATM



**Figure 2: Broad functions for ATMs in the regulation of adipose tissue homeostasis.** ATMs have a broad range of functions in different aspects of adipose tissue remodeling. Novel functions for ATMs include mitochondrial uptake which is a protective mechanisms that is suppressed with obesity, induction of glycolysis, induction of phagocytic capacity, generation and reception of extracellular vesicles (EV), and generation of inflammatory cytokines.

mitochondrial capture increasing circulating mitochondrial load from adipocytes for delivery to other organs [37]. Obese mice with myeloid-specific deletion of COX-2 had decreased expression of *LAMP2*, *CD36*, and *Gas6* expression in adipose tissue, indicative of decreased phagocytotic capacity [33], suggesting that COX-2 is essential for phagocytosis by ATMs.

### 3.3. ATM extracellular vesicles and intercellular communication

Another form of intercellular communication in adipose tissue that involves ATMs is the production and uptake of extracellular vesicles (EV). Adipose tissue derived EV were shown to be taken up by monocytes and promote pro-inflammatory cytokine production by monocyte-derived macrophages [38]. Such EV were shown to promote insulin resistance when injected into mice. Adipocyte derived EV carrying miR-34a has been shown to be induced by obesity and transferred to ATMs where it suppressed M2 polarization pathways and promote adipose tissue inflammation and insulin resistance [39]. Beyond receiving signals through EV, ATMs have been shown to potentially modulate metabolic inflammation via exosomes they generate. miR-690 is enriched in M2 polarized macrophage EV that are sufficient to improve insulin sensitivity and glucose tolerance in obese mice [40]. Insulin sensitivity has also been shown to be suppressed by the delivery of miR-155 containing EVs from ATMs to adipocytes which target PPAR $\gamma$ . While body weight was not different between mice treated with obese ATM EV in lean mice, treated mice had impaired insulin signaling and  $\sim$ 61% attenuated glucose uptake in adipocytes, myocytes, and hepatocytes. Treating mice with ATM EV from lean mice repaired insulin resistance [41].

### 3.4. Metabolic regulation of ATM function

While much of the focus of immunometabolism research has centered on how ATMs alter adipose tissue and systemic metabolism, metabolic regulation of ATM function may also play a role in the response to obesity. ATMs from obese mice express gene pathways involved in glycolysis and oxidative phosphorylation, making them distinct from LPS-activated ATMs [42]. In mice, ATM activation and cytokine production are highly dependent on the bioenergetic profile of ATMs. ATMs isolated from obese have high glycolytic and oxidative capacity [43].

Mitochondrial function and biogenesis in adipose tissue have been shown to be impaired with obesity and Type 2 Diabetes [44,45]. The activation state of macrophages is known to significantly alter mitochondrial function in macrophages with classical M1 activation making macrophages more reliant on glycolysis than oxidative phosphorylation (OXPHOS) [46]. Gaps remain in our understanding of specific mitochondrial activity in ATMs in mice or humans. However, mice with reduced mitochondrial OXPHOS due to myeloid-specific deletion of CR6-interacting factor 1 (*Crif1*) had increased M1 polarization of macrophages, AT inflammation, and insulin resistance, suggesting a role for ATM mitochondrial function in metabolic dysfunction [47]. In addition, adenine nucleotide translocase 2 (*ANT2*) is induced in obese ATMs leading to increased mitochondrial reactive oxygen species production and damage due to modulation of free fatty acid-induced mitochondrial permeability alterations [40].

Efforts to understand the role of ATM mitochondria in cytokine production led to the testing of the near-infrared dye, IR-61, as a potential candidate to treat AT inflammation. IR-61 is a small molecule that localizes to the mitochondria of ATMs, suppressing inflammatory genes in macrophages [36]. Bone marrow-derived macrophages (BMDM) treated with IR-61 had decreased TNF, IL-6, and IL1 $\beta$  mRNA

and protein expression upon LPS activation. This was paired with increased oxygen consumption rate and mitochondrial ATP production. Treatment of obese mice with IR-61 suppressed pro-inflammatory gene expression and reduced crown like structures in adipose tissue accompanied by weight loss and improvements in insulin sensitivity. Direct action of hormones altered in metabolic disease such as insulin can also shape ATM function. Insulin receptor (IR) signaling in ATMs has been shown in several studies to contribute to insulin resistance. Deletion of IR in myeloid cells led to an improvement in insulin sensitivity with obesity related to preservation of IRS2/IL-4 signaling that preserves M2 activation profiles [48]. Aligned with this observation are studies showing that activation of the IR in ATMs promotes the secretion of IL-10, which has been shown to regulate hepatic glucose metabolism, suppressing gluconeogenic genes *G6pc* and *Pck* and significantly reducing glucose concentration [49]. Insulin signaling and IL-10 production in ATMs are both stunted under obese conditions [49,50] which can promote hyperglycemia. Myeloid IR signaling may also play a role in the accumulation of ATMs and adipose tissue dysfunction with obesity. Myeloid IR deletion in obese mice decreased basal hepatic glucose production, increased insulin-stimulated glucose disposal in skeletal muscle, and blocked the accumulation of ATMs in adipose tissue with obesity [51].

