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Plasticity and metabolic switching in adipose tissue macrophages

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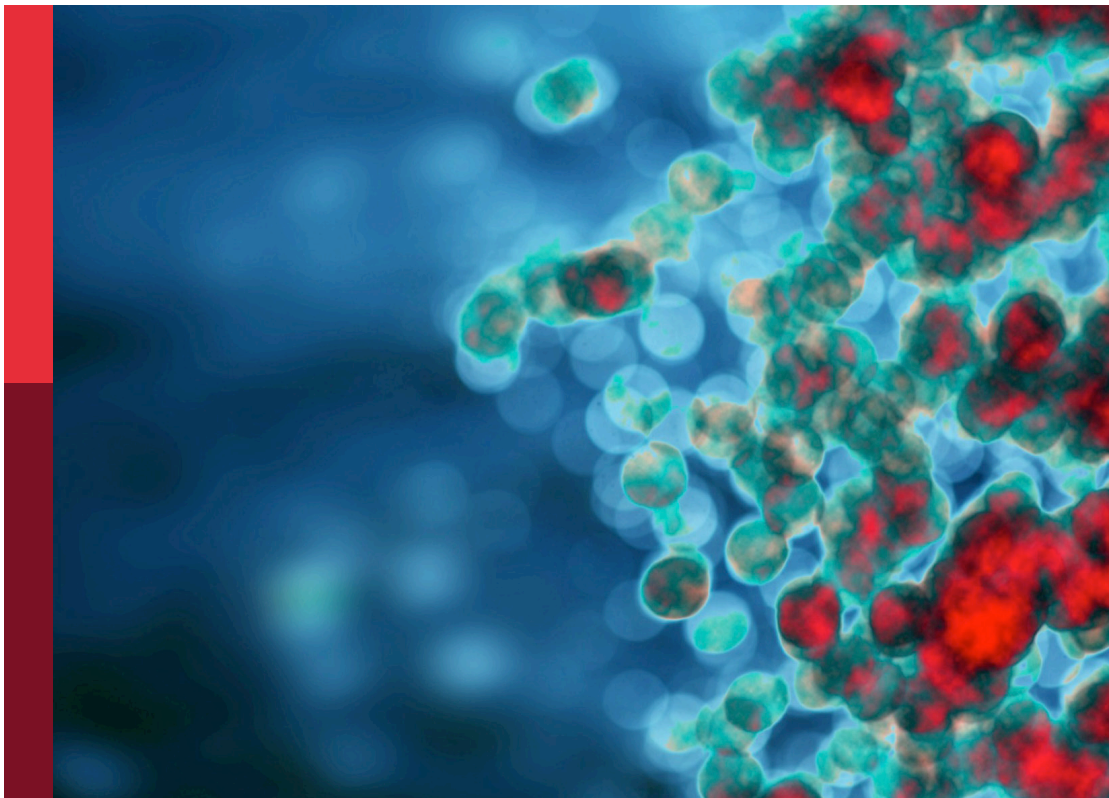

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Plasticity and metabolic switching in adipose tissue macrophages

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Editorial: Plasticity and metabolic switching in adipose tissue macrophages

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KEYWORDS

innate immunity, adipose tissue macrophages, plasticity, phenotype, metabolism, therapeutic target, obesity, metabolic diseases

Editorial on the Research Topic
Plasticity and metabolic switching in adipose tissue macrophages

Macrophages are frontier soldiers of innate immunity. Adipose tissue macrophages (ATMs), originally identified by the expression of the macrophage marker F4/80 in murine fat depots, are the most abundant immune cells in adipose tissue, representing more than half of leukocytes in depots from lean and obese animals (1). ATMs have an important role in maintaining adipose tissue homeostasis and contributing to the metabolically harmful chronic inflammation in obesity associated diseases (2). ATMs are a heterogenous population of cells with 'hard wired' diversity brought upon by distinct developmental lineages in the adipose tissue of lean and obese animals (3). ATMs exhibit phenotypic plasticity requiring polarization switching between pro- and anti-inflammatory phenotypes and functional diversity between innate and adaptive immunity in lean and obese mice and humans (4). ATMs also have complex functions in metabolism inflammation and important adaptive functions in lipid homeostasis in obesity-related diseases (2). Although research on ATMs is accumulating, there is still much to uncover regarding the developmental origin, phenotypic plasticity, functional diversity, and metabolism regulation of ATMs in obesity-related diseases.

This Research Topic "*Plasticity and Metabolic Switching in Adipose Tissue Macrophages*" highlights 10 recent studies that investigate the metabolic regulation, phenotype, developmental origin, and polarization regulation of ATMs, and summarize the plasticity, regulatory mechanisms, and therapeutic targets of ATMs in obesity-related diseases.

The study of the mechanisms underlying the conversion from the metabolically healthy obese (MHO) to the metabolically unhealthy obese (MUO) represents a substantial opportunity for the development of personalized stratified risk therapies in obesity-related diseases (5). Johnson et al. investigated the mechanisms underlying the transition from MHO to MUO and found a novel subset of CD95+ proinflammatory macrophages may mediate the switch from MHC to MUC. The authors showed the MHO mice ($ApoE^{-/-}$ $miR155^{-/-}$ mouse model) shift to MUO with increased vascular inflammation and atherosclerosis after extended (24 weeks) high-fat diet (HFD) feeding. Mechanistically,

they found the $CD95^+CD86^-$ subset of proinflammatory ATMs were increased to activate aortic endothelial cells for promoting vascular inflammation in MUO mice.

The heterogeneity, phenotype, and developmental origin of ATMs remain unknown in obese individuals. Felix et al. analyzed white adipose tissue (WAT) during homeostasis and diet interventions using single-cell mass cytometry and genetic lineage tracking models. The authors found there were eight kinetically evolving $CD206^+$ ATMs (defined by TIM4, CD163 and MHCII) and two $CD206^-$ ATMs in WAT of lean mice. They showed $TIM4^-CD163^+$, $TIM4^-CD163^-$ and $CD206^-$ ATMs were mainly bone marrow-derived, whereas the proliferating $TIM4^+CD163^+$ ATMs were of embryonic origin. Additionally, a HFD induced massive infiltration of $CD206^-$ ATMs and selective down-regulation of MHC II on $TIM4^+$ ATMs, suggesting that the development origin and environment jointly regulate the functional malleability of resident ATMs.

The underlying mechanisms of tissue remodeling, immunomodulation, and polarization of ATMs in obese patients with tumor are still elusive. Micallef et al. identified the C1q/TNF-related protein family member C1qtnf3 as one of the most regulated genes in tumor-associated inguinal adipose tissue from HFD-induced obese mice. They showed administration of C1QTNF3 neutralizing antibodies inhibited ATMs accumulation in tumor-associated inguinal adipose tissue while tumor growth was unaffected. The C1QTNF3 treatment promoted polarization of M2-type macrophages to M1-like macrophages through activation ERK and Akt pathway. These results suggest the immunomodulatory effects of C1qTNF3 in polarization of ATMs and adipose tissue remodeling. Thibaut et al. reported the relationship of cellular metabolism and macrophage polarization. They showed that disruption of cellular metabolism influenced cytokine secretion and expression of crucial inflammatory genes in M1 and M2 macrophages, highlighting the need for specific metabolic functions in regulating macrophage polarization. Pan et al. reviewed the potential regulatory mechanisms underlying ATMs polarization induced by autocrine and paracrine factors. A better understanding of how ATMs polarize may provide novel therapeutic strategies for obesity-related diseases.

ATMs infiltration into adipose tissue plays pathogenic role in inducing adipose tissue dysfunction and contributes to obesity-induced inflammation and metabolic diseases. Li et al. summarized the latest research on the heterogeneity of ATMs in adipose tissue and presented the identities of the newly discovered ATMs subtypes. They also discussed macrophage-targeting strategies to ameliorate obesity-related inflammation and metabolic diseases. Liang et al. summarized the factors affecting the polarization of ATMs and the pathogenic mechanisms of ATMs in metabolic diseases like obesity and diabetes. They also reviewed the progression of ATMs as a potential therapeutic target for treating obesity and diabetes. Matz et al. reviewed current knowledge on regulatory networks critical to plasticity and multifaceted response of ATMs in the complex adipose tissue microenvironment. This will give a clue on how to target ATMs to lessen obesity-associated health risks.

The above description shows the important roles of ATMs in obesity and metabolic disorders. However, recent studies have identified the unique role and regulation of ATMs in thermogenic adipose tissue to regulate energy expenditure and systemic energy homeostasis. Rahman and Jun summarized the current understanding of ATMs in thermogenic fat niches and the critical roles of four distinct subsets of ATMs in adaptive thermoregulation. Hepatic glucose production (HGP) is fine-regulated to maintain physiological concentration of blood glucose, whereas aberrant HGP leads to hyperglycemia in obesity-associated diabetes. Tao et al. reviewed several pathways by which ATMs remotely regulate HGP and summarized emerging therapeutic targets to treat metabolic disorders in morbid obesity or diabetes based on ATMs-HGP axis.

Finally, we would like to thank all the authors for entrusting us with their discoveries, and all the referees for their careful and insightful review. We believe that all the articles included in this Research Topic will be of interest to all researchers studying the role of ATMs in obesity-related diseases and will make them aware of how a clearer understanding of these mechanisms can guide future therapeutic treatments for obesity-related diseases.

Author contributions

JZ and JX performed literature research and wrote the manuscript. All authors contributed to the manuscript and approved it for publication.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* (2003) 112:1796–808. doi: 10.1172/JCI200319246

2. Chavakis T, Alexaki VI, Ferrante AW. Macrophage function in adipose tissue homeostasis and metabolic inflammation. *Nat Immunol* (2023) 24:757–66. doi: 10.1038/s41590-023-01479-0

3. Cox N, Geissmann F. Macrophage ontogeny in the control of adipose tissue biology. *Curr Opin Immunol* (2020) 62:1–8. doi: 10.1016/j.coi.2019.08.002

4. Caslin HL, Bhanot M, Bolus WR, Hasty AH. Adipose tissue macrophages: unique polarization and bioenergetics in obesity. *Immunol Rev* (2020) 295:101–13. doi: 10.1111/imr.12853

5. Phillips CM. Metabolically healthy obesity: personalised and public health implications. *Trends Endocrinol Metab* (2016) 27:189–91. doi: 10.1016/j.tem.2016.02.001

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ORIGINAL RESEARCH
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A Novel Subset of CD95$^+$ Pro-Inflammatory Macrophages Overcome miR155 Deficiency and May Serve as a Switch From Metabolically Healthy Obesity to Metabolically Unhealthy Obesity

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Metabolically healthy obesity (MHO) accounts for roughly 35% of all obese patients. There is no clear consensus that has been reached on whether MHO is a stable condition or merely a transitory period between metabolically healthy lean and metabolically unhealthy obesity (MUO). Additionally, the mechanisms underlying MHO and any transition to MUO are not clear. Macrophages are the most common immune cells in adipose tissues and have a significant presence in atherosclerosis. Fas (or CD95), which is highly expressed on macrophages, is classically recognized as a pro-apoptotic cell surface receptor. However, Fas also plays a significant role as a pro-inflammatory molecule. Previously, we established a mouse model (ApoE$^{-/-}$/miR155$^{-/-}$; DKO mouse) of MHO, based on the criteria of not having metabolic syndrome (MetS) and insulin resistance (IR). In our current study, we hypothesized that MHO is a transition phase toward MUO, and that inflammation driven by our newly classified CD95$^+$CD86$^-$ macrophages is a novel mechanism for this transition. We found that, with extended (24 weeks) high-fat diet feeding (HFD), MHO mice became MUO, shown by increased atherosclerosis. Mechanistically, we found the following: *1)* at the MHO stage, DKO mice exhibited increased pro-inflammatory markers in adipose tissue, including CD95, and serum; *2)* total adipose tissue macrophages (ATMs) increased; *3)* CD95$^+$CD86$^-$ subset of ATMs also increased; and *4)* human aortic endothelial cells (HAECs) were activated (as determined by upregulated ICAM1 expression) when incubated with conditioned media from CD95$^+$-containing DKO ATMs and human peripheral blood mononuclear

cells-derived macrophages in comparison to respective controls. These results suggest that extended HFD in MHO mice promotes vascular inflammation and atherosclerosis *via* increasing $CD95^+$ pro-inflammatory ATMs. In conclusion, we have identified a novel molecular mechanism underlying MHO transition to MUO with HFD. We have also found a previously unappreciated role of $CD95^+$ macrophages as a potentially novel subset that may be utilized to assess pro-inflammatory characteristics of macrophages, specifically in adipose tissue in the absence of pro-inflammatory miR-155. These findings have provided novel insights on MHO transition to MUO and new therapeutic targets for the future treatment of MUO, MetS, other obese diseases, and type II diabetes.

Keywords: Macrophage, CD95 (Fas), atherosclerosis, metabolic disease, obesity, metabolic, miR-155, adipose tissue

INTRODUCTION

Since the mid-1970s, the United States has seen a consistent rise in obesity in men and women, with rates of approximately 35% and 40%, respectively (1). Likewise, direct and indirect medical costs of obesity have steadily risen from a 39.3 billion dollar estimate in 1986 to 147 billion dollars in recent years (2, 3). Behind much of the high cost of obesity are the commonly resulting ailments, such as type 2 diabetes (T2DM), coronary heart disease, hypertension, dyslipidemia, atherosclerosis, non-alcoholic fatty liver disease (NAFLD), sleep apnea (4), osteoarthritis, gallbladder disease, stroke, several forms of cancer, and depression (2). However, not all obese individuals develop these associated metabolic dysfunctions. While the classical model of obesity (metabolically unhealthy obesity) is often associated with the comorbidities mentioned above, there exists a subset of obese individuals who do not present with these metabolic dysfunction-related comorbidities and are thus referred to as metabolically healthy obese (5).

Defining metabolically healthy obesity (MHO) has proven challenging due to a lack of consensus in the field (6, 7). As a result, determining the prevalence of MHO proves difficult. In 2017, a meta-analysis of 40 population-based MHO studies found the global rate to be an estimated 35% of obese individuals (8). We previously introduced an MHO mouse model, which maintained insulin sensitivity despite obesity. Additionally, our model did not meet the criteria for metabolic syndrome (MetS), which is the presence of at least three of five risk factors: enlarged waistline, hypertension, low high-density lipoprotein (HDL) levels, high triglyceride levels, and high fasting blood glucose level. Apolipoprotein E-knockout ($ApoE^{-/-}$) mice are a well-accepted atherogenic mouse model (9). As we previously reviewed (10), the discovery of non-coding RNAs, including microRNAs (miRs), has revolutionized the way that we examine the genome, RNA products, and the regulation of transcription and translation. By facilitating mRNA degradation and translation repression, miRs regulate inflammatory responses, endothelial cell activation, atherosclerosis, obesity, non-alcoholic fatty liver disease (NAFLD), and other diseases (11). Our $ApoE^{-/-}/miR155^{-/-}$ (DKO) mice on high-fat diet (HFD) maintained normal plasma triglyceride levels, normal fasting blood glucose level, and had increased plasma HDL level compared with $ApoE^{-/-}$ mice on normal chow diet (NC) and high-fat diet (HFD) as well as with DKO mice on NC (11). Several studies agree that, compared with metabolically unhealthy obesity (MUO), MHO is associated with a reduced risk for developing cardiovascular diseases (CVDs), type 2 diabetes mellitus (T2DM) (12, 13) and with lower mortality (14, 15). However, MHO has an increased risk compared with a metabolically healthy (MH) lean state, which remains the case no matter the stringency of criteria applied (16). Moreover, several studies demonstrate that MHO patients often switch to being MUO. This ranged from approximately 30 to 50% of MHO subjects and took place within approximately three to ten years (8, 17, 18). However, the underlying mechanisms for how MHO transition may occur are unknown.

It is well-documented that obesity is characterized by chronic, low-grade, sterile inflammation, and that macrophages are the predominant immune cells in adipose tissue (6, 19–21). In fact, human adipose tissue macrophages (ATMs) increase from between 5% and 10% in lean adipose tissue to between 40% and 50% in obese adipose tissue. Moreover, macrophages play a key role in adipose tissue inflammation *via* pro-inflammatory cytokine/adipokine secretion (22) and pro-inflammatory miR155-containing exosomes (23), which contribute to systemic inflammation (24). However, more studies are needed to decipher the many macrophage subtypes and their roles in systemic inflammation.

Fas, or CD95, is a member of the death receptor family, which also includes the tumor necrosis factor α (TNFα) cognate receptor, TNF receptor type 1 (TNFR1). As the family name suggests, Fas was initially discovered as a death receptor, specifically inducing apoptosis (25). However, its role in non-apoptotic inflammation has also been demonstrated (26–29). In fact, several papers document pro-inflammatory cytokine production (interleukin-1β (IL-1β), IL-6, IL-8, TNFα) *via* Fas signaling in adipocytes and monocytes/monocyte-derived macrophages (30–33). Moreover, Fas mRNA levels are significantly higher in circulating blood monocytes of obese subjects compared with lean subjects. Obese subject stratification into those with normal glucose tolerance versus those with T2DM showed that the latter expressed higher levels of Fas in monocytes (30). In addition, Fas protein showed a trend of increase in the adipose tissue of obese subjects compared with lean subjects, and was more highly expressed in adipose tissue of T2DM obese subjects compared with non-T2DM obese subjects

(33). Furthermore, plasma TNFα levels were significantly reduced in myeloid-specific Fas-depleted mice on high-fat diet (HFD). Monocyte-derived Fas mRNA levels and circulating TNFα protein levels were positively correlated in human serum (33), supporting a link between Fas in myeloid cells and increased TNFα levels. These data support the idea of a pro-inflammatory role for Fas in obesity, with and without metabolic diseases. However, issue of whether CD95 serves as a marker for pro-inflammatory macrophages remains.

In our current study, we hypothesized that MHO is a transition phase toward MUO, and that inflammation driven by ATMs is a novel mechanism for this transition. We found that, with extended HFD (24 weeks of HFD), MHO mice became MUO, as judged by increased atherosclerosis. At the MHO stage, DKO mice exhibited increased pro-inflammatory markers in adipose tissue, including CD95, and serum. We found that total adipose tissue macrophages were increased, and that a new $CD95^{+}CD86^{-}$ subset of adipose tissue macrophages (ATMs) was increased. These results highlight a role for $CD95^{+}$ macrophages as a player in MHO and its transition to MUO. We hope that these findings will propel the field of MHO forward toward greater understanding as well as lead to and enhance clinical therapy.

METHODS

Animal Care

All animal experiments were performed in accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines and were approved by the IACUC of Lewis Katz School of Medicine (LKSOM) at Temple University. Apolipoprotein E (ApoE, B6.129P2-*ApoE*^{tm1Unc}/J, stock no. 002052) knockout mice, microRNA-155 (miR-155, B6.Cg-*Mir155*^{tm1Rsky}/J, stock no. 007745) knockout mice, and wild-type (WT) mice were of a C57BL/6J background, and were purchased from the Jackson Laboratory (Bar Harbor, ME, United States). Mice were housed under controlled conditions in the LKSOM Animal Facility, where they had *ad libitum* access to standard chow diet/HFD, water, and were subject to a 12-h light-dark cycle. DKO mice were generated as previously reported (11) by crossing $ApoE^{-/-}$ mice with $miR155^{-/-}$ mice. Mice were age-matched and gender-specific in all experiment groups, unless otherwise stated. At eight weeks old, mice either remained on normal chow diet (10.7% fat, 23.9% protein, 5.1% fiber, 58.7% carbohydrate/other, 200ppm cholesterol; Labdiet 5001) or switched to HFD [20% (w/w) fat, 17.4% protein, 5% fiber, 49.9% carbohydrate/other, 2027 ppm cholesterol (0.15% (w/w) cholesterol); TestDiet AIN-76A] (34) for 12 weeks or 24 weeks, specified in each experiment.

Mouse Genotyping

Mouse genotype was confirmed using end-point polymerase chain reaction (PCR) on genomic DNA obtained from mouse tail sample. Briefly, DNA was extracted using 50µl of extraction solution on tail samples, followed by incubation at 95°C for 30 min. Afterwards, 50µl of stabilization solution was added (Extracta™ DNA Prep for PCR, QuantaBio, cat. No. 97065-350, VWR, Radnor, PA). PCR was then performed (**Supplementary Table 1**), followed by 1.5% agarose gel electrophoresis. The ethidium bromide-containing gel was then imaged by ultraviolet using Foto® analyst image system (Fotodyne, Fisher Scientific, Hartland, Wisconsin).

Gonadal White Adipose Tissue Single-Cell Suspension

Gonadal white adipose tissue (gWAT) was isolated and mechanically digested, followed by enzymatic digestion with collagenase type II (Sigma, cat. No. C6885, St. Louis, MO) at 37°C. Following filtration steps and centrifugation, the remaining immune cell-containing stromal vascular fraction (SVF) was stained in preparation for flow cytometry.

Flow Cytometry

Following single-cell suspension, SVF cells were stained with live/dead dye (ThermoFisher, Waltham, MA) for 30 min at room temperature in the dark. After washing with Hank's balanced salt solution (HBSS) (Corning, Corning, NY) supplemented with 2% fetal bovine serum (FBS) (GE Life Sciences, Marlborough, MA), Fc receptor block (ThermoFisher) was added to cells. Following a 5-min incubation, cells were incubated with surface antibodies for 15 min at room temperature in the dark. Intracellular markers: After washing, cells were fixed (ThermoFisher) for 30 min, washed and permeabilized (ThermoFisher) for 15 min. Cells were then incubated with antibodies for 20 min at room temperature in the dark, followed by washing (**Supplementary Table 2**). Data was collected using BD LSRII flow cytometer and DIVA software (BD Biosci., Woburn, MA). Data were analyzed using FlowJo (BD Biosci.).

Cytokine Array

Mouse blood was allowed to clot for 4 h at room temperature. After centrifuging for 15 min at 2,000xg, supernatant (i.e., serum) was carefully obtained and stored at −80°C until further use. Cytokine array experiment was conducted according to the manufacturer's protocol (R&D, Minneapolis, MN, cat. No. ARY028). Blots were imaged *via* chemiluminescence method; X-ray film exposure in a dark room and development with SRX-101A medical film processor (Konica, Tokyo, Japan). Protein levels were quantified using ImageJ software.

Murine Aortic Single-Cell Suspension

After perfusion, whole aortas were isolated and collected in DMEM-low medium (GE Life Sciences) supplemented with 20% FBS. Aortas were rinsed in PBS (Corning), dissected, and then enzymatically digested with cocktail consisting of FBS, HEPES (Gibco, Gaithersburg, MD), hyaluronidase type I-S (Sigma), collagenase types I (Sigma) and XI (Sigma) at 37°C for 30 min. Next, the suspension was filtered, then washed and resuspended in HBSS supplemented with 2% FBS, before staining for flow cytometry.

Human Aortic Endothelial Cell Culture

Human aortic endothelial cells (HAECs) (Lonza, Basel, Switzerland) were cultured on gelatin-coated flask in Medium

199 (GE Life Sciences) supplemented with FBS, PSA (ThermoFisher), ECGS (endothelial cell growth serum, Corning), and heparin (Sigma). The medium was changed every two days and cells were passaged at 70% confluency, not exceeding more than two subcultures.

Adipose Tissue Macrophage Culture

SVF cells were cultured in murine macrophage differentiation medium [RPMI 1640 medium supplemented with 25 ng/ml macrophage colony-stimulating factor (R&D), HEPES (Gibco), sodium pyruvate (Sigma), non-essential amino acids (Gibco), GlutaMAX (Gibco)] for six to seven days at $5x10^5$ cells per well in six-well plates (Falcon, VWR).

Human Adipose Tissue

We acknowledge Dr. Zsolt Bagi for generously donating IRB-approved de-identified human pericardial adipose tissue (**Supplementary Table 3**).

Human Peripheral Blood Mononuclear Cell Isolation and Culture

Whole blood from healthy male donors (**Supplementary Table 4**) was collected in anticoagulant solution [aqueous solution of sodium citrate (Fisher Scientific), citric acid (Sigma), and dextrose (Sigma)]. Blood was then gently layered onto Histopaque-1077 (Sigma), followed by centrifugation at room temperature. The peripheral blood mononuclear cell (PBMC)-containing phase was gently isolated then washed with PBS, followed by ammonium-chloride-potassium (ACK) lysis. Following centrifugation, PBMCs were resuspended in human macrophage differentiation medium [RPMI 1640 medium supplemented with 50 ng/ml human macrophage colony-stimulating factor (R&D), HEPES, sodium pyruvate, non-essential amino acids, glutamax] for six to seven days at $1x10^6$ cells per well in six-well plates.

Human Peripheral Blood Mononuclear Cell Stimulation Assay

After six to seven days of culture, human PBMCs remained unstimulated or were stimulated with TNFα (10 ng/ml) (R&D) for 24 h, followed by assessment of Fas expression.

Human Aortic Endothelial Cells Activation With CD95$^+$ Macrophage-Conditioned Medium

HAECs were plated at $5x10^5$ cells per well in six-well plates for 24 h. Following, HAECs were treated with macrophage-conditioned medium, with macrophage differentiation medium, or with endothelial cell medium for 24 h. HAECs were then assessed for EC activation *via* flow cytometry.

RNA Extraction and Quantification

Briefly, 100 to 200 mg of liquid nitrogen-frozen adipose tissue was homogenized using mortar and pestle, followed by the addition of QIAzol lysis reagent (Qiagen, Hilden, Germany). After homogenate was brought to room temperature, chloroform (Sigma) was added, then solution was vigorously shaken. Following centrifugation and aqueous phase retrieval, 100% ethanol (PHARMACO-AAPER, Thermal Scientific) was added and mixed. Next, following the manufacturer's protocol (miRNeasy Mini Kit, Qiagen), ethanol-aqueous phase solution was added to RNeasy Mini columns and subjected to a series of buffer washes and centrifugation steps. RNA was resuspended in nuclease-free water. RNA quality and concentration were determined using NanoDrop 2000 (ThermoFisher).

RNA Reverse Transcription and Quantitative Real-Time PCR

Per the manufacturer's instruction, total RNA was reverse transcribed to generate complementary DNA (cDNA) using the miScript II RT Kit (Qiagen). Briefly, template RNA in tubes containing buffer, Nucleics Mix, Reverse Transcriptase Mix and RNase-free water was reverse transcribed at 37°C for 60 min and 95°C for five min to generate cDNA.

Quantitative PCR (qPCR) was performed using the StepOnePlus PCR System (Applied Biosystems, Foster City, CA), following preparation with miScript SYBR Green PCR Kit for Use with miScript Primer Assays (Qiagen). Primers for human and mouse miRNA-155 and the housekeeping gene RNU6 (miScript Primer Assay) were purchased from Qiagen. Cycling conditions were as follows: 40 cycles at 95°C for 15 min, 94°C for 15 s, 55°C for 30 s, and 70°C for 30 s. Data were analyzed using the delta-delta Ct method.

Atherosclerotic Lesion Analysis

Following perfusion with PBS, mouse aortas were excised and fixed overnight in 4% paraformaldehyde (Sigma). Next, aortas were placed in 20% sucrose (Sigma) for 24 h. Aortas were then stored in PBS at 4°C. For *en face* staining, aortas were stained in Sudan IV (Sigma) for 40 min at 37°C, followed by incubation in 70% isopropanol for five min. Afterwards, aortas were opened longitudinally and imaged using AxioCam camera mounted to Stemi 2000-C microscope (Carl Zeiss Inc., Jena, Germany).

Protein Extraction and Western Blot

Adipose tissue and aortas were sonicated in an aqueous sample buffer consisting of sodium dodecyl sulfate (SDS, Sigma), Tris-hydrochloride [Tris (Fisher Scientific)-HCl (Sigma)], glycerol (ThermoFisher), PBS, EDTA, phenylmethylsulfonyl fluoride (PMSF, Sigma), and cOmplete Protease Inhibitor Cocktail (Sigma). Following centrifugation and retrieval of the protein-containing supernatant, protein concentration was determined using the Pierce BCA Protein Assay Kit (ThermoFisher). For gel electrophoresis, 15 to 30 μg of protein were loaded into wells and run for 90 min. Next, proteins were transferred onto polyvinylidene fluoride (PVDF) membrane (Bio-Rad, Hercules, CA) for 90 min at 400mA. Following Ponceau S (Sigma) staining, the membrane was blocked with 5% non-fat milk (Lab Scientific) for 1 h at room temperature and washed. The membrane was then incubated with primary antibody in non-fat milk or bovine serum albumin (BSA, Gemini Bioproducts, West Sacramento, CA) overnight at 4°C (**Supplementary Table 5**). Afterwards, the membrane was washed and incubated with horseradish

peroxidase (HRP)-linked secondary antibody at room temperature between 30 and 120 min (**Supplementary Table 5**). Membranes were washed and incubated in enhanced chemiluminescence substrate (ThermoFisher) for five min prior to imaging. Next, protein bands were imaged on X-ray film (AGFA, Mortsel, Belgium) after development with SRX-101A medical film processor (Konica).

Statistical Analysis

Statistical analyses were performed using the GraphPad Prism software. Two-tailed Student's *t*-test was used for statistical comparison between two groups. One-way ANOVA with Tukey Multiple Comparison test was used for three or more groups. Data presented as mean ± SEM (standard error of the mean). Statistical significance was defined as $p<0.05$.

RESULTS

MicroRNA-155 Transcripts Are Decreased in White Adipose Tissue of Obese Wild-Type Mice Compared With White Adipose Tissue of ApoE$^{-/-}$ Mice

Our previous data showed that miR-155 was significantly increased in the aortas of the well-established atherosclerotic mouse model, ApoE$^{-/-}$ mice, following 12 weeks of HFD versus normal chow (11). In addition, following 12 weeks of HFD, DKO (ApoE$^{-/-}$/miR155$^{-/-}$) mice (i.e., our MHO model) showed significant reduction of atherosclerotic plaque deposition in aortas compared with ApoE$^{-/-}$ mice. DKO mice also presented with obesity without insulin resistance (11, 35), as shown by a normal glucose tolerance test (GTT) and insulin tolerance test (ITT) (11). These results led to the classification of these mice as MHO. In our current study, to corroborate our finding of miR-155 suppressing obesity, we found reduced miR-155 transcript levels in the classical obese model (WT mice on 12 weeks of HFD) compared with atherosclerotic mice (**Figure 1A**), which correlated well with our data mining findings in **Figure 3D** of our previous report (11). Furthermore, we found that human pericardial adipose tissue from obese patients with T2DM showed a trend of increased miR-155 transcripts compared with obese patients without T2DM (**Figure 1B**) (see additional information in the Discussion). Taken together, these results demonstrate that WT mice on HFD and obese patients without T2DM have reduced miR-155 levels compared with atherogenic mice and obese patients with T2DM. Since pro-inflammatory miR-155 suppresses adipogenic transcription factors such as peroxisome proliferator-activated receptor gamma (PPARγ), and CCAAT/enhancer-binding protein alpha (C/EBPα), miR155 reduction and/or deficiency allow MHO establishment as we reported (11, 36).

ApoE$^{-/-}$/miR155$^{-/-}$ (DKO) Mice Exhibit Resurgent Atherosclerosis Following Extended HFD Feeding

As earlier mentioned, at least 30% of obese subjects eventually develop MUO (17, 18). MUO is associated with an inflammatory environment perpetuated by ATMs (36, 37). We sought to uncover, first, whether our MHO model transitioned to MUO over time; and, second, whether DKO mice at the MHO stage have increased pro-inflammatory macrophages and cytokine secretion. In order to define whether MHO mice can transition to MUO, we extended HFD from 12 to 24 weeks (**Figure 2A**) and determined whether MHO mice developed MUO as judged by atherosclerosis development. We found that there were no significant differences in total body weight or in gWAT weight between ApoE$^{-/-}$ and DKO mice following 24 weeks of HFD (**Figures 2B, C**). This is in contrast to the significant differences in total body weight as well as gWAT weight following 12 weeks of HFD between the two genotypes (11). Previously, we reported that following 12 weeks of HFD, DKO mice showed a significant

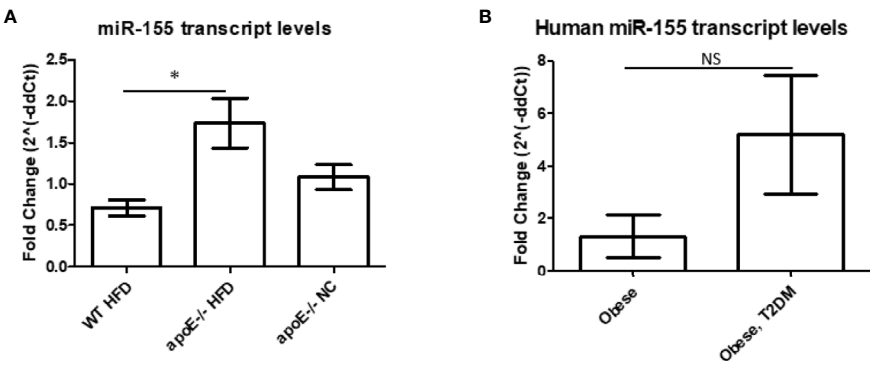

FIGURE 1 | MicroRNA-155 transcripts are decreased in white adipose tissue (WAT) of obese wild-type (WT) mice compared with WAT of apolipoprotein E (ApoE)$^{-/-}$ mice. **(A)** Mice were kept on either normal chow (NC) or switched to high-fat diet (HFD) at 8 weeks old and fed for an additional 12 weeks. ApoE$^{-/-}$ NC (n=6); apoE$^{-/-}$ HFD (n=7); WT HFD (n=4). *$p < 0.05$. **(B)** Obese patients with or without type 2 diabetes mellitus (T2DM). Donor information detailed in Table 3. Obese (n=2), Obese, T2DM (n=4). $p > 0.05$. NS, not significant.

reduction in aortic plaque deposition compared with ApoE$^{-/-}$ mice (11). Here, that difference was lost as shown by increased atherosclerosis in DKO mice following 24 weeks of HFD. DKO mice exhibited extensive aortic plaque deposition, signifying a resurgent atherosclerosis phenotype (**Figure 2D**). Moreover, there were no significant differences in blood glucose levels in response to insulin challenge between ApoE$^{-/-}$ and DKO male mice, which were similar to our previous findings following 12 weeks of HFD. However, DKO mice exhibited a lower blood glucose level at baseline, before insulin challenge (**Figure 2E**). These data show that MHO status was lost over time as DKO mice developed MUO following 24 weeks of HFD and that miR-155 deficiency in ApoE$^{-/-}$ background with extended HFD promotes obesity.

High-Fat Diet-Fed DKO Mice (Metabolically Healthy Obesity Stage) Exhibit White Adipose Tissue Inflammation

After establishing that MHO mouse on extended HFD becomes MUO and is therefore an appropriate model to study MHO switch to MUO, we assessed whether DKO mice at the MHO stage exhibited a pro-inflammatory profile, which would contribute to these mice developing MUO over time. Cytokine array analysis showed that the pro-inflammatory cytokine, TNFα, and the endothelial cell (EC) activation markers, soluble intercellular adhesion molecule 1 (Icam1) and soluble E-selectin, were induced in the serum of DKO mice, showing a trend of increase with HFD feeding (**Figure 3A**). Previously, we reported that the pro-inflammatory adipokines, leptin and resistin, were increased in the plasma of DKO mice compared with ApoE$^{-/-}$ mice after 12 weeks of HFD (11). We also previously found that resistin was significantly increased in DKO gonadal WAT (gWAT) compared with WT gWAT and ApoE$^{-/-}$ gWAT (36). However, the changes in leptin were not significant in the gWAT of WT, ApoE$^{-/-}$ and DKO mice after 12 weeks of HFD (**Supplementary Figure 1**).

We next examined the pro-inflammatory marker, TNFα, and Fas, which were previously reported to be elevated in obese adipose tissue (33). We also examined monocyte chemoattractant protein 1 (Mcp1/chemokine C-C motif ligand 2, CCL2), a chemokine that recruits macrophages to tissues. We found that TNFα showed higher expression at 12 weeks of HFD compared with 3 weeks of HFD, though this difference was not statistically significant. Fas, but not Mcp1, was significantly increased in gWAT of DKO mice following 12 weeks of HFD versus three weeks of HFD (**Figures 3B–D**). These data indicate that although DKO mice at the MHO stage are protected from or have delayed atherosclerosis development, a miR155-independent pro-inflammatory and pro-atherogenic environment is present.

High-Fat Diet-Fed DKO Mice (Metabolically Healthy Obesity Stage) Exhibit Increased Macrophages in White Adipose Tissue

Next, we assessed the percentages of various immune cell types within the gWAT. In male ApoE$^{-/-}$ and DKO mice, there was no significant difference in total leukocyte percentage (**Figure 4A**). Likewise, we found no significant differences in Ly6C^{+}

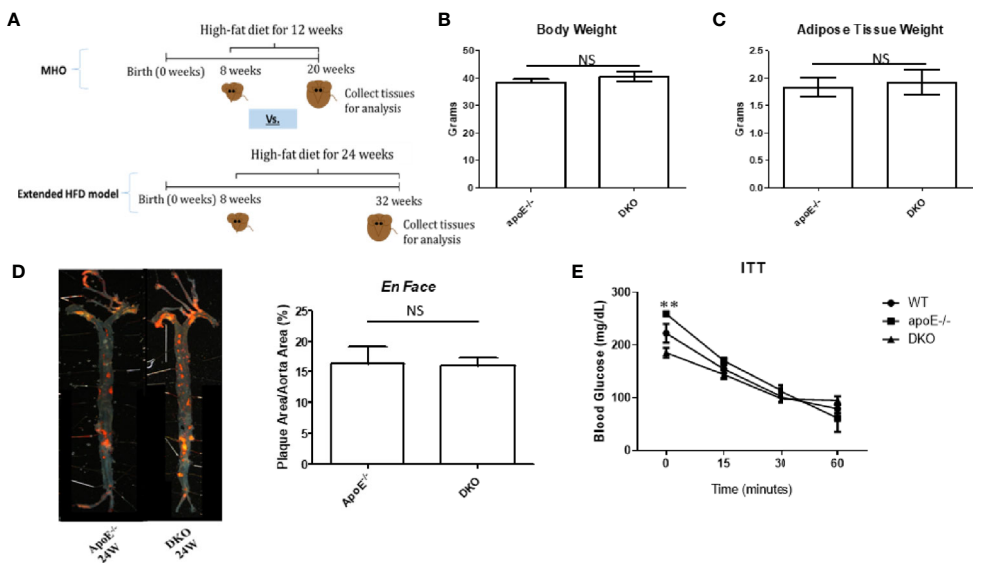

FIGURE 2 | miR155-/-/ApoE-/- double gene knock-out (DKO) mice exhibit resurgent atherosclerosis following extended high fat diet (HFD) feeding. **(A)** Extended HFD experiment design. DKO mice at MHO stage are fed on HFD for 12 weeks beginning at 8 weeks. DKO mice at extended HFD stage (when MUO develops) are fed on HFD for 24 weeks beginning at 8 weeks. **(B)** Total body weight (grams); ApoE-/- (n=15), DKO (n=12). **(C)** Gonadal WAT (grams); apoE$^{-/-}$ (n=10), DKO (n=11). **(D)** *En Face* analysis of whole aortas; male ApoE$^{-/-}$ (n=5); male DKO (n=8). Previous data showed that aortas from 12 weeks of HFD-fed DKO mice compared with ApoE$^{-/-}$ mice had significantly less plaque deposition (PMID: 27856635). Here, following 24 weeks of HFD, both ApoE$^{-/-}$ and DKO mice have significant plaque deposition. (E) Insulin Tolerance Test (ITT); WT (n=3), ApoE$^{-/-}$ (n=2), DKO (n=3). $^{**}p<0.01$. NS, not significant.

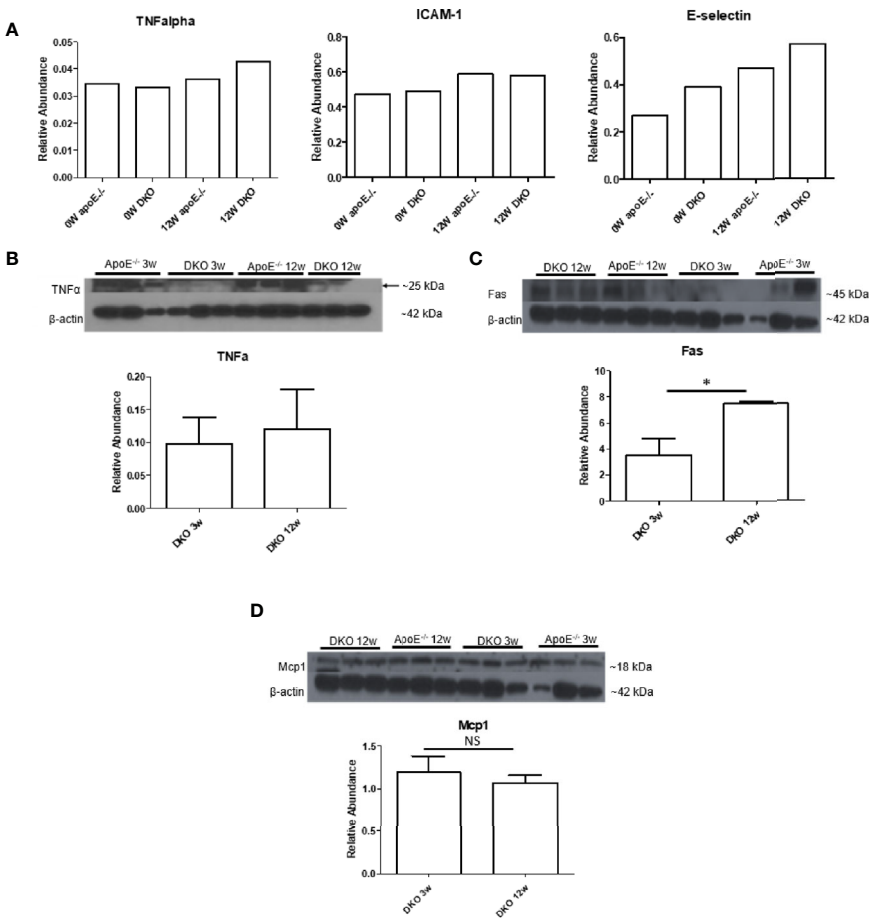

FIGURE 3 | Pro-inflammatory tumor necrosis factor-a (TNFa) and Fas are increased while leptin and Mcp1 show no change. **(A)** 0W: 8-week old male mice on normal chow (NC). 12W: 20-week old male mice that began HFD at 8 weeks old and continued for 12 weeks; n=3 per sample. Each group is composed of samples from 3 mice combined into 1 sample for analysis. **(B–D)** 3W: 11-week old male mice that began HFD at 8 weeks old and continued for 3 weeks. 12W: 20-week old male mice that began HFD at 8 weeks old and continued for 12 weeks; n=3 per group. *$p<0.05$. NS, not significant.

monocytes (38), $CD4^+$ T cells (39), or $CD19^+$ B cell populations (40) (**Figures 4D–F**). In contrast, $F4/80^+$ macrophages and $F4/80^+CD11b^+$ monocyte/macrophages were significantly increased in DKO gWAT compared with $ApoE^{-/-}$ gWAT (**Figures 4B, C**). Interestingly, we found that $CD5^+$ T lymphocytes/B lymphocytes showed a trend of decrease in DKO mice (**Figure 4G**), a finding that correlates well with miR-155's role in promoting lymphoproliferative B cell disorder (41, 42). When we assessed the total leukocytes and $F4/80^+$ macrophages in female mice, we saw that there were no significant differences (**Figures 4H, I**). Moreover, we did not find a significant change in total leukocyte population between $miR155^{-/-}$ gWAT and WT gWAT (**Figure 4J**). However, when we examined $F4/80^+$ macrophages in male $miR155^{-/-}$ gWAT compared with WT gWAT, we saw a significant increase in the former (**Figure 4K**), suggesting that miR-155 deletion supports macrophage proliferation and/or infiltration.

As previously mentioned, an increase in macrophage number is a hallmark of obesity (20). In addition, macrophages exist as varying subsets in adipose tissue, most notably pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages (43). We next assessed whether these macrophages could be broadly classified as pro-inflammatory or anti-inflammatory. We found that there were no significant changes in pro-inflammatory (M1) ATMs or anti-inflammatory (M2) ATMs in $ApoE^{-/-}$ and DKO male and female mice as well as WT and $miR155^{-/-}$ male mice (**Supplementary Figure 2**). Interestingly, we found that the majority of the ATMs expressed both M1 and M2 markers in male $ApoE^{-/-}$ and DKO mice. Previous papers have reported similar findings, where double-positive macrophages are hypothesized to be pro-inflammatory (44, 45). These results—suggesting M1 and M2 double-positive macrophages as a feature of ATMs in obesity conditions—are well correlated with our recent report (37).

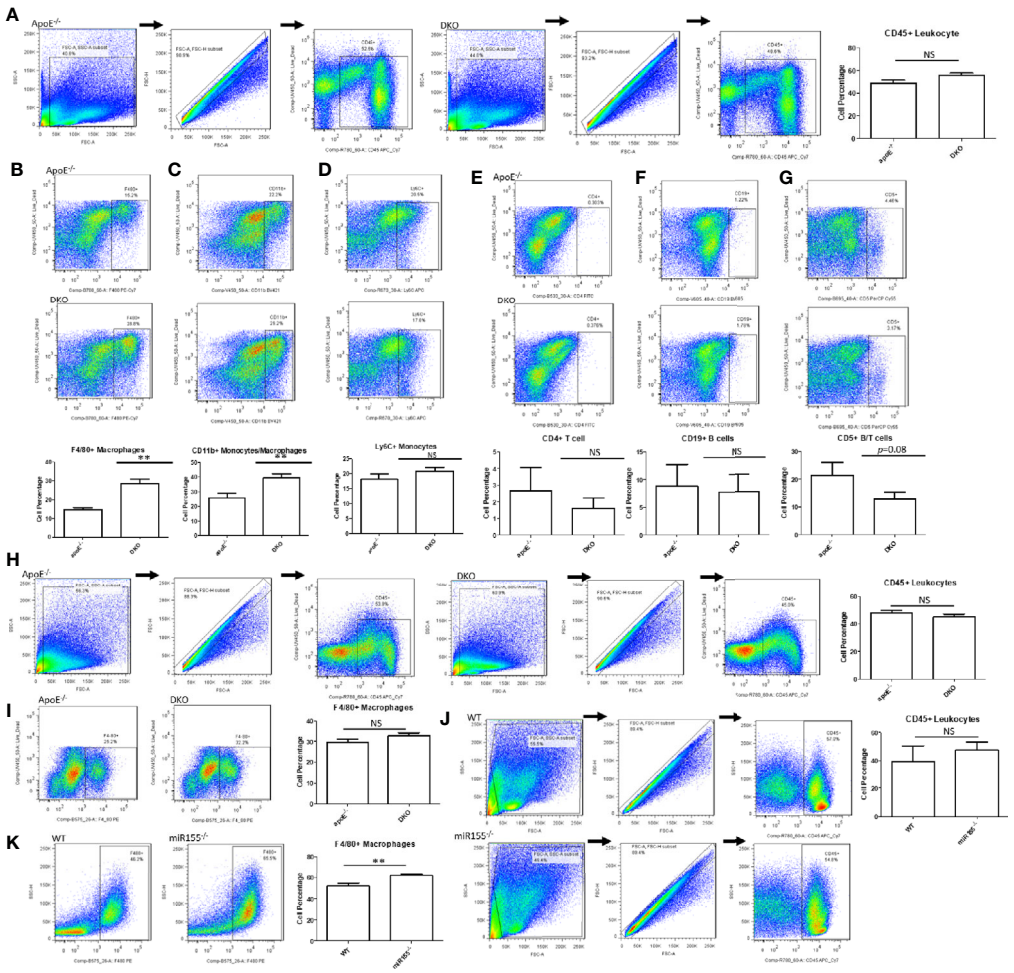

FIGURE 4 | Macrophages and monocytes are increased in miR155-deficient, high fat diet (HFD)-fed male mice in the presence or absence of ApoE background. Mice were fed on HFD from 8 weeks old to 20 weeks old. **(A)** Male mice: Total leukocytes were defined as CD45+. Macrophages/monocytes were defined as **(B)** CD45+F4/80+ or **(C)** CD45+CD11b+. **(D)** Inflammatory monocytes were defined as CD45+Ly6C+. **(E)** CD4+ T lymphocytes were defined as CD45+CD4+. **(F)** B lymphocytes were defined as CD45+CD19+. **(G)** B/T lymphocyte subsets were defined as CD45+CD5+; ApoE$^{-/-}$ (n=7), DKO (n=11). **(H)** Female mice: Total leukocytes were defined as CD45+. **(I)** Macrophages were defined as CD45+F4/80+; ApoE$^{-/-}$ (n=5), DKO (n=4). **(J)** Male mice: Total leukocytes were defined as CD45+. **(K)** Macrophages were defined as CD45+F4/80+; WT (n=8), miR155$^{-/-}$ (n=9). $^{**}p<0.01$. NS, not significant.

High-Fat Diet-Fed DKO Mice (Metabolically Healthy Obesity Stage) Exhibit Increased CD95^{+} CD86^{-} Macrophage Subset in White Adipose Tissue

In our previous publication, we found that pro-inflammatory macrophage markers in metabolic disease were lacking compared with markers for CVD and infectious diseases (46), thereby showing the need for a novel marker. Earlier, we showed that Fas was increased in DKO mice over time with HFD feeding. We examined whether CD95^{+} (Fas) macrophages in gWAT may be involved as a player in MHO mice. We found that while there was no significant difference in total CD95^{+} macrophages between ApoE$^{-/-}$ and DKO male and female mice at MHO stage (**Figures 5A, C**), there was a significant increase in the CD95^{+}CD86^{-} subset of CD95^{+} macrophages in both male and female DKO mice compared with ApoE$^{-/-}$ mice (**Figures 5B, D**). However, there was no difference in the CD95^{+}CD86^{-} macrophage population of ApoE$^{-/-}$ and DKO male mice fed on NC for 20 weeks (**Supplementary Figure 3**). These data identify a potential novel subset of CD95^{+} macrophages that could play a role in MHO development, maintenance or progression.

We also assessed CD95^{+} macrophages in WT and miR155$^{-/-}$ mice. We found that CD95^{+} macrophages were lower in miR155$^{-/-}$ gWAT compared with WT gWAT, showing that the WT gWAT may have a more pro-inflammatory environment than miR155$^{-/-}$ gWAT (**Supplementary Figure 4A**). In contrast, CD95^{+}CD86^{-} macrophage population in WT versus miR155$^{-/-}$ male mice did not show a robust population or significant difference (**Supplementary Figure 4B**). However, in extended HFD (24

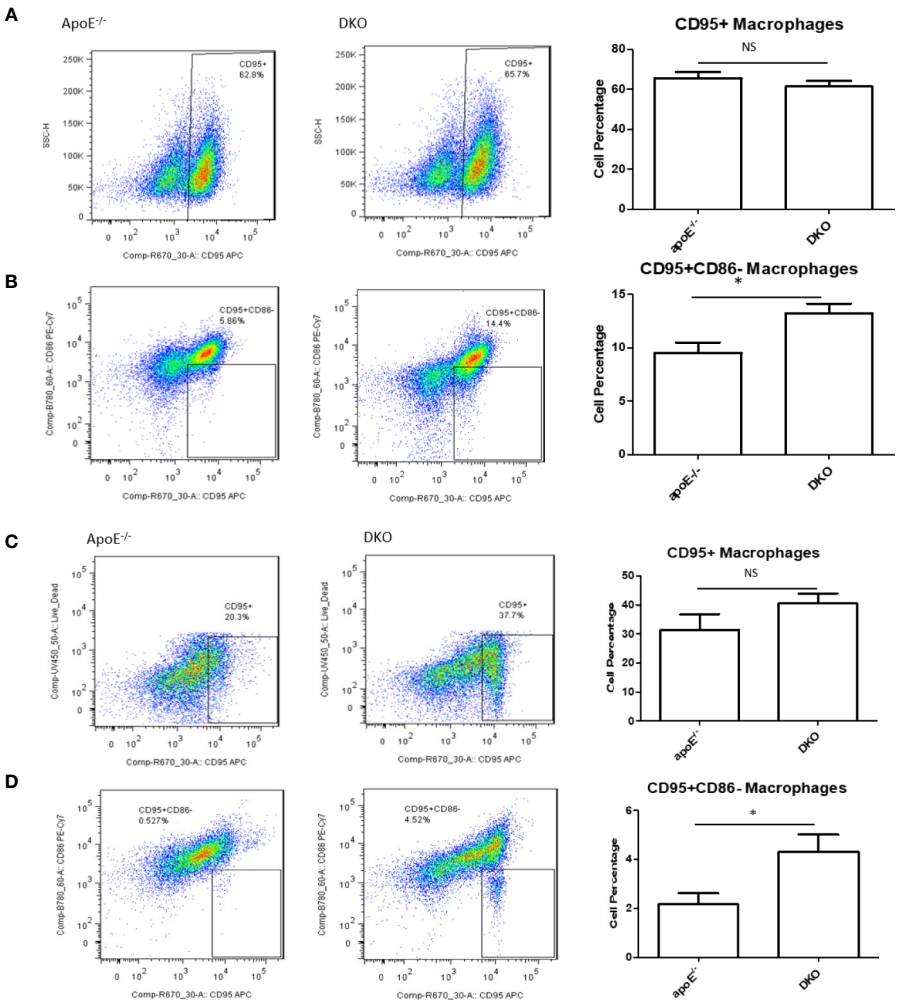

FIGURE 5 | A novel subset of CD95$^+$CD86$^-$ macrophages is increased in the white adipose tissues of DKO versus that of ApoE$^{-/-}$ mice on high-fat diet. Mice were fed on HFD from 8 weeks old to 20 weeks old. Macrophages were defined as CD45$^+$F4/80$^+$ and then evaluated for the markers, CD95 and CD86. **(A, B)** Male mice: ApoE$^{-/-}$ (n=5), DKO (n=4). **(C, D)** Female mice: ApoE$^{-/-}$ (n=5), DKO (n=4). *p<0.05. NS, not significant.

weeks of HFD), we see that miR155$^{-/-}$ gWAT had significantly more CD95$^+$ macrophages than WT mice, unlike the 12-week HFD time point (**Supplementary Figure 4C**), which emphasized the roles of extended HFD. Additionally, while miR155$^{-/-}$ mice exhibited fewer CD95$^+$CD86$^-$ macrophages than WT mice (**Supplementary Figure 4D**), these mice had a higher percentage of CD95$^+$CD86$^-$ macrophages at this extended time point compared with 12-week HFD. We recently reported that many of 28 T cell co-stimulatory receptors (CSRs), such as CD40, 4–1BBL, TL1A, CD30L, SLAM, CD48, SEMA4A, B7–1 (CD80), B7–2 (CD86), and CD155, are significantly upregulated in M1 macrophage polarization (47). This suggests a possibility that missing single CSR CD86, macrophages could still be polarized into pro-inflammatory macrophages such as CD95$^+$CD86$^-$ macrophages as demonstrated here; and that although Fas/CD95 induces apoptosis, CD40 expressed in M1 macrophages may rescue pro-inflammatory macrophages from CD95-mediated apoptosis, as reported in B cells (48).

The analyses of the expressions of 1376 innate immune genes in the CD95 KO microarray (NIH-NCBI-Geo Datasets database GSE111244) showed that 34 out of 1376 (2.5%) innate immune genes from the Innate Immune Database (https://www.innatedb.com/) were significantly downregulated (fold changes |log2|>1, p<0.05) compared with WT control cells (**Supplementary Figure 5A**). Further analysis of the downregulated innate immune genes in CD95 KO with the Ingenuity Pathway Analysis (IPA) showed that the top eight pathways of inflammation, including Th1 pathway, NF-κB signaling, systemic lupus erythematosus in B cells, cardiac hypertrophy signaling, production of nitric oxide and reactive oxygen species, neuroinflammation, systemic lupus erythematosus in T cells, and hepatic fibrosis signaling, were significantly downregulated (z score ≤1) compared with WT controls (**Supplementary Figure 5B**). Taken together, these results have demonstrated that CD95 may promote inflammation signaling in CD95$^+$ macrophages,

which were well correlated with our findings in ATM in gWAT in DKO mice and others' reports (33, 49).

Metabolically Healthy Obesity Aortas Exhibit Higher Expression of IL-1β and Fas Than miR155$^{-/-}$ Aortas, and Metabolically Healthy Obesity Aortas Have No Reduction in Pro-Inflammatory Mediators Compared With Those of ApoE$^{-/-}$ Mice

A pro-inflammatory function for Fas has been discovered in both atherosclerosis and obesity (33, 49). We next examined whether Fas and other pro-inflammatory molecules were changed in the aorta at the MHO stage compared with ApoE$^{-/-}$ aortas. We found that the expression of IL-1b was increased in DKO aortas compared with miR155$^{-/-}$ single KO aortas. DKO aortas showed no reduction in pro-inflammatory mediators, IL-1β, TLR4 (50) [previous studies show a link with atherosclerosis in ApoE$^{-/-}$ mice (51, 52)], and Fas compared with ApoE$^{-/-}$ aortas (**Figure 6**). To determine whether aortic expressions of Fas and TLR4 were partially contributed by aortic monocytes and macrophages, the mouse aortic single cell RNA-Seq (scRNA-Seq) data were analyzed on the Single Cell^{Beta} Portal database (https://singlecell.broadinstitute.org/single_cell) of the Broad Institute of MIT and Harvard. The results in **Supplementary Figure 6** showed that aortic expressions of Fas and TLR4 were partially contributed by aortic monocytes and macrophages (53). Taken together, these findings show that MHO mouse aortas have higher expressions of IL-1b than miR155$^{-/-}$ aortas; and MHO aortas are not less pro-inflammatory compared with ApoE$^{-/-}$ mouse aortas. Of note, we previously reported that IL-17A promotes endothelial cell activation but not atherosclerosis (54); that deficiency of mRNA-decaying protein tristetraprolin (TTP) in bone marrow cells promote strong systemic inflammation but not atherosclerosis in low-density lipoprotein receptor (LDLR)-deficient mice (55). Thus, during MHO transition to MUO, inflammatory status and atherosclerosis may not always progress at the same pace. In addition, we recently proposed a new working model that miR-155-suppressed "secondary wave inflammatory state (SWIS)" may be characteristic of MHO transition to MUO (36).

The Culture Medium of DKO Stromal Vascular Fraction Containing CD95+ ATMs Promotes Aortic Endothelial Cell Activation

We next assessed whether CD95^{+} macrophages could promote EC activation, which is the early stage of atherosclerotic development (56, 57). Previous studies' results support a role for ATMs in atherosclerosis development and metabolic vascular disease (23, 58). We first performed a data mining analysis on a dataset of single cell RNA-Seq of mouse SVF from mouse adipose tissue deposited in the scRNA-Seq database (the Single Cell^{Beta} Portal database) in the Broad Institute of MIT and Harvard to verify CD95 (Fas) expression on the monocytes and macrophages. As shown in **Supplementary Figure 7A**, FAS transcript expressions were detected in the scRNA-Seq dataset of the monocytes and macrophages in the SVF of adipose tissues, which were well correlated with our FACS data in **Figure 5**. In addition, proinflammatory cytokines TNF-α and IL-1β transcript expressions were also detected in the monocytes and macrophages in the mouse adipose tissue SVF (**Supplementary Figures 7B, C**), which were well correlated with the reported proinflammatory function of FAS in obesity (33, 49). Moreover, as shown in **Supplementary Figure 8**, the expressions of FAS, TNFα and IL-1β were also found in the scRNA-Seq datasets from the four monocyte clusters in human peripheral blood (59) in the scRNA-Seq database (the Single Cell^{Beta} Portal database). Then, using cultured human aortic endothelial cells (HAECs), we found that HAECs incubated with DKO SVF-conditioned medium (which contains CD95^{+} macrophages) for 24 h resulted in increased EC activation, shown by a significant increase in ICAM1 expression compared with untreated ECs (**Figure 7A**). Moreover, incubating HAECs with human macrophage-conditioned

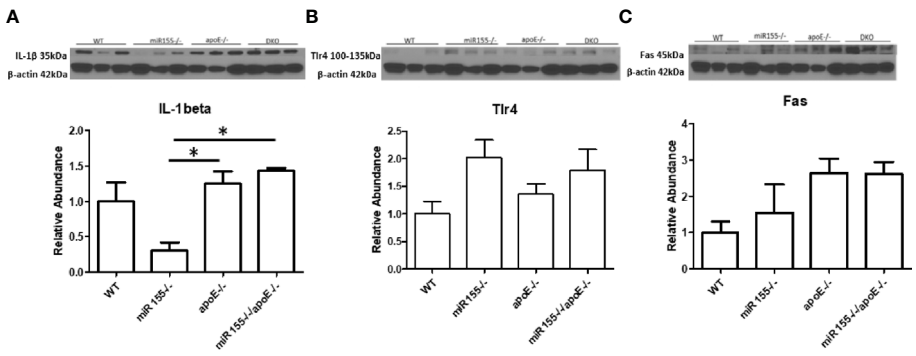

FIGURE 6 | There are no differences in the expressions of three pro-inflammatory markers such as IL-1b, TLR4 and Fas in DKO aortas compared with ApoE-/- aortas following HFD. The expressions of IL-1b and Fas are significantly increased in DKO aortas compared with miR155-/- single KO aortas. A-C. Male mice were fed on HFD for 12 weeks from 8 weeks old to 20 weeks old. **(A)** Il-beta. **(B)** Tlr4. **(C)** Fas; n=3 per group. *p<0.05.

medium resulted in increased ICAM1 expression compared with HAECs incubated with control EC medium or macrophage differentiation medium only (**Figure 7B**). Our findings were well correlated with others' reports (60). Seminal studies of obesity have helped us to understand that macrophages secrete TNFα, which further enhances lipolysis, thereby driving metabolic dysfunction (61). Moreover, when this occurs in the visceral adipose tissue (VAT), collateral damage to nearby organs, such as liver and pancreas, can lead to metabolic dysfunction of these organs (62, 63). Regarding the mechanism of MHO, a previous report showed that TNFα can increase Fas expression (64). Additionally, we showed that TNFα was induced in our DKO mouse WAT at MHO over time. We found that TNFα-treated human peripheral blood mononuclear cells (PBMCs) resulted in increased Fas expression detected by flow cytometry (**Figure 8**). Taken together, these results suggest that in addition to $CD95^+$ monocytes/macrophages found in aortas and peripheral blood by scRNA-Seq data (**Supplementary Figures 7** and **8**), DKO ATMs containing $CD95^+$ macrophages may secrete pro-inflammatory cytokines, including TNFα, and induce Fas expression *via* potential autocrine manner in ATMs and activate aortic endothelial cells. Future work is needed to determine whether $CD95^+$ macrophages are essential for the culture medium of DKO SVF to promote HAEC activation, and to assess what cytokines from DKO SVF-containing $CD95^+$ macrophages are responsible for activating HAECs.

DISCUSSION

Despite its recognition since the 1980s (65), the mechanisms underlying why some obese individuals maintain MHO status as well as how MHO individuals progress toward MUO remain understudied. Several correlations have been determined to help explain what prompts MHO switch to MUO. For example, Schröder *et al.* reported that increasing waist-to-hip ratio, waist circumference and BMI are factors (66), in addition to increasing age (6). Moreover, MHO patients more likely to transition to MUO were women (67). Although a molecular mechanism for MHO switch to MUO status (68, 69) has not been discovered, genetic mechanisms have been proposed, since MUO has been linked to genes (70). Likewise, a number of mechanisms have been suggested to explain how some instances of obesity can be MH. Some include reduced immune cell infiltration in adipose tissue; conserved insulin sensitivity; MHO patients' proclivity to deposit lipids in subcutaneous adipose tissue (SAT) depot along with lower visceral adipose tissue (VAT) and lower ectopic fat (in skeletal muscle and liver). Additionally, greater level of fitness and exercise have been proposed (15). Furthermore, lower levels of C-reactive protein, TNFα, and IL-6 were reported in patients with MHO compared with MUO individuals (71–76). Additionally, MHO individuals had higher adiponectin (anti-inflammatory adipokine) level (71, 77), lower white blood cell count, and lower plasminogen activator inhibitor-1 (PAI-1) level

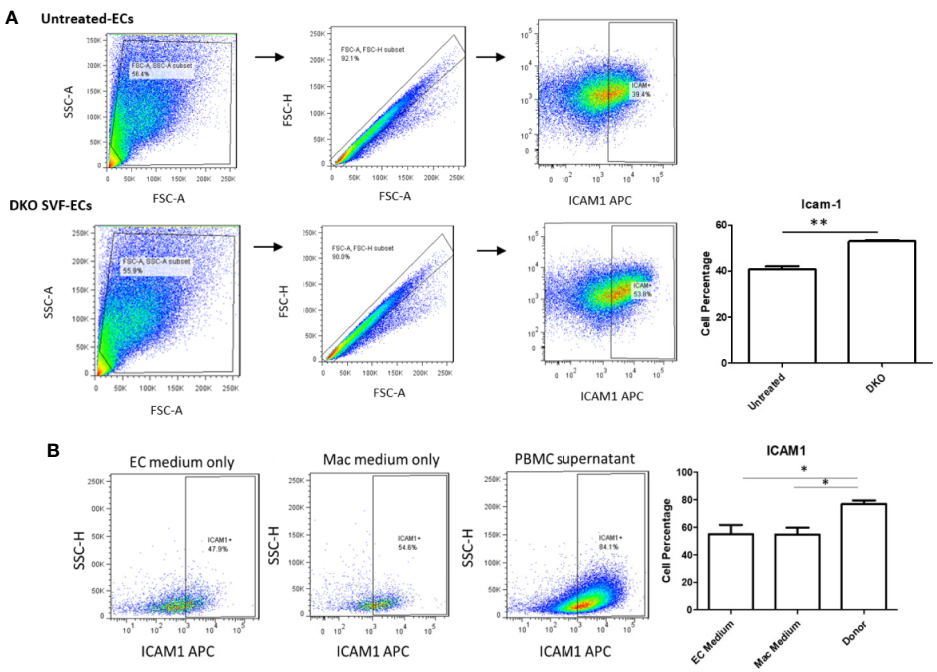

FIGURE 7 | DKO SVF and human macrophage-conditioned medium promote HAEC activation. **(A)** Stromal vascular fraction (SVF) from 20-week old DKO male mice on NC was cultured overnight in macrophage differentiation medium. Centrifuged supernatant was incubated with human aortic ECs (HAECs) for 24 hours. Untreated HAECs were incubated with EC medium; untreated (n=3), DKO-treated (n=3). **(B)** Isolated human peripheral blood mononuclear cells (PBMCs) were grown for 7 days in macrophage differentiation medium. Afterwards, cultured HAECs were incubated with centrifuged supernatant (macrophage-conditioned medium) for 24 hours. HAECs in EC medium group were incubated with EC medium only for 24 hours and HAECs in Mac medium group were incubated with macrophage medium only for 24 hours; EC medium (n=11), Mac medium (n=11), Cond Mac medium (n=12). *p<0.05; **p<0.01.

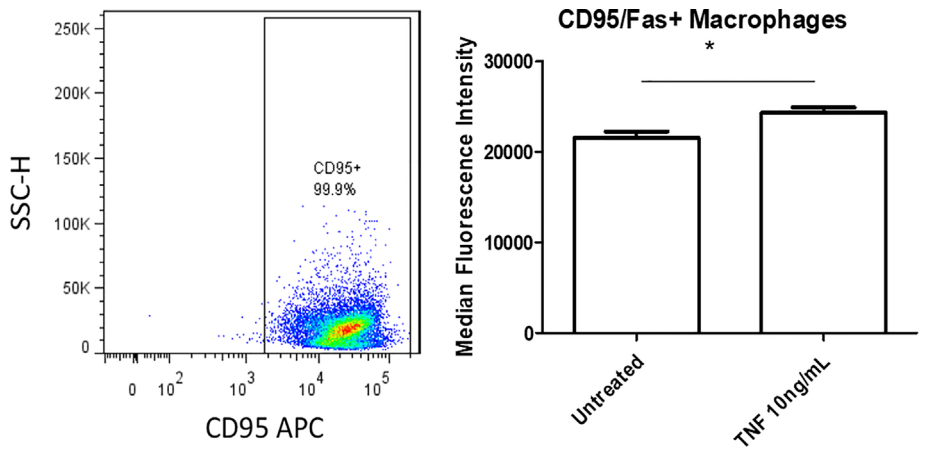

FIGURE 8 | TNFα-treated human peripheral blood mononuclear cells (PBMCs) show increased Fas expression. Isolated human PBMCs were grown for 7 days in macrophage differentiation medium. Cells were treated with TNFα (10ng/mL) for 24 hours. Untreated was incubated with fresh medium; Untreated (n=4), TNFα (n=4). *p<0.05.

(71). We previously reported the first mouse model of MHO with miR155 deficiency (11) (**Figure 9A**). As a prototypic master regulator, miR155 promotes inflammation and atherosclerosis but inhibits the adipogenesis of WATs. Thus, the deficiency of miR155 in ApoE$^{-/-}$ background leads to increased obesity, increased non-alcoholic fatty liver disease (NAFLD), decreased atherosclerosis, no insulin resistance and no type II diabetes mellitus (T2DM) (11). Given what is already known, it is clear that there exists the need to improve our understanding of MHO and the underlying mechanisms of its transition to MUO.

To this end, we have utilized our miR155$^{-/-}$/ApoE$^{-/-}$ (DKO) male mice fed on HFD as a suitable MHO model (11). Not only did we find a role for miR-155 in MHO in mice, we found that miR155 transcripts showed a trend of increase in pericardial adipose tissue from obese-type 2 diabetic patients compared with obese patients. A limitation of our study is the difference in visceral adipose tissue depot: *in vivo* mouse studies utilized gonadal adipose tissue, while our human adipose tissue was pericardial. Although literature comparing the pericardial and gonadal/epididymal fat depots, specifically, were not found, both fat depots exhibit inflammatory and/or metabolic dysfunction parameters (78–80). Future work is needed to verify a similar inflammatory profile between the two adipose tissue depots. Several studies report that approximately one-third to one-half of MHO individuals develop MUO, showing that MHO may not be a stable condition for a significant portion of obese patients. We therefore aimed to better understand how MHO transitions to MUO (36). We determined whether MHO mice develop MUO with extended HFD, which mimics the MHO patient continuing an HFD lifestyle. We saw that over time, extended HFD-fed MHO model developed atherosclerosis, although aortic plaque deposition was not significantly different between ApoE$^{-/-}$ and DKO mice at 24 weeks (**Figure 2**). This demonstrated MHO transition to MUO, which has been delayed due to miR155 deficiency (**Figure 9A2**). We recently proposed a new concept of second wave of inflammation status, in which a proinflammatory master regulator such as miR155 promotes the first wave of inflammation but inhibits the second wave inflammation so that when it is deficient, the second wave of inflammation is increased (36). In our recent report (Front. Immunol. doi: 10.3389/fimmu.2020.554301), we further found that proinflammatory cytokine blockages induce inflammatory regulators. In the circulation, we found that TNFα and soluble forms of E-selectin and Icam1 were induced in DKO mice at the MHO stage (**Figure 3A**). It is possible that TNFα levels may be significantly attributed to gWAT, which we showed produced increasing amounts of TNFα over time (**Figure 3B**). Additionally, the soluble forms of E-selectin and ICAM1 are signatures of EC activation, suggesting that ECs at the MHO stage may be activated (81, 82) (**Figure 9B**). We next assessed the pro-inflammatory environment (if any) in DKO mice at the MHO stage and found that TNFα and Fas expression in gWAT were increased in DKO mice at the MHO stage compared with DKO mice at an earlier stage of HFD feeding. The levels of Mcp1, however, were unchanged, suggesting that macrophage recruitment was not changed, at least at this stage (83) (**Figures 3B–D**). We found that macrophages were significantly increased in male DKO versus ApoE$^{-/-}$ mice as well as miR155$^{-/-}$ vs WT mice, as assessed by flow cytometry (**Figures 4B, C, K**). Moreover, analysis of the macrophages as pro-inflammatory (CD45^{+}F4/80^{+}CD86^{+}) or anti-inflammatory (CD45^{+}F4/80^{+}CD206^{+}) showed no difference between ApoE$^{-/-}$ and DKO male and female mice. No difference was observed between WT and miR155$^{-/-}$ mice as well (**Supplementary Figure 2**). Our recent publication highlights that metabolic disease markers for pro-inflammatory macrophages are lacking, especially when compared with markers for CVD and infectious diseases (Table 3a in our recent publication) (46). Therefore, novel markers are needed. Fas (or CD95) is well-known for its pro-apoptotic role (25) but also has a non-apoptotic function as a pro-inflammatory mediator in cells such as macrophages (30–33). We examined whether CD95^{+}

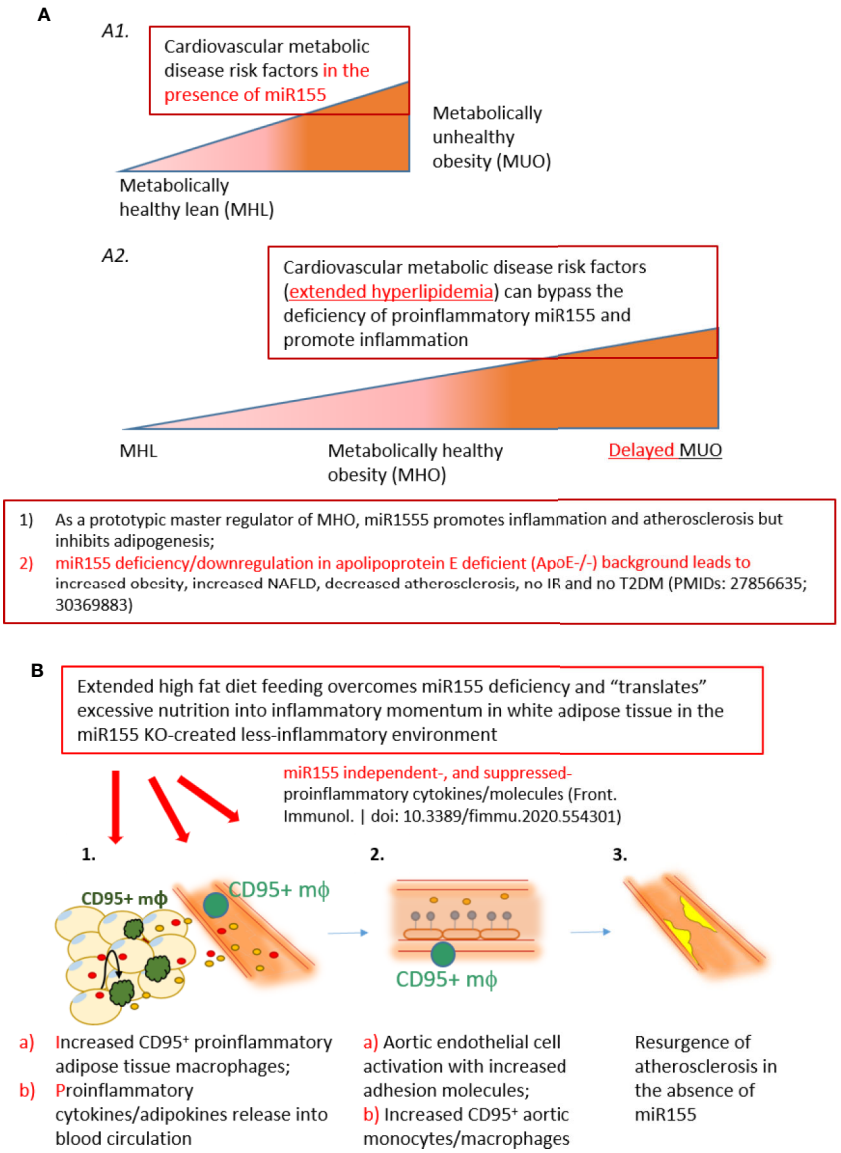

FIGURE 9 | A new working model: Extended high fat diet feeding overcomes the deficiency of proinflammatory miR155, generates CD95+ adipose tissue macrophages and secretes miR155 independent-, or miR155-suppressed proinflammatory cytokines/adipokines (second wave of inflammation), promotes aortic endothelial cell activation and increases atherosclerosis, which makes MHO transition to delayed MUO. **(A)** MHO is an intermediate stage in the development of delayed MUO. ***(A1)*** in the presence of wild-type miR155, cardiovascular metabolic disease risk factors accelerate the progression of MHO from metabolically healthy lean; ***(A2)*** In the absence of miR155, development of MUO need to go through an intermediate stage of MHO. **(B)** ***(B1)*** Adipocyte (pale yellow with light blue nuclei)-secreted and macrophage-secreted TNFα (red dots) may promote CD95+CD86- macrophage phenotype (green). This macrophage subset together with peripheral blood CD95+ monocytes/macrophages may secrete pro-inflammatory cytokines and chemokines into the circulation (yellow dots), which in turn induce aortic EC activation, shown by ICAM1 upregulation (***B2***, gray dots). The CD95+ monocytes/macrophages in aortas may also secrete proinflammatory cytokines and contribute to EC activation ***(B2b)***. **(C)** Over time, EC activation progresses towards atherosclerosis development (***B3***, bright yellow). Red dots: TNFα. Yellow dots: pro-inflammatory cytokines/chemokines/other molecules.

macrophages are increased in MHO mice. We found that while total $CD95^+$ macrophages were not increased in MHO mice compared with $ApoE^{-/-}$ mice, the subset CD95+CD86- was (**Figures 5A, B**). This was also observed in female DKO mice compared with $ApoE^{-/-}$ mice, albeit with a smaller percentage compared with male mice (**Figure 5D**). These differences were unsurprising since female DKO mice exhibited a significant yet smaller difference in body and gWAT weights compared with female $ApoE^{-/-}$ mice in our previous report (11). In WT and $miR155^{-/-}$, we observed that $CD95^+$ macrophages were significantly reduced in $miR155^{-/-}$ mice following 12 weeks of HFD and that there was no difference in $CD95^+CD86^-$

macrophage population (**Supplementary Figures 4A, B**). With extended HFD feeding (24 weeks HFD), we saw that $CD95^+$ macrophages significantly increased in $miR155^{-/-}$ mice compared to WT mice (**Supplementary Figure 4C**). While $CD95^+CD86^-$ macrophages are significantly lower in $miR155^{-/-}$ mice compared with the population in WT mice after extended HFD, the overall numbers of $CD95^+CD86^-$ $miR155^{-/-}$ macrophages between 12 weeks and 24 weeks have increased; from approximately 2% to 12.5%, respectively (**Supplementary Figures 4B, D**). Additional experiment is needed to determine the extent to which CD95+ macrophage deletion can block MHO-MUO transition. Previous study showed that deletion of CD95 in adipocytes did not affect mouse weight, but led to improved insulin sensitivity, reduced plasma free fatty acid level, and reduced plasma pro-inflammatory cytokine IL-6. Also, adipocyte-specific CD95 deletion in mice had decreased Mcp1 and Cd11b mRNA levels, showing macrophages might be reduced (33).

Moving beyond, we found that there were no differences in the expression of pro-inflammatory IL-1β, TLR4 and Fas between the well-established atherosclerotic model, $ApoE^{-/-}$ mice, and our MHO mice (**Figure 6**). In other words, MHO mice maintained a pro-inflammatory environment, despite very minimal plaque manifestation. We and collaborators recently report a similar finding that deficiency of mRNA-decaying protein, TTP, in bone marrow cells promotes strong systemic inflammation but not atherosclerosis in $LDLR^{-/-}$ mice (55). Macrophages may exert their pro-inflammatory effects in a paracrine manner or *via* cell-cell contact. Using the culture medium of DKO SVF, which includes $CD95^+$ macrophages, as well as using macrophage differentiation culture medium of PBMCs from human blood (84), we found increased ICAM1 expression on HAECs compared with respective controls (**Figures 7A, B**). Additionally, we found that treatment with TNFα, which was increased in DKO WAT, for 24 h increased Fas (CD95) detection in PBMCs, showing that TNFα in WAT of DKO model may increase the expression of $CD95^+$ on macrophages (**Figure 8**). Our single cell RNA-Seq data analysis results showed that CD95, TNF-α and IL-1β are co-expressed in $CD95^+$ monocytes and macrophages in aortas and peripheral blood (**Supplementary Figures 7** and **8**), and CD95 expressions are increased in MHO aortas in hyperlipidemia conditions (**Figure 6**). We performed Cytoscape database analysis (https://cytoscape.org/) on four pro-inflammatory molecules identified in our MHO model here (FAS, TNFα, IL-1β, TLR4), as well as on two adipogenesis regulators (C/EBP, PPARγ), and on two adipokines (leptin, resistin) in our reports (11, 36), which indicates that these pro-inflammatory pathways in MHO interplay with lipid storage and adipogenesis (**Supplementary Figure 9**). Taken together, our study suggests that $CD95^+CD86^-$ ATMs may represent a novel subset for driving MHO transition to MUO *via* secretion of proinflammatory adipokines and cytokines in the absence of miR155, as shown in our new working model (**Figure 9**). Practically, it may represent the degree of transition to MUO in a clinical setting. Furthermore, this subset defines a previously unappreciated role for $CD95^+$ macrophages in obesity, which may have similar pro-inflammatory roles in other disease conditions.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/ **Supplementary Material**.

ETHICS STATEMENTS

The studies involving human participants were reviewed and approved by the Temple University Institutional Review Board (IRB) and the Institutional Biosafety Committee (IBC). Human samples were de-identified patient samples. The animal study was reviewed and approved by the Temple University Laboratory Animal Resources (ULAR) and the Institutional Animal Care & Use Committee (IACUC).

AUTHOR CONTRIBUTIONS

CJ carried out the experiments, data analysis, and drafted the manuscript. CDIV, HS, YSh, YSu, and YL aided in the data collection. FS, KX, GN, PF, ZB, XJ, EC, and HW provided material input. XY supervised the experimental design and data analysis, and edited the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2020.619951/full#supplementary-material

SUPPLEMENTARY FIGURE 1 | Leptin is not significantly changed in DKO mice. 20-week old male mice were fed on HFD for 12 weeks beginning at 8 weeks old; n=3 per group. $p>0.05$. NS, not significant.

SUPPLEMENTARY FIGURE 2 | There is no significant difference in CD86+ (M1) or CD206+ (M2) ATMs. Pro-inflammatory macrophages were defined as CD45+F4/80+CD86+. Anti-inflammatory macrophages were defined as CD45+F4/80+ CD206+. **(A)** male $ApoE^{-/-}$ (n=4), DKO (n=6). **(B)** male WT (n=10), $miR155^{-/-}$ (n=8). **(C)**. female $ApoE^{-/-}$ (n=5), DKO (n=4). $p>0.05$. NS, not significant.

SUPPLEMENTARY FIGURE 3 | A novel subset of macrophages shows no significant difference in DKO vs $ApoE^{-/-}$ male mice on NC. Male mice were fed on NC and analyzed at 20 weeks old. $ApoE^{-/-}$ (n=5), DKO (n=7). $p>0.05$. NS, not significant.

SUPPLEMENTARY FIGURE 4 | CD95+ macrophage percentage increases with HFD feeding. **(A, B)** Male mice were fed on HFD from 8 weeks old to 20 weeks old. **(A)** Macrophages were defined as CD45+F4/80+CD95+. **(B)** Macrophages were defined as CD45+F4/80+CD95+CD86-; WT (n=5), miR155$^{-/-}$ (n=6). **(C, D)** Male mice were fed on HFD from 8 to 32 weeks old. **(C).** Macrophages were defined as CD45+F4/80+CD95+. **(B)** Macrophages were defined as CD45+F4/80 +CD95+CD86-; WT (n=7), miR155$^{-/-}$ (n=10). *, p<0.05. NS, not significant.

SUPPLEMENTARY FIGURE 5 | CD95 (Fas) promotes inflammation signaling pathways. **(A)** The expressions of 34 genes out of 1376 innate immune genes from the Innate Immune Database (https://www.innatedb.com/) with the methods reported in our recent paper (https://www.frontiersin.org/articles/10.3389/fimmu.2020.554301/abstract) were decreased in the FAS knock-out microarray dataset (NIH-NCBI-Geo Datasets database GSE111244, FAS KO versus control cells). **(B)** The Ingenuity Pathway Analysis (IPA) with the Fas KO-decreased genes showed that eight inflammation signaling pathways were significantly downregulated (Z score ≥ 1 or Z score ≤ -1).

SUPPLEMENTARY FIGURE 6 | Fas mRNA transcripts are found in mouse aortic monocyte 1, monocyte 2 and macrophages. The data mining analyses were performed on the Single Cell RNA-Seq database of the Broad Institute of MIT and Harvard (Single Cell^{Beta} Portal; https://singlecell.broadinstitute.org/single_cell/study/SCP289/single-cell-analysis-of-the-normal-mouse-aorta-reveals-functionally-distinct-endothelial-cell-populations#study-summary, PMID: 31146585). **(A)** Fas expressions in monocytes and macrophages were circled in red in the Scatter; **(B)** Fas expressions in monocytes and macrophages were boxed in red in the Distribution; **(C)** Toll-like receptor 4 (TLR4) expressions in monocytes and macrophages were also boxed in read in the Distribution.

SUPPLEMENTARY FIGURE 7 | The expressions of Fas, TNFα, and IL-1b are found in all the four clusters of monocytes/macrophages in mouse adipose tissue stromal vascular fraction (SVF). The data mining analyses were performed on the Single Cell RNA-Seq database of the Broad Institute of MIT and Harvard (Single Cell^{Beta} Portal; https://singlecell.broadinstitute.org/single_cell/study/SCP708/mouse-adipose-stromal-vascular-fraction#study-summary). **(A)** Fas expressions in monocytes and macrophages were boxed in red in the Scatter; **(B)** TNFα expressions in monocytes and macrophages were boxed in red in the Distribution; **(C)** IL-1b expressions in monocytes and macrophages were also boxed in read in the Distribution.

SUPPLEMENTARY FIGURE 8 | The expressions of Fas, TNFα, and IL-1b are found in all the four clusters of monocytes/macrophages in human blood. The data mining analyses were performed on the Single Cell RNA-Seq database of the Broad Institute of MIT and Harvard (Single Cell^{Beta} Portal; https://singlecell.broadinstitute.org/single_cell/study/SCP43/atlas-of-human-blood-dendritic-cells-and-monocytes#study-summary; PMID: 28428369). **(A)** Fas expressions in monocytes and macrophages were boxed in red in the Scatter; **(B)** TNFα expressions in monocytes and macrophages were boxed in red in the Distribution; **(C)** IL-1b expressions in monocytes and macrophages were also boxed in read in the Distribution.

SUPPLEMENTARY FIGURE 9 | Extended high fat diet (HF) feeding-upregulated proinflammatory regulators including Fas (CD95), TNFα, IL-1b, and TLR4 overcome miR155 deficiency and promote resurgence of atherosclerosis. We reported that the expressions of C/EBP, PPARg, leptin (Lep), and resistin are upregulated in HF-fed MHO mice (PMIDs: 27856635; 30369883); and we also found that the expressions of CD95, TNFα, IL-1b and TLR4 are increased in HF-fed MHO mice in this study. The Cytoscape analyses (https://cytoscape.org/; the network data integration, analysis and visualization database) indicate that pro-inflammatory and pro-immune, lipid storage and white adipose tissue differentiation pathways are interplayed and promote resurgence of atherosclerosis and MHO transition to MUO.

REFERENCES

1. Fryar CD, Carroll MD, Ogden CL. *Prevalence of overweight, obesity, and severe obesity among adults aged 20 and over: United States, 1960–1962 through 2015–2016*. (2018) National Center for Health Statistics, Centers for Disease Control and Prevention.
2. Bigelow S. Food for Thought: Exploring Contributors to Obesity in Nurses *Adult Obesity Causes and Consequences*. (2018). Available: https://www.cdc.gov/obesity/adult/causes.html. 4 February 2020. [Online].
3. Colditz GA. Economic costs of obesity. *Am J Clin Nutr* (1992) 55(2):503S–7S. doi: 10.1093/ajcn/55.2.503s
4. Oliveros E, Somers VK, Sochor O, Goel K, Lopez-Jimenez F. The concept of normal weight obesity. *Prog Cardiovasc Dis* (2014) 56(4):426–33. doi: 10.1016/j.pcad.2013.10.003
5. Bluher M. Metabolically Healthy Obesity. *Endocr Rev* (2020) 41(3):405–20. doi: 10.1210/endrev/bnaa004
6. Smith GI, Mittendorfer B, Klein S. Metabolically healthy obesity: facts and fantasies. *J Clin Invest* (2019) 129(10):3978–89. doi: 10.1172/JCI129186
7. Bala C, Craciun A, Hancu N. Updating the concept of metabolically healthy obesity. *Acta Endocrinol (Bucharest)* (2016) 12(2):197. doi: 10.4183/aeb.2016.197
8. Lin H, Zhang L, Zheng R, Zheng Y. The prevalence, metabolic risk and effects of lifestyle intervention for metabolically healthy obesity: a systematic review and meta-analysis: a PRISMA-compliant article. *Medicine* (2017) 96(47). doi: 10.1097/MD.0000000000008838
9. Yin Y, Li X, Sha X, Xi H, Li Y-F, Shao Y, et al. Early hyperlipidemia promotes endothelial activation via a caspase-1-sirtuin 1 pathway. *Arterioscler Thromb Vasc Biol* (2015) 35(4):804–16. doi: 10.1161/ATVBAHA.115.305282
10. Virtue A, Wang H, Yang XF. MicroRNAs and toll-like receptor/interleukin-1 receptor signaling. *J Hematol Oncol* (2012) 5:66. doi: 10.1186/1756-8722-5-66
11. Virtue A, Johnson C, Lopez-Pastraña J, Shao Y, Fu H, Li X, et al. MicroRNA-155 Deficiency Leads to Decreased Atherosclerosis, Increased White Adipose Tissue Obesity, and Non-alcoholic Fatty Liver Disease: A NOVEL MOUSE MODEL OF OBESITY PARADOX. *J Biol Chem* (2017) 292(4):1267–87. doi: 10.1074/jbc.M116.739839
12. Eckel N, Meidtner K, Kalle-Uhlmann T, Stefan N, Schulze MB. Metabolically healthy obesity and cardiovascular events: a systematic review and meta-analysis. *Eur J Prevent Cardiol* (2016) 23(9):956–66. doi: 10.1177/2047487315623884
13. Bell JA, Kivimaki M, Hamer M. Metabolically healthy obesity and risk of incident type 2 diabetes: a meta-analysis of prospective cohort studies. *Obes Rev* (2014) 15(6):504–15. doi: 10.1111/obr.12157
14. Phillips CM. Metabolically healthy obesity: definitions, determinants and clinical implications. *Rev Endocr Metab Disord* (2013) 14(3):219–27. doi: 10.1007/s11154-013-9252-x
15. Bluher M. Are metabolically obese individuals are really healthy? in 16th European Congress of Endocrinology. *BioScientifica* (2014) 171:R209–R219. doi: 10.1530/endoabs.35.S5.3
16. Caleyachetty R, Thomas GN, Toulis KA, Mohammed N, Gokhale KM, Balachandran K, et al. Metabolically healthy obese and incident cardiovascular disease events among 3.5 million men and women. *J Am Coll Cardiol* (2017) 70(12):1429–37. doi: 10.1016/j.jacc.2017.07.763
17. Appleton SL, Seaborn CJ, Visvanathan R, Hill CL, Gill TK, Taylor AW, et al. Diabetes and cardiovascular disease outcomes in the metabolically healthy obese phenotype: a cohort study. *Diabetes Care* (2013) 36(8):2388–94. doi: 10.2337/dc12-1971
18. Soriguer F, Gutiérrez-Repiso C, Martín E, Fuentes E, Almaraz MC, Colomo N, et al. Metabolically healthy but obese, a matter of time? Findings from the prospective Pizarra study. *J Clin Endocrinol Metab* (2013) 98(6):2318–25. doi: 10.1210/jc.2012-4253
19. Russo L, Lumeng CN. Properties and functions of adipose tissue macrophages in obesity. *Immunology* (2018) 155(4):407–17. doi: 10.1111/imm.13002
20. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, et al. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* (2003) 112(12):1796–808. doi: 10.1172/JCI200319246
21. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* (2003) 112(12):1821–30. doi: 10.1172/JCI200319451

22. Scheja L, Heeren J. The endocrine function of adipose tissues in health and cardiometabolic disease. *Nat Rev Endocrinol* (2019) 15(9):507–24. doi: 10.1038/s41574-019-0230-6
23. Ying W, Riopel M, Bandyopadhyay G, Dong Y, BirminghamA, Seo JB, et al. Adipose Tissue Macrophage-Derived Exosomal miRNAs Can Modulate In Vivo and In Vitro Insulin Sensitivity. *Cell* (2017) 171(2):372–84.e12. doi: 10.1016/j.cell.2017.08.035
24. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* (1995) 95(5):2409–15. doi: 10.1172/JCI117936
25. Wang JLB, Nanayakkara G, Yang Q, Sun Y, Lu Y, Shao Y, et al. Experimental data-mining analyses reveal new roles of low-intensity ultrasound in differentiating cell death regulatome in cancer and non-cancer cells via potential modulation of chromatin long-range interactions. *Front Oncol* (2019) 2019:00600. doi: 10.3389/fonc.2019.00600
26. Ashkenazi A, Dixit VM. Death receptors: signaling and modulation. *science* (1998) p:1305–8. doi: 10.1126/science.281.5381.1305
27. Krammer PH. CD95's deadly mission in the immune system. *Nature* (2000) 407(6805):789–95. doi: 10.1038/35037728
28. Schumann DM, Maedler K, Franklin I, Konrad D, Størling J, Böni-Schnetzler M, et al. The Fas pathway is involved in pancreatic β cell secretory function. *Proc Natl Acad Sci* (2007) 104(8):2861–6. doi: 10.1073/pnas.0611487104
29. Wajant H, Pfizenmaier K, Scheurich P. Non-apoptotic Fas signaling. *Cytokine Growth Factor Rev* (2003) 14(1):53–66. doi: 10.1016/S1359-6101(02)00072-2
30. Wueest S, Mueller R, Blüher M, Item F, Chin ASH, Wiedemann MSF, et al. Fas (CD95) expression in myeloid cells promotes obesity-induced muscle insulin resistance. *EMBO Mol Med* (2014) 6(1):43–56. doi: 10.1002/emmm.201302962
31. Park DR, Thomsen AR, Frevert CW, Pham U, Skerrett SJ, Kiener PA, et al. Fas (CD95) induces proinflammatory cytokine responses by human monocytes and monocyte-derived macrophages. *J Immunol* (2003) 170(12):6209–16. doi: 10.4049/jimmunol.170.12.6209
32. Wang F, Lu Z, Hawkes M, Yang H, Kain KC, Liles WC. Fas (CD95) induces rapid, TLR4/IRAK4-dependent release of pro-inflammatory HMGB1 from macrophages. *J Inflammation (Lond)* (2010) 7:30. doi: 10.1186/1476-9255-7-30
33. Wueest S, Rapold RA, Schumann DM, Rytka JM, Schildknecht A, Nov O, et al. Deletion of Fas in adipocytes relieves adipose tissue inflammation and hepatic manifestations of obesity in mice. *J Clin Invest* (2010) 120(1):191–202. doi: 10.1172/JCI38388
34. Getz GS, Reardon CA. Diet and murine atherosclerosis. *Arterioscler Thromb Vasc Biol* (2006) 26(2):242–9. doi: 10.1161/01.ATV.0000201071.49029.17
35. Pansuria M, Xi H, Li L, Yang X-F, Wang H. Insulin resistance, metabolic stress, and atherosclerosis. *Front Biosci (Schol Ed)* (2012) 4:916–31. doi: 10.2741/s308
36. Johnson C, Drummer C IV, Virtue A, Gao T, Wu S, Hernandez M, et al. Increased Expression of Resistin in MicroRNA-155-Deficient White Adipose Tissues May Be a Possible Driver of Metabolically Healthy Obesity Transition to Classical Obesity. *Front Physiol* (2018) 9:1297. doi: 10.3389/fphys.2018.01297
37. Lai B, Wang J, Fagenson A, Sun Y, Saredy J, Lu Y, et al. Twenty Novel Disease Group-Specific and 12 New Shared Macrophage Pathways in Eight Groups of 34 Diseases Including 24 Inflammatory Organ Diseases and 10 Types of Tumors. *Front Immunol* (2019) 10:2612. doi: 10.3389/fimmu.2019.02612
38. Fang P, Li X, Shan H, Saredy JJ, Cueto R, Xia J, et al. Ly6C(+) Inflammatory Monocyte Differentiation Partially Mediates Hyperhomocysteinemia-Induced Vascular Dysfunction in Type 2 Diabetic db/db Mice. *Arterioscler Thromb Vasc Biol* (2019) 39(10):2097–119. doi: 10.1161/ATVBAHA.119.313138
39. Xu K, Yang WY, Nanayakkara G, Shao Y, Yang F, Hu W, et al. gaTa3, hDac6, and Bcl6 regulate FOXP3+ Treg Plasticity and Determine Treg conversion into either novel antigen-Presenting cell-like Treg or Th1-Treg. *Front Immunol* (2018) 9:45. doi: 10.3389/fimmu.2018.00045
40. Ke X, Wang J, Li L, Chen IH, Wang H, Yang X-F. Roles of CD4+CD25(high) FOXP3+ Tregs in lymphomas and tumors are complex. *Front Biosci* (2008) 13:3986–4001. doi: 10.2741/2986
41. Zhang Y, Roccaro AM, Rombaoa C, Flores L, Obad S, Fernandes SM, et al. LNA-mediated anti–miR-155 silencing in low-grade B-cell lymphomas. *Blood J Am Soc Hematol* (2012) 120(8):1678–86. doi: 10.1182/blood-2012-02-410647
42. Kluiver J, Slezak-Prochazka I, Winkle M, Visser L, Diepstra A, Kroesen B-J, et al. Mir-155 Enhances B-Cell Lymphoma Growth By Targeting TBRG1. *Blood* (2015) 126(23):4820.
43. Castoldi A, de Souza CN, Câmara NOS, Moraes-Vieira PM. The macrophage switch in obesity development. *Front Immunol* (2016) 6:637. doi: 10.3389/fimmu.2015.00637
44. Serbulea V, Upchurch CM, Schappe MS, Voigt P, DeWeese DE, Desai BN, et al. Macrophage phenotype and bioenergetics are controlled by oxidized phospholipids identified in lean and obese adipose tissue. *Proc Natl Acad Sci* (2018) 115(27):E6254–63. doi: 10.1073/pnas.1800544115
45. Wentworth JM, Naselli G, Brown WA, Doyle L, Phipson B, Smyth GK, et al. Pro-inflammatory CD11c+ CD206+ adipose tissue macrophages are associated with insulin resistance in human obesity. *Diabetes* (2010) 59(7):1648–56. doi: 10.2337/db09-0287
46. Lai B, Wang J, Fagenson A, Sun Y, Saredy J, Lu Y, et al. 20 novel disease group-specific-, and 12 new shared-macrophage pathways have been identified in eight groups of 34 diseases including 24 inflammatory organ diseases and 10 types of tumors. *Front Immunol* (2019) 10:2612. doi: 10.3389/fimmu.2019.02612
47. Shen H, Wu N, Nanayakkara G, Fu H, Yang Q, Yang WY, et al. Co-signaling receptors regulate T-cell plasticity and immune tolerance. *Front Biosci (Landmark Ed)* (2019) 24:96–132. doi: 10.2741/4710
48. Benson RJ, Hostager BS, Bishop GA. Rapid CD40-mediated rescue from CD95-induced apoptosis requires TNFR-associated factor-6 and PI3K. *Eur J Immunol* (2006) 36(9):2535–43. doi: 10.1002/eji.200535483
49. de Claro RA, Zhu X, Tang J, Morgan-Stevenson V, Schwartz BR, Iwata A. Hematopoietic Fas deficiency does not affect experimental atherosclerotic lesion formation despite inducing a proatherogenic state. *Am J Pathol* (2011) 178(6):2931–7. doi: 10.1016/j.ajpath.2011.02.011
50. Yang XF, Yin Y, Wang H. VASCULAR INFLAMMATION AND ATHEROGENESIS ARE ACTIVATED VIA RECEPTORS FOR PAMPs AND SUPPRESSED BY REGULATORY T CELLS. *Drug Discovery Today Ther Strateg* (2008) 5(2):125–42. doi: 10.1016/j.ddstr.2008.11.003
51. Libby P. Interleukin-1 Beta as a Target for AtherosclerosisáTherapy: Biological Basis of CANTOSáandáBeyond. *J Am Coll Cardiol* (2017) 70(18):2278–89. doi: 10.1016/j.jacc.2017.09.028
52. Singh RK, Haka AS, Asmal A, Barbosa-Lorenzi VC, Grosheva I, Chin HF, et al. TLR4 (toll-like receptor 4)-dependent signaling drives extracellular catabolism of LDL (low-density lipoprotein) aggregates. *Arteriosclerosis Thrombosis Vasc Biol* (2020) 40(1):86–102. doi: 10.1161/ATVBAHA.119.313200
53. Kalluri AS, Vellarikkal SK, Edelman ER, Nguyen L, Subramanian A, Ellinor PT, et al. Single-Cell Analysis of the Normal Mouse Aorta Reveals Functionally Distinct Endothelial Cell Populations. *Circulation* (2019) 140(2):147–63. doi: 10.1161/CIRCULATIONAHA.118.038362
54. Mai J, Nanayakkara G, Lopez-Pastrana J, Li X, Li Y-F, Wang X, et al. Interleukin-17A Promotes Aortic Endothelial Cell Activation via Transcriptionally and Post-translationally Activating p38 Mitogen-activated Protein Kinase (MAPK) Pathway. *J Biol Chem* (2016) 291(10):4939–54. doi: 10.1074/jbc.M115.690081
55. Saaoud F, Wang J, Iwanowycz S, Wang Y, Altomare D, Shao Y, et al. Bone marrow deficiency of mRNA decaying protein Tristetraprolin increases inflammation and mitochondrial ROS but reduces hepatic lipoprotein production in LDLR knockout mice. *Redox Biol* (2020) p:101609. doi: 10.1016/j.redox.2020.101609
56. Mai J, Virtue A, Shen J, Wang H, Yang X. An evolving new paradigm: endothelial cells–conditional innate immune cells. *J Hematol Oncol* (2013) 6:61. doi: 10.1186/1756-8722-6-61
57. Shao Y, Saredy J, Yang G, Sun Y, Lu Y, Saaoud F, et al. Vascular Endothelial Cells and Innate Immunity. *Arterioscler Thromb Vasc Biol* (2020) 40(6):e138–52. doi: 10.1161/ATVBAHA.120.314330
58. Öhman MK, Shen Y, Obimba CI, Wright AP, Warnock M, Lawrence DA, et al. CLINICAL PERSPECTIVE. *Circulation* (2008) 117(6):798–805. doi: 10.1161/CIRCULATIONAHA.107.717595
59. Villani AC, Satija R, Reynolds G, Sarkizova S, Shekhar K, Fletcher J, et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. *Science* (2017) 356(6335). doi: 10.1126/science.aah4573

60. Antonelli A, Bianchi M, Crinelli R, Gentilini L, Magnani M. Modulation of ICAM-1 expression in ECV304 cells by macrophage-released cytokines. *Blood Cells Mol Dis* (2001) 27(6):978–91. doi: 10.1006/bcmd.2001.0470
61. Ibrahim MM. Subcutaneous and visceral adipose tissue: structural and functional differences. *Obes Rev* (2010) 11(1):11–8. doi: 10.1111/j.1467-789X.2009.00623.x
62. Bijnen M, Josefs T, Cuijpers I, Maalsen CJ, van de Gaar J, Vroomen M, et al. Adipose tissue macrophages induce hepatic neutrophil recruitment and macrophage accumulation in mice. *Gut* (2018) 67(7):1317–27. doi: 10.1136/gutjnl-2016-313654
63. Cildir G, Akincilar SC, Tergaonkar V. Chronic adipose tissue inflammation: all immune cells on the stage. *Trends Mol Med* (2013) 19(8):487–500. doi: 10.1016/j.molmed.2013.05.001
64. Maciejewski JP, Selleri C, Sato T, Anderson S, Young NS. Increased expression of Fas antigen on bone marrow CD34+ cells of patients with aplastic anaemia. *Br J Haematol* (1995) 91(1):245–52. doi: 10.1111/j.1365-2141.1995.tb05277.x
65. Andres R. Effect of obesity on total mortality. *Int J Obes* (1980) 4(4):381.
66. Schröder H, Ramos R, Baena-Díez JM, Mendez MA, Canal DJ, Fíto M, et al. Determinants of the transition from a cardiometabolic normal to abnormal overweight/obese phenotype in a Spanish population. *Eur J Nutr* (2014) 53 (6):1345–53. doi: 10.1007/s00394-013-0635-2
67. Hwang Y, Hayashi T, Fujimoto WY, Kahn SE, Leonetti DL, McNeely MJ, et al. Visceral abdominal fat accumulation predicts the conversion of metabolically healthy obese subjects to an unhealthy phenotype. *Int J Obes* (2015) 39 (9):1365–70. doi: 10.1038/ijo.2015.75
68. Ortega FB, Lavie CJ, Blair SN. Obesity and Cardiovascular Disease. *Circ Res* (2016) 118(11):1752–70. doi: 10.1161/CIRCRESAHA.115.306883
69. Targher G, Byrne CD. Obesity: Metabolically healthy obesity and NAFLD. *Nat Rev Gastroenterol Hepatol* (2016) 13(8):442–4. doi: 10.1038/nrgastro.2016.104
70. Huang LO, Loos RJ, Kilpeläinen TO. Evidence of genetic predisposition for metabolically healthy obesity and metabolically obese normal weight. *Physiol Genomics* (2018) 50(3):169–78. doi: 10.1152/physiolgenomics.00044.2017
71. Phillips CM, Perry IJ. Does inflammation determine metabolic health status in obese and nonobese adults? *J Clin Endocrinol Metab* (2013) 98(10):E1610–9. doi: 10.1210/jc.2013-2038
72. Jung CH, Lee MJ, Kang YM, Hwang JY, Kim EH, Park J-Y, et al. The risk of chronic kidney disease in a metabolically healthy obese population. *Kidney Int* (2015) 88(4):843–50. doi: 10.1038/ki.2015.183
73. Knigge A, Klöting N, Schön MR, Dietrich A, Fasshauer M, Gärtner D, et al. ADCY5 gene expression in adipose tissue is related to obesity in men and mice. *PloS One* (2015) 10(3):e0120742. doi: 10.1371/journal.pone.0120742
74. Karelis AD, Faraj M, Bastard J-P, St-Pierre DH, Brochu M, Prud'homme D, et al. The metabolically healthy but obese individual presents a favorable inflammation profile. *J Clin Endocrinol Metab* (2005) 90(7):4145–50. doi: 10.1210/jc.2005-0482
75. Koster A, Stenholm S, Alley DE, Kim LJ, Simonsick EM, Kanaya AM, et al. Body fat distribution and inflammation among obese older adults with and without metabolic syndrome. *Obesity* (2010) 18(12):2354–61. doi: 10.1038/oby.2010.86
76. Shin M-J, Hyun YJ, Kim OY, Kim JY, Jang Y, Lee JH, et al. Weight loss effect on inflammation and LDL oxidation in metabolically healthy but obese (MHO) individuals: low inflammation and LDL oxidation in MHO women. *Int J Obes* (2006) 30(10):1529–34. doi: 10.1038/sj.ijo.0803304
77. Aguilar-Salinas CA, García EG, Robles L, Riaño D, Ruiz-Gomez DG, García-Ulloa AC, et al. High adiponectin concentrations are associated with the metabolically healthy obese phenotype. *J Clin Endocrinol Metab* (2008) 93 (10):4075–9. doi: 10.1210/jc.2007-2724
78. Francisqueti FV, Nascimento AF, Minatel IO, Dias MC, de Azevedo Melo Luvizotto R, Berchieri-Ronchi C, et al. Metabolic syndrome and inflammation in adipose tissue occur at different times in animals submitted to a high-sugar/fat diet. *J Nutr Sci* (2017) 6:e41. doi: 10.1017/jns.2017.42
79. Rosito GA, Massaro JM, Hoffmann U, Ruberg FL, Mahabadi AA, Vasan RS, et al. Pericardial fat, visceral abdominal fat, cardiovascular disease risk factors, and vascular calcification in a community-based sample: the Framingham Heart Study. *Circulation* (2008) 117(5):605–13. doi: 10.1161/CIRCULATIONAHA.107.743062
80. Ong KL, Ding J, McClelland RL, Cheung BMY, Criqui MH, Barter PJ, et al. Relationship of pericardial fat with lipoprotein distribution: The Multi-Ethnic study of atherosclerosis. *Atherosclerosis* (2015) 241(2):664–70. doi: 10.1016/j.atherosclerosis.2015.06.027
81. Harrington EO, Stefanec T, Newton J, Rounds S. Release of soluble E-selectin from activated endothelial cells upon apoptosis. *Lung* (2006) 184(5):259–66. doi: 10.1007/s00408-005-2589-5
82. Videm V, Albrigtsen M. Soluble ICAM-1 and VCAM-1 as markers of endothelial activation. *Scand J Immunol* (2008) 67(5):523–31. doi: 10.1111/j.1365-3083.2008.02029.x
83. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K-I, Kitazawa R, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* (2006) 116(6):1494–505. doi: 10.1172/JCI26498
84. Zhang R, Saredy J, Shao Y, Yao T, Liu L, Saaoud F, et al. End-stage renal disease is different from chronic kidney disease in upregulating ROS-modulated proinflammatory secretome in PBMCs - A novel multiple-hit model for disease progression. *Redox Biol* (2020) 34:101460. doi: 10.1016/j.redox.2020.101460

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Single-Cell Proteomics Reveals the Defined Heterogeneity of Resident Macrophages in White Adipose Tissue

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Adipose tissue macrophages (ATMs) regulate homeostasis and contribute to the metabolically harmful chronic inflammation in obese individuals. While evident heterogeneity of resident ATMs has been described previously, their phenotype, developmental origin, and functionality remain inconsistent. We analyzed white adipose tissue (WAT) during homeostasis and diet interventions using comprehensive and unbiased single-cell mass cytometry and genetic lineage tracking models. We now provide a uniform definition of individual subsets of resident ATMs. We show that in lean mice, WAT co-harbors eight kinetically evolving CD206$^+$ macrophage subpopulations (defined by TIM4, CD163, and MHC II) and two CD206$^-$ macrophage subpopulations. TIM4$^-$CD163$^+$, TIM4$^-$CD163$^-$ and CD206$^-$ macrophage populations are largely bone marrow-derived, while the proliferating TIM4$^+$CD163$^+$ subpopulation is of embryonic origin. All macrophage subtypes are active in phagocytosis, endocytosis, and antigen processing *in vitro*, whereas TIM4$^+$CD163$^+$ cells are superior in scavenging *in vivo*. A high-fat diet induces massive infiltration of CD206$^-$ macrophages and selective down-regulation of MHC II on TIM4$^+$ macrophages. These changes are reversed by dietary intervention. Thus, the developmental origin and environment jointly regulate the functional malleability of resident ATMs.

Keywords: adipose tissue, mass cytometry (CyTOF), developmental origin, obesity, macrophage

INTRODUCTION

Obesity and impaired metabolic health are escalating worldwide, and obesity is a significant risk factor for metabolic disorders, including type II diabetes, non-alcoholic fatty liver disease, atherosclerosis, and ischemic cardiovascular disease (1, 2). The tissue most strongly associated with the pathogenesis of obesity is white adipose tissue (WAT). Adipose tissue macrophages (ATMs), the most abundant immune cell type in WAT, have been associated with the development

and expansion of adipose tissue, as well as with antigen presentation, iron metabolism, and catecholamine synthesis (3–8). Immunological profiling of obesity has mainly focused on the contribution of the recruited bone marrow-derived macrophages to WAT (9–16), showing that the CCR2-mediated recruitment of monocytes and their differentiation into macrophages (17–19) forms the basis of harmful obesity-associated chronic inflammation of WAT (20, 21). The behavior and identity of the recruited macrophage populations that accumulate in obese adipose tissue are well characterized (15, 18, 19, 22). Diet-induced obesity (DIO) has been suggested to alter ATMs phenotype from an anti-inflammatory (M2) state towards a more pro-inflammatory (M1) activation state that contributes to insulin resistance (15). Inhibition of the accumulation of pro-inflammatory macrophages in WAT results in amelioration of obese conditions and improved metabolic status (18, 23–28). However, further studies are needed to unravel the function and diversity of resident macrophages, especially in steady state lean adipose tissue, to provide knowledge that would shift intervention approaches away from targeting the inflammatory ATMs towards targeting their metabolic reprogramming in order to maintain adipose tissue homeostasis.

Not all tissue-resident macrophages originate from bone marrow-derived monocyte precursors. In numerous tissues, resident macrophages are established during early embryonic development and are self-maintaining locally throughout adult life, with minimal input from circulating monocytes (29–35). Likewise, adipose tissue also contains self-renewing macrophages, which populate adipose tissue independently of the establishment of bone marrow hematopoiesis (10–12, 36, 37). Recently, several studies have addressed the identity of the steady state ATM subpopulations in mice, both at RNA level with single-cell sequencing and protein level with conventional flow cytometry, demonstrating an evident heterogeneity among the resident ATMs in WAT (15, 38–41). However, it is unclear how these different subsets described in various studies using different techniques and markers are related to each other, and detailed analyses of developmental origin and maintenance of resident macrophages in adipose tissue are still lacking. Defining the identity of different resident ATMs in lean WAT is essential to dissect more precisely their role in homeostatic or obesity-related functions.

In the present study, we aimed to unravel the development, identity, and origin of resident ATM populations in lean WAT and their responsiveness to dietary interventions. Using comprehensive and unbiased single-cell analyses in wild type and genetically modified mouse models, we identified the co-existence of multiple distinct resident ATM populations in the lean WAT defined by CD206, TIM4, CD163, and MHC II cell surface markers. The results showed that resident ATMs are a complex pool of embryonic and infiltrating monocyte-derived macrophages. Our analyses reveal for the first time that resident ATM subsets exist both in lean and obese WAT in relatively steady numbers and have unique phenotypic switches compared to the obesity-induced bone marrow-derived macrophages. Our findings provide a unifying approach for using distinct markers to identify resident ATM populations in a lean WAT and suggest a critical role for the origin of macrophages in regulating the function of ATM subpopulations in WAT.

MATERIALS AND METHODS

Mice

The genetic mouse models used in this study were: *Ccr2*$^{-/-}$ (stock 004999), *Nur77*$^{-/-}$ (stock 006187), *R26R-EYFP* (stock 006148), *Cx3cr1*^{CreERT2} (stock 020940), and *Csf1r*^{CreEsr1} (stock 019098), purchased from Jackson Laboratories, and *Plvap*^{tm1Salm} (*Plvap*$^{-/-}$) mice (42, 43). Wild type (WT) mice, C57BL/6J and C57BL/6N, were acquired from Janvier labs. All mice were kept under 22°C and 12 hours of light and 12 hours dark cycles at the animal facilities of the University of Turku (Turku, Finland). Unless stated otherwise, the animals were fed with standard pellet chow and reverse osmosis water. Only males were used in all experiments, and age-matched WT mice were used as controls in each experiment. Embryonic development was estimated considering the day of a vaginal plug as embryonic day 0.5 (E0.5). Animal experiments were conducted under the revision and approval of the Regional Animal Experiment Board in Finland, according to the 3R-principle and under Animal license numbers 6211/04.10.07/2017 and 14685/2020. All experiments were regulated according to the Finnish Act on Animal Experimentation (497/2013).

Mass and Flow Cytometry

Both sides of the epididymal white adipose depots (eWAT) were collected to Hank's Buffered Saline (Sigma-Aldrich, H9394-500ML). The fat depots were minced with scissors and then digested for 1 hour at 37°C, with 1 mg/ml collagenase D (Roche, 1108886601) and 50 µg/ml DNase 1 (Roche, 10104159001). During the digestion, the samples were shaken 2 to 3 times. After homogenization by pipetting, the cell suspension was left for 10 minutes at room temperature (RT) for gravitational separation of the fractions. The stromal vascular fraction (SVF) fraction was filtered through silk (pore size 77 µm), pelleted, washed twice with Hank's Buffered Saline, and suspended in PBS or FACS buffer.

For mass cytometry analysis, eWAT from both sides of seven 2-week-old and three 5-week-old mice were pooled. For analyses of 8-, 12-, 16- and 23-week-old mice, two donors per data point were used to get a sufficient number of leukocytes. Samples were stained in U-bottom 96-well plates. They were incubated in 2.5 µM Cell-ID Cisplatin (Fluidigm 201064; 5 min, RT) to exclude dead cells and then with anti-CD16/32 (clone 2.4G2, Bio X Cell; 10 min, RT) to block the Fc-receptors. Target antigens were labeled with pools of metal-tagged antibodies listed in **Supplementary Table 1** (RT, 30 min). The samples were finally stained with DNA intercalation solution (1:1000 Cell ID Intercalator-103Rh in MaxPar® Fix and Perm Buffer, cat. 201067; Fluidigm; 1 hour, RT), and fixed by 4% paraformaldehyde solution (PFA; Santa Cruz Biotechnology,

cat. sc-281692; overnight, 4°C). Before the acquisition, the cells were washed and resuspended in purified H_2O.

The samples were run using Fluidigm Helios Mass Cytometer. Data were analyzed using Cytobank software (Cytobank, Inc) or Cytosplore visual analysis system (44, 45). Bead normalized data were gated for single ($191Ir^+$) live ($Cisplatin^-$) cells, analyzed by viSNE (dimensionality reduction algorithm t-SNE), and clustered in an unsupervised manner using FlowSOM algorithm (Cytobank, https://www.cytobank.org) or HSNE [Hierarchical Stochastic Neighbor Embedding (44)] using Cytosplore.

For fluorescent flow cytometry, the Fc-receptors were blocked the same way as for CyTOF. The staining with fluorochrome-conjugated antibodies was performed at 4°C for 20 min (the antibodies used are listed in **Supplementary Table 1**). Data were acquired with LSR Fortessa flow cytometer (Becton Dickinson) and analyzed using the FlowJo software (FlowJo LLC).

Lineage Tracking Experiments

To study the embryonic origin of macrophages, $Csf1r^{CreEsr1}$ male mice were crossed with R26R-EYFP female mice. The plugged females were injected intraperitoneally (i.p.) with tamoxifen (1.5 mg) and progesterone (0.75 mg) at E8.5 to fluorescently label the offspring's $CSF1R^+$ yolk sac (YS) -derived macrophages. Additionally, $Cx3cr1^{CreERT2}$ male mice were crossed with R26R-EYFP female mice. Pregnant dams were injected i.p. with the same dose of tamoxifen and progesterone as above at E9.5 or E13.5 to induce reporter recombination on the offspring. Both E9.5 and E13.5 tamoxifen pulsings selectively label only YS-derived macrophages since CX3CR1 is not expressed in the fetal liver-derived monocytes or their precursors (31, 46).

Histologic Analysis

Epididymal fat pad samples were fixed for 48 to 72 hours at RT with 10% Normal Buffered Formalin (Sigma, cat.:HT501128-4L). The specimens were then dehydrated in 50% EtOH to 70% EtOH gradient and kept at 4°C in 70% EtOH until paraffin embedding at Histology core facility of the Institute of Biomedicine, University of Turku, Finland. Four µm sections were cut, dried overnight at 37°C, deparaffinized, rehydrated, stained with Periodic Acid Schiff (Sigma-Aldrich, cat.:395B-1KT), and counterstained with hematoxylin. Slides were mounted with Dibutylphthalate Polystyrene Xylene (DPX; Sigma, cat.:06522-100ML). Stained sections were imaged using Pannoramic 1000 (3DHISTECH) scanner (with a 40x lens) and analyzed using CaseViewer software (3DHISTECH – CaseCenter 2.9 SP1).

Proliferation Experiments

5- and 16-week-old mice were injected i.p. with 1.2 mg of bromodeoxyuridine (BrdU, BD Pharmingen, cat.: 51-2420KC). Two hours after injection, fat pads were collected and processed as described above for flow cytometry. After standard FACS staining for leukocyte markers, cells were fixed and stained with FITC-conjugated BrdU antibody (BrdU Flow kit, BD Bioscience).

Diet Experiments

High-fat diet (HFD; Research diets, cat.: D12492, 5.24 kcal/g, 60 kcal% fat) was used to induce obesity, and it was compared with a natural ingredient-based standard diet [Chow; CRM (E), Special Diet Services, cat.: 801730, 3.62 kcal/g, 9.12 kcal% fat] and purified ingredient normal-fat diet (NFD; Research diets, cat.: D12450J, 3.85 kcal/g, 10 kcal% fat). 7 to 8-week-old male mice were weighed and randomly divided into the specific diet groups fed *ad libitum*. After eight weeks of the experimental diets (at the age of 16 weeks), eWAT from Chow and HFD fed group was analyzed to establish a baseline to study the effects of weight loss in obese mice (n = 8). As weight loss models, last 8 weeks of the experiment (from 16 to 23 weeks), a cohort of HFD-fed mice started a metformin treatment incorporated in the diet (HFD + Met, n = 8; Research diets, cat.: D18120701, 60 kcal% fat, average dose 353 mg/kg calculated based on food intake) and another cohort was reversed to Chow (HFD to Chow, n = 10). The remaining three groups [Chow (n = 8), NFD (n = 8), and HFD (n = 16)] continued with their original diets to the end of the experiment.

All diet experiment mice were weighed every two weeks. Glucose tolerance test (GGT) was performed after 14 weeks of diet interventions (22 weeks of age). Before GTT, mice were fasted for four hours, and the basal blood glucose level was measured. Glucose (2.5 g/kg lean mass) was injected intraperitoneally (i.p.), and glucose was measured from a blood sample using a glucometer (Contour XT, Bayer) obtained from the tail vein 20, 40, 60, and 90 minutes after the glucose injection. The fat mass gain was analyzed two times during the diet interventions (8 and 15 weeks from the beginning of diet intervention) by quantitative nuclear magnetic resonance (NMR) scanning (EchoMRI-700, Echo Medical Systems). After euthanasia, eWAT samples were collected, processed, and analyzed as described above.

Scavenging Experiments

For *in vitro* endocytosis assays, SVF was isolated from eWAT of 5-week-old WT mice (digested as described in "*Flow and Mass Cytometry*"). Negative control cells were kept at +4°C for 1 hour before exposure to the fluorescent cargoes. SVF of one animal was diluted to 200 µl of RPMI-1640 (Sigma, cat.: R5886-500mL) supplemented with 10% FCS and 2 mM L-glutamine (Glutamax, Gibco, cat.: 35050-038), and the cells were seeded to U-bottom 96-well plates. Fluorescein-conjugated dextran (1 mg/mL; 500 kDa; Invitrogen, cat.: D7136) or fluorescent beads (1:1000, 0.002% of solids; FluoSpheres carboxylate, 0.5 µm, yellow-green 505/515; Invitrogen, cat.: F8813) were added to cells. The plates were incubated at +37°C or +4°C for 1 h. After that, the cells were washed with ice-cold PBS to inactivate them metabolically. Flow cytometric staining was performed as described before. To calculate the phagocytosis/endocytosis capacity of $TIM4^+CD163^+$, $TIM4^+$, $CD163^+$, and $TIM4^-CD163^-$ ATM subsets, the mean intensity fluorescence (MFI) of samples incubated at +37°C was divided by the MFI of samples incubated at +4 °C.

For *in vivo* endocytosis assays, 5- or 22-week-old mice were injected in the lateral tail vein, with 0.8 mg of 500 kDa dextran Fluorescein (Invitrogen, cat.: D7136) or with 0.1 mg Ovalbumin Fluorescein (Invitrogen, cat.: O23020) – 0,02mg chicken egg albumin antibody (Sigma, cat.: C6534-2ML) complex (47). One (for dextran) or two (for the immunocomplex) hours after the injections, the fat pads were collected, processed, and analyzed by flow cytometry, as described above.

Antigen Processing Experiment

DQ ovalbumin (DQ-OVA, 1mg/ml; Invitrogen, cat.: D12053) is a self-quenched conjugate of ovalbumin that exhibits green fluorescence after proteolytic degradation in the cells. SVF was isolated from eWAT of 5-week-old WT mice and diet experiment mice (Chow, HFD and HFD to Chow) at 30 weeks of age and incubated with the reporter under the conditions described in the "Scavenging experiments". Preparation for this assay was performed as mentioned for the *in vitro* scavenging tests. DQ-OVA was added into 200 µL of cell suspension at a concentration of 10 µg/mL. Flow cytometric staining was performed as described before. To calculate the proteolytic capacity of ATM subsets, the MFI of samples incubated at +37°C was divided by the MFI of samples incubated at +4°C.

Statistics

Adult mice were allocated to experimental groups without specific randomization methods. Numeric data are given as mean ± SEM. Comparisons between the genotypes or treatment groups were made using the nonparametric two-tailed Mann-Whitney test, parametric two-tailed t-test, one- or two-way ANOVA test with Bonferroni post-hoc test. GraphPad Prism 9 (GraphPad Software, LLC) was used for the statistical analyses. P-values ≤ 0.05 are considered to be statistically significant.

RESULTS

TIM4 and CD163 Define the Resident Macrophage Populations in Adipose Tissue

We applied a single-cell mass cytometry (CyTOF) approach for immune profiling of white adipose tissue (WAT) macrophage populations. Based on previous studies (11, 15, 38, 39, 41, 48), we first designed an extensive antibody panel including the signature markers associated with resident macrophages. We then carried out comprehensive profiling of epididymal white adipose tissue (eWAT) immune cell composition in 2, 5, 8, 12, 16, and 23 weeks old wild type (WT) male mice on a standard diet (referred hereafter as Chow; **Supplementary Figure 1A**). To visualize the overall immune cell profile of eWAT, we applied the t-Distributed Stochastic Neighbor Embedding (tSNE; 49) dimensionality reduction approach to all $CD45^+$ cells (**Supplementary Figures 1B, C**). Across all samples, $CD11b^+$ myeloid cells, including macrophages ($CD64^+F4/80^+$), monocytes ($Ly6C^+$), dendritic cells ($CD11c^+$), granulocytes ($Ly6G^+$), and eosinophils ($SiglecF^+$), were identified as the dominant leukocyte population (together representing 53 to 60% of all $CD45^+$ cells). In addition, T- ($CD4^+$ and $CD8^+$) and B-lymphocytes ($B220^+$), $CD11b^-$ dendritic cells ($CD11b^-CD11c^+$), and unclassified $CD45^+$ cells were detectable in eWAT at all time points studied (**Supplementary Figure 1C**).

We then focused on the immunophenotypic characteristics and the kinetics of eWAT tissue-resident macrophages in more detail from juvenile (2- to 5-week-old) to adult (8- to 23-week-old) WT mice. We manually gated the macrophages from $CD45^+$ cells based on the commonly used canonical macrophage markers, $CD64^+$ and $F4/80^+$ (**Supplementary Figure 1B**). As expected, most steady state eWAT macrophages from 2 to 23 weeks expressed CD206, a well-characterized M2-like macrophage marker, and only a minor fraction of $CD64^+F4/80^+$ macrophages were CD206 negative in a lean eWAT (**Figure 1A**). To avoid bias through manual gating, we performed unsupervised clustering of $CD64^+F4/80^+$ cells with the FlowSOM algorithm (50). This self-organizing map on top of tSNE visualization distinguished several macrophage subclusters, each expressing unique surface marker profiles (**Figures 1A, B** and **Supplementary Figure 2**). Among the $CD206^+CD64^+F4/80^+$ tissue-resident macrophages, four main clusters of cells were observed based on their differential expression of TIM4 and CD163. For brevity, the $TIM4^+CD163^+$, $TIM4^+CD163^-$, $TIM4^-CD163^+$ and $TIM4^-CD163^-$ populations will be called hereafter $TIM4^+CD163^+$, $TIM4^+$, $CD163^+$, and $TIM4^-CD163^-$, respectively. Each of these four major populations was further subdivided into two clusters based on major histocompatibility complex II (MHC II) expression (**Figure 1B** and **Supplementary Figure 2**). All MHC II^+ clusters also expressed higher levels of Siglec1 (CD169) than MHC II^- sub-clusters (**Supplementary Figure 2**). Interestingly, CX3CR1 expression was detected mainly in $TIM4^-CD163^-$MHC II^+ cluster and in a small proportion of cells in the $CD163^+$MHC II^+ cluster (**Supplementary Figure 2**).

Kinetic analyses showed that several macrophage subpopulations underwent characteristic alterations in frequency between the different time points (**Figure 1C** and **Supplementary Figures 3A–F**). In both $TIM4^+CD163^+$ and $TIM4^+$ ATM populations, the frequency of MHC II positive macrophages increased from 2 to 5 weeks of age and thereafter started to decrease slowly. In contrast, the MHC II negative $TIM4^+CD163^+$ and $TIM4^+$ ATMs, the major subpopulations at juvenile (2- to 5-week-old) time points, strongly diminished throughout the postnatal development (**Figure 1C** and **Supplementary Figures 3A, B**). Among $CD163^+$ and $TIM4^-CD163^-$ macrophages, on the other hand, the frequency of MHC II positive cells grew significantly by age (**Figure 1C** and **Supplementary Figures 3C, D**). While the majority of $CD64^+F4/80^+$ cells were CD206 positive, a minor percentage of CD206 negative cells were also identified (**Figures 1A–C**). Consistent with other reports (15, 39), the $CD206^-CD64^+F4/80^+$ cells in lean mice included a small CD11c and $Ly6C^+$ positive ATM clusters (**Figures 1A–C** and **Supplementary Figure 2**), likely representing monocyte-derived cells. The frequency of

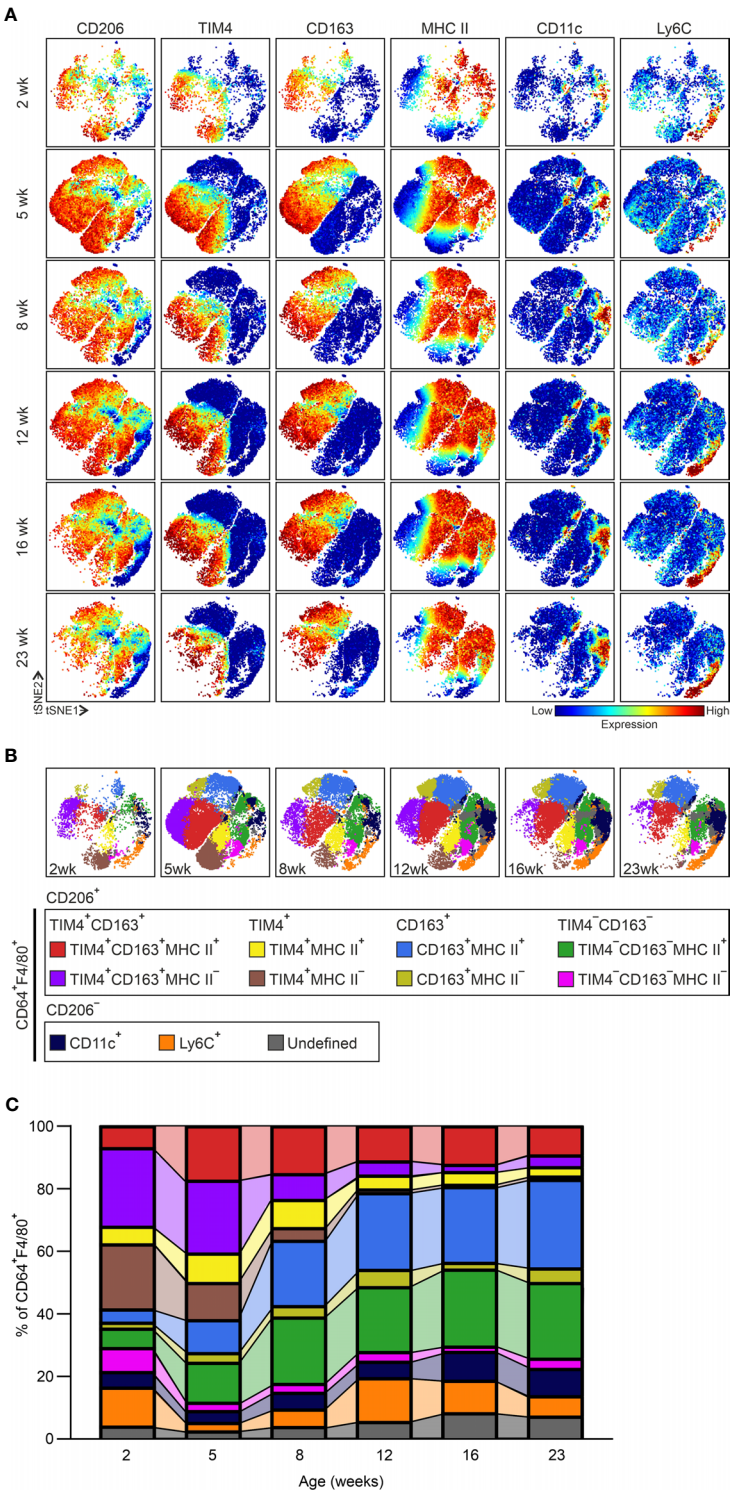

FIGURE 1 | Adipose tissue macrophages (ATMs) evolve during postnatal development. **(A)** Representative viSNE plots of $CD45^+CD64^+F4/80^+$ macrophages of epididymal white adipose tissue (eWAT) in 2-, 5-, 8-, 12-, 16- and 23-week-old wild-type (WT) mice. The color code indicates the expression level of a given marker from low (blue) to high (red). **(B)** Representative unsupervised FlowSOM analysis of eWAT $CD45^+CD64^+F4/80^+$ macrophage cell clusters at the indicated time points. Each FlowSOM metacluster (subpopulations) is indicated in different colors. **(C)** Frequencies of the macrophage subpopulations in WT eWAT based on the FlowSOM analyses at the indicated time points. Each FlowSOM metacluster (subpopulation) is represented by an individual color depicted in the columns (from **B**). Data are from n = 14 (2 wk), 12 (5 wk), n = 6 (8, 12, and 16) or n = 8 (23 wk) mice.

$CD11c^+$ ATMs remained relatively steady, while the frequency of $Ly6C^+$ ATMs fluctuated throughout the postnatal development (**Figure 1C** and **Supplementary Figures 3E, F**). Notably, although most $CD11c^+$ cells were observed to be CD206 negative, low numbers of $TIM4^+CD163^+$ and $CD163^+$ cells also expressed CD11c (**Supplementary Figure 2**).

Previous work has reported that most of the resident ATMs in lean mice are associated with adipose tissue vasculature. The vasculature-associated macrophages (VAMs) were identified with the expression of CD206, TIM4, and MHC II surface markers (11). To compare our data with the identified VAM macrophage populations, we manually gated our mass cytometry data with the same gating strategy used to determine the VAMs (**Supplementary Figure 4A**). We observed that all the populations recognized previously, VAM1, VAM2, pre-VAM and double positive (DP) macrophages, actually included cells from all main $CD206^+$ ($TIM4^+CD163^+$, $TIM4^+$, $CD163^+$, and $TIM4^-CD163^-$) clusters identified with our unsupervised computational approach (**Supplementary Figure 4B**). In many tissues, LYVE1 positive macrophages are assigned to be the vessel-associated macrophages (51), and scRNA-seq experiments have correspondingly shown that the ATMs in adult lean mice express *Lyve1* (11, 38). Therefore, we next studied LYVE1 from our data. We found that $LYVE1^+$ macrophages did not represent a separate cluster but were identified in small subpopulations within each of the four major $CD206^+$ ATM populations at all time points studied (**Supplementary Figures 4C, D**). Likewise, the LYVE1 positivity was found among all VAM1, VAM2, pre-VAM, and DP macrophage populations (**Supplementary Figure 4B**). Together these single-cell mass cytometric analyses show that the division of ATM subclasses in lean WAT is much more complex than previously appreciated and pointed out the need for higher dimensional flow cytometry and computational methods to capture the full heterogeneity of ATMs. Our mass cytometric analyses provide the first high-resolution map of the development of ATMs in eWAT and identify the optimal surface markers for identifying the distinct macrophage subtypes.

Bone Marrow-Derived CCR2 Dependent Monocytes Account for TIM4 Negative Tissue-Resident Macrophages in White Adipose Tissue

The circulating bone marrow-derived monocytes are known to heavily replenish macrophage pools in WAT in obesity (19). To address the contribution of bone marrow-derived monocytes to eWAT tissue-resident macrophage subpopulations in steady state, we used *Ccr2*- and *Nur77*-deficient mice manifesting with low numbers in circulating classical ($Ly6C^{High}$) and patrolling ($Ly6C^{Low}$) monocytes, respectively (52, 53). The total body weights and eWAT morphology of WT and $Ccr2^{-/-}$ male mice were comparable (**Figures 2A, B** and **Supplementary Figure 5A**), except for lighter eWAT fat pads in over 12-week-old $Ccr2^{-/-}$ mice (**Supplementary Figure 5B**), which is a known phenotype of *Ccr2* deficient mice (18). FlowSOM clustering analysis of 5- and 8-week-old $Ccr2^{-/-}$ mice eWAT showed that the frequency of $TIM4^+CD163^+$ and $TIM4^+$ ATMs (MHC II positive and negative subpopulations) was increased (**Figures 2C–E**; and **Supplementary Figure 5C**). On the other hand, *Ccr2* deletion resulted in a striking loss of $CD163^+$MHC II^+ and $TIM4^-CD163^-$MHC II^+ ATM populations that were not recovered upon aging (**Figures 2C, F, G**). Additionally, $CD206^-CD11c^+$ and $CD206^-Ly6C^+$ macrophage populations were significantly diminished in *Ccr2* deficient mice compared to WT controls (**Figures 2H, I**). On the contrary, the contribution of bone marrow-derived patrolling $Ly6C^{Low}$ monocytes in the generation of ATMs was not substantial. A slight decrease in the frequency of $CD163^+$MHC II^+ and $TIM4^-CD163^-$MHC II^+ ATMs was seen only at 8-week time point in *Nur77*-deficient mice. Interestingly, the $Ly6C^+$ cell populations increased in $Nur77^{-/-}$ mice (**Supplementary Figures 5C–J**). These genetic models collectively show that the bone marrow-derived CCR2 dependent $Ly6C^{High}$ monocytes contributed greatly not only to the $CD206^-CD11c^+$ and $CD206^-Ly6C^+$ ATM populations but also control the generation of the two TIM4 negative ATM populations ($CD163^+$MHC II^+ and $TIM4^-CD163^-$MHC II^+) in lean mice.

Adipose Tissue $TIM4^+CD163^+$ Macrophages Are Embryonically Derived

The embryonic-derived macrophage progenitors, either yolk sac or fetal liver-derived, contribute to tissue-resident macrophage populations in multiple organs (46, 54–59). Therefore, we carried out the cell-fate mapping experiments to address the contribution of the yolk sac-derived precursor to the ATM populations in lean eWAT. To identify yolk sac-derived macrophages (60), we induced YFP conversion in $Csf1r^{CreEsr1}$; *R26R-EYFP* -reporter mice by a single tamoxifen injection at E8.5 (**Figure 3A**) and used flow cytometry for the disentanglement of YFP positive cells in adult mice (**Supplementary Figure 6A**). We found YFP^+ cells solely in $TIM4^+CD163^+$ positive macrophage population in 5-week-old mice (over 80% of $YFP^+CD64^+F4/80^+$ cells were $TIM4^+CD163^+$ macrophages). From the rest of the ATMs, only the $TIM4^+$ population had a small percentage of YFP^+ cells, while $CD163^+$, $TIM4^-CD163^-$, and $CD11c^+$ ATMs were completely YFP negative (**Figure 3B**). As an alternative model to label yolk sac-derived macrophages, we pulsed $Cx3cr1^{CreERT2}$;*R26R-EYFP* -reporter mice (31, 46, 61) with tamoxifen at E9.5 (**Figure 3C**). The results confirmed the embryonic origin of $TIM4^+CD163^+$ cells and the lack of contribution of yolk sac-derived cells to the other macrophage populations (**Figure 3D**). When cells were converted later (at E13.5) in $Cx3cr1^{CreERT2}$;*R26R-EYFP* (31, 46, 61) mice (**Figure 3E**), the vast majority of YFP^+ cells were still found in $TIM4^+CD163^+$ subpopulation, both in 5- and 10-week-old mice (**Figure 3F** and **Supplementary Figure 6B**). Interestingly, the conversion at E13.5 resulted in a slight YFP positivity among $TIM4^-CD163^-$ ATMs in 5-week-old mice (**Figure 3F**). We noticed that the absolute cell number of YFP^+ cells dramatically decreased in the 10-week-old mice (**Supplementary Figure 6C**), indicating restricted survival of yolk sac-derived macrophages in aging mice.

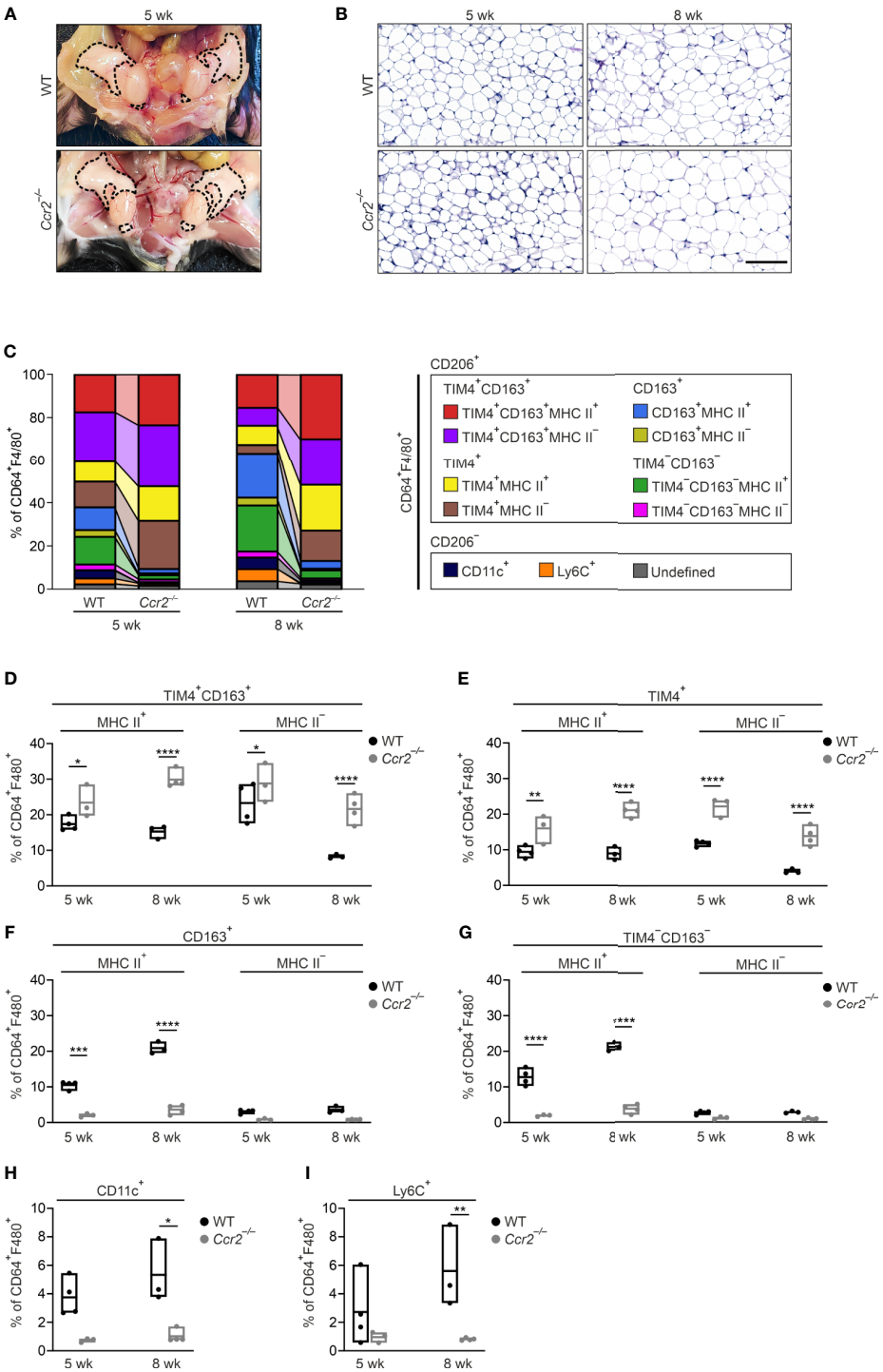

FIGURE 2 | CCR2-dependent trafficking of bone marrow-derived monocytes contributes differentially to eWAT macrophage subpopulations. **(A)** Representative macroscopic images of eWAT in 5-week-old wild type (WT) and *Ccr2*$^{-/-}$ mice. Epididymal fat pads are outlined with a dashed line. **(B)** Representative PAS-stained histology of eWAT in 5- and 8-week-old WT and *Ccr2*$^{-/-}$ mice. Scale bar 100 µm. **(C)** Frequencies of macrophage subpopulations in eWAT of 5- and 8-week-old WT and *Ccr2*$^{-/-}$ mice based on the FlowSOM analyses at the indicated time points. Each FlowSOM metacluster (subpopulation) is represented by an individual color depicted in the columns. **(D–I)** Frequencies of eWAT macrophage subpopulations in *Ccr2*$^{-/-}$ and WT mice. The quantitative data are shown as mean ± SEM (*$P \leq 0.0332$, **$P \leq 0.0021$, ***$P \leq 0.0002$, ****$P \leq 0.0001$, two-way ANOVA with Bonferroni post-hoc test). Each data point represents a pooled eWAT from 3 mice for 5 wk and 2 mice for 8 wk. All mass cytometry data are from 2 (8 wk WT) or 3 (5 wk WT, and 5 and 8 wk *Ccr2*$^{-/-}$) independent experiments.

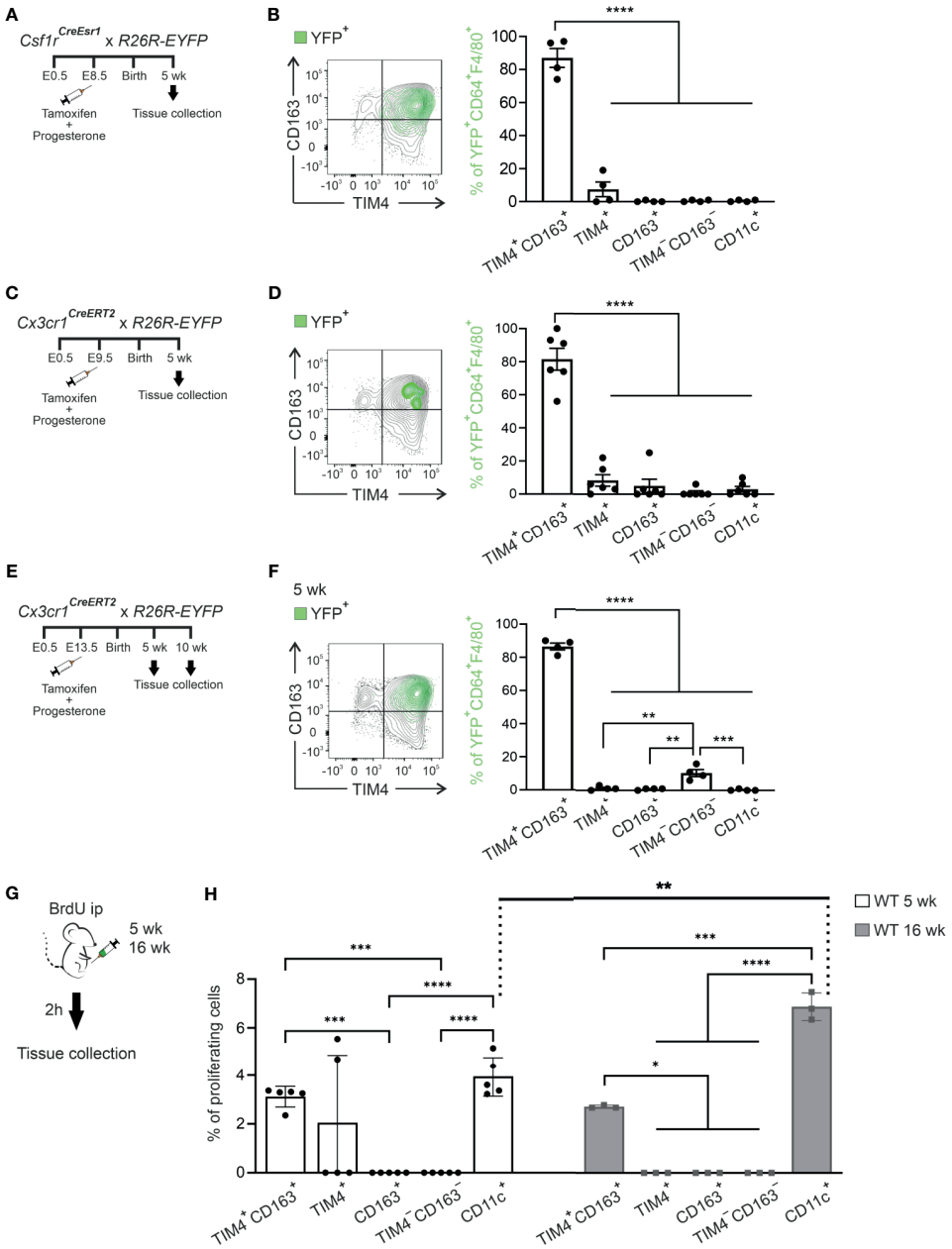

FIGURE 3 | Yolk sac-derived macrophages give rise to self-renewing tissue-resident ATMs. **(A, B)** The experimental outline **(A)** and representative FACS plot and kinetic quantifications **(B)** of yolk sac-derived macrophages in ATMs of *Csf1r*^{CreEsr1}*; R26R-EYFP* mice. **(C, D)** The experimental outline **(C)** and representative FACS plot and quantifications **(D)** of yolk sac-derived macrophages in ATMs with the early conversion of *Cx3cr1*^{CreERT2}*; R26R-EYFP* reporter mice. **(E, F)** The experimental outline **(E)** and representative FACS plot and quantifications **(F)** of yolk sac-derived macrophages in ATMs of the *Cx3cr1*^{CreERT2}*; R26R-EYFP* mice with the late conversion of *Cx3cr1*^{CreERT2}*; R26R-EYFP* reporter mice. **(B, D, F)** The plots show the backgating of the YFP$^+$ cells (green) on the different ATM populations. The quantifications show the frequency of CD45$^+$YFP$^+$CD64$^+$F4/80$^+$ cells in each macrophage population. **(G)** The experimental outline shows i.p. administration of BrdU to 5- and 16-week-old wild type (WT) mice and harvesting of ATMs after 2 hours. **(H)** Frequencies of BrdU positive cells in ATM populations. The quantitative data are shown as mean ± SEM [$^*P \leq 0.0332$, $^{**}P \leq 0.0021$, $^{***}P \leq 0.0002$, $^{****}P \leq 0.0001$, one-way ANOVA **(B, D, F)** and two-way ANOVA **(H)** with Bonferroni post-hoc test]. Each data point represents one mouse. All flow cytometry data are from 2 (**B, D, F**, and **H** for 5 wk) or 1 (**H** for 16 wk) independent experiments.

To gain additional insight into the development of ATMs, we analyzed *Plvap*$^{-/-}$ mice, which shows a selective reduction of fetal liver-derived macrophages in many tissues (42, 43). At the age of 5 weeks, *Plvap*$^{-/-}$ mice had significantly lower body weight but comparable relative weight and morphology of eWAT to WT mice (**Supplementary Figures 6D–F**). We found an overall reduction of CD64$^+$F4/80$^+$ macrophages in *Plvap*$^{-/-}$ mice eWAT (**Supplementary Figure 6G**). More detailed analysis

revealed that the frequency of the TIM4$^+$CD163$^+$ positive macrophage population was reduced in *Plvap* deficient mice compared to the WT littermates (**Supplementary Figures 6H, I**). Together with the lineage tracking experiments, these results indicate that embryonic macrophage precursors, both from the yolk sac and fetal liver origin, enter the eWAT during embryonic development and persist in eWAT after birth. Collectively, the data support the embryonic origin of the TIM4$^+$CD163$^+$ ATMs.

The dynamic changes in ATM subpopulations during aging might be due to local proliferation. To assess this possibility, we quantified the proportion of proliferating ATM cells *in vivo* with 5-bromo-2`-deoxyuridine (BrdU) labeling (**Figure 3G**). TIM4$^+$CD163$^+$ and CD11c$^+$ ATM subpopulations consistently had BrdU$^+$ cells at the age of 5 and 16 weeks. At the age of 16 weeks, resident CD11c$^+$ cells proliferated faster than any other ATMs. In contrast, we did not observe any reproducible macrophage proliferation in TIM4$^+$, CD163$^+$, or TIM4$^-$CD163$^-$ ATM populations (**Figure 3H**). The data collectively reveal that TIM4$^+$CD163$^+$ and CD11c$^+$ macrophages in lean eWAT undergo *in situ* proliferation to retain the population density at a steady state. Conversely, the expansion of TIM4$^-$CD163$^-$ and CD163$^+$ populations depends mainly on CCR2-dependent recruitment of bone marrow-derived circulating blood monocytes rather than local proliferation.

The Major Tissue-Resident Macrophage Subpopulations Persist in Adipose Tissue During Metabolic Alterations

To study the influence of different diet interventions on eWAT resident ATM populations, we set up several models. We fed WT male mice for 16 weeks (from 8 weeks to 23 weeks of age) with an obesogenic high-fat diet (HFD) and compared them with mice fed with natural ingredient-based chow diet (Chow) and purified ingredient normal-fat diet resembling chow in the levels of macronutrients, but HFD in the sources of other nutrients (NFD; **Figure 4A** and **Supplementary Figures 7A–C**). Moreover, we established two different weight loss models to explore further how weight loss will impact the ATM subpopulations. To this end, mice were first fed with HFD to induce obesity (8-week diet) and, after that, mice were assigned either to metformin treatment (HFD + Met), known to reduce body weight and improve insulin sensitivity (62), or switched to standard chow (HFD to Chow; **Figure 4A** and **Supplementary Figures 7A–C**).

To identify ATM populations under different diets and to avoid the possible effect of downsampling, we first implemented the Hierarchical Stochastic Neighboring Embedding (HSNE) for the mass cytometry data (44, 63). ATM cells from the diet experiments were analyzed with the Cytosplore single-cell analysis framework. At the overview level, the CD64$^+$F4/80$^+$ ATMs analysis verified that the same major CD206 positive macrophage populations TIM4$^+$CD163$^+$, TIM4$^+$, and CD163$^+$ identified in steady state, also existed in all diet groups (**Figures 4B, C**). However, in HFD and HFD + Met, the TIM4$^-$CD163$^-$ population was merged with a considerably increased inflammatory CD11c cell population (**Figures 4B–D**).

We then selected and explored all subpopulations separately by generating new higher resolution embeddings (**Figures 4E, F**). The result showed that the frequency of CD163$^+$ macrophages decreased in HFD and HFD + Met groups and TIM4$^+$CD163$^+$ cells showed a similar tendency compared to Chow (**Supplementary Figure 8A**). However, this was caused by the influx of inflammatory CD11c$^+$ cells as, despite the large individual variability, the cell number stayed relatively steady in TIM4$^+$CD163$^+$, TIM4$^+$, and CD163$^+$ populations, regardless of the diet intervention (**Supplementary Figure 8B**). The absolute cell number data suggested that tissue-resident ATMs do not respond to HFD with increased proliferation or apoptosis. Interestingly, only the NFD diet showed a trend towards increased cell counts in TIM4$^+$CD163$^+$ and CD163$^+$ populations (**Supplementary Figure 8B**).

Overlayed visualization of different diet groups showed several diet-specific regions in TIM4$^+$CD163$^+$, TIM4$^+$, and CD163$^+$ATM subpopulations (**Figures 4E, G** and **Supplementary Figure 8C**). For instance, the NFD ATMs clustered separately because of their high Ly6C expression (**Figures 4E, G**), indicating that monocyte differentiation to macrophages occurs during the NFD diet. In the Chow, NFD, and HFD to Chow groups, we found that the TIM4$^+$CD163$^+$, TIM4$^+$ and CD163$^+$ ATMs had high MHC II expression. In contrast, the HFD and HFD + Met treatment induced a phenotypic switch in ATMs, resulting in a downregulation of MHC II expression (**Figures 4E, G**). Furthermore, in the HFD, HFD + Met, and NFD groups, the TIM4$^+$CD163$^+$ MHC II$^-$ and CD163$^+$MHC II$^-$ macrophage populations had higher expression of CD206 (**Figures 4E, G**), which is commonly considered as an M2-like macrophage marker (64). The TIM4$^-$CD163$^-$ together with CD206$^-$ ATM populations underwent remarkable remodeling during the diet alterations (**Figures 4F, H**). Among these cells, HFD induced a vast expansion of inflammatory CD11c$^+$ ATM cells, which is in line with earlier published literature (22, 65, 66). However, a similar effect was also seen in the HFD + Met group (**Figures 4F, H**).

Interestingly, the expression of CD115 and CX3CR1 was induced within the merged TIM4$^-$CD163$^-$ and CD206$^-$ ATMs in HDF and HFD + Met groups (**Supplementary Figure 8D**). In contrast, the switch from HFD to Chow decreased the number of TIM4$^-$CD163$^-$ and CD206$^-$ ATM cells back to the normal state (**Supplementary Figure 8B**). Together these results indicate that while the HFD induces a major influx of macrophages, the resident ATMs persist in the eWAT despite the nutritional challenges or dietary or pharmacological anti-obesity treatments.

Obesity Induces TIM4$^+$CD163$^+$ and TIM4$^+$ Macrophages to Undergo MHC II Reprogramming

For quantifying the MHC II expression, we applied viSNE followed by an unsupervised FlowSOM algorithm to cluster the CD64$^+$F4/80$^+$ ATMs from the different diet interventions. FlowSOM identified 13 metaclusters, of which main TIM4$^+$CD163$^+$, TIM4$^+$, CD163$^+$, TIM4$^-$CD163$^-$ and CD11c$^+$ ATM populations were identified (**Figure 5A** and

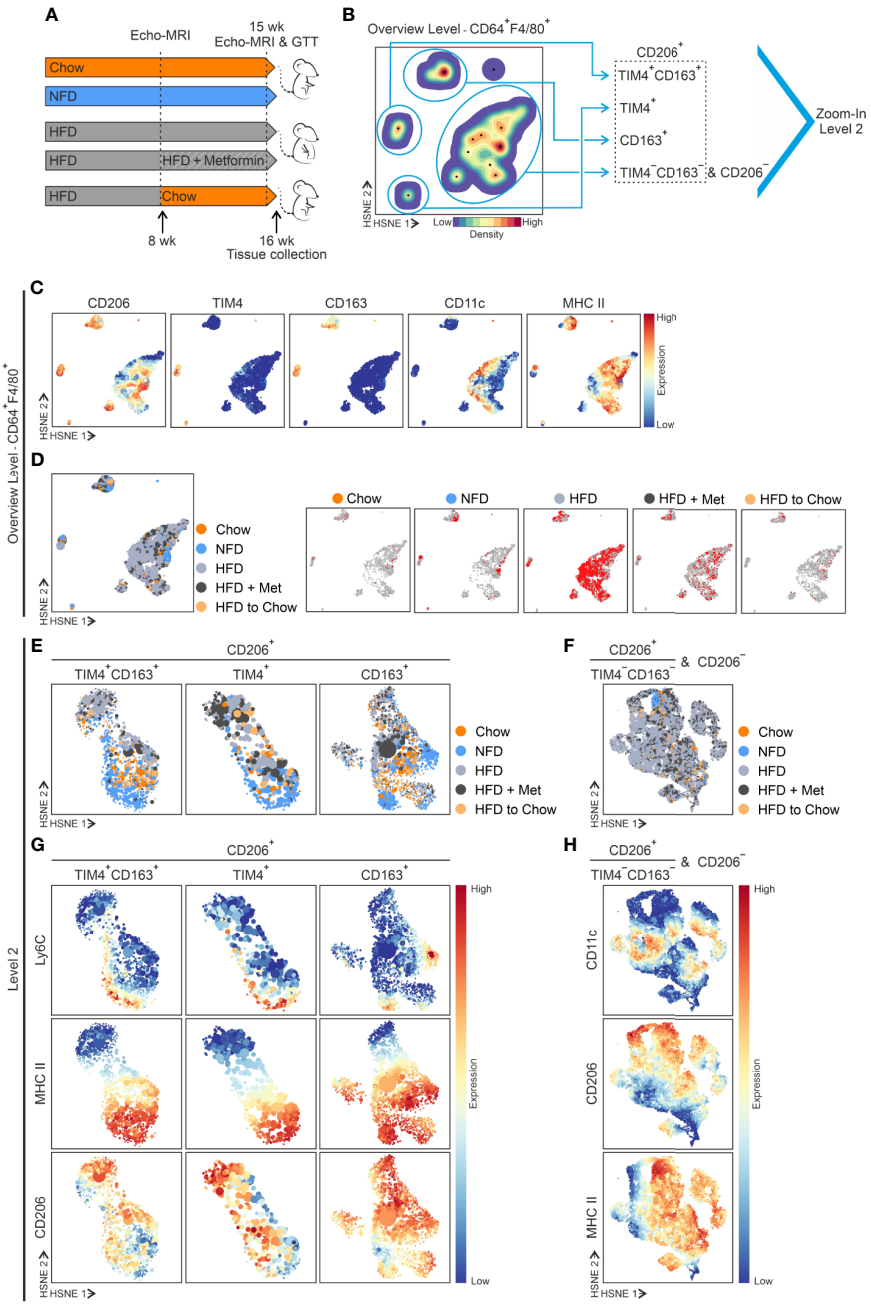

FIGURE 4 | Diet interventions induce alterations in ATMs. **(A)** Experimental setup of diet interventions. The standard diet is referred to as Chow, normal-fat diet as NFD, and high-fat diet as HFD. In the weight loss models, HFD fed mice were administrated with metformin (referred to as HFD + Met), or the diet was switched to Chow (referred as HFD to Chow) after 8 weeks on HFD (at 16 weeks of age). **(B)** Overview HSNE level (Level 1) embedding of $CD64^{+}F4/80^{+}$ ATMs in eWAT at the end of the dietary experiments (cells from all study groups superimposed). HSNE level embedding shows density features (blue, low density; red, high density) depicting the local probability density of cells. The black dots indicate the centroids of identified cluster partitions. Blue encirclement indicates manual selection of the major ATM populations representing $TIM4^{+}CD163^{+}$, $TIM4^{+}$, $CD163^{+}$, and the merged $TIM4^{-}CD163^{-}$ and $CD206^{-}$ ATMs. **(C)** Expression of the indicated markers. **(D)** The left-hand blot: Contribution of cells from the five different diet groups (color-coded as indicated) to the different HSNE density clusters (dark orange for Chow, light orange for HFD to Chow, light blue for NFD, light gray for HFD, and dark gray for HFD + Met). In the five other plots: Each diet is embedded individually, and the red color presents the cells in the indicated diet group, and the gray color shows cells in the whole data set. **(E)** HSNE embedding representing a selection of $TIM4^{+}CD163^{+}$, $TIM4^{+}$, and $CD163^{+}$ ATMs (Zoom level 2), demonstrating different diet groups with different colors as indicated. **(F)** Selection of merged $CD206^{+}TIM4^{-}CD163^{-}$ and $CD206^{-}$ ATMs in HSNE embedding representing different diet groups with different colors as indicated. **(G)** Expression of the indicated markers in $TIM4^{+}CD163^{+}$, $TIM4^{+}$, and $CD163^{+}$ ATMs. **(H)** Expression of the indicated markers in merged $CD206^{+}TIM4^{-}CD163^{-}$ and $CD206^{-}$ ATMs. Indicated markers' expression intensity is shown from low (blue) to high (red). All mass cytometry data are from n = 8 (Chow), n = 8 (NFD), n = 16 (HFD), n = 8 (HFD + Met), or n= 10 (HFD to Chow) mice.

Supplementary Figure 9A). Quantitative analyses revealed the expected increase in the frequency of CD11c positive cell populations, while Ly6C cells stayed steady (**Supplementary Figures 9B, C**). More interestingly, quantitative analyses showed that all ATM subpopulations were mainly MHC II positive in Chow and NFD diets (**Figures 5B–E**). In contrast, the HFD induced a robust phenotype switch in TIM4$^+$CD163$^+$ and TIM4$^+$ macrophages with significant downregulation of MHC II expression (**Figures 5B, C**). This phenotypic change was already detectable after an 8-week HDF diet (**Supplementary Figure 9D**). This switch appeared to be specific as the alterations in the expression of MHC II in the

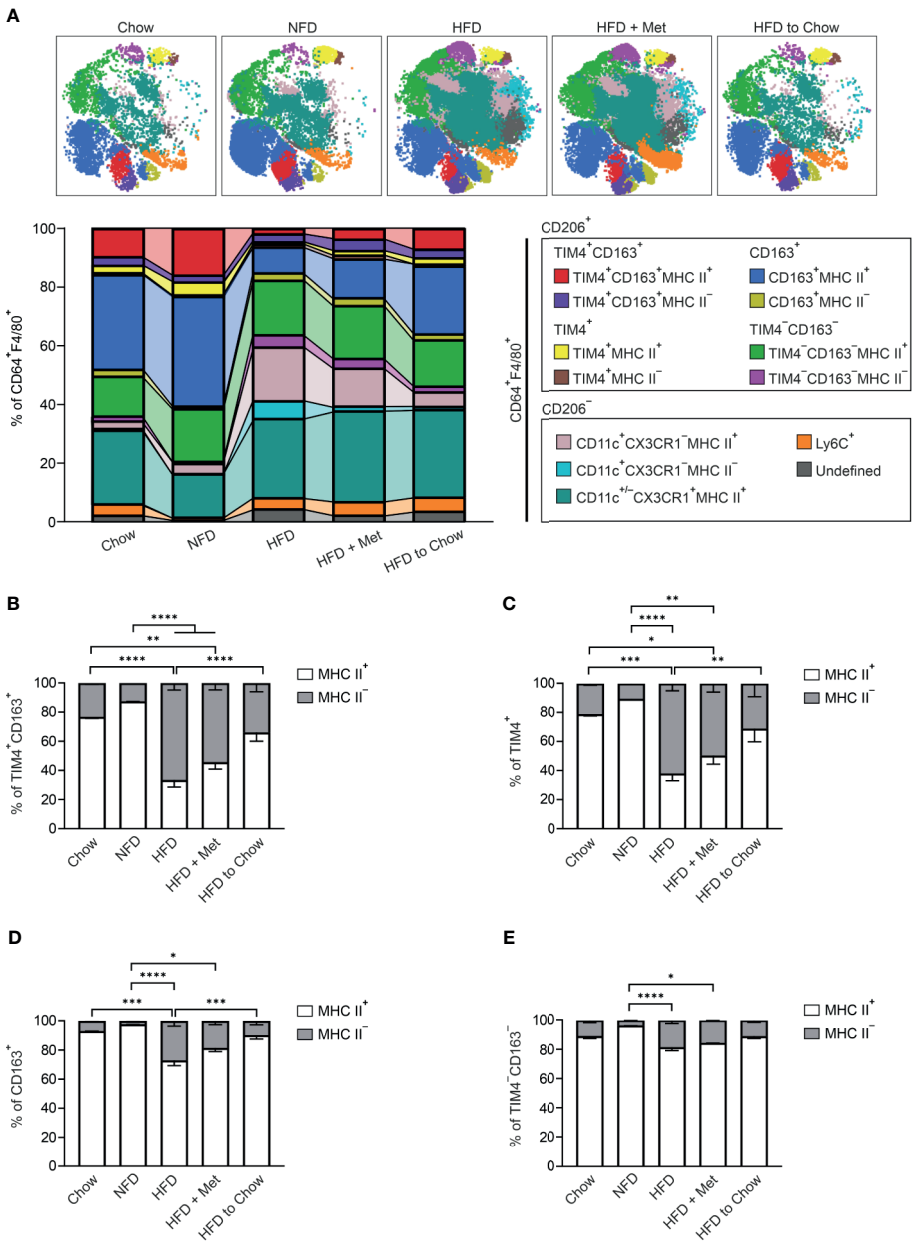

FIGURE 5 | Obesity induces reversible down-regulation of MHC II in resident ATMs. **(A)** Representative FlowSOM maps and frequencies of CD45$^+$CD64$^+$F4/80$^+$ macrophage cell clusters in eWAT of wild type (WT) mice with different dietary interventions; standard diet (Chow), normal-fat diet (NFD), high-fat diet (HFD), or weight loss models; metformin-treated mice on HFD (HFD + Met) or HFD fed mice switched to standard diet after 8 weeks (HFD to Chow). Individual FlowSOM metaclusters (subpopulations) are indicated in different colors. **(B–E)** Frequencies of MHC II$^+$ and MHC II$^-$ cells in different ATM subpopulations of mice on different dietary interventions. The quantitative data are shown as mean ± SEM (*$P \leq 0.0332$, **$P \leq 0.0021$, ***$P \leq 0.0002$, ****$P \leq 0.0001$, two-way ANOVA with Bonferroni post-hoc test). All mass cytometry data are from n = 8 (Chow), n = 8 (NFD), n = 16 (HFD), n = 8 (HFD + Met), or n = 10 (HFD to Chow) mice and from 2 independent experiments.

bone marrow-derived CD163$^+$ and TIM4$^-$CD163$^-$ macrophages, although significant, were substantially smaller (**Figures 5D, E**). Interestingly, we observed that the diet switch from HFD to Chow returned the MHC II expression of TIM4$^+$CD163$^+$ and TIM4$^+$ macrophages to steady state, while the metformin treatment did not (**Figures 5B, C**). This macrophage phenotype change was associated with a significant decrease in adipocyte size (**Supplementary Figure 9E**). Together these results suggest that resident ATM populations have an origin-dependent MHC II response to diet-induced obesity.

All WAT Macrophage Subtypes Are Phagocytic and Endocytic Cells With the Capacity to Process Antigens

To study the phagocytic and endocytic ability of ATMs *in vitro*, we incubated the isolated cells with fluorescent beads (0.5µm) or fluorescent dextran (500 kDa; **Figure 6A** and **Supplementary Figure 10A**). The results revealed that all four main ATM subpopulations in the eWAT were able to phagocytose the fluorescent beads (**Supplementary Figure 10B**). Furthermore, when the cells were incubated with dextran *in vitro*, endocytosis took place in all ATM subpopulations, although the TIM4$^+$CD163$^+$ cells had higher endocytic capacity than the TIM4$^-$CD163$^-$ cells (**Figure 6B** and **Supplementary Figure 10C**). Within TIM4$^+$, CD163$^+$, and TIM4$^-$CD163$^-$ subpopulations, the MHC II positive cells showed higher efficacy in endocytosis of dextran, while in TIM4$^+$CD163$^+$ ATMs, both MHC II$^+$ and MHC II$^-$ cells showed equal endocytic capacity (**Supplementary Figure 10D**). Altogether, these *in vitro* results support that all populations we have assigned as macrophages in eWAT are indeed genuine macrophages with phagocytic and endocytic capacity.

To study the ability of isolated resident ATMs to process soluble antigens, we used DQ-ovalbumin conjugated with BODIPY FL dye (DQ-OVA) as a surrogate antigen. This self-quenched reporter emits fluorescence upon proteolytic

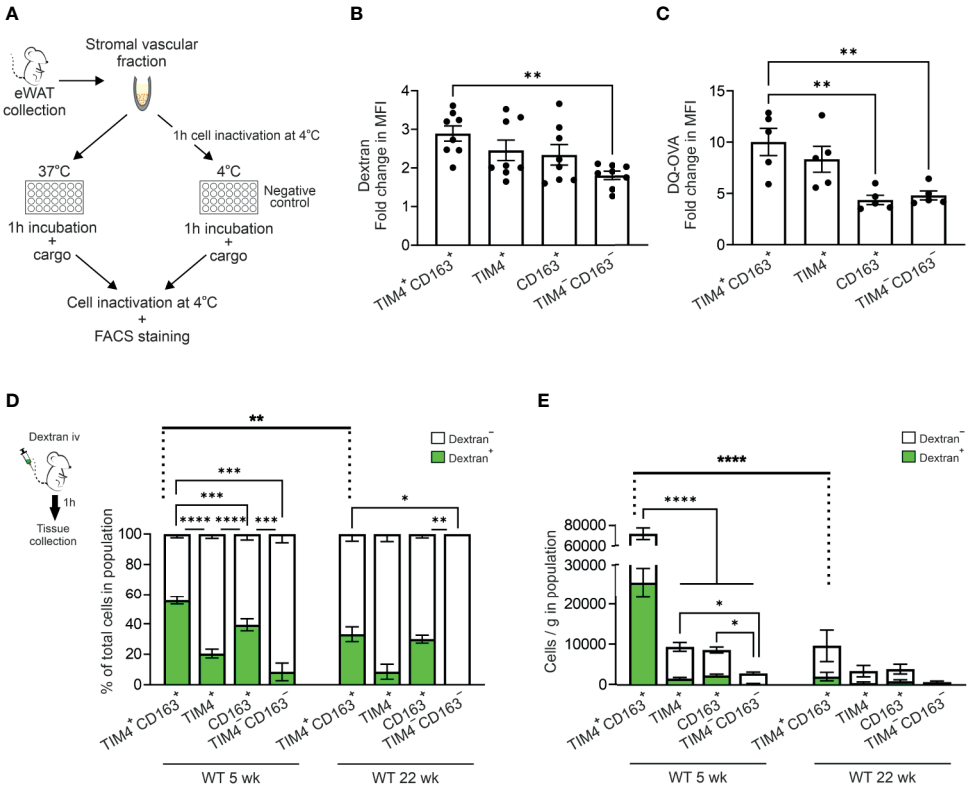

FIGURE 6 | TIM4$^+$CD163$^+$ and TIM4$^+$ ATMs are efficient in scavenging and have an antigen processing capacity. **(A)** The experimental setup for measuring the *in vitro* engulfment of 0.5 µm fluorescent beads, 500 kDa fluorescent dextran, or DQ ovalbumin by different macrophage subtypes isolated from eWAT of 5 or 30-week-old wild type (WT) mice. **(B)** Endocytosis of dextran by the different macrophage subpopulations [fold change in mean fluorescence intensity (MFI) at 37°C versus background at 4°C]. **(C)** Antigen processing capacity of the different macrophage subpopulations (fold change in MFI of DQ-OVA at 37°C versus background at 4°C). **(D)** Scavenging of dextran *in vivo*. The experimental setup for studying scavenging (intravenously administered fluorescent 500 kDa dextran). The quantifications show the frequencies of dextran positive and dextran negative cells within TIM4$^+$CD163$^+$, TIM4$^+$, CD163$^+$, and TIM4$^-$CD163$^-$ ATM populations of 5- and 22-week-old WT mice. **(E)** Analyses of the absolute numbers of dextran positive and dextran negative cells from **(D)** The quantitative data are shown as mean ± SEM (*$P \leq 0.0332$, **$P \leq 0.0021$, ***$P \leq 0.0002$, ****$P \leq 0.0001$, one- **(B, C)** or two-way **(D, E)** ANOVA with Bonferroni post-hoc test). Each data point represents one mouse in **(B, C)** Dextran *in vivo* experiment **(D, E)** data are from n = 9 (5 wk) or n = 4 (22 wk) mice. All flow cytometry data are from 1 (**D** for 22 wk, and **E** for 22 wk) or 2 (**B–E** for 5 wk) independent experiments.

degradation (67). Interestingly, all ATM subsets appeared to have a capacity to endocytose and process antigens when studied *in vitro* after one-hour incubation with DQ-OVA (**Figure 6C** and **Supplementary Figure 10E**). However, at the age of 5 weeks, especially TIM4$^+$CD163$^+$ ATMs degraded DQ-OVA significantly higher degree than CD163$^+$ or TIM4$^-$CD163$^-$ cells (**Figure 6C**). The MHC II$^+$ cells displayed more predominant proteolytic capacity in the TIM4$^+$ resident ATM population, while in all the other resident ATMs, both MHC II$^+$ and MHC II$^-$ cells showed equal antigen processing capability (**Supplementary Figure 10F**). We similarly performed *in vitro* DQ-OVA experiment with ATMs isolated from different diet experiments. The result revealed that contrary to earlier timepoint, CD163$^+$ and TIM4$^-$CD163$^-$ ATMs showed the most active antigen processing in the aged animals (Chow group; **Supplementary Figure 10G**). However, no difference in antigen processing between resident ATMs in the different diet intervention groups was observed (**Supplementary Figures 10G–I**). Thus, all ATM subpopulations are capable of antigen processing. While aging impacts the antigen processing capability of resident ATMs, it is not chanced due to metabolic challenges.

Embryonic-Derived TIM4$^+$CD163$^+$ Macrophages Are Superior in Scavenging *In Vivo*

Unlike *in vitro*, macrophages' scavenging properties *in vivo* are also regulated by the proximity of macrophage subsets to the antigen's entrance routes into adipose tissue. Therefore, to evaluate possible functional differences of macrophage subpopulations *in vivo*, we intravenously (i.v.) administered fluorescently labeled 500 kDa dextran or fluorescent immune complex (OVA-SIC) to 5-week-old WT mice (**Figure 6D** and **Supplementary Figure 10J**). Interestingly, TIM4$^+$CD163$^+$ and CD163$^+$ populations were the most effective ATMs to scavenge dextran from the blood (**Figure 6D**). When the total cell numbers were analyzed, only the embryonic-derived TIM4$^+$CD163$^+$ ATM population showed noteworthy scavenging of dextran *in vivo* at 5 weeks of age (**Figure 6E**). Notably, the ability of TIM4$^+$CD163$^+$ ATMs to bind/uptake dextran was significantly reduced at 22 weeks of age, indicating an age-dependent adjustment in the functionality of embryonic-derived ATMs (**Figures 6D, E**). When analyzing *in vivo* OVA-SIC, the binding/up-take was readily detected in F4/80$^+$ macrophages in the spleen, but there was only minimal OVA-SIC accumulation in eWAT macrophages (**Supplementary Figure 10J**). Nevertheless, the only OVA-SIC binding cells visible in eWAT were TIM4$^+$CD163$^+$ macrophages (**Supplementary Figure 10J**). These *in vivo* findings demonstrate a superior scavenging capacity of embryonic-derived TIM4$^+$CD163$^+$ tissue-resident macrophages *in vivo* in steady state conditions.

DISCUSSION

Although resident adipose tissue macrophages are central to tissue homeostasis (68), resident ATMs' origin, identity, and kinetics are still not well-understood. This study determined the development of resident macrophage subsets in adipose tissue at a steady state and their response to metabolic challenges. Using extensive marker selection with unsupervised high-dimensional single-cell analyses, we distinguished six different resident ATM populations in lean mice. Four of them expressed CD206, a classical marker for alternatively activated M2-like macrophages, while the two CD206-negative populations were identified by CD11c and Ly6C positivity. In addition, TIM4 and CD163 were identified as dichotomic markers for separating the four major CD206 positive macrophage populations. All four CD206 positive ATM populations were further subdivided by the MHC II expression status and displayed characteristic kinetic changes during aging. Combining cell-fate mapping and macrophage deficient mouse models, we show that resident ATM macrophages represent in lean mice a mixture of cells originating from the yolk sac, fetal liver, and adult bone marrow. Our diet interventions showed that the numbers of CD206 positive resident ATMs in WAT are largely diet independent, yet obesity induced reversible changes in MHC II expression of resident ATMs.

Adipose tissue forms already *in utero*, and new adipocytes are generated continuously at a substantial level, even in adults (69). ATMs are critical during the early postnatal life when *de novo* generation of adipocytes is elevated (12, 69). We found that all major ATM populations were identifiable already at two weeks of age and that the overall number of ATMs grew during postnatal development. While the frequency of the TIM4$^+$CD163$^+$ and TIM4$^+$ subsets decreased upon aging, the two other CD206$^+$ macrophage populations, CD163$^+$, and TIM4$^-$CD163$^-$ cells, steadily increased in postnatal mice. Whereas the MHC II negative subpopulations dominated the TIM4$^+$CD163$^+$ and TIM4$^+$ cells at the early postnatal age, MHC II positive subpopulations became dominant in the adults. In contrast, CD163$^+$ and TIM4$^-$CD163$^-$ ATMs were mainly MHC II$^+$ throughout postnatal life.

Recently many groups have studied the ATMs in lean mice with different techniques (3, 11, 15, 38–41, 48, 70). While these studies have identified various resident ATM populations in eWAT, comparing the differences between macrophage populations from the different studies has been challenging. Thus, there was an obvious need for a more uniform and precise definition of ATMs before studying the subset-specific function of these heterogenic resident ATMs in the future. Most ATM studies have been done using conventional fluorimetric flow cytometry with a limited set of classical macrophage markers F4/80, CD11b, and CD64 (8, 39, 48), while other studies have used CD11c, CD206, or CX3CR1 instead (3, 15, 41, 71). Major limitations in all of these studies have been the suboptimal marker repertoire for dissecting the true heterogeneity of resident ATMs, challenges in controlling the high autofluorescence of myeloid cells, and the bias introduced by manual gating strategies. More comprehensive but still limited marker selection was used when the vascular-associated macrophage (VAM) nomenclature was proposed (11).

Few RNA studies have also addressed the ATMs in lean mice (38, 40, 70). In scRNAseq analyses of lean mice, Jaitin et al.

identified only one macrophage cluster that expressed *Cd163* and *Lyve-1*, markers commonly associated with perivascular macrophages (38, 51). Bulk RNA results of VAM2 confirmed the transcription of the same markers in lean mice (11). In contrast, Weinstock et al. identified six different ATM populations in lean adult mice (40). Two prominent macrophage clusters in lean mice, designed as resident macs and activated macs, prospectively resemble our TIM4$^+$CD163$^+$ (both MHC II$^+$ and MHC II$^-$ subpopulations) cells, as *Cd163*, *Lyve-1*, and *H2-Ab* were expressed in these clusters. Interestingly, *Tim4* was not present in differentially expressed genes in any of the clusters (40). One of the clusters identified, named stem-like macs, highly expressed genes related to proliferation, such as *Ube2c*, *Top2a*, and *Mki67*. Likewise, we saw that proportion of TIM4$^+$CD163$^+$ and CD11c$^+$ ATM cells consistently proliferated at both 5 and 16 week time points. From our data, we identified a smaller CD206$^-$Ly6C$^+$ macrophage population that was TIM3 and CD43 positive. Similar cells have been previously called B cell-like and Heme macs. Moreover, the monocyte cluster identified in scRNAseq analyses highly expressed *Fcgr1* (CD64) and thus likely includes cells similar to our CD206$^-$Ly6C$^+$ macrophages. In obesity, resident macrophages called major macs were found to expand along with newly appearing phagocytic macs (40). Together, these two clusters likely correspond to our TIM4$^-$CD163$^-$ ATM population mingling with the incoming CD11c population in obese mice. Weinstock et al. found that only the number of major macs increased in obese animals, while the other resident macrophage clusters remained relatively stable in obese mice, which is consistent with our observations. Clearly, further studies are warranted to precisely correlate RNA and protein levels in ATM macrophages for an unambiguous definition of different subpopulations in lean and obese mice.

Macrophages internalize extracellular antigens through endocytosis and phagocytosis and process them for antigen presentation. Our *in vitro* analyses showed that each of the four main ATM macrophage subtypes is capable of endocytosing and phagocyting several different cargos and are hence bona fide macrophages. *In vitro*, TIM4$^+$CD163$^+$ and TIM4$^+$ macrophages, compared to the other main ATMs, showed an approximately two-fold increase in endocytosis of dextran. However, the *in vivo* binding/endocytosis assays with dextran and immunocomplex revealed TIM4$^+$CD163$^+$ macrophages as the main interacting population. The result may reflect superior scavenging capacity or physiological exposure of TIM4$^+$CD163$^+$ cells to the blood-borne ligands. Nevertheless, all macrophage populations contained LYVE1$^+$ subsets, tentatively identified as vessel-associated macrophages (72, 73), and all populations had cells highly expressing CD206, which is the primary scavenger receptor for dextran (74, 75). Therefore, other mechanisms may contribute to the better capacity of TIM4$^+$CD163$^+$ macrophages for *in vivo* scavenging. Of note, *in vitro* endocytosis/phagocytosis assays revealed that also MHC II negative subsets are effective in these processes. It may imply a functional specialization in which the ingested antigens are not presented to boost the immune system but are possibly silently disposed.

Recent studies of macrophage ontogeny have highlighted macrophage heterogeneity and have revealed that various tissue-resident macrophage compartments are already established during embryogenesis (43, 46, 51, 76). Under homeostatic conditions, each adult tissue has its unique composition of coexisting embryonic-derived and adult bone marrow-generated macrophages (55–57, 59, 77). Thus, ATM expansion can be driven by *in situ* proliferation and/or by the influx of new bone marrow-derived monocytic precursors. Our *Ccr2* knockout data collectively suggest that the recruitment of CCR2-mediated monocytes from the blood is crucial for expanding the resident CD163$^+$ and TIM4$^-$CD163$^-$ populations. Our results are well in line with lineage tracking studies done with Ms4a3-Cre-dtTomato reporter, labeling all bone marrow-derived monocytes but not embryonically derived macrophages, showing that 50% of ATMs are bone marrow origin (38). Resident ATMs are proposed to be predominantly derived from embryonic yolk sac precursors (12). Using fate mapping models and *Plvap* deficient mice, we showed that long-lived embryonic-derived, both yolk sac and liver origin, macrophages exist in ATMs but almost solely within the TIM4$^+$CD163$^+$ subpopulation. These results are consistent with a previous study showing that yolk sac-derived macrophages are necessary to expand adipocytes postnatally (12).

The adaptation of adipose tissue to diet variations is critical to the maintenance of metabolic control. In obese adipose tissue, the high influx of recruited monocyte-derived CD11c$^+$ ATMs leads to low-grade systemic chronic inflammation and insulin resistance (19, 66). In line with published data, we observed the HFD induced increase of total macrophages as a cause of the massive expansion of the inflammatory CD11c$^+$ ATM cells. However, the cell numbers of resident TIM4$^+$CD163$^+$, TIM4$^+$, and CD163$^+$ populations remained surprisingly steady despite the diet or treatment interventions, suggesting a homeostatic role for tissue-resident macrophages in white adipose tissue.

Diet-induced obesity promotes the differentiation of ATMs towards a more classically activated M1-like phenotype reflected by an increased MHC II expression. In contrast to previously published papers (48, 78), our studies revealed that although diet-induced obesity might increase MHC II expression in ATMs generally (3), both TIM4$^+$ resident ATM populations decreased their MHC II expression significantly during the HFD. After this phenotypic switch, these cells started to resemble the early postnatal CD206$^+$TIM4$^+$CD163$^+$ MHCII$^-$ cells. The loss of MHC II from TIM4$^+$ ATMs during HFD notably did not impact antigen processing capability. Remarkably, after eight weeks of the diet switch from HFD to Chow, the TIM4$^+$CD163$^+$ ATMs had regained the high MHC II expression. Although the functional implications of the phenotypic switch remain to be studied, it may be related to adipose tissue remodeling, to which yolk sac-derived macrophages contribute in early life (79, 80).

In conclusion, we show here that tissue-resident macrophage subsets in white adipose tissue consist of four main populations defined by CD206, TIM4, and CD163 markers. The contribution of embryonic and adult bone marrow varies in the generation of the distinct macrophage populations, and at least the yolk

sac-derived macrophages do not show any tendency towards phenotypic conversion to other macrophage types. Furthermore, tissue-resident macrophage subpopulations respond differently to metabolic challenges. These results imply that both ontogeny-derived and tissue-derived signals control the plasticity of resident ATMs. Moreover, the diet intervention studies suggest that the pro-inflammatory macrophage phenotype induced by HFD is not a fixed state but can be reverted to a normal anti-inflammatory phenotype by dietary therapy. We thus reveal previously unknown heterogeneity of tissue-resident macrophage subsets in white adipose tissue with advanced single-cell proteomics technology and unbiased single-cell analyses. Our data should be useful for defining individual subsets of resident macrophages when dissecting their metabolic and other functional roles in the future.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Regional Animal Experiment Board in Finland.

AUTHOR CONTRIBUTIONS

IF contributed to experiment design, conducted experiments, analyzed data, and contributed to the preparation of the manuscript. HJ designed experiments, conducted experiments, supervised the study and wrote the manuscript. JK performed experiments and contributed to data analyses. NK and ES provided samples and contributed to the preparation of the manuscript. MS and PR conceived and supervised the study and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2021.719979/full#supplementary-material

REFERENCES

1. Dagfinn A, Abhijit S, Teresa N, Imre J, Pål R, Serena T, et al. Body Mass Index, Abdominal Fatness, and Heart Failure Incidence and Mortality. *Circulation* (2016) 133:639–49. doi: 10.1161/CIRCULATIONAHA.115.016801
2. Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH. The Incidence of Co-Morbidities Related to Obesity and Overweight: A Systematic Review and Meta-Analysis. *BMC Public Health* (2009) 9:88. doi: 10.1186/1471-2458-9-88
3. Morris DL, Cho KW, DelProposto JL, Oatmen KE, Geletka LM, Martinez-Santibanez G, et al. Adipose Tissue Macrophages Function as Antigen-Presenting Cells and Regulate Adipose Tissue Cd4 $^{+}$ T Cells in Mice. *Diabetes* (2013) 62:2762–72. doi: 10.2337/db12-1404
4. Nguyen KD, Qiu Y, Cui X, Goh YPS, Mwangi J, David T, et al. Alternatively Activated Macrophages Produce Catecholamines to Sustain Adaptive Thermogenesis. *Nature* (2011) 480:104–8. doi: 10.1038/nature10653
5. Nishimura S, Manabe I, Nagasaki M, Hosoya Y, Yamashita H, Fujita H, et al. Adipogenesis in Obesity Requires Close Interplay Between Differentiating Adipocytes, Stromal Cells, and Blood Vessels. *Diabetes* (2007) 56:1517–26. doi: 10.2337/db06-1749.Additional
6. Orr JSS, Kennedy A, Anderson-Baucum EKK, Webb CDD, Fordahl SCC, Erikson KMM, et al. Obesity Alters Adipose Tissue Macrophage Iron Content and Tissue Iron Distribution. *Diabetes* (2014) 63:421–32. doi: 10.2337/db13-0213
7. Russo L, Lumeng CN. Properties and Functions of Adipose Tissue Macrophages in Obesity. *Immunology* (2018) 155:407–17. doi: 10.1111/imm.13002
8. Hubler MJ, Erikson KM, Kennedy AJ, Hasty AH. Mfehi Adipose Tissue Macrophages Compensate for Tissue Iron Perturbations in Mice. *Am J Physiol Physiol* (2018) 315:C319–29. doi: 10.1152/ajpcell.00103.2018
9. Huber J, Kiefer FW, Zeyda M, Ludvik B, Silberhumer GR, Prager G, et al. CC Chemokine and CC Chemokine Receptor Profiles in Visceral and Subcutaneous Adipose Tissue Are Altered in Human Obesity. *J Clin Endocrinol Metab* (2008) 93:3215–21. doi: 10.1210/jc.2007-2630
10. Satoh T, Kidoya H, Yamamoto M, Takemura N, Nakagawa K, Yoshioka Y, et al. Critical Role of Trib1 in Differentiation of Tissue-Resident M2-Like Macrophages. *Nature* (2013) 495:524–8. doi: 10.1038/nature11930
11. Silva HM, Báfica A, Rodrigues-Luiz GF, Chi J, D'Emery Alves Santos P, Reis BS, et al. Vasculature-Associated Fat Macrophages Readily Adapt to Inflammatory and Metabolic Challenges. *J Exp Med* (2019) 216:786–806. doi: 10.1084/jem.20181049
12. Hassnain Waqas SF, Noble A, Hoang AC, Ampem G, Popp M, Strauß S, et al. Adipose Tissue Macrophages Develop From Bone Marrow–Independent Progenitors in Xenopus Laevis and Mouse. *J Leukoc Biol* (2017) 102:845–55. doi: 10.1189/jlb.1A0317-082RR
13. Zeyda M, Farmer D, Todoric J, Aszmann O, Speiser M, Györi G, et al. Human Adipose Tissue Macrophages Are of an Anti-Inflammatory Phenotype But Capable of Excessive Pro-Inflammatory Mediator Production. *Int J Obes* (2007) 31:1420–8. doi: 10.1038/sj.ijo.0803632

14. Hume DA. The Many Alternative Faces of Macrophage Activation. *Front Immunol* (2015) 6:370. doi: 10.3389/fimmu.2015.00370
15. Lumeng CN, Bodzin JL, Saltiel AR. Obesity Induces a Phenotypic Switch in Adipose Tissue Macrophage Polarization. *J Clin Invest* (2007) 117:175–84. doi: 10.1172/JCI29881
16. Mclaughlin T, Ackerman SE, Shen L, Engleman E. Role of Innate and Adaptive Immunity in Obesity-Associated Metabolic Disease. *J Clin Invest* (2017) 127:5–13. doi: 10.1172/JCI88876
17. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, et al. MCP-1 Contributes to Macrophage Infiltration Into Adipose Tissue, Insulin Resistance, and Hepatic Steatosis in Obesity. *J Clin Invest* (2006) 116:1494–505. doi: 10.1172/JCI26498.1494
18. Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, et al. CCR2 Modulates Inflammatory and Metabolic Effects of High-Fat Feeding. *J Clin Invest* (2006) 116:115–24. doi: 10.1172/JCI24335
19. Weisberg S, McCann D, Desai M, Rosenbaum M, Leibel R, Ferrante A. Obesity is Assciated With Macrophage Accumulation in Adipose Tissue. *J Clin Invest* (2003) 112:1796–808. doi: 10.1172/JCI200319246
20. Coats BR, Schoenfelt KQ, Rhodes CJ, Maxfield FR, Becker L, Barbosa-lorenzi C, et al. Metabolically Activated Adipose Tissue Macrophages Perform Detrimental and Beneficial Functions During Diet-Induced Obesity. *Cell Rep* (2017) 20:3149–61. doi: 10.1016/j.celrep.2017.08.096
21. Hill AA, Bolus WR, Hasty AH. A Decade of Progress in Adipose Tissue Macrophage Biology. *Immunol Rev* (2014) 262:134–52. doi: 10.1111/imr.12216
22. Li P, Lu M, Nguyen MTA, Bae EJ, Chapman J, Feng D, et al. Functional Heterogeneity of CD11c-Positive Adipose Tissue Macrophages in Diet-Induced Obese Mice. *J Biol Chem* (2010) 285:15333–45. doi: 10.1074/jbc.M110.100263
23. Gong Z, Zhang X, Su K, Jiang R, Sun Z, Chen W, et al. Deficiency in AIM2 Induces Inflammation and Adipogenesis in White Adipose Tissue Leading to Obesity and Insulin Resistance. *Diabetologia* (2019) 62:2325–39. doi: 10.1007/s00125-019-04983-x
24. Liu Y, Lu X, Li X, Du P, Qin G. High-Fat Diet Triggers Obesity-Related Early Infiltration of Macrophages Into Adipose Tissue and Transient Reduction of Blood Monocyte Count. *Mol Immunol* (2020) 117:139–46. doi: 10.1016/j.molimm.2019.11.002
25. Miyachi Y, Tsuchiya K, Shiba K, Mori K, Chikara K, Ogasawara N, et al. A Reduced M1-Like / M2-Like Ratio of Macrophages in Healthy Adipose Tissue Expansion During SGLT2 Inhibition. *Sci Rep* (2018) 8:16113. doi: 10.1038/s41598-018-34305-x
26. Oh DY, Morinaga H, Talukdar S, Bae EJ, Olefsky JM. Increased Macrophage Migration Into Adipose Tissue in Obese Mice. *Diabetes* (2012) 61:346–54. doi: 10.2337/db11-0860
27. Parekh PI, Petro AE, Tiller JM, Feinglos MN, Surwit RS. Reversal of Diet-Induced Obesity and Diabetes in C57BL/6J Mice. *Metabolism* (1998) 47:1089–96. doi: 10.1016/S0026-0495(98)90283-9
28. Patsouris D, Li P-P, Thapar D, Chapman J, Olefsky JM, Neels JG. Ablation of CD11c-Positive Cells Normalizes Insulin Sensitivity in Obese Insulin Resistant Animals. *Cell Metab* (2008) 8:301–9. doi: 10.1016/j.cmet.2008.08.015
29. Dick SA, Macklin JA, Nejat S, Momen A, Clemente-Casares X, Althagafi MG, et al. Self-Renewing Resident Cardiac Macrophages Limit Adverse Remodeling Following Myocardial Infarction. *Nat Immunol* (2019) 20:29–39. doi: 10.1038/s41590-018-0272-2
30. Guilliams M, De Kleer I, Henri S, Post S, Vanhoutte L, De Prijck S, et al. Alveolar Macrophages Develop From Fetal Monocytes That Differentiate Into Long-Lived Cells in the First Week of Life Via GM-CSF. *J Exp Med* (2013) 210:1977–92. doi: 10.1084/jem.20131199
31. Hashimoto D, Chow A, Noizat C, Teo P, Beasley MB, Leboeuf M, et al. Tissue-Resident Macrophages Self-Maintain Locally Throughout Adult Life With Minimal Contribution From Circulating Monocytes. *Immunity* (2013) 38:792–804. doi: 10.1016/j.immuni.2013.04.004
32. Hoeffel G, Wang Y, Greter M, See P, Teo P, Malleret B, et al. Adult Langerhans Cells Derive Predominantly From Embryonic Fetal Liver Monocytes With a Minor Contribution of Yolk Sac–Derived Macrophages. *J Exp Med* (2012) 209:1167–81. doi: 10.1084/jem.20120340
33. Jacome-Galarza CE, Percin GI, Muller JT, Mass E, Lazarov T, Eitler J, et al. Developmental Origin, Functional Maintenance and Genetic Rescue of Osteoclasts. *Nature* (2019) 568:541–5. doi: 10.1038/s41586-019-1105-7
34. Kim K-W, Williams JW, Wang Y-T, Ivanov S, Gilfillan S, Colonna M, et al. Mhc II $^{+}$ Resident Peritoneal and Pleural Macrophages Rely on IRF4 for Development From Circulating Monocytes. *J Exp Med* (2016) 213:1951–9. doi: 10.1084/jem.20160486
35. Shaw TN, Houston SA, Wemyss K, Bridgeman HM, Barbera TA, Zangerle-Murray T, et al. Tissue-Resident Macrophages in the Intestine Are Long Lived and Defined by Tim-4 and CD4 Expression. *J Exp Med* (2018) 215:1507–18. doi: 10.1084/jem.20180019
36. Amano SU, Cohen JL, Vangala P, Tencerova M, Sarah M, Yawe JC, et al. Local Proliferation of Macrophages Contributes to Obesity- Associated Adipose Tissue Inflammation Shinya. *Cell Metab* (2014) 19:162–71. doi: 10.1016/j.cmet.2013.11.017.Local
37. Ginhoux F, Schultze JL, Murray PJ, Ochando J, Biswas SK. New Insights Into the Multidimensional Concept of Macrophage Ontogeny, Activation and Function. *Nat Immunol* (2016) 17:34–40. doi: 10.1038/ni.3324
38. Jaitin DA, Adlung L, Thaiss CA, Weiner A, Li B, Descamps H, et al. Lipid-Associated Macrophages Control Metabolic Homeostasis in a Trem2-Dependent Manner. *Cell* (2019) 178:686–98.e14. doi: 10.1016/j.cell.2019.05.054
39. Hill DA, Lim H, Hoon Y, Ho WY, Foong YH, Nelson VL, et al. Distinct Macrophage Populations Direct Inflammatory Versus Physiological Changes in Adipose Tissue. *PNAS* (2018) 115:E5096–105. doi: 10.1073/pnas.1802611115
40. Weinstock A, Brown EJ, Garabedian ML, Pena S, Lafaille J, Moore KJ, et al. Single-Cell RNA Sequencing of Visceral Adipose Tissue Leukocytes Reveals That Caloric Restriction Following Obesity Promotes the Accumulation of a Distinct Macrophage Population With Features of Phagocytic Cells. *Immunometabolism* (2019) 1:1–26. doi: 10.20900/immunometab20190008
41. Nawaz A, Aminuddin A, Kado T, Takikawa A, Yamamoto S, Tsuneyama K, et al. Cd206+ M2-Like Macrophages Regulate Systemic Glucose Metabolism by Inhibiting Proliferation of Adipocyte Progenitors. *Nat Commun* (2017) 8:286. doi: 10.1038/s41467-017-00231-1
42. Rantakari P, Auvinen K, Jäppinen N, Kapraali M, Valtonen J, Karikoski M, et al. The Endothelial Protein PLVAP in Lymphatics Controls the Entry of Lymphocytes and Antigens Into Lymph Nodes. *Nat Immunol* (2015) 16:386–96. doi: 10.1038/ni.3101
43. Rantakari P, Jäppinen N, Lokka E, Mokkala E, Gerke H, Peuhu E, et al. Fetal Liver Endothelium Regulates the Seeding of Tissue-Resident Macrophages. *Nature* (2016) 538:392–6. doi: 10.1038/nature19814
44. van Unen V, Höllt T, Pezzotti N, Li N, Reinders MJTT, Eisemann E, et al. Visual Analysis of Mass Cytometry Data by Hierarchical Stochastic Neighbour Embedding Reveals Rare Cell Types. *Nat Commun* (2017) 8:1740. doi: 10.1038/s41467-017-01689-9
45. Höllt T, Pezzotti N, van Unen V, Koning F, Eisemann E, Lelieveldt B, et al. Cytosplore: Interactive Immune Cell Phenotyping for Large Single-Cell Datasets. *Comput Graph Forum (Proc EuroVis)* (2016) 35:171–80. doi: 10.1111/cgf.12893
46. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate Mapping Reveals Origins and Dynamics of Monocytes and Tissue Macrophages Under Homeostasis. *Immunity* (2013) 38:79–91. doi: 10.1016/j.immuni.2012.12.001
47. Stamatiades EG, Tremblay M, Bohm M, Crozet L, Bisht K, Kao D, et al. Immune Monitoring of Trans-Endothelial Transport Article Immune Monitoring of Trans-Endothelial Transport by Kidney-Resident Macrophages. *Cell* (2016) 166:991–1003. doi: 10.1016/j.cell.2016.06.058
48. Cho KW, Morris DL, delProposto JL, Geletka L, Zamarron B, Martinez-Santibanez G, et al. An MHC II-Dependent Activation Loop Between Adipose Tissue Macrophages and CD4+ T Cells Controls Obesity-Induced Inflammation. *Cell Rep* (2014) 9:605–17. doi: 10.1016/j.celrep.2014.09.004
49. Amir EAD, Davis KL, Tadmor MD, Simonds EF, Levine JH, Bendall SC, et al. ViSNE Enables Visualization of High Dimensional Single-Cell Data and Reveals Phenotypic Heterogeneity of Leukemia. *Nat Biotechnol* (2013) 31:545–52. doi: 10.1038/nbt.2594
50. Van Gassen S, Callebaut B, Van Helden MJ, Lambrecht BN, Demeester P, Dhaene T, et al. Flowsom: Using Self-Organizing Maps for Visualization and Interpretation of Cytometry Data. *Cytom Part A* (2015) 87:636–45. doi: 10.1002/cyto.a.22625
51. Chakarov S, Lim HY, Tan L, Lim SY, See P, Lum J, et al. Two Distinct Interstitial Macrophage Populations Coexist Across Tissues in Specific Subtissular Niches. *Science* (2019) 363:6432. doi: 10.1126/science.aau0964

52. Boring L, Gosling J, Chensue SW, Kunkel SL, Farese RV, Broxmeyer HE, et al. Impaired Monocyte Migration and Reduced Type 1 (Th1) Cytokine Responses in C-C Chemokine Receptor 2 Knockout Mice. *J Clin Invest* (1997) 100:2552–61. doi: 10.1172/JCI119798
53. Hanna RN, Carlin LM, Hubbeling HG, Nackiewicz D, Green AM, Punt JA, et al. The Transcription Factor NR4A1 (Nur77) Controls Bone Marrow Differentiation and the Survival of Ly6C-Monocytes. *Nat Immunol* (2011) 12:778–85. doi: 10.1038/ni.2063
54. Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, et al. Tissue-Resident Macrophages Originate From Yolk-Sac-Derived Erythro-Myeloid Progenitors. *Nature* (2015) 518:547–51. doi: 10.1038/nature13989
55. Lokka E, Lintukorpi L, Cisneros-Montalvo S, Mäkelä JA, Tyystjärvi S, Ojasalo V, et al. Generation, Localization and Functions of Macrophages During the Development of Testis. *Nat Commun* (2020) 11:4375. doi: 10.1038/s41467-020-18206-0
56. Jokela H, Lokka E, Kiviranta M, Tyystjärvi S, Gerke H, Elima K, et al. Fetal-Derived Macrophages Persist and Sequentially Maturate in Ovaries After Birth in Mice. *Eur J Immunol* (2020) 50:1500–14. doi: 10.1002/eji.202048531
57. Zhu Y, Herndon JM, Sojka DK, Fields RC, Randolph GJ, DeNardo DG, et al. Tissue-Resident Macrophages in Pancreatic Ductal Adenocarcinoma Originate From Embryonic Hematopoiesis and Promote Tumor Progression. *Immunity* (2017) 47:323–38. doi: 10.1016/j.immuni.2017.07.014
58. Epelman S, Lavine KJ, Beaudin AE, Sojka DK, Carrero JA, Calderon B, et al. Embryonic and Adult-Derived Resident Cardiac Macrophages Are Maintained Through Distinct Mechanisms at Steady State and During Inflammation. *Immunity* (2014) 40:91–104. doi: 10.1016/j.immuni.2013.11.019
59. Jäppinen N, Félix I, Lokka E, Tyystjärvi S, Pynttäri A, Lahtela T, et al. Fetal-Derived Macrophages Dominate in Adult Mammary Glands. *Nat Commun* (2019) 10:281. doi: 10.1038/s41467-018-08065-1
60. Schulz C, Gomez Perdiguero E, Chorro L, Szabo-Rogers H, Cagnard N, Kierdorf K, et al. A Lineage of Myeloid Cells Independent of Myb and Hematopoietic Stem Cells. *Science* (2012) 336:86–90. doi: 10.1126/science.1219179
61. Molawi K, Wolf Y, Kandalla PK, Favret J, Hagemeyer N, Frenzel K, et al. Progressive Replacement of Embryo-Derived Cardiac Macrophages With Age. *J Exp Med* (2014) 211:2151–8. doi: 10.1084/jem.20140639
62. Yerevanian A, Soukas AA. Metformin: Mechanisms in Human Obesity and Weight Loss. *Curr Obes Rep* (2019) 8:156–64. doi: 10.1007/s13679-019-00335-3
63. Pezzotti N, Höllt T, Lelieveldt B, Eisemann E, Vilanova A. Hierarchical Stochastic Neighbor Embedding. *Comput Graph Forum* (2016) 35:21–30. doi: 10.1111/cgf.12878
64. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage Activation and Polarization: Nomenclature and Experimental Guidelines. *Immunity* (2014) 41:14–20. doi: 10.1016/j.immuni.2014.06.008
65. Nguyen MTA, Favelyukis S, Nguyen AK, Reichart D, Scott PA, Jenn A, et al. A Subpopulation of Macrophages Infiltrates Hypertrophic Adipose Tissue and is Activated by Free Fatty Acids Via Toll-Like Receptors 2 and 4 and JNK-Dependent Pathways. *J Biol Chem* (2007) 282:35279–92. doi: 10.1074/jbc.M706762200
66. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic Inflammation in Fat Plays a Crucial Role in the Development of Obesity-Related Insulin Resistance. *J Clin Invest* (2003) 112:1821–30. doi: 10.1172/JCI200319451
67. Boonacker E, Van Noorden CJF. Enzyme Cytochemical Techniques for Metabolic Mapping in Living Cells, With Special Reference to Proteolysis. *J Histochem Cytochem* (2001) 49:1473–86. doi: 10.1177/002215540104901201
68. Boutens L, Stienstra R. Adipose Tissue Macrophages: Going Off Track During Obesity. *Diabetologia* (2016) 59:879–94. doi: 10.1007/s00125-016-3904-9
69. Wang QA, Tao C, Gupta RK, Scherer PE. Tracking Adipogenesis During White Adipose Tissue Development, Expansion and Regeneration. *Nat Med* (2013) 19:1338–44. doi: 10.1038/nm.3324
70. Burl RB, Ramseyer VD, Rondini EA, Pique-Regi R, Lee Y-H, Granneman JG. Deconstructing Adipogenesis Induced by β3-Adrenergic Receptor Activation With Single-Cell Expression Profiling. *Cell Metab* (2018) 28:300–9. doi: 10.1016/j.cmet.2018.05.025
71. Serbulea V, Upchurch CM, Schappe MS, Voigt P, DeWeese DE, Desai BN, et al. Macrophage Phenotype and Bioenergetics Are Controlled by Oxidized Phospholipids Identified in Lean and Obese Adipose Tissue. *Proc Natl Acad Sci USA* (2018) 115:E6254–63. doi: 10.1073/pnas.1800544115
72. Munro DA, Wineberg Y, Tarnick J, Vink CS, Li Z, Pridans C, et al. Macrophages Restrict the Nephrogenic Field and Promote Endothelial Connections During Kidney Development. *Elife* (2019) 8:1–27. doi: 10.7554/eLife.43271
73. Schyns J, Bai Q, Ruscitti C, Radermecker C, De Schepper S, Chakarov S, et al. Non-Classical Tissue Monocytes and Two Functionally Distinct Populations of Interstitial Macrophages Populate the Mouse Lung. *Nat Commun* (2019) 10. doi: 10.1038/s41467-019-11843-0
74. Chieppa M, Bianchi G, Doni A, Del Prete A, Sironi M, Laskarin G, et al. Cross-Linking of the Mannose Receptor on Monocyte-Derived Dendritic Cells Activates an Anti-Inflammatory Immunosuppressive Program. *J Immunol* (2003) 171:4552–60. doi: 10.4049/jimmunol.171.9.4552
75. Sallusto F, Cella M, Danieli C, Lanzavecchia A. Dendritic Cells Use Macropinocytosis and the Mannose Receptor to Concentrate Macromolecules in the Major Histocompatibility Complex Class 1I Compartment: Downregulation by Cytokines and Bacterial Products. *J Exp Med* (1995) 182:389–400. doi: 10.1084/jem.182.2.389
76. Hoeffel G, Ginhoux F. Fetal Monocytes and the Origins of Tissue-Resident Macrophages. *Cell Immunol* (2018) 330:5–15. doi: 10.1016/j.cellimm.2018.01.001
77. Mass E, Ballesteros I, Farlik M, Halbritter F, Günther P, Crozet L, et al. Specification of Tissue-Resident Macrophages During Organogenesis. *Science* (2016) 353:6304. doi: 10.1126/science.aaf4238
78. Deng T, Lyon C, Minze L, Lin J, Zou J, Liu J, et al. Class II Major Histocompatibility Complex Plays an Essential Role in Obesity-Induced Adipose Inflammation. *Cell Metab* (2013) 17:411–22. doi: 10.1016/j.cmet.2013.02.009.Class
79. Pridans C, Raper A, Davis GM, Alves J, Sauter KA, Lefevre L, et al. Pleiotropic Impacts of Macrophage and Microglial Deficiency on Development in Rats With Targeted Mutation of the Csf1r Locus. *J Immunol* (2018) 201:2683–99. doi: 10.4049/jimmunol.1701783
80. Pollard JW, Dominguez MG, Mocci S, Cohen PE, Stanley ER. Effect of the Colony-Stimulating Factor-1 Null Mutation, Osteopetrotic (Csfm(Op)), on the Distribution of Macrophages in the Male Mouse Reproductive Tract. *Biol Reprod* (1997) 56:1290–300. doi: 10.1095/biolreprod56.5.1290

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The Adipose Tissue Macrophages Central to Adaptive Thermoregulation

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White fat stores excess energy, and thus its excessive expansion causes obesity. However, brown and beige fat, known as adaptive thermogenic fat, dissipates energy in the form of heat and offers a therapeutic potential to counteract obesity and metabolic disorders. The fat type-specific biological function is directed by its unique tissue microenvironment composed of immune cells, endothelial cells, pericytes and neuronal cells. Macrophages are major immune cells resident in adipose tissues and gained particular attention due to their accumulation in obesity as the primary source of inflammation. However, recent studies identified macrophages' unique role and regulation in thermogenic adipose tissues to regulate energy expenditure and systemic energy homeostasis. This review presents the current understanding of macrophages in thermogenic fat niches with an emphasis on discrete macrophage subpopulations central to adaptive thermoregulation.

Keywords: adipose tissue macrophage, beige adipocyte, brown adipocyte , obesity, thermogenesis

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INTRODUCTION

Obesity is directly linked to the onset of many diseases, including type 2 diabetes, hypertension, cardiovascular diseases and some types of cancer (1, 2). Therefore, weight loss is suggested as a fundamental approach to ameliorate those disorders (3, 4). Obesity develops when energy intake chronically exceeds energy expenditure, and adipose tissue is the center of the regulation of systemic energy homeostasis. An expansion of white adipose tissue (WAT) in which energy-storing white adipocytes grow in number (hyperplasia) and size (hypertrophy) is the major characteristic of obesity (5). Conversely, brown adipocytes found in brown adipose tissue (BAT) and beige adipocytes inducible in particular WAT depots dissipate excess energy as heat, termed adaptive thermogenesis (5, 6). Since the biological functions of thermogenic adipocytes include regulating lipid and glucose metabolism and insulin sensitivity beyond strengthened energy expenditure, enhancing thermogenic adipocyte activity and amount holds promise to combat obesity and related disorders (7–10).

The distinct biological functions of white and thermogenic adipocytes are directed by their unique tissue microenvironments composed of multiple cell types, including immune cells, adipocyte progenitors, endothelial cells, pericytes and neuronal cells (11). In particular, immune cells have received considerable attention owing to their role in WAT inflammation, key pathophysiology of obesity. Macrophages are the most plentiful WAT-infiltrating immune cells

in obesity and are crucial mediators of inflammation in adipose tissues (12). Adipose tissue macrophages (ATMs) are a heterogeneous population but can be conventionally classified as M1 (classically activated or pro-inflammatory) or M2 (anti-inflammatory or alternatively activated) cells. In obese WAT, M1 macrophages are recruited in response to pro-inflammatory mediators (lipopolysaccharides, interferon-gamma among others) and release pro-inflammatory cytokines [tumor necrosis factor-alpha (TNFα), inducible nitric oxide synthase) (13). Oppositely, M2 macrophages are abundantly found in lean WAT, express specific biomarkers (e.g., arginase) and secrete anti-inflammatory cytokines [e.g., transforming growth factor-beta (TGFβ), interleukin (IL)-10, IL-1 decoy receptor] (13).

In contrast to WAT, there was a limited research interest in the role of ATMs in regulating thermogenic adipose tissue characteristics and functions. The accumulation of pro-inflammatory M1 macrophages is observed in thermogenic adipose tissues in the state of obese and impairs thermogenic machinery of brown and beige adipocytes (14). Intriguingly, recent studies identified and highlighted the non-canonical roles of ATMs found in thermogenic adipose niches. Certain ATM subsets are specialized to mediate thermogenic programs of brown or beige adipocytes through driving local noradrenergic or cholinergic tone (15–20). This review covers the roles of ATMs in thermogenic adipose tissues, emphasizing distinct macrophage subpopulations central to adaptive thermoregulation.

A DISTINCT ROLE OF THERMOGENIC FAT IN ENERGY HOMEOSTASIS

In humans, thermogenic fat was believed to exist restrictively to infancy to generate heat and fight against hypothermia under cold environments. However, in 2009 a series of milestone publications identified the presence of functional thermogenic fat in the upper supraclavicular region of adult humans (21–23). Thermogenic activity, determined by 18F-fluorodeoxyglucose uptake, was induced in the supraclavicular area following short cold exposure, and its biopsy specimens showed the molecular and morphological features of thermogenic fat. So far, adult humans have been known to have thermogenic fat in several anatomic areas, including cervical, axillary, paraspinal, mediastinal, and abdominal, as well as supraclavicular (24). A recent study implied the significance of thermogenic fat in humans by demonstrating a lower prevalence of cardiometabolic disorders, including type 2 diabetes, coronary artery disease, hypertension and congestive heart failure, in individuals with thermogenic fat (25).

To date, three types of adipocytes have been identified in mammals: white, brown and beige adipocytes. While white adipocytes found in WAT possess few mitochondria and a large unilocular lipid droplet to store energy efficiently, brown and beige adipocytes contain multilocular lipid droplets and numerous mitochondria to generate heat. However, despite their similarities, brown and beige adipocytes are distinct cell types due to the main differences in their anatomical location and developmental origin (26). Therefore, brown adipocytes are specified as "classic" in distinction from beige adipocytes. Classic brown adipocytes cluster in dedicated depots, such as interscapular BAT of infants and mice. Thermogenic function of brown adipocytes is active at birth and relatively stable because they develop prenatally from precursors in the dermomyotome that expresses myogenic factor 5 (*Myf5*), paired-box protein 7 (*Pax7*) and engrailed 1 (27–29). However, beige adipocytes are inducible in WAT, particularly subcutaneous WAT, in response to cold exposure, catecholamines, thiazolidinediones and exercise. They derive from *Myf5*-absent precursors during postnatal development in mice, and upon cold exposure, beige adipocytes can be recruited by differentiation of precursors expressing alpha-smooth muscle actin (*αSma*), *Cd81, Pax3* or platelet-derived growth factor receptor, alpha polypeptide (*Pdgfrα*) (30–33).

The long-standing paradigm in adaptive thermogenic mechanism has held that heat is generated by uncoupling protein 1 (UCP1) located in the mitochondrial inner membrane of brown or beige adipocytes. During mitochondrial respiration, UCP1 uncouples ATP synthesis and catalyzes proton leak across mitochondrial membrane, resulting in heat generation. However, recent studies discovered UCP1-independent adaptive thermogenic pathways mediated by futile creatine or calcium cycling in mammals (34–36). The futile cycles dissipate energy as the form of heat by consumption of ATP derived from lipid or glucose oxidation and are mainly found in beige adipocytes rather than brown adipocytes due to their high ATP synthetase expression. Like traditional UCP1-dependent thermogenesis, the UCP1-independent thermogenic machinery is also sufficient to regulate whole-body energy homeostasis and protect against diet-induced obesity and related metabolic dysfunction (35–38).

Adaptive thermogenic activation is directed by intercellular crosstalk within adipose niches. Neuronal-thermogenic adipocyte communication through catecholamines is the most well-known. The innervation of sympathetic nervous system (SNS) in thermogenic adipose tissues connects the central nervous system (CNS)-originated efferent signals with brown and beige adipocytes. Upon cold environment, catecholamines, particularly norepinephrine, released from sympathetic nerve terminals activates thermogenic programs of existing brown and beige adipocytes *via* β3-adrenergic receptor (AR) and induce *de novo* beige fat biogenesis *via* β1-AR in rodents (39–42). It is of note that in humans, β2 subtype has been recently reported to play a dominant role in β-ARs-dependent thermogenic fat activation (43). Besides sympathetic innervation or denervation, the local noradrenergic tone within thermogenic adipose tissues has been proposed to be mediated by adipose resident immune cells, such as subpopulations of ATM synthesizing or degrading catecholamine (17, 19). Intriguingly, recent studies also identified cholinergic adipose macrophages (ChAMs) that secrete acetylcholine to selectively activate beige fat thermogenesis under cold exposure (15). Hence, the regulation and function of thermogenic fat need to be understood

considering its heterogeneity and complexity stemming from the tissue microenvironment as a bona fide platform for developing thermogenic fat-targeting therapeutic interventions.

A CLASSICAL VIEW OF MACROPHAGES IN THERMOGENIC ADIPOSE NICHES: INFLAMMATION

Emerging evidence of macrophages' involvement in controlling thermogenic fat function has recently drawn enormous attention to the non-canonical roles and regulation of ATMs (discussed details in the next section). However, recruitment of pro-inflammatory macrophages in thermogenic adipose tissues has been accepted as a pathophysiological phenomenon of obesity, even though it is less profound than in visceral WAT – the fat depot containing mainly white adipocytes to store energy (14, 44, 45). Chronic inflammation stemming from prolonged calorie overload contributes to the whitening of thermogenic adipocytes that transforms the cells to possess intensive energy-storing unilocular lipid droplets and lose unique characteristic of energy expenditure (14).

Gene expression landscapes of obese mouse BAT revealed enriched expression of markers for macrophages-derived pro-inflammatory cytokines, such as *Tnfα*, C-C motif chemokine ligand (*Ccl*) 2, and *Ccl5* (46, 47). Independent studies showed that the activated pro-inflammatory markers were accompanied by reduced expression of thermogenic genes in obese BAT (14, 48–50). As direct evidence, pro-inflammatory macrophage infiltration and related cytokines, such as TNFα and IL-1β, were seen to inhibit the induction of *Ucp1* expression in response to thermogenic stimuli in mouse thermogenic adipose tissues and differentiated C3H10T1/2 stem cells (14, 51, 52). Genetic deletion of TNF receptors in obese mice led to reduced apoptosis and induced transcriptional activation of *Ucp1* in BAT, suggesting a significant role of TNFα-mediated inflammatory response in thermogenic adipobiology during obesity (53). Besides, TNFα appeared to cause insulin receptor substrate 2-mediated insulin resistance in brown adipocytes, supporting that pro-inflammatory signaling could affect a broad spectrum of metabolic processes beyond thermogenic fat cell activity (54).

M1 macrophage infiltration in subcutaneous WAT in obese mouse models shows deleterious effects on beige thermogenesis. Interestingly, a recent study found a self-sustained cycle of inflammation-driven beiging inhibitory mechanism in which pro-inflammatory macrophages expressing α4 integrin directly interact with beige adipocytes and their precursors through vascular cell adhesion molecule-1 (55). Genetic or pharmacological inhibition of the adhesive interaction enhanced beige adipogenesis and whole-body energy expenditure, thereby attenuating obese phenotype. In addition, genetic ablation of IκB kinase ε that amplifies inflammation signal by its elevation in ATMs enhanced UCP1 expression in subcutaneous WAT of mice (56). Hence, in obesity, recruitment of pro-inflammatory macrophages occurs in thermogenic adipose tissues, as seen in WAT, as a hallmark of obesity and negatively influences the biological functions of brown and beige adipocytes.

THE ADIPOSE MACROPHAGES CENTRAL TO ADAPTIVE THERMOREGULATION

Since Nguyen et al. first reported catecholamine-producing ATMs in BAT in 2011, non-classical roles of ATMs in the regulation of thermogenesis and systemic energy homeostasis had been received significant attention and extensively studied as an innovative view to explain complex thermogenic mechanisms (17). To date, the research effort discovered four subpopulations of ATMs that played a direct role in controlling thermogenic fat function and broadened our understanding of the significance of adipose resident immune cells in systemic energy homeostasis.

Alternatively Activated Macrophages

In contrast to M1 macrophages, M2 or alternatively activated macrophages are predominant in lean adipose tissues and release anti-inflammatory cytokines, such as TGFβ and IL-10. In an obese state, a phenotypic transformation from anti-inflammatory M2 to pro-inflammatory M1-like macrophages occurs in adipose tissues leading to insulin resistance. M2 macrophages are required for tissue repair, tissue homeostasis and anti-helminthic activities. Interestingly, helminth infection attenuated high-fat diet-induced obesity along with induction of adaptive thermogenic capacity through enhanced M2 macrophage polarization (57).

Accumulated evidence shows the association between the expansion of alternatively activated macrophages and thermogenic activation (58–62). Many studies have highlighted how M2 macrophages are polarized and activated during adipose thermogenesis, such as through C-X-C motif chemokine ligand 14 (CXCL14) and meteorin-like (58, 61). Particularly, it is fascinating that CXCL14 is released from brown adipocytes to recruit M2 macrophages upon thermogenic activation, indicating an active interplay between thermogenic adipocytes and M2 macrophages during adaptive thermogenesis. However, the mechanisms by which M2 macrophages induce thermogenic responses have not been well established. Nguyen et al. first identified that upon cold stress M2 macrophages expressing tyrosine hydroxylase (TH), key catecholamine synthesizing enzyme, release norepinephrine *via* IL-4 signaling to activate BAT thermogenesis (17). Serial studies from the same group have found catecholamine-producing M2 macrophage-mediated thermogenesis in beige adipocytes as well and completed its whole mechanistic machinery. In mouse subcutaneous WAT, IL-5 secreted from stimulated type 2 innate lymphoid (ILC2) cells by IL-33 were identified to activate and recruit eosinophils, and subsequently, eosinophils activated M2 macrophages by secreting IL-4 (63, 64). Additionally, ILC2- and eosinophil-secreted type 2 cytokines, including IL-13 and IL-4, promoted beige differentiation of PDGFRα$^+$ precursors through IL-4Rα (64). However, in 2017, Fischer et al. presented contradictory data that M2 macrophages do not express TH enough to produce

norepinephrine in BAT and subcutaneous WAT (20). It is still controversial whether alternatively activated macrophages synthesize NE during thermogenesis based on published independent studies (60, 65–67). It has been advances in identifying thermogenic ligands for brown and beige adipocytes within adipose niches. However, whether alternatively activated macrophages can produce any known ligands other than NE for thermogenic fat activation is still unclear (68).

A recent study provided a novel aspect of M2 macrophage-dependent catecholamine secretion and beiging by discovering the mechanism by which M2 macrophages enhanced the local SNS activation in subcutaneous WAT upon cold exposure. Slit guidance ligand 3 (SLIT3) secreted from M2 macrophages induced sympathetic innervation and TH activation by binding to sympathetic neurons *via* roundabout guidance receptor 1 (ROBO1), thereby promoting NE synthesis and beiging to sustain adaptive thermogenesis (16). Consistent with this notion, an independent study found that IL-25-induced M2 macrophage polarization increased outgrowth of sympathetic nerves in subcutaneous WAT (60). It is conceivable that M2 macrophages contribute to adaptive thermogenesis, and dissecting the mechanisms by which M2 macrophages induce thermogenesis and maintain systemic energy homeostasis may empower the specific immune cell type as a novel therapeutic target for obesity.

Sympathetic Innervation-Regulatory Macrophages

Under a cold environment, TH-expressing sympathetic axons are the primary source of NE that binds to β3-ARs on the surface of mature brown and beige adipocytes and activates thermogenic programs. A recent mouse study indicated that beige adipocytes could be differentiated from αSMA-expressing progenitors through β1-ARs in response to NE (39). Of note, beige adipocytes are greatly inducible and heterogeneous due to their remarkable plasticity at the adipocyte and progenitor levels in response to external cues or genetic disposition. In mice lacking β-ARs, beige adipocytes were activated by directly sensing cold temperature or differentiated from myogenic differentiation 1-expressing progenitors (known as glycolytic beige adipocytes) (40, 69).

Local sympathetic innervation and activity in rodent BAT and subcutaneous WAT are enhanced by adipocyte-derived neurotrophic factors, such as nerve growth factor (NGF), neuregulin 4 and S-100 protein β-chain, and by vascular endothelial growth A factor secreted from vascular cells and brown adipocytes (70–74). Recent studies highlight the contribution of immune cells resident in thermogenic adipose niches to sympathetic innervation under cold conditions. In mouse BAT, γδ T cells induced TGFβ1 secretion from adipocytes through IL-17 receptor C signaling and thus increased outgrowth of sympathetic nerves and adaptive thermogenesis (75). Eosinophils maintained by IL-5 also secreted NGF, thereby promoting sympathetic innervation during cold-induced beiging (76).

Methyl-CpG binding protein 2 (*Mecp2*)-expressing, $CX3CR1^+$ macrophages have been suggested as a subpopulation of BAT-resident macrophages that directly influences sympathetic innervation (18). *Mecp2* is well-known to show a mutation in the postnatal neurodevelopmental disorder Rett syndrome. Both *Mecp2*-null and brain-restricted *Mecp2*-deleted mice demonstrated neurological defects related to Rett syndrome, such as irregular breathing and hindlimb clasping (77). Wolf et al. genetically depleted *Mecp2* in all tissue macrophages using *Cx3cr1-Cre* mice or in a macrophage subpopulation expressing CX3CR1 using tamoxifen-inducible *Cx3cr1-CreER* mice (18, 78). Upon chow diet feeding, the mutant mice developed obese phenotypes with excessive body weight gain and enlarged fat mass without Rett-like symptoms in adulthood (18). Thermogenic dysfunction of BAT and resulted decrease in whole-body energy expenditure at a steady-state were seen in the mutant animals due to reduced NE input by impaired local sympathetic innervation. Mechanistically, Plexin A4 overexpressed in *Mecp2*-deleted $CX3CR1^+$ macrophages seemed to interact with and block outgrowth of Semaphorin 6A-positive sympathetic axons in the tissue.

Interestingly, *Mecp2*-expressing macrophages were found to be insignificant in coordinating the responsiveness to acute thermogenic stimulation, such as brief cold exposure (18). Instead, it was linked to sustaining sympathetic innervation and adaptive thermogenesis at a steady-state for homeostasis. As discussed, alternatively activated macrophages have also been reported to involve sympathetic nerve outgrowth in subcutaneous WAT during cold adaptation (16, 60). Therefore, available evidence indicates that a discrete subpopulation of ATMs may reshape local sympathetic innervation to mediate energy expenditure and maintain metabolic homeostasis.

Catecholamine-Scavenging Macrophages

Over the controversy surrounding the presence and significance of catecholamine-producing macrophages within thermogenic adipose tissues, Pirzgalska et al. identified a subpopulation of ATMs that contains catecholamine by its uptake, not by its biosynthesis, in both rodents and humans and that mediates thermogenesis and obesity (19). The macrophage subset, named sympathetic neuron-associated macrophages (SAMs), was initially found in sympathetic nerve bundles of subcutaneous WAT and confirmed its existence in BAT macrophages in mice. SAMs have been characterized as hematopoietic lineage cells that have enriched expression of macrophage-specific markers and as a functionally distinct population that uniquely expresses solute carrier family 6 member 2 (*Slc6a2*) responsible for NE transport. NE was detectable in SAMs, and the NE uptake depended on extracellular catecholamine availability. Additionally, the absence of TH in SAMs supported the notion that intercellular NE accumulation was due to its uptake, not biosynthesis. To coordinate the cellular catecholamine scavenging process, SAMs also highly expressed monoamine oxidase A (*Maoa*) for NE degradation along with *Slc6a2*. Previous studies also prove that macrophages are capable of NE uptake and degradation (79, 80). Interestingly, beige adipocytes served as a NE clearance route *via*

organic cation transporter 3 (*Oct3*) in subcutaneous WAT, and fat-specific *Oct3*-deleted mice showed enhanced beige thermogenesis in response to NE (81).

Adipose SAMs have been found to be excessively recruited in obese conditions, suggesting their possible role in pro-inflammation (19). However, when SAM-mediated catecholamine uptake was blocked by bone-marrow transplantation from *Slc6a2* KO mice into obese recipients, the chimeric mice showed elevated serum NE levels and adaptive thermogenic capacity in both BAT and subcutaneous WAT under cold exposure. Furthermore, SNS activation *via* food restriction resulted in body weight loss in the chimeric animals due to activation of adipocyte lipolysis, the first step to generate energy substrates for thermogenesis in thermogenic adipocytes. An independent study uncovered a *Maoa*-enriched macrophage subset within visceral WAT (82). The distinct subpopulation was found to lower the bioavailability of NE and blunt adipocyte lipolysis in the elderly. Mechanistically, its catecholamine-degradation machinery was activated by NLRP3 inflammasome-induced growth differentiation factor 3 (82).

This potential integrative explanation involving the coexistence of catecholamine scavenging and producing ATM subpopulations within thermogenic adipose niches indicates the significance of ATMs in maintaining local noradrenergic tone and related metabolic homeostasis. Furthermore, the catecholamine scavenging ATMs may also play a role in regulating systemic NE homeostasis upon sustained hypernoradrenergic conditions.

Cholinergic Adipose Macrophages (ChAMs)

Acetylcholine is arguably accepted as the most crucial neurotransmitter in the CNS, autonomic nervous system and somatic nervous system. Parasympathetic nerves are the primary source of acetylcholine. However, non-neuronal cell types also contain cellular machinery that modulates acetylcholine availability and utilization, called the non-neuronal cholinergic system (NNCS), to maintain physiological functions and homeostasis of key organs (83–85). Furthermore, dysfunctional NNCS is directly linked to the pathogenesis of diseases (84, 85). Hence, identifying and understanding NNCS in various types of cells provides new insights to treat diseases.

NNCS includes an acetylcholine synthesizing enzyme [choline acetyltransferase (ChAT)], transporters [vesicular acetylcholine transporter (VaChT)], receptors [nicotinic acetylcholine receptors (nAChRs), muscarinic acetylcholine receptors (mAChRs)] and degrading enzymes [acetylcholinesterase, (AChE), butyrylcholinesterase (BChE)] (84, 85). Immune cells have been known to express NNCS components, particularly ChAT. Recent findings highlight distinct biological roles of acetylcholine-synthesizing immune cells, particularly T cells and B cells, in maintaining innate immunity and blood pressure and responding to chronic viral infection (86–88).

ChAT has been reported to be expressed in macrophages; however, the physiological significance of the acetylcholine-producing macrophages has not been elucidated (86, 89–91). Jun et al. recently identified ChAMs responsible for acetylcholine secretion and adaptive thermogenesis in subcutaneous WAT where parasympathetic innervation is absent (15, 92, 93). Using ChAT reporter mice to monitor functional ChAT-expressing cells *in vivo*, acetylcholine-producing cell populations were defined as $CD45^+$ hematopoietic immune cells, not neurons, and they were composed mainly of B cells, T cells and macrophages (92). Among the $ChAT^+$ immune subsets, macrophages were the only population to show induction in ChAT abundance and acetylcholine secretion in response to cold stress (15). Macrophage-specific ChAT deleted mice showed impaired cold-induced thermogenesis in subcutaneous WAT. ChAMs were activated by NE through β2-AR signaling, demonstrating their role in linking sympathetic signals to thermogenic beige fat activation (15). As suggested by transcriptome analysis, the functionally distinct ChAMs displayed unique molecular machinery fulfilling their role in neurotransmitter metabolism (15).

It has been found that neuronal acetylcholine receptor subunit alpha 2 (CHRNA2) senses ChAMs-produced acetylcholine in beige adipocytes and activates thermogenesis through UCP1- and creatine futile cycling-mediated pathways (92, 94). CHRNA2 has been identified as a unique marker for activated beige adipocytes. However, brown adipocytes do not have functional CHRNA2 to receive acetylcholine (92). Besides, the abundance of $ChAT^+$ macrophages in BAT was very low and not responsive to cold exposure (15). Therefore, CHRNA2-dependent thermogenic activation was not seen in BAT. CHRNA2 signaling was induced during cold exposure, and its deficiency at the whole-body or adipocyte level in mice was unable to fully activate cold-induced thermogenic programs in subcutaneous WAT and systemic energy expenditure (92, 94). Importantly, this acetylcholine-mediated CHRNA2 signaling showed physiological significance to combat obesity and related metabolic dysfunction by demonstrating profound fat accumulation and impaired whole-body glucose metabolism after chronic calorie overload in the absence of *Chrna2*. The discovery of ChAMs that secrete acetylcholine *via* β2-AR signaling and induce beige thermogenesis provides novel evidence of the neuro-immune-beige fat axis and may offer a new approach to counteract obesity and metabolic disorders.

CONCLUDING REMARKS

Thermogenic fat in adult humans was first found and described as the interscapular gland in 1908 (95). Around a hundred years later, its rediscovery, in which thermogenic fat is inducible and functional in adult humans upon external stimuli, fueled scientific interest in the field and contributed to recent advances in understanding thermogenic adipobiology (21–23). Over the last decade, immune-thermogenic adipose interaction has received particular attention in the field due to its unexpected critical roles in shaping thermogenic fat function. Macrophages have been identified as a major resident immune cell type in thermogenic adipose niches. Four distinct subsets of ATMs central to thermoregulation have been identified so far, as summarized in **Figure 1**. Secretory response to cold

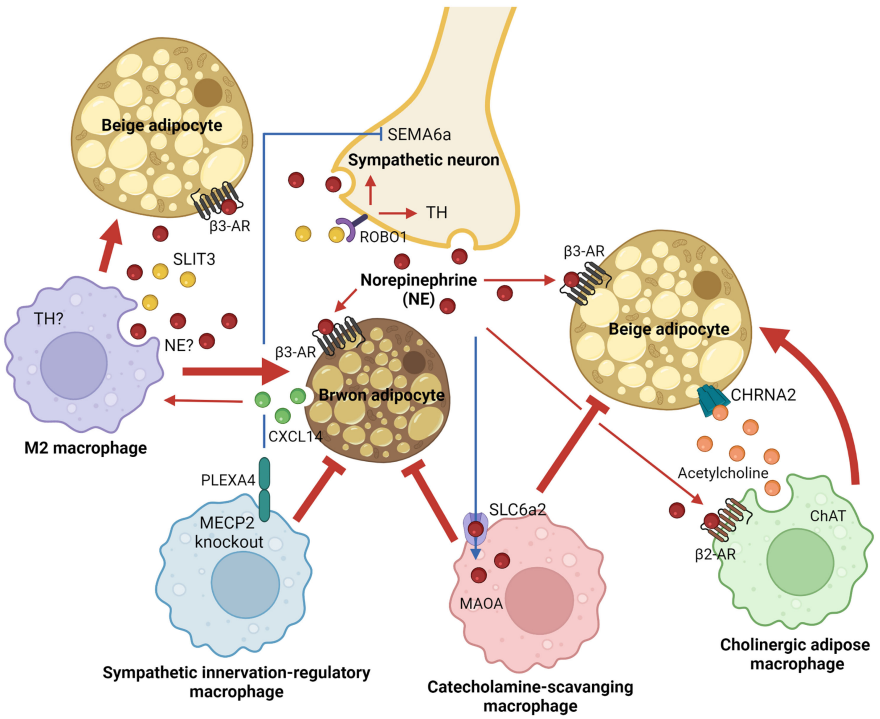

FIGURE 1 | Adipose tissue macrophage-mediated adaptive thermogenesis in brown and beige adipocytes. Distinct macrophage subpopulations within thermogenic adipose tissues, including brown and subcutaneous white fat, support functions of brown or beige adipocytes to dissipate energy and regulate systemic energy homeostasis. Alternatively activated M2 and cholinergic adipose macrophages activate thermogenic responses in brown or beige adipocytes through paracrine mechanisms in a cold environment (red arrows). On the other hand, thermogenic inhibitory ATM subsets that block sympathetic innervation or import/degrade catecholamine have been identified in thermogenic adipose niches (red blunt-ended lines). AR, adrenergic receptor; ChAT, choline acetyltransferase; CHRNA2, neuronal acetylcholine receptor subunit alpha 2; CXCL14, C-X-C motif chemokine ligand 14; MAOA, monoamine oxidase A; MECP2, methyl-CpG binding protein 2; NE, norepinephrine; PLEXA4, plexin A4; ROBO1, roundabout guidance receptor 1; SEMA6a, semaphoring 6a; SLC6a2, solute carrier family 6 member 2; SLIT3, slit guidance ligand 3; TH, tyrosine hydroxylase.

exposure was seen in both M2 macrophages and ChAMs to rapidly stimulate thermogenic machinery in a paracrine manner. Surprisingly, M1 macrophages-derived inflammatory cytokine CXCL5 has been reported to induce beige thermogenesis (96). On the contrary, there are ATM subsets that inhibit adaptive thermogenesis and exert pathogenesis of obesity. The four ATM subpopulations have been characterized as distinct populations that express their unique markers or cellular machinery. However, it is still unknown whether they share their molecular natures or developmental origins, at least in part, and interact with each other in a particular environment. In addition, their distribution across tissues and tissue-specific functions is worth studying to understand their biological significance better at the whole-body level.

The unexpected discovery of acetylcholine-secreting ChAMs in subcutaneous WAT raises the possibility that existing distinct macrophage subpopulations within thermogenic adipose niches might be more diverse than we think. ChAMs are the only ATM subset so far known to target a non-canonical thermogenic pathway, CHRNA2 signaling, in beige adipocytes among the four thermogenic ATMs. In addition, ChAMs responded to β2-AR agonist or fibroblast growth factor 21 mediated by miRNA-182-5p from beige adipocytes to induce acetylcholine secretion in a cold environment (15, 97). The other three ATM subpopulations are linked to the canonical β-AR mechanism in thermogenic adipocytes *via* regulating local noradrenergic tone. Further work studying mechanistic details that modulate activation and target thermogenic pathways of the ATM subsets may contribute to defining their distinct features and functions from each other in regulating adipose thermogenesis and metabolism. For example, it is reasonable to question whether and how the thermoregulatory ATMs' functions are mediated by the dynamic regulators of macrophage activation that control cytokine secretion and innate immunity in response to external cues and also affect the recruitment of subtypes of thermogenic adipocytes (98–100). In addition, since chronic excessive systemic acetylcholine or catecholamine causes disease conditions, such as acetylcholine-induced nicotinic/muscarinic toxicity and catecholamine-induced hypertension, we need to investigate whether their levels mediated by the thermogenic ATM subsets are limited at the local site level or affect systemic levels (101–103). Furthermore, these ATM subsets' presence and biological significance need to be assessed in human thermogenic adipose tissues.

There is growing awareness that brown and beige adipocytes improve lipid metabolism, glucose metabolism and insulin sensitivity beyond enhanced energy expenditure. However, underlying regulatory mechanisms of thermogenic adipocytes are complex due to their remarkable plasticity and heterogeneity depending on external cues, genetic disposition and microenvironment. Although understanding of adaptive thermogenic mechanisms still remains incomplete, discovering the ATM subpopulations central to thermoregulation has broadened our knowledge of immune-thermogenic adipose interaction in metabolic adaptation and brought new insights into the development of therapeutic strategies to enhance energy expenditure. Further research in dissecting ATM populations to identify a specific subset that supports the functions of thermogenic adipocytes may provide exciting perspectives in the field and establish an immune-targeting metabolic drug to improve systemic energy homeostasis and reverse the pathophysiology of obesity and other metabolic disorders.

AUTHOR CONTRIBUTIONS

HJ structured the manuscript and made the figure. MR and HJ reviewed literature and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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REFERENCES

1. Grundy SM. Obesity, Metabolic Syndrome, and Cardiovascular Disease. *J Clin Endocrinol Metab* (2004) 89(6):2595–600. doi: 10.1210/jc.2004-0372
2. De Pergola G, Silvestris F. Obesity as a Major Risk Factor for Cancer. *J Obes* (2013) 2013:291546. doi: 10.1155/2013/291546
3. Lingvay I, Sumithran P, Cohen RV, le Roux CW. Obesity Management as a Primary Treatment Goal for Type 2 Diabetes: Time to Reframe the Conversation. *Lancet* (2022) 399(10322):394–405. doi: 10.1016/S0140-6736(21)01919-X
4. Han TS, Lean ME. A Clinical Perspective of Obesity, Metabolic Syndrome and Cardiovascular Disease. *JRSM Cardiovasc Dis* (2016) 5:2048004016633371. doi: 10.1177/2048004016633371
5. Giralt M, Villarroya F. White, Brown, Beige/Brite: Different Adipose Cells for Different Functions? *Endocrinology* (2013) 154(9):2992–3000. doi: 10.1210/en.2013-1403
6. Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, et al. Beige Adipocytes Are a Distinct Type of Thermogenic Fat Cell in Mouse and Human. *Cell* (2012) 150(2):366–76. doi: 10.1016/j.cell.2012.05.016
7. Bartelt A, Bruns OT, Reimer R, Hohenberg H, Ittrich H, Peldschus K, et al. Brown Adipose Tissue Activity Controls Triglyceride Clearance. *Nat Med* (2011) 17(2):200–5. doi: 10.1038/nm.2297
8. Bartelt A, John C, Schaltenberg N, Berbee JFP, Worthmann A, Cherradi ML, et al. Thermogenic Adipocytes Promote HDL Turnover and Reverse Cholesterol Transport. *Nat Commun* (2017) 8:15010. doi: 10.1038/ncomms15010
9. Dallner OS, Chernogubova E, Brolinson KA, Bengtsson T. Beta3-Adrenergic Receptors Stimulate Glucose Uptake in Brown Adipocytes by Two Mechanisms Independently of Glucose Transporter 4 Translocation. *Endocrinology* (2006) 147(12):5730–9. doi: 10.1210/en.2006-0242
10. Seale P, Conroe HM, Estall J, Kajimura S, Frontini A, Ishibashi J, et al. Prdm16 Determines the Thermogenic Program of Subcutaneous White Adipose Tissue in Mice. *J Clin Invest* (2011) 121(1):96–105. doi: 10.1172/JCI44271
11. Shamsi F, Wang CH, Tseng YH. The Evolving View of Thermogenic Adipocytes - Ontogeny, Niche and Function. *Nat Rev Endocrinol* (2021) 17(12):726–44. doi: 10.1038/s41574-021-00562-6
12. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity Is Associated With Macrophage Accumulation in Adipose Tissue. *J Clin Invest* (2003) 112(12):1796–808. doi: 10.1172/JCI200319246
13. Thomas D, Apovian C. Macrophage Functions in Lean and Obese Adipose Tissue. *Metabolism* (2017) 72:120–43. doi: 10.1016/j.metabol.2017.04.005
14. Sakamoto T, Nitta T, Maruno K, Yeh YS, Kuwata H, Tomita K, et al. Macrophage Infiltration Into Obese Adipose Tissues Suppresses the Induction of UCP1 Level in Mice. *Am J Physiol Endocrinol Metab* (2016) 310(8):E676–87. doi: 10.1152/ajpendo.00028.2015
15. Knights AJ, Liu S, Ma Y, Nudell VS, Perkey E, Sorensen MJ, et al. Acetylcholine-Synthesizing Macrophages in Subcutaneous Fat Are Regulated by Beta2 -Adrenergic Signaling. *EMBO J* (2021) 40(24):e106061. doi: 10.15252/embj.2020106061
16. Wang YN, Tang Y, He Z, Ma H, Wang L, Liu Y, et al. Slit3 Secreted From M2-Like Macrophages Increases Sympathetic Activity and Thermogenesis in Adipose Tissue. *Nat Metab* (2021) 3(11):1536–51. doi: 10.1038/s42255-021-00482-9
17. Nguyen KD, Qiu Y, Cui X, Goh YP, Mwangi J, David T, et al. Alternatively Activated Macrophages Produce Catecholamines to Sustain Adaptive Thermogenesis. *Nature* (2011) 480(7375):104–8. doi: 10.1038/nature10653
18. Wolf Y, Boura-Halfon S, Cortese N, Haimon Z, Sar Shalom H, Kuperman Y, et al. Brown-Adipose-Tissue Macrophages Control Tissue Innervation and Homeostatic Energy Expenditure. *Nat Immunol* (2017) 18(6):665–74. doi: 10.1038/ni.3746
19. Pirzgalska RM, Seixas E, Seidman JS, Link VM, Sanchez NM, Mahu I, et al. Sympathetic Neuron-Associated Macrophages Contribute to Obesity by Importing and Metabolizing Norepinephrine. *Nat Med* (2017) 23(11):1309–18. doi: 10.1038/nm.4422
20. Fischer K, Ruiz HH, Jhun K, Finan B, Oberlin DJ, van der Heide V, et al. Alternatively Activated Macrophages do Not Synthesize Catecholamines or Contribute to Adipose Tissue Adaptive Thermogenesis. *Nat Med* (2017) 23(5):623–30. doi: 10.1038/nm.4316
21. Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, et al. Identification and Importance of Brown Adipose Tissue in Adult Humans. *N Engl J Med* (2009) 360(15):1509–17. doi: 10.1056/NEJMoa0810780
22. Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, et al. Functional Brown Adipose Tissue in Healthy Adults. *N Engl J Med* (2009) 360(15):1518–25. doi: 10.1056/NEJMoa0808949
23. van Marken Lichtenbelt WD, Vanhommerig JW, Smulders NM, Drossaerts JM, Kemerink GJ, Bouvy ND, et al. Cold-Activated Brown Adipose Tissue in Healthy Men. *N Engl J Med* (2009) 360(15):1500–8. doi: 10.1056/NEJMoa0808718
24. Leitner BP, Huang S, Brychta RJ, Duckworth CJ, Baskin AS, McGehee S, et al. Mapping of Human Brown Adipose Tissue in Lean and Obese Young Men. *Proc Natl Acad Sci USA* (2017) 114(32):8649–54. doi: 10.1073/pnas.1705287114
25. Becher T, Palanisamy S, Kramer DJ, Eljalby M, Marx SJ, Wibmer AG, et al. Brown Adipose Tissue Is Associated With Cardiometabolic Health. *Nat Med* (2021) 27(1):58–65. doi: 10.1038/s41591-020-1126-7
26. Cohen P, Kajimura S. The Cellular and Functional Complexity of Thermogenic Fat. *Nat Rev Mol Cell Biol* (2021) 22(6):393–409. doi: 10.1038/s41580-021-00350-0
27. Seale P, Bjork B, Yang W, Kajimura S, Chin S, Kuang S, et al. PRDM16 Controls a Brown Fat/Skeletal Muscle Switch. *Nature* (2008) 454(7207):961–7. doi: 10.1038/nature07182

28. Lepper C, Fan CM. Inducible Lineage Tracing of Pax7-Descendant Cells Reveals Embryonic Origin of Adult Satellite Cells. *Genesis* (2010) 48(7):424–36. doi: 10.1002/dvg.20630
29. Atit R, Sgaier SK, Mohamed OA, Taketo MM, Dufort D, Joyner AL, et al. Beta-Catenin Activation Is Necessary and Sufficient to Specify the Dorsal Dermal Fate in the Mouse. *Dev Biol* (2006) 296(1):164–76. doi: 10.1016/j.ydbio.2006.04.449
30. Oguri Y, Shinoda K, Kim H, Alba DL, Bolus WR, Wang Q, et al. CD81 Controls Beige Fat Progenitor Cell Growth and Energy Balance *via* FAK Signaling. *Cell* (2020) 182(3):563–77.e20. doi: 10.1016/j.cell.2020.06.021
31. Lee YH, Petkova AP, Mottillo EP, Granneman JG. *In Vivo* Identification of Bipotential Adipocyte Progenitors Recruited by Beta3-Adrenoceptor Activation and High-Fat Feeding. *Cell Metab* (2012) 15(4):480–91. doi: 10.1016/j.cmet.2012.03.009
32. Berry DC, Jiang Y, Graff JM. Mouse Strains to Study Cold-Inducible Beige Progenitors and Beige Adipocyte Formation and Function. *Nat Commun* (2016) 7:10184. doi: 10.1038/ncomms10184
33. Liu W, Shan T, Yang X, Liang S, Zhang P, Liu Y, et al. A Heterogeneous Lineage Origin Underlies the Phenotypic and Molecular Differences of White and Beige Adipocytes. *J Cell Sci* (2013) 126(Pt 16):3527–32. doi: 10.1242/jcs.124321
34. Kazak L, Chouchani ET, Jedrychowski MP, Erickson BK, Shinoda K, Cohen P, et al. A Creatine-Driven Substrate Cycle Enhances Energy Expenditure and Thermogenesis in Beige Fat. *Cell* (2015) 163(3):643–55. doi: 10.1016/j.cell.2015.09.035
35. Ikeda K, Kang Q, Yoneshiro T, Camporez JP, Maki H, Homma M, et al. UCP1-Independent Signaling Involving SERCA2b-Mediated Calcium Cycling Regulates Beige Fat Thermogenesis and Systemic Glucose Homeostasis. *Nat Med* (2017) 23(12):1454–65. doi: 10.1038/nm.4429
36. Rahbani JF, Roesler A, Hussain MF, Samborska B, Dykstra CB, Tsai L, et al. Creatine Kinase B Controls Futile Creatine Cycling in Thermogenic Fat. *Nature* (2021) 590(7846):480–5. doi: 10.1038/s41586-021-03221-y
37. Kazak L, Chouchani ET, Lu GZ, Jedrychowski MP, Bare CJ, Mina AI, et al. Genetic Depletion of Adipocyte Creatine Metabolism Inhibits Diet-Induced Thermogenesis and Drives Obesity. *Cell Metab* (2017) 26(4):693. doi: 10.1016/j.cmet.2017.08.009
38. Kazak L, Rahbani JF, Samborska B, Lu GZ, Jedrychowski MP, Lajoie M, et al. Ablation of Adipocyte Creatine Transport Impairs Thermogenesis and Causes Diet-Induced Obesity. *Nat Metab* (2019) 1(3):360–70. doi: 10.1038/s42255-019-0035-x
39. Jiang Y, Berry DC, Graff JM. Distinct Cellular and Molecular Mechanisms for Beta3 Adrenergic Receptor-Induced Beige Adipocyte Formation. *Elife* (2017) 6:e30329. doi: 10.7554/eLife.30329
40. Chen Y, Ikeda K, Yoneshiro T, Scaramozza A, Tajima K, Wang Q, et al. Thermal Stress Induces Glycolytic Beige Fat Formation *via* a Myogenic State. *Nature* (2019) 565(7738):180–5. doi: 10.1038/s41586-018-0801-z
41. Bartness TJ, Vaughan CH, Song CK. Sympathetic and Sensory Innervation of Brown Adipose Tissue. *Int J Obes (Lond)* (2010) 34(Suppl 1):S36–42. doi: 10.1038/ijo.2010.182
42. Jiang H, Ding X, Cao Y, Wang H, Zeng W. Dense Intra-Adipose Sympathetic Arborizations Are Essential for Cold-Induced Beiging of Mouse White Adipose Tissue. *Cell Metab* (2017) 26(4):686–92.e3. doi: 10.1016/j.cmet.2017.08.016
43. Blondin DP, Nielsen S, Kuipers EN, Severinsen MC, Jensen VH, Miard S, et al. Human Brown Adipocyte Thermogenesis Is Driven by Beta2-AR Stimulation. *Cell Metab* (2020) 32(2):287–300.e7. doi: 10.1016/j.cmet.2020.07.005
44. Fitzgibbons TP, Kogan S, Aouadi M, Hendricks GM, Straubhaar J, Czech MP. Similarity of Mouse Perivascular and Brown Adipose Tissues and Their Resistance to Diet-Induced Inflammation. *Am J Physiol Heart Circ Physiol* (2011) 301(4):H1425–37. doi: 10.1152/ajpheart.00376.2011
45. Roberts-Toler C, O'Neill BT, Cypess AM. Diet-Induced Obesity Causes Insulin Resistance in Mouse Brown Adipose Tissue. *Obes (Silver Spring)* (2015) 23(9):1765–70. doi: 10.1002/oby.21134
46. Bae J, Ricciardi CJ, Esposito D, Komarnytsky S, Hu P, Curry BJ, et al. Activation of Pattern Recognition Receptors in Brown Adipocytes Induces Inflammation and Suppresses Uncoupling Protein 1 Expression and Mitochondrial Respiration. *Am J Physiol Cell Physiol* (2014) 306(10):C918–30. doi: 10.1152/ajpcell.00249.2013
47. McGregor RA, Kwon EY, Shin SK, Jung UJ, Kim E, Park JH, et al. Time-Course Microarrays Reveal Modulation of Developmental, Lipid Metabolism and Immune Gene Networks in Intrascapular Brown Adipose Tissue During the Development of Diet-Induced Obesity. *Int J Obes (Lond)* (2013) 37(12):1524–31. doi: 10.1038/ijo.2013.52
48. Martins FF, Bargut TCL, Aguila MB, Mandarim-de-Lacerda CA. Thermogenesis, Fatty Acid Synthesis With Oxidation, and Inflammation in the Brown Adipose Tissue of Ob/Ob (-/-) Mice. *Ann Anat* (2017) 210:44–51. doi: 10.1016/j.aanat.2016.11.013
49. Xu F, Zheng X, Lin B, Liang H, Cai M, Cao H, et al. Diet-Induced Obesity and Insulin Resistance Are Associated With Brown Fat Degeneration in SIRT1-Deficient Mice. *Obes (Silver Spring)* (2016) 24(3):634–42. doi: 10.1002/oby.21393
50. Sanchez-Infantes D, Cereijo R, Peyrou M, Piquer-Garcia I, Stephens JM, Villarroya F. Oncostatin M Impairs Brown Adipose Tissue Thermogenic Function and the Browning of Subcutaneous White Adipose Tissue. *Obes (Silver Spring)* (2017) 25(1):85–93. doi: 10.1002/oby.21679
51. Sakamoto T, Takahashi N, Sawaragi Y, Naknukool S, Yu R, Goto T, et al. Inflammation Induced by RAW Macrophages Suppresses UCP1 mRNA Induction *via* ERK Activation in 10T1/2 Adipocytes. *Am J Physiol Cell Physiol* (2013) 304(8):C729–38. doi: 10.1152/ajpcell.00312.2012
52. Goto T, Naknukool S, Yoshitake R, Hanafusa Y, Tokiwa S, Li Y, et al. Proinflammatory Cytokine Interleukin-1beta Suppresses Cold-Induced Thermogenesis in Adipocytes. *Cytokine* (2016) 77:107–14. doi: 10.1016/j.cyto.2015.11.001
53. Nisoli E, Briscini L, Giordano A, Tonello C, Wiesbrock SM, Uysal KT, et al. Tumor Necrosis Factor Alpha Mediates Apoptosis of Brown Adipocytes and Defective Brown Adipocyte Function in Obesity. *Proc Natl Acad Sci USA* (2000) 97(14):8033–8. doi: 10.1073/pnas.97.14.8033
54. Valverde AM, Teruel T, Navarro P, Benito M, Lorenzo M. Tumor Necrosis Factor-Alpha Causes Insulin Receptor Substrate-2-Mediated Insulin Resistance and Inhibits Insulin-Induced Adipogenesis in Fetal Brown Adipocytes. *Endocrinology* (1998) 139(3):1229–38. doi: 10.1210/endo.139.3.5854
55. Chung KJ, Chatzigeorgiou A, Economopoulou M, Garcia-Martin R, Alexaki VI, Mitroulis I, et al. A Self-Sustained Loop of Inflammation-Driven Inhibition of Beige Adipogenesis in Obesity. *Nat Immunol* (2017) 18(6):654–64. doi: 10.1038/ni.3728
56. Chiang SH, Bazuine M, Lumeng CN, Geletka LM, Mowers J, White NM, et al. The Protein Kinase IKKepsilon Regulates Energy Balance in Obese Mice. *Cell* (2009) 138(5):961–75. doi: 10.1016/j.cell.2009.06.046
57. Su CW, Chen CY, Li Y, Long SR, Massey W, Kumar DV, et al. Helminth Infection Protects Against High Fat Diet-Induced Obesity *via* Induction of Alternatively Activated Macrophages. *Sci Rep* (2018) 8(1):4607. doi: 10.1038/s41598-018-22920-7
58. Rao RR, Long JZ, White JP, Svensson KJ, Lou J, Lokurkar I, et al. Meteorin-Like Is a Hormone That Regulates Immune-Adipose Interactions to Increase Beige Fat Thermogenesis. *Cell* (2014) 157(6):1279–91. doi: 10.1016/j.cell.2014.03.065
59. Brestoff JR, Kim BS, Saenz SA, Stine RR, Monticelli LA, Sonnenberg GF, et al. Group 2 Innate Lymphoid Cells Promote Beiging of White Adipose Tissue and Limit Obesity. *Nature* (2015) 519(7542):242–6. doi: 10.1038/nature14115
60. Li L, Ma L, Zhao Z, Luo S, Gong B, Li J, et al. IL-25-Induced Shifts in Macrophage Polarization Promote Development of Beige Fat and Improve Metabolic Homeostasis in Mice. *PloS Biol* (2021) 19(8):e3001348. doi: 10.1371/journal.pbio.3001348
61. Cereijo R, Gavalda-Navarro A, Cairo M, Quesada-Lopez T, Villarroya J, Moron-Ros S, et al. CXCL14, a Brown Adipokine That Mediates Brown-Fat-To-Macrophage Communication in Thermogenic Adaptation. *Cell Metab* (2018) 28(5):750–63.e6. doi: 10.1016/j.cmet.2018.07.015
62. Henriques F, Bedard AH, Guilherme A, Kelly M, Chi J, Zhang P, et al. Single-Cell RNA Profiling Reveals Adipocyte to Macrophage Signaling

Sufficient to Enhance Thermogenesis. *Cell Rep* (2020) 32(5):107998. doi: 10.1016/j.celrep.2020.107998
63. Qiu Y, Nguyen KD, Odegaard JI, Cui X, Tian X, Locksley RM, et al. Eosinophils and Type 2 Cytokine Signaling in Macrophages Orchestrate Development of Functional Beige Fat. *Cell* (2014) 157(6):1292–308. doi: 10.1016/j.cell.2014.03.066
64. Lee MW, Odegaard JI, Mukundan L, Qiu Y, Molofsky AB, Nussbaum JC, et al. Activated Type 2 Innate Lymphoid Cells Regulate Beige Fat Biogenesis. *Cell* (2015) 160(1-2):74–87. doi: 10.1016/j.cell.2014.12.011
65. Ruiz de Azua I, Mancini G, Srivastava RK, Rey AA, Cardinal P, Tedesco L, et al. Adipocyte Cannabinoid Receptor CB1 Regulates Energy Homeostasis and Alternatively Activated Macrophages. *J Clin Invest* (2017) 127 (11):4148–62. doi: 10.1172/JCI83626
66. Spadaro O, Camell CD, Bosