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INVITED REVIEW

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Adipose tissue macrophages: Unique polarization and bioenergetics in obesity

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Abstract

Macrophages comprise a majority of the resident immune cells in adipose tissue (AT) and regulate both tissue homeostasis in the lean state and metabolic dysregulation in obesity. Since the AT environment rapidly changes based upon systemic energy status, AT macrophages (ATMs) must adapt phenotypically and metabolically. There is a distinct dichotomy in the polarization and bioenergetics of in vitro models, with M2 macrophages utilizing oxidative phosphorylation (OX PHOS) and M1 macrophages utilizing glycolysis. Early studies suggested differential polarization of ATMs, with M2-like macrophages predominant in lean AT and M1-like macrophages in obese AT. However, recent studies show that the phenotypic plasticity of ATMs is far more complicated, which is also reflected in their bioenergetics. Multiple ATM populations exist along the M2 to M1 continuum and appear to utilize both glycolysis and OX PHOS in obesity. The significance of the dual fuel bioenergetics is unclear and may be related to an intermediate polarization, their buffering capacity, or the result of a mixed population of distinct polarized ATMs. Recent evidence also suggests that ATMs of lean mice serve as a substrate buffer or reservoir to modulate lipid, catecholamine, and iron availability. Furthermore, recent models of weight loss and weight cycling reveal additional roles for ATMs in systemic metabolism. Evaluating ATM phenotype and intracellular metabolism together may more accurately illuminate the consequences of ATM accumulation in obese AT, lending further insight into obesity-related comorbidities in humans.

KEYWORDS

bioenergetics, immunometabolism, phenotype, plasticity, polarization

1 | INTRODUCTION

Obesity afflicts millions of people worldwide. According to the World Health Organization, the prevalence of obesity has almost tripled since 1975 with approximately 650 million people characterized as obese in 2016. The prevalence of obesity is projected to increase to 1.12 billion people in 2030 when accounting for secular trends.[1] Obesity carries a significant risk for comorbidities including, but not limited to, cardiometabolic diseases such as atherosclerosis, hypertension, myocardial infarction, stroke, insulin resistance (IR), type 2 diabetes mellitus (T2DM), dyslipidemia, and non-alcoholic fatty liver disease. Obesity also imparts a profound economic burden due to healthcare costs. Weight loss has been associated with

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improvements in the aforementioned obesity-associated metabolic complications.[2,3] However, for many, these weight loss efforts are not maintained, resulting in a cycle of weight gain and loss termed weight cycling. In several recent studies, weight cycling was shown to increase the risk of cardiometabolic diseases even beyond obesity itself, [reviewed in ref. 4]. The inflammatory nature of obesity is thought to contribute to many of its comorbidities and much recent work has focused on immune changes in weight loss and weight cycling. Specifically, obesity is associated with chronic adipose tissue (AT) inflammation.

Over 15 years have passed since the first reports of increased macrophage infiltration into AT in obese compared to lean mice and humans.[5,6] Since that time, almost every known immune cell type has been found in AT. Cumulatively, we now know that in addition to macrophages, T cells (CD4, CD8, and T regulatory cells), B cells, natural killer T cells, dendritic cells, neutrophils, eosinophils, and mast cells also reside in AT. Overall, during the transition from the lean to the obese state, the AT milieu becomes more inflammatory. Changes in these immune populations have been thoroughly discussed in many outstanding reviews [7-9] and will not be detailed at length here. Taken together, however, the knowledge that cells of the innate and adaptive immune system co-exist in AT is important and raises many questions regarding the immunogenicity of obesity. Furthermore, most of the immune cell populations change in their numbers and inflammatory status within AT during weight gain, weight loss, and weight cycling. In obesity, the inflammatory response in AT contributes to—if not drives—IR and dysregulated adipocyte lipolysis, which promote ectopic lipid storage and T2DM. The intracellular fuel utilization of immune cells and the interaction of immune cells within metabolic tissues are termed intrinsic and extrinsic immunometabolism, respectively. Both are important for understanding mechanisms by which immune cells contribute to immune-mediated diseases. We recently reviewed the interactions between intrinsic and extrinsic immunometabolism[10]; however, there is still much to uncover regarding the phenotype, function, and metabolism of AT macrophages (ATMs) in obesity. While extrinsic immunometabolism has been studied by many groups, including ours, much less is known about intrinsic immunometabolism of ATMs. Targeting the associated metabolic pathways may help improve our understanding of and treatments for obesity, cardiovascular disease (CVD), and T2DM. In the current review article, recent advances in our understanding of ATM phenotypes and immunometabolism are emphasized.

2 | ADIPOSE TISSUE MACROPHAGE POLARIZATION AND POPULATIONS

In pivotal reports published in 2003, Xu et al and Weisberg et al[5,6] showed that macrophages reside in AT, increase in obesity in proportion to body weight, and coincide with systemic IR in both mice and humans.[6] Subsequently, many studies in mice and humans have characterized mechanisms by which immune cell populations contribute to inflammation and dysregulation of metabolism in AT. Macrophages are the predominant cell type to accumulate in AT in obesity, and their role in inflammation has been strongly linked to both obesity and IR.[11] Early work focused on the chemokine-dependent recruitment of macrophages to AT. In mouse models null for specific chemokines, the results were mixed and have been reviewed in detail by others[12,13] and us.[14,15] Additional work has shown that resident yolk sac derived ATMs can self-renew and proliferate,[16] while hematopoietic-derived ATMs can infiltrate and proliferate in situ[17,18] and can even egress from the tissue.[19,20] Other features of ATMs have been discovered over the years, and we will focus the current article on those that may directly affect, or be impacted by, intrinsic immunometabolism.

2.1 | Polarization

The first report of ATM polarization in obese vs lean mice was published by Lumeng et al.[11] They showed that ATMs from lean mice expressed higher levels of genes such as *Ym1*, *Arg1*, and *Il10*, traditionally attributed to M2-like or "alternatively activated" macrophages. Conversely, ATMs from obese mice expressed higher levels of genes such as *TNFa*, *Nos2*, and *Ccr2*, which are associated with M1-like or "classically activated" macrophages. Two of the key enzymes that distinguish M1-like from M2-like macrophages are iNOS and arginase, respectively. In other contexts, iNOS utilizes arginine for the generation of nitric oxide (NO) and reactive oxygen species (ROS) for bacteriostatic purposes; however, in obese AT, NO contributes to mitochondrial dysfunction and ROS production by macrophages and contributes to AT IR and inflammation in obese mice.[21,22] In M2 macrophages, the arginine used by arginase is ultimately converted for use in collagen biosynthesis during tissue remodeling. Interestingly, obese ATMs have been reported to express *Nos2*, not *Arg1*, despite their role in extracellular matrix remodeling with obesity.[11,23] Others' early reports, dependent on real-time (RT) PCR and flow cytometry revealed a more nuanced and mixed phenotype.[24,25] For example, Shoelson et al[24] found that ATMs from high fat diet (HFD)-fed mice had increased expression of *Tnfα*, *Il1β*, *Cxcl10*, and *Il6*, but downregulation of *Nos2* and *Il12α*—all considered to be markers of M1-like cells. Conversely, traditional M2 markers *Il10* and *Tgfβ* were upregulated in the obese AT. Obin et al[25] reported that all ATM subsets they evaluated displayed an enhanced M2 profile in mice fed HFD for 12 compared to 8 weeks. Taken together, these data suggest a mixed phenotype of macrophages in obese AT.

As technologies have advanced, so has our ability to discover additional nuances in ATM phenotypes. Becker et al[26] used proteomics of membrane proteins to identify the ATMs from obese mice as being unique from other classically activated macrophages in vivo, and from in vitro polarized M1-like or M2-like macrophages. They named these macrophages "MMe," for metabolically activated, and showed that exposure of BMDMs to a metabolic cocktail (palmitic acid, glucose, and insulin) creates an in vitro phenotype that closely mimics ATMs from HFD mice. MMe's were defined by expression of lipid-related genes, *Plin2* and *Abca1*, as well as typical

M1-inflammatory cytokines like *Tnfα*, *Il1β*, and *Il6*. In a follow-up paper, this group found that MMe macrophages promote not only inflammation, but dead adipocyte clearance through lysosomal exocytosis driven by NADPH oxidase 2 (NOX2).[27] A recent paper by Stienstra's group supported this mixed phenotype with obese ATMs expressing elevated inflammatory genes like *Cd11c*, *Tnf*, *Nlrp3*, and *Il6* as well as anti-inflammatory genes like *Il1ra*, *Il10*, *Akt1*, *Stat6*, *Socs1*, and *Tgfb1*.[28] Interestingly, the inflammatory genes *Ifny*, *Nos2*, *Myd88*, and *Il1β* were downregulated in obese compared with lean ATMs. The differences between ATMs and in vitro polarized macrophages have been further supported by Zhou et al who studied visceral ATMs from chow and HFD-fed mice and directly compared their transcriptomes.[29] They developed a high-resolution data analysis platform, named "MacSpectrum," which accounts for a polarization index and an activation-induced differentiation index. Their results demonstrated that ATMs in lean and obese mice cluster separately from in vitro polarized M2 and M1 BMDMs. In addition, they revealed that ATMs and circulating monocytes have a unique signature that correlates with BMI and homeostasis model assessment of IR in humans. Thus, the field at large is accepting that simple M1/M2 nomenclature does not accurately describe ATMs, although the simplified categorization can make phenotypic and metabolic descriptions easier to describe and categorize.

Moreover, additional data are surfacing that human ATM polarization is also more variable than the M1 or M2 paradigm. Human ATMs have phenotypic plasticity or a mixed activation state with the differential expression of both M1 and M2 markers [reviewed in ref. 30]. In lean humans, a presumably anti-inflammatory state, markers for both M1 and M2 were identified comprising approximately 60% of interstitial macrophages with a shift in obese humans to more of an M2 phenotype in fibrotic areas.[31] Moreover, an M2 marker in mice, CD206, is found on ATMs of both lean and obese humans. Higher expression of CD206 correlated with another M2 marker CD163 and M1 markers CD11c, CD80, CD86, and HLA-DR.[32] The CD11c+CD206+ ATMs described by Wentworth et al[33] are another example of macrophages harboring both markers, existing on the continuum of M1/M2. It is interesting to note differential expression of other markers on human CD11c+ and CD206+ ATMs, such as the CD45 leukocyte marker, CD1c lipid antigen-presenting cell marker, and CD86 general antigen-presenting cell marker that likely assists with handling excess lipid.[33]

Macrophage lipid production and utilization is also linked to their polarization. For example, M1 macrophages synthesize lipids and produce inflammatory lipid mediators like eicosanoids while M2 macrophages tend to take up and oxidize lipids.[34] This relationship appears in the context of obesity as well. Chawla et al demonstrated that the transcription factor peroxisome proliferator-activated receptor (PPAR) gamma is required for alternative activation of ATMs and that mice deficient in macrophage PPARγ have accelerated development of obesity and IR.[35] In similar studies by Lee et al, macrophages lacking PPARδ were not able to increase lipid handling and the mice developed IR and hepatic steatosis.[36] Further evidence of the importance of lipid handling in obese ATMs was provided by Becker et al in their description of MMe ATMs.[26] This phenotype was characterized as having an upregulation of lipid metabolism typically observed in M2-like macrophages including the cholesterol export protein, ABC-A1, and the lipid droplet protein, perilipin 2. In particular, palmitic acid recreated the obesogenic phenotype, indicating that the lipid-rich milieu of AT shapes the identity of the macrophages. In addition, PPARγ and p62 were shown to promote a lipid metabolism phenotype in MMe macrophages.[26] Thus, M2-like ATMs in lean AT require lipid-related transcriptional regulation for their protective function, while at the same time, the lipid-rich environment of obese AT drives a pro-inflammatory MMe phenotype, indicating the importance of lipid balance for ATM phenotype and function.

2.2 | Newly identified populations

Single-cell data has propelled the field forward by verifying, further characterizing, and identifying novel ATMs and other immune cell populations (Table 1). Using flow cytometry coupled with single-cell RNA sequencing (scRNA-seq) followed by imaging, Lazar et al identified CD11b+Ly6c-CD9- ATMs to be interstitially spaced, while CD11b+Ly6c-CD9+ ATMs were within crown-like structures and increased with obesity—this was true in both mouse and human AT.[37] The CD9+ ATMs expressed genes related to lipid metabolism (including *Lpl*, *Plin2*, *Cd63*), lysosomal pathways (*Acp5*, *Ctss*, *Lamp2*, *Lipa*), pro-inflammatory mediators (*Il1α*, *Il18*, *Tnfα*, *Ccl2*), and intracellular vesicle formation. In contrast, the CD9- ATMs had tissue-homeostatic gene programs, such as vascular development and organization. This group also performed assay for transposase-accessible chromatin (ATAC-seq) to assess chromatin regulation and demonstrated Ly6c+ and CD9+ ATMs displayed distinct profiles: CD9+ cells were enriched for binding at transcription sites for inflammatory mediators while Ly6C+ cells were enriched for tissue regulatory gene expression pathways, data that confirmed their scRNA-seq results. CD9+ ATMs were also found in human visceral AT, and as in mice, had higher lipid content and localized to CLSs.

This past year, Amit et al published another comprehensive scRNA-seq analysis of CD45+ immune cells from lean and obese mice.[38] This group performed a time course analysis of AT from mice placed on HFD for 6, 12, or 18 weeks; each time point was compared to age-matched chow-fed controls. They detected the most extensive reorganization of immune cells in mice on diet for 12 weeks. As expected, the largest changes were an expansion of ATMs and a reduction in T regulatory cells and type 2 innate lymphoid cells. Importantly, ATMs from leptin-deficient obese mice fed a chow diet also showed these changes. They identified two monocyte and three macrophage clusters increased in obese mice. One cluster had a perivascular phenotype similar to a recent report,[39] and another had elevated CD9 as was shown by Lazar's group.[37] A CD9+CD63+ subset they called lipid-associated macrophages (LAM) was characterized by an expression pattern reflecting lipid metabolism and phagocytosis. LAMs were also found in AT from obese but not lean

TABLE 1 Populations of adipose tissue macrophages identified by single cell RNA sequencing

Citation	Mouse or human	Populations	Function/Role	Obesity phenotype	Associated genes/Other info
37	Both	Cd11b+Ly6c+	monocyte derived cells	Increased	
		CD11b+Ly6C−F480+ CD64+ CD9−	interstitially spaced, vascular development/ organization	No difference	
		CD11b+Ly6C−F480+ CD64+ CD9+	surrounding CLS, lipid metabolism	Increased	*Lpl*, *Plin2*, *CD63*, *ACp5*, *Ctss*, *Lamp2*, *Lipa*, *IL1ra*, *IL18*, *Tnf*, *Ccl2*
39	Mouse	Lyve lo MHCII hi	nerve associated macs	Unknown	
		Lyve hi MHC II lo	vascular associated macs	Unknown	
38	Mouse	"Mon1"		Decreased	*Retnla*, *Fn1*
		"Mon2"		Decreased	*Plac8*, *Clec4e*
		"Mac1"		Decreased	Retnla, Lyve1, CD209f, Cd163 associated with perivascular macrophages from Chakarov[39]
		"Mac 2"	surrounding CLS	Increased	*Nche1*, *Cd9*
		"Mac 3"	surrounding CLS, lipid metabolism/ phagocytosis (LAMs)	Increased	*Nche1*, *Cd9*, *Spp1*, *Trem2*, *Lipa*, *Lpl*, *Ctsb*, *Ctsl*, *Fabp5*, *Fabp5*, *Lgals1/3*, *Cd36* associated with CD9+ from Hill[37] KO Trem2 increased weight, body fat, glucose intolerance, insulin, cholesterol, LDL

humans. This scRNA-seq technique allowed them to identify the gene *Trem2* to be uniquely identified in this LAM population. They showed Trem2 was required to generate LAMs during obesity and was essential for AT remodeling, as knockout mice displayed adipocyte hypertrophy, accelerated AT accumulation, and dramatically impaired systemic glucose tolerance.

3 | RELATIONSHIP BETWEEN POLARIZATION AND BIOENERGETICS

3.1 | In vitro polarized macrophage energetics

The concept that "fuel feeds function," was first proposed by Craig Thompson[40] for T cell responses, and we now know that intracellular bioenergetic pathways are intricately linked to differentiation and effector function for all immune cells. Indeed, in vitro, M1-like and M2-like macrophages display very different metabolic profiles, whereby M1-like macrophages utilize glycolysis for rapid ATP generation while M2-like macrophages use oxidative phosphorylation (OX PHOS).[41] These differences were first shown by Boscá et al who utilized tracer-based metabolomics to show that classically activated macrophages are glycolytic.[42] We also know that macrophages, like other immune cells, utilize many metabolic pathways and fuel sources for purposes beyond ATP generation, summarized nicely by Viola et al.[43] In addition to ATP generation from glycolysis, M1-like macrophages rely on the oxidative pentose phosphate pathway (PPP) for both ATP and NADPH generation, contributing to ROS generation as well as glutathione reduction. Additionally, breaks in the TCA cycle allow for accumulation of itaconate and succinate, thereby facilitating microbial killing and HIF1α stabilization, respectively. Citrate and acetyl-coA facilitate the acetylation of histones and proteins that regulate M1 function. Finally, lipid signaling mediators are produced by fatty acid synthesis, and additional ROS is generated by the inhibition of the electron transport chain (ETC). In M2 macrophages, the TCA cycle is intact and respiration is coupled efficiently in the ETC, supporting enhanced OX PHOS and diminished ROS production. Additionally, glutamine-derived α-ketoglutarate drives epigenetic reprogramming on the promoters of M2-specific marker genes,[44] favors prolyl hydroxylase activity that inhibits HIF1α expression, and provides a substrate for UDP-GlcNAc synthesis, a process essential for the glycosylation of many M2-associated proteins.[45]

Interestingly, while M1 macrophages require glucose utilization for cytokine production, ROS formation, and bacterial killing,[46] M2 macrophages appear fairly flexible in their fuel utilization. Several studies have shown that M2 cells breakdown glucose and that glycolytic blockade with 2-deoxyglucose (2-DG) may inhibit M2 polarization and function[47,48]; however, it appears that M2 differentiation does not require glycolysis, as long as OX PHOS remains intact.[46] Moreover, fatty acid oxidation has long been considered the primary source of energy for M2 cells. In 2014, Pearce et al showed that CD36-mediated uptake of fatty acids and their subsequent lipolysis in lysosomes via lysosomal acid lipase was required for the elevated OX PHOS in IL-4 differentiated M2 macrophages.[49] Further, pharmacological inhibition of carnitine palmitoyltransferase (CPT), the transporter for fatty acids into the mitochondria, impairs M2

polarization.[50,51] However, subsequent studies challenge the importance of CPT utilization, as high doses of etomoxir, a CPT inhibitor, suppress M2 polarization by reducing coenzyme A, and genetic ablation of CPT2 does not prevent M2 differentiation by IL-4.[52] Interestingly, the same study showed that direct OX PHOS blockade did not impair IL-4-associated genes or cell surface markers, suggesting our understanding of M2 metabolic requirements is incomplete.

3.2 | Metabolic profiles of ATMs

While utilization of glycolysis vs OX PHOS in in vitro polarized or transfected cells appears relatively straight forward, much less is known about in vivo ATM metabolism. ATMs from obese mice appear to have a unique metabolic phenotype, partially M1 and M2 like, similar to their polarization as previously discussed. Leitinger's group was one of the first to assess the bioenergetic profile of ATMs from lean and obese mice.[53] Linking their work to oxidized phospholipids and even iron handling (via heme oxygenase), they show that Cd11b+ "Mox" macrophages[54] predominate in lean AT and have low glycolytic capacity and low OX PHOS. In contrast, Cd11b+CD11c+CD206+ mixed-phenotype ATMs from obese mice are characterized by elevated glycolysis and OX PHOS. Their studies uniquely showed that treatment of macrophages with certain truncated oxidized phospholipids results in suppressed bioenergetics, phenocopying ATMs from lean mice, while treatment with full-length oxidized phospholipids results in enhanced bioenergetics, phenocopying ATMs from obese mice. More recent work by Stienstra et al utilized transcriptome and metabolic flux analysis of ATMs from lean and obese mice, as well as ex vivo co-culture of BMDMs with AT explants to determine the metabolic signature of macrophages from various conditions.[28,54] Similar to Leitinger's work, they showed F480+ ATMs from obese mice or from co-culture with obese AT explants had increases in both glycolysis and OX PHOS. In fact, expression of genes in these fuel utilization pathways was sufficient for them to distinguish between macrophages from lean and obese mice. Interestingly, these changes were only noted in macrophages from AT, and not those from the peritoneal cavity. We have included example data from our laboratory of both the oxidative and glycolytic profiles of in vitro polarized M1 and M2 BMDMs and of lean and obese ATMs (Figure 1). These closely recapitulate what has been shown by others,[28,49,53] and draw attention to the idea that obese ATMs are not specifically M1- or M2-like, similar to what was suggested by gene expression and flow cytometry results discussed above.

Interestingly, the study by Stienstra's group also suggests that cytokine production in lean ATMs is supported by fatty acid, glucose, and glutamine utilization (more similar to M2 cells), while cytokine production in obese ATMs is supported by glycolysis (more similar to M1 cells).[28] Microarray analysis also highlighted genes associated with many metabolic pathways, including carbon metabolism, amino acid metabolism, OX PHOS, glycolysis, amino/nucleotide sugar metabolism, PPP, fructose/mannose metabolism, mTOR signaling, glycerolipid metabolism, and sphingolipid metabolism; however, little is known about the requirements of these pathways for metabolic and inflammatory function. Only two studies modulate metabolic proteins in ATMs. Makowski's group found a similar phenotype to Stienstra's group, with glycolytic macrophages expressing high levels of the glucose transporter, Glut 1; however, paradoxically, Glut1 myeloid knockout mice were not protected against diet-induced obesity.[55] Moreover, while obese ATMs have increased expression of the fatty acid transporter Fatp1, *Fatp1* deficient mice have worsened weight gain and glucose tolerance following HFD-feeding.[56] *Fatp*1 deficiency increases glycolysis, *Nos2*, and the prostaglandin 15-HETE in BMDMs, and IL-6, IL-1β, NLRP3 activation, and oxidative stress following HFD-feeding in vivo; however, *Fatp1* deficiency also increased OX PHOS, despite suppressing fatty acid uptake and oxidation in BMDMs. Together, these results suggest that obese ATM metabolism is not unequivocal and ATMs may be metabolically flexible; however, the contribution of each metabolic pathway to ATM function in lean and obese settings is not known. Lipid metabolism has also been assessed in ATMs by Ferrante et al, due to the lipid-rich environment of AT.[57,58] HFD-feeding results in lysosome biogenesis and lipid catabolism in ATMs induced by factors secreted by AT, but is not dependent on autophagy. Further understanding of mechanistic links between obese ATM metabolism and function may suggest potential targets to alleviate macrophage-associated AT inflammation and dysfunction.

No studies have examined human ATM bioenergetics, although a few studies link macrophages with adipocyte metabolism. One study showed an increase in ATP-linked respiration, but not glycolysis, in a human adipocyte cell line treated with LPS/IFN-gamma activated macrophage-conditioned media. Conversely, there was a decrease in ATP-linked respiration in the context of IL-10 and TGFβ treatment.[59] As these cytokines are present in AT during obesity and can be released by ATMs, this suggests a role for macrophage function influencing adipocyte function. Moreover, gene expression analysis of human subcutaneous AT from subjects with varying BMIs show a negative correlation between the macrophage markers CD163 and CD40 and the mitochondrial genes UQCRC2 (encoding complex III core protein of the ETC) and NDUFB8 (encoding complex I subunit of the ETC) primarily found in adipocytes, which also supports the idea that macrophages may influence adipocyte metabolism. DNA microarray analysis in AT from human obese subjects undergoing calorie restriction showed decreased gene expression related to mitochondrial metabolism, namely OX PHOS; however, it is unclear if these genes change in macrophages, adipocytes, or both.[60] Concomitantly there was an increase in macrophage markers during calorie restriction indicating a possible activation of ATMs by FFA released during increased lipolysis in the local environment, but this did not translate to systemic inflammation, as there was a decrease in serum C-reactive protein.[60] No work has directly examined bioenergetics in obese human macrophages, which may be especially important to our overall understanding of ATM metabolism in obesity. Moreover, newer work seeks to understand not

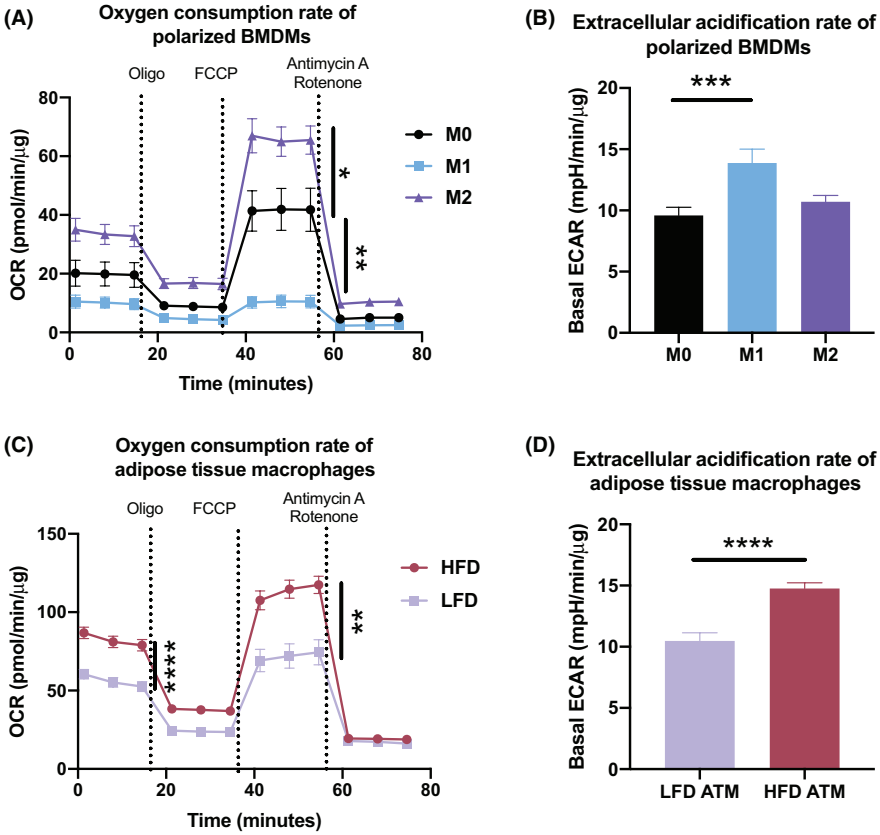

FIGURE 1 Obese adipose tissue macrophages (ATMs) display a hypermetabolic phenotype, different from M1 or M2 polarized bone marrow-derived macrophages (BMDMs). A, B, BMDMs from C57BL/6J mice were polarized for 24 h with 10 ng/mL LPS/IFNy (M1) or IL-4/IL1-3 (M2). A, A mitochondrial stress test was completed on a Seahorse metabolic flux analyzer for basal and maximal oxygen consumption rate (OCR). B, Basal extracellular acidification rate (ECAR). C, D, ATMs were isolated from C57BL/6J mice on a low-fat diet (LFD) or high-fat diet (HFD) for 9 wk, and (C) OCR and (D) ECAR were assessed. $^{*}P < .05$, $^{**}P < .01$, $^{***}P < .001$, $^{***}P < .0001$ by two-way ANOVA (A and C), one-way ANOVA (B), or *t* test (C and D) and post hoc testing where appropriate

only ATMs in obesity, but the role and function of ATMs in lean healthy AT as well as following weight loss, calorie restriction, bariatric surgery, and weight cycling.

4 | ROLE OF RESIDENT IMMUNE CELLS IN AT HOMEOSTASIS

For many years, investigators in the field of extrinsic immunometabolism have focused on mechanisms by which the inflammatory milieu of obese AT promotes local IR resulting in increased basal lipolysis, ectopic lipid storage, and concomitant impairment in systemic insulin action. However, more recent work is exploring the concept that resident immune cells in lean AT play important homeostatic roles. These roles could include anti-inflammatory cytokine secretion, antigen presentation, and extracellular matrix maintenance. Newer areas of investigation have highlighted the role of ATMs in buffering concentrations of locally required substrates such as catecholamines, lipid, and iron. Whether the bioenergetics of these special ATMs influences their buffering may be important for these functions as well.

4.1 | Catecholamine buffering

Sympathetic innervation of white AT is important for lipolysis, beiging, and thermogenesis, and this occurs via norepinephrine (NE) signaling. While one early study reported that ATMs themselves can produce NE,[61] several other groups have refuted this.[62-64] Now, the prevailing thought is that a special population of ATMs can recycle NE delivered from sympathetic nerves. These specialized ATMs have variously been called sympathetic neuron-associated macrophages [SAMs; ref. 63] and nerve-associated macrophages [NAMs; ref. 64]. These cells do not express a key enzyme required for NE synthesis, but rather, have a unique gene expression profile characterized by increases in neural- and adrenergic-related genes.[63] Thus, they can import and degrade NE, thereby controlling local sympathetic tone. Interestingly, depletion of the NE importer in SAMs resulted in increased serum NE, thermogenesis, lipolysis, and weight loss during food restriction.[63] In contrast to iron-recycling ATMs discussed below, SAMs have a pro-inflammatory phenotype that is exaggerated in obesity.[63] The metabolic phenotype of these cells has not been assessed; however, given the intricate link between metabolism and function, it may be of interest to understand how catecholamine buffering influences the metabolic phenotype of these cells and vice versa.

4.2 | Lipid buffering

As mentioned above, obese ATMs express transcription factors important for lipid metabolism and have a distinct fatty acid-mediated metabolic phenotype. However, whether the ATMs actually contribute to lipid metabolism in AT in the lean or obese state, or whether their phenotype was merely a consequence of their lipid-rich environment was not known. Ferrante et al answered this question in

a series of papers starting in 2013. First, they showed that obesity induces a program of lysosomal biogenesis in ATMs.[57] Like Becker, they defined a lipid catabolism phenotype, rather than a classically activated M1-like phenotype. Importantly, when this lysosome-dependent lipid metabolism was inhibited, adipocyte lipolysis was also depressed. In Ferrante's subsequent work, his group demonstrated that adipocytes can release exosome-sized, lipid-filled vesicles they refer to as "AdExos".[65] These AdExos are actually the vehicle for lipid transfer from adipocytes to ATMs and they also can convert BMDMs into the MMe phenotype. Using electron microscopy, they revealed invaginated lipid structures budding off adipocytes and many vesicular structures that looked like exosomes.[65] We also detect similar structures in transmission electron microscopy work from our group (Figure 2). These exosomes are released at more than twice the rate from obese leptin-deficient AT compared to lean, a rate that is suggested to turn over the entire lipid content of adipocytes from obese mice every 42 days compared to the turnover from lean mice every 104 days[65]! In other related work, Maxfield et al have shown that the ATMs can actually exocytose lysosomal enzymes in extracellular synapses that join ATMs with dead adipocytes, in a process called exophagy.[66] Thus, this work cumulatively reveals ATMs as a buffer and recycler of lipids in AT; however, we have little understanding of the contribution of lipid uptake and metabolism to the production of ATP, metabolic intermediates, and inflammatory lipid mediators.

4.3 | Iron buffering

Macrophages also play an important role in iron homeostasis by sequestering iron from pathogens, recycling iron for use to generate new red blood cells, and preventing toxicity as unbound iron can act as an oxidant [reviewed in ref. 67]. Consistent with the plasticity of macrophages and their ability to adapt to the tissue microenvironment, we have recently published data suggesting that a special population of ATMs might recycle iron.[68,69] We identified phenotypic M2-like macrophages in the AT with increased intracellular iron and iron-handling genes including iron uptake (CD163, transferrin receptor), metabolism (heme oxygenase), storage (ferritin light and heavy chains), and export (ferroportin and ceruloplasmin) from the stromal vascular fraction of lean mice fed normal chow.[68] These ferromagnetic ATMs were isolated via magnetic column separation and are termed MFe^{hi} with the non-ferromagnetic population termed MFe^{lo}. In obesity, we observed the expected increase in total ATMs from obese mice fed HFD, with a majority representing MFe^{lo} macrophages that were of the M1-phenotype. Moreover, there was also a phenotypic shift in the MFe^{hi} cells from the M2-like phenotype identified in lean mice to a more pro-inflammatory phenotype in obese mice. These inflammatory MFe^{hi} cells also had a decrease in iron content as well as the aforementioned iron-handling genes. Concomitantly, atomic absorption spectrometry showed an increase in iron content in adipocytes of mice fed HFD. These data suggest that in addition to the role of ATMs in protecting adipocytes from lipotoxicity and promoting tissue repair, they also may play a role in protecting adipocytes from iron overload. However, during obesity, inflammatory ATM polarization impairs homeostatic iron handling in MFe^{hi} cells and promotes adipocyte iron overload. Consistent with this hypothesis, we have shown that MFe^{hi} cells from lean AT increase their iron content and upregulate iron-handling genes following a high iron diet or intraperitoneal injection of iron, protecting the adipocytes from iron overload.[69] The phenomenon of iron-handling macrophages is not unique to AT as other studies have shown increased iron uptake by M2 macrophages in human atherosclerotic plaque hemorrhages and in the context of iron overload.[70] In addition, emerging evidence suggests that macrophage iron handling plays a critical role in other settings such as muscle, brain, bone marrow, and pancreas [reviewed in ref. 71].

Since iron can affect the polarization of macrophages and macrophage polarization dictates fuel metabolism, it seems logical that iron affects immunometabolism. One study has shown that iron depletion of human macrophages results in increased glycolysis, enhanced extracellular acidification rate, and citrate accumulation, all of which characterize metabolic flux deviation from the TCA cycle, likely due to inhibition of iron-dependent mitochondrial aconitase.[71] Conversely, there was a decrease in genes involved in mitochondrial oxidation.[72] This is logical given that one of functions of the mitochondria is the formation of Fe-S clusters that acts as a co-factor

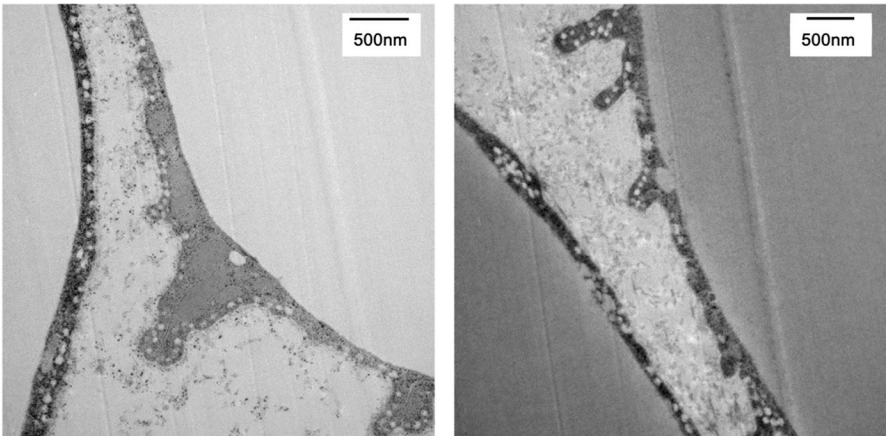

FIGURE 2 Electron microscopy of closely interacting adipocytes and macrophages: invaginated lipid structures budding off adipocytes and many vesicular structures resembling exosomes. Adipose tissue was collected from lean chow-fed C57BL/6J mice, cut into 1 mm pieces, and immediately fixed in 2.5% glutaraldehyde, before being prepared by Vanderbilt Imaging Core for TEM imaging. Images were captured on a Philips/FEI T-12 transmission electron microscope

in many cellular pathways, namely in energy production. More specifically, the Fe-S clusters are necessary for the efficient function of Complexes I-III of the ETC.[73] However, a recent study of hemoglobin-haptoglobin-treated monocytes shows that glucose transporters, fatty aldehyde dehydrogenase, and glucose-6-phosphate-1 dehydrogenase are also within the top 50 upregulated proteins.[74] More work will need to be done to determine how these genes and their associated pathways influence cellular iron uptake and cycling.

5 | WEIGHT LOSS

Weight loss is the most logical and effective way to ameliorate the metabolic effects of obesity. Weight loss has been associated with an improvement in obesity-associated metabolic derangements such as T2DM and cardiovascular disease [reviewed in refs 2,3]. Weight loss can be accomplished by calorie restriction such as switching from a HFD to a low fat diet (LFD), exercise, medications, or bariatric surgery. Despite the systemic benefits, emerging studies in mice and humans suggest the immune activation of obesity is not readily resolved, which has implications for weight cycling as discussed below.

The first mouse study investigating ATMs after weight loss was published by Ferrante et al in 2010.[75] They discovered an interesting dichotomy, whereby early phases of weight loss via caloric restriction—marked by increased lipolysis—resulted in an increase in ATMs, and only after extended weight loss did the ATM numbers decrease. Mechanistically, these investigators demonstrated that the increase in fatty acid flux due to lipolysis resulted in increased adipocyte chemokines and thereby the initial increase in ATMs. One of the conclusions of this work was that ATMs can act as buffers for fatty acids lipolyzed from adipocytes—an idea corroborated by their later work discussed above. In a later study, Lumeng et al showed that IR was not completely ameliorated even though mice had reached a lean weight after 8 weeks of weight loss by switching to a chow diet.[76] With regard to immune cell populations, ATMs and T cells persisted even after weight loss and the ATMs retained an inflammatory phenotype.[76] In this report, even 24 weeks after switching the diet, the weight loss animals had increased inflammatory ATMs compared to weight-matched controls that had never been obese. CD40 and CD80, co-stimulatory molecules, were also maintained in an elevated state in the weight loss mice. Their data suggest that T cell activation is also maintained after weight loss. Finally, they showed that ATM populations were maintained in weight loss via proliferation. Bruning et al found a similarly sustained inflammatory ATM phenotype in mice that lost weight for 14 weeks with a combination of switching to a diet with intermediate fat content and pair-feeding.[77] Similar to what Lumeng found, Bruning's group showed that some inflammatory cytokines were even higher in the weight loss mice than in obese mice maintained on HFD for the entire study. Together, these studies demonstrate persistent macrophage-dominated inflammation in AT even after prolonged weight loss, an observation not found for the liver, in which inflammation and fat content were normalized after weight loss.

Fisher et al used both weight gain and weight loss models to assess visceral AT immune cell populations.[78] They detected 15 different leukocyte populations, all of which were changed in obesity. However, macrophages were the most abundant (seven different clusters) and were also the most diverse in their gene expression profiles. Similar to what was shown by Jaitin et al,[38] in obesity, the predominant macrophage subpopulation in obese AT was characterized by a lipid binding and metabolism phenotype. Interestingly after only 2 weeks of caloric restriction, this population was lost, and a new population—characterized by phagocytosis—was gained, indicating not only that ATMs do not revert to a lean phenotype after caloric restriction, they take on a completely different phenotype. With regard to metabolic processes, they showed that many of the macrophage clusters were enriched for genes related to OX PHOS and also lipid metabolism.

In a model of 24 weeks of weight loss via voluntary exercise wheel running, Vieira-Potter and Padilla demonstrated a decrease in ATMs and inflammatory cytokines at the end of the study.[79] Interestingly, this decrease was not found in mice that were allowed to exercise only intermittently, despite having the same body weight and adiposity as the regularly exercised animals. More work should examine how different mechanisms of exercise influence ATM inflammation, and more broadly, how other mechanisms of weight loss may influence immunometabolism.

There have also been some studies investigating the consequence of weight loss on ATMs in humans. Most of these studies have analyzed AT from patients undergoing bariatric surgery as this intervention has been shown to result in an average decrease of total body weight by 30 to 35% after 1 year.[80,81] There is increasing evidence that the total number of macrophages in the AT decreases after bariatric surgery with a decrease in pro-inflammatory ATMs.[82-84] Specifically, Aron-Wisnewsky et al[85] showed an increase in classical M2 markers CD163 and CD206 from subcutaneous ATMs after 15% weight loss induced 3 months after bariatric surgery. Further studies showed a significant association between *Il10* mRNA in visceral AT and the magnitude of weight loss at 1-3 months after bariatric surgery when compared to baseline and that a higher expression of *Il1* and *leptin* mRNA, Th2 cells, and total macrophages could be a predictor of greater improvement of BMI within months after bariatric surgery.[82] In addition to ATMs, neutrophils were also found to decrease in both visceral and subcutaneous tissue after weight loss due to bariatric surgery; however, no changes in T cell populations were observed.[83] Together, these results suggest that while weight loss may improve physiological dysfunction, the effects on AT immune cells are not all restorative and should be further classified.

Another way to model the negative energy balance associated with weight loss is via activating lipolysis. Dr Granneman et al performed scRNA-seq on over 33 000 cells from the stromal vascular fraction of epididymal and inguinal AT in lean mice with or without β-adrenergic stimulation.[86] The purpose of this study was to evaluate the recruitment of brown/beige adipocytes during lipolytic stimulation, and they indeed found that they could classify a proliferating population of cells that would differentiate into brown/

beige adipocytes. In addition to lipolysis, adrenergic stimulation also causes adipocyte death, and thereby resident ATMs take on a role in efferocytosis, the phagocytosis and clearance of dead cells. Lipolysis is considered one of the mechanisms by which weight loss, as in the studies above, changes the phenotype of macrophages and other immune cells in the AT. Granneman's group performed scRNA-seq also on the Lin+ immune cells from eWAT and iWAT of control and stimulated mice. Lipolysis increased the number of proliferating macrophage/dendritic cells, while reducing the number of NKT cells. Interestingly, their data showed that some M2-like markers, *Chil3*, *Clec10a*, and *Arg1*, segregated on different cell populations, indicating more heterogeneity that was previously appreciated. Finally, a macrophage population identified as proliferating was also enriched for genes involved in lipid metabolism: *Cd36*, *Fabp5*, *Lpl*, and *Lipa*—possibly the population acting as efferocytes. In addition, this population was associated with expression of genes involved in extracellular matrix remodeling, migration, and adhesion. There have been no studies to determine how intrinsic immunometabolism changes with weight loss, which may elucidate interesting pathways tied to the function of AT immune populations.

6 | WEIGHT CYCLING

Weight cycling, also termed "weight fluctuation" or "weight variability" is not a new phenomenon in human weight patterns. A recent meta-analysis sought to evaluate the effect of weight cycling on obesity-related metabolic risk factors and T2DM from studies conducted between 1994 and 2015. Mackie et al[87] concluded that 11 out of 19 publications showed a positive correlation between weight cycling and increased body fat and deposition of AT centrally but did not find a correlation with increased risk of T2DM. Many other studies have seen a correlation between weight cycling and worsened cardiometabolic disease, atherosclerosis, and even mortality,[88-91] suggesting that it is still an important area of study.

Our laboratory and others have consistently shown that weight-cycled obese mice have impaired glucose tolerance, increased AT IR, and increased pro-inflammatory T cell infiltration compared to non–weight-cycled obese mice. Our mouse model is of extreme weight cycling[92]:9 weeks of HFD (weight gain), followed by 9 weeks of LFD (weight loss), followed by an additional 9 weeks of HFD (weight regain). Control mice were on HFD for the final 18 weeks of the study. Both the weight cycling and the obese models were on HFD for a total of 18 weeks, and their body and fat mass were equivalent for the last 4-5 weeks of the study. Despite similar body mass, the weight-cycled mice had dramatically reduced glucose clearance during intraperitoneal glucose tolerance tests and also displayed severely impaired insulin signaling in the AT. In our initial report, we demonstrated increased CD8+ T cells and memory T cells, a finding we can reproduce and are pursuing. Aguilas's group has also shown that alternating 8 weeks high-fat diet bouts increase glucose intolerance, blood lipids, and TNFα[93] and Gao's group has published that weight-cycled mice regain weight faster and also have an increase in CD4+ T cells.[94] Moreover, transcriptome analyses suggested that weight regain increases macrophages, T cells, CLS, and dead/dying adipocytes.[95] An unanswered question is whether the intrinsic immunometabolism of T cells and/or ATMs is altered during weight cycling and further, whether these potential changes contribute to the accelerated metabolic disease noted in these models.

7 | CONCLUSIONS AND EMERGING QUESTIONS

One thing that is clear from the studies detailed in this review is that resident macrophages in lean AT do not have transcriptional profiles of in vitro M2-polarized macrophages and recruited or proliferated macrophages in obese AT do not have transcriptional profiles of in vitro M1-polarized macrophages. Nor do they recapitulate the metabolic profiles of in vitro polarized cells. As ATMs in obesity have both increased glycolysis and OX PHOS, this represents a novel finding in the field of immunometabolism as compared to previously published data regarding fuel utilization in M1 favoring glycolysis and M2 favoring OX PHOS. Typically, cells upregulate one pathway over the other in order to facilitate the production of both ATP and other mediators. This dichotomy may be due to: (a) analyzing different ATM subpopulations cumulatively, with some subpopulations having increased glycolysis (possibly associated with CLS) whereas other populations have increased OX PHOS (possibly representing interstitial ATMs) within the same tissue or (b) the same cell is utilizing both glycolysis and OX PHOS (Figure 3). The latter hypothesis may seem less likely; however, the dual metabolic phenotype appears to parallel the studies that suggest ATMs secrete both inflammatory and anti-inflammatory mediators and have both M1- and M2-related gene and protein expression. Moreover, there is currently evidence that Th17 cells (at least those polarized in vitro) as well as atherogenic monocytes can increase both pathways simultaneously.[96-100] Interestingly, isolating ATMs by CD11b,[53] F480+,[28] or adherence all produce the same hyperinflammatory phenotype (Figure 1); however, these are likely still mixed populations. Future work with ATMs sorted by a marker such as CD9 will help to determine whether the different functional phenotypes have similar or different metabolic pathway utilization. Furthermore, cell sorting or deeper analysis of single-cell data may help to determine whether the cell pathways are upregulated similarly in the monocyte-macrophage differentiation following recruitment or whether the hypermetabolic phenotype is due to change in function. Assays like metabolomics, metabolic flux analysis, or tracer studies can also be used to determine the fuel sources pathways and intermediates that contribute to ATM function.

Furthermore, a deep dive into recent literature suggests that at least one of the primary roles of macrophages in AT is to store and/or recycle a variety of substances from other cells in the AT: lipids from adipocytes, catecholamines from neurons, and iron from the local environment. Another thing that is quite clear is that lipid

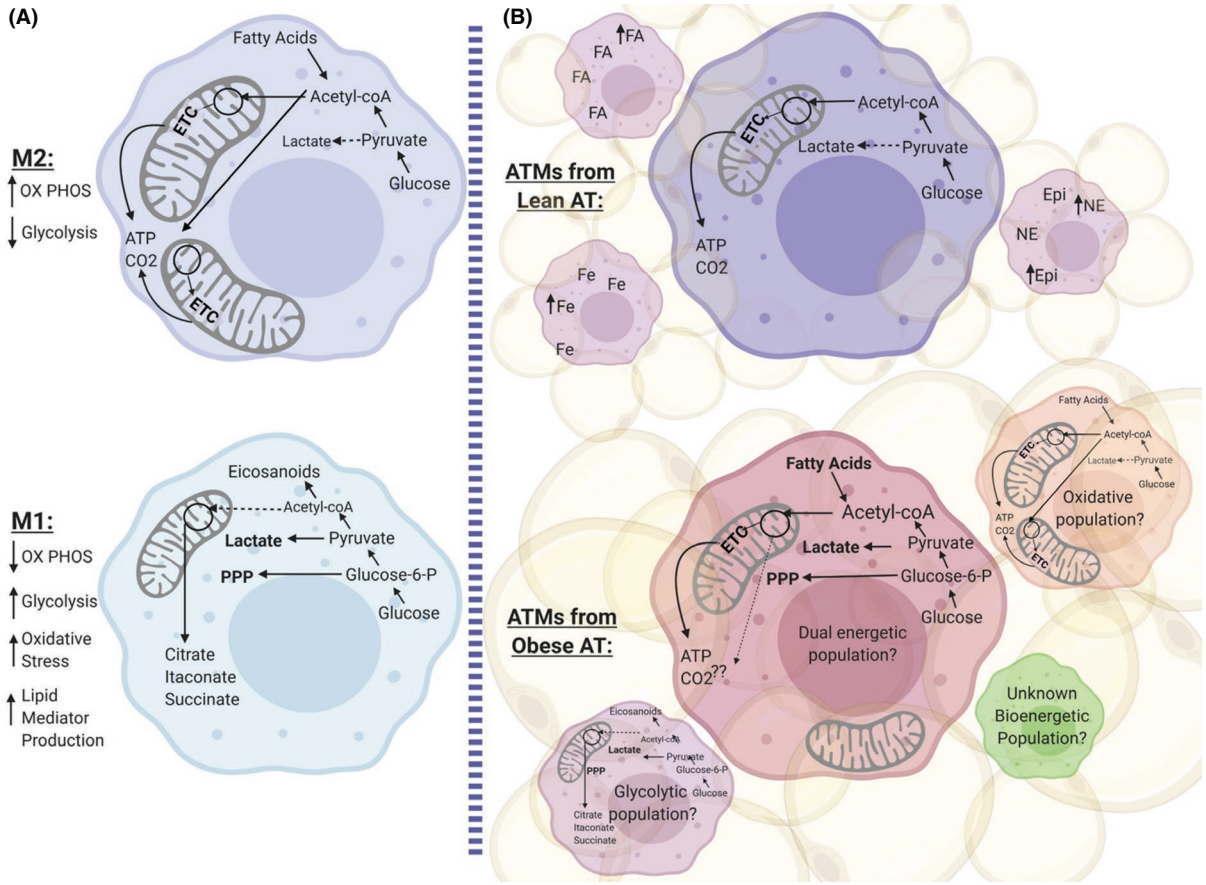

FIGURE 3 Obese ATMs have a unique hypermetabolic phenotype. A, Metabolic pathways are well-defined in BMDM polarized to M1 and M2 macrophages in vitro. B, Lean ATMs have low metabolic utilization and may have multiple populations that handle catecholamines, iron, and lipids. In contrast, obese ATMs are hypermetabolic. Recent single-cell RNA sequencing data suggests that obese ATM also have multiple mixed populations. Future work should define (1) if the hypermetabolic phenotype is a result of pooled populations or if it is present in single populations and (2) how obese ATMs use glycolysis and OX PHOS for energy production, intermediate mediators, and both phagocytic and inflammatory functions. ATMs, adipose tissue macrophages; BMDMs, bone marrow-derived macrophages

metabolism is a major defining feature of macrophages from AT as compared to macrophages from other tissues or those that are in vitro polarized. From the oxidized phospholipids in Leitinger's work, to the palmitate-driven MMe phenotype of Becker's work, to the lipid recycling ATM phenotype of Ferrante's work and the scRNA-seq studies discussed here, lipids clearly define the phenotype, function, and bioenergetics of ATMs. Not surprising given the tissue within which they reside. Thus, future studies should examine not only the role of obese inflammatory ATMs, but tissue-resident homeostatic functions as well. And, as function is linked closely to bioenergetic utilization, understanding the contribution of different pathways to buffering/recycling may expand our knowledge of macrophage functions.

Finally, new studies examining weight loss and weight cycling, human AT immune populations, and new technologies like single-cell sequencing are dramatically progressing the field of knowledge regarding extrinsic immunometabolism. Further studies regarding intrinsic immunometabolism should help provide a mechanistic understanding of how AT immune tissue function is changed with weight; however, there is much to still uncover. Technologies like single-cell sequencing, CITE-sequencing, and CyTOF may help to uncover how many immune cell populations change along with body weight. Technologies like CODEX and MALDI-TOF Mass Spectrometry may help to determine how these populations change spatially within the AT, and technologies like ATAC-sequencing may help to define if these changes are driven by epigenetic regulation. Additionally, future studies should continue to examine how AT immune cells contribute to weight loss and regain with different diets, with exercise, and following both pharmacological and surgical weight loss interventions, both in mice and in humans. Finally, there has not been clear work studying the influence of age or sex on the outcomes reviewed, which may also influence not only the results, but may have implications for how we evaluate and treat obesity-associated diseases.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

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