A primary response to obesity and a differentiator of ATM subtypes is their accumulation and metabolism of lipids. Lipid uptake by ATMs increases with obesity in mice, with ATMs from obese mice had increased levels of triglycerides and expression of lipoprotein lipase (LPL) [19,52]. Silencing LPL in ATMs from VAT of obese mice inhibited lipid accumulation in ATMs and increased serum FFA levels but did not effect serum triglyceride levels [53]. ATMs also express hypoxia inducible lipid droplet associated (Hilpda), a protein that mediates lipid accumulation in adipose tissue and whose expression is increased with obesity and FFA exposure [54]. Overexpression of acyl CoA: diacylglycerol acyltransferase 1 (DGAT1) protects mice from adipose tissue inflammation suggesting that inflammatory activation and lipid storage may be decoupled in some situations [55].

TREM2<sup>+</sup> LAM ATMs have been shown to play a functional role in adipose tissue lipid metabolism in both mouse and human VAT [21]. LAMs from *Trem2*<sup>-/-</sup> mice on HFD effectively store lipids and failed to accumulate in adipose tissue suggesting a requirement for *Trem2* in LAM ATM accumulation [21]. We note that studies on the metabolic role of TREM2 in mice have been somewhat mixed. *Trem2*<sup>-/-</sup> mice have been shown to have worse insulin resistance and hepatic steatosis related to increased serum ceramide levels [56]. Bone marrow transplantation suggested that these effects of *Trem2* deficiency were not related to immune cell TREM2 function. Overall, while TREM2 appears to be a strong marker of LAM ATMs, the functional role of TREM2 in metabolic inflammation remains not fully understood.

## 4. BAT ATMS AND REMODELING WITH ACTIVATION

The role of resident ATMs in brown adipose tissue (BAT) is a prominent feature of BAT remodeling upon activation. Single cell RNA sequencing identifies dramatic remodeling of BAT macrophages and dendritic cells upon activation [57,58]. BAT ATMs include populations of TREM2<sup>+</sup> LAMs and localize to regions of new adipocyte formation suggesting a critical role for ATMs in remodeling BAT upon activation. Although the percentage of ATMs from BAT decreases with obesity [59], they have increased expression of pro-inflammatory cytokines induced by the activation of toll-like receptors [60]. Upon cold exposure, inflammatory

monocytes differentiate into CD45<sup>+</sup>CD11b<sup>+</sup>F4/80<sup>+</sup> ATMs in BAT, expressing markers of M2-like or anti-inflammatory ATMs [35]. Recent studies identified methyl-CpG binding protein 2 (*Mecp2*) as a potential regulator of BAT ATM activation. Mice with mutated nuclear transcription regulator *Mecp2* in BAT macrophages had increased weight gain, increased fat mass, and increased leptin secretion compared to controls. *Mecp2* knockout mice also exhibited decreased heat production evidenced by decreased expression of UCP-1 in BAT [61]. These findings indicate a role for ATMs from BAT in regulating adiposity and energy expenditure.

## 5. HUMAN ATMS VERSUS MOUSE ATMS

There remain many gaps in our understanding of human ATM diversity and its relationship with obesity and metabolic disease. Recent RNA-seq studies identify analogous ATM populations between humans and mice, however, the proportions of these cells are dramatically different in obese humans and mouse models [33,62] (Figure 1). In general, the profound induction of LAMs in obese mice is not strongly recapitulated in human obesity. While CD11c<sup>+</sup> LAM ATMs dominate in obese and insulin resistant mice, this does not appear to be a prominent feature in human adipose tissue. Initial studies suggested that CD206<sup>+</sup>CD11c<sup>+</sup> ATMs were increased in subcutaneous compared to visceral/omental adipose tissue depots in humans [14]. The finding of double positive CD206<sup>+</sup>CD11c<sup>+</sup> ATMs, a population that is less prominent in mice has been a consistent finding in human ATMs in several studies as noted below.

Unlike mice where metabolic responses to high fat diet induced obesity are relatively uniform, challenges remain in characterizing ATMs in the context of variation in metabolic dysfunction in obese humans [63]. CD206<sup>+</sup>CD11c<sup>+</sup> inflammatory ATMs in subcutaneous adipose tissue (SAT) were reported to be increased in obese individuals with non-alcoholic fatty liver disease (NAFLD) compared to lean and obese individuals without NAFLD [64]. Macrophage concentration and Plasminogen activator inhibitor-1 (PAI-1) in SAT were inversely correlated with hepatic and systemic insulin sensitivity. Metabolic activation of ATMs was more robust in ATMs from obese, diabetic humans compared to obese, non-diabetic controls [42].

Using similar markers from mouse studies (CD206 and CD11c), flow cytometry analysis of human adipose tissue from obese subjects showed that visceral adipose tissue (VAT) has significantly more M1-like (CD206<sup>+</sup>CD11c<sup>+</sup>) than M2-like (CD206<sup>+</sup>CD11c<sup>-</sup>) ATMs compared to subcutaneous adipose tissue (SAT) [65]. These M1-like ATMs were differentiated from M2-like ATMs by cell-surface markers CCR2, CD44, HLA-DR, and CD40, reinforcing their pro-inflammatory phenotype (Figure 1). This study observed a correlation between M1-like ATMs and the ratio of M1/M2-like ATMs in VAT with measures of insulin resistance. Additionally, human M1-like macrophages are identified by cell surface markers CD38, CD274, and CD319 [7]. While traditionally we understood that ATMs undergo a phenotypic switch from resident M2 to pro-inflammatory M1 ATMs during obesity, there is evidence that obesity produces a distinct subset of metabolically activated ATMs (MMes), ATMs isolated from obese human SAT and VAT did not express markers of classical M1 activation as expected, but expressed markers for a unique subset of MMes. While both M1 ATMs and MMes produce pro-inflammatory cytokines, MMes are differentiated from M1 ATMs by cell-surface markers ABCA1, CD36, and PLIN2, regulated by PPAR $\gamma$  [7]. However, many early studies did not delineate or differentiate ATMs from other myeloid cells such as adipose tissue dendritic cells which are prominent in adipose tissue and express many of the same

markers such as CD11c and HLA-DR [66]. Using a more stringent separation of ATMs from dendritic cells, our group identified three primary ATM populations in human adipose tissue in both SAT and VAT depots - CD206<sup>+</sup>CD11c<sup>-</sup>, CD206<sup>+</sup>CD11c<sup>+</sup>, and CD206<sup>-</sup>CD11c<sup>+</sup> [15]. CD206<sup>-</sup>CD11c<sup>+</sup> dendritic cells and CD206<sup>+</sup>CD11c<sup>+</sup> ATMs had gene expression signatures that overlapped with LAMs and were more lipid laden. In contrast, CD206<sup>+</sup>CD11c<sup>-</sup> resident ATMs had low lipid staining and were enriched for scavenger receptor genes. Importantly, all three populations were found in lean and obese individuals indicating that the LAMs are not dependent on obesity to reside in adipose tissue. In addition, LAM ATMs were increased in SAT compared to VAT - again a contrast from mouse models. Surprisingly, there was no significant difference in LAM-like CD11c<sup>+</sup> ATMs between obese and lean subjects. Instead, the quantity of CD206<sup>+</sup>CD11c<sup>-</sup> resident ATMs were found to be increased in VAT in obese diabetic compared to lean and obese non-diabetic individuals. It is unclear if these quantitative differences contribute to metabolically unhealthy obese phenotypes or a homeostatic reaction to the obese-diabetic state.

Both human and mouse ATMs from obese adipose tissue significantly express TNF $\alpha$ , IL1 $\beta$ , ABCA1, and PLIN2. In addition, ATMs from obese VAT have greater expression of scavenger receptor CD36 compared to obese SAT in humans. A positive correlation between CD36 expression and BMI has been reported [7]. scRNA-seq of human AT identified an uncharacterized subset of inflammatory ATMs, named IM, expressing CCL3L1, TNF, and CXCL3 that accumulate in AT of obese subjects [67]. In this report, ~30% of CD11b<sup>+</sup>CD14<sup>+</sup> ATMs from obese VAT are IMs, which are presumably derived from a specific subset of monocytes. A wider diversity of human ATM types was reported in other single cell studies but has not been experimentally validated [62].

## 6. CONCLUSION

As the most prominent resident immune cell in adipose tissue, ATMs helped shine a spotlight on immunometabolic regulation of obesity and metabolic disease. Future needs in the field are to close many of the gaps in our understanding of ATM biology in mouse models and human physiology. The vast diversity of adipose tissue depots and their local influences and effects makes human research on ATMs a continued challenge. The possibility that ATMs may be directly targeted for pharmacologic manipulation is still an outstanding question and there is evidence that many existing treatments for insulin resistance such as thiazolidinediones mediate their effects via anti-inflammatory influences on macrophage activation [68]. In addition, recent advances in identifying phenotypic subtypes of type 2 diabetes provide an increased diversity in human metabolic disease that future studies on ATM biology need to take into account [69].

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## DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## DATA AVAILABILITY

No data was used for the research described in the article.

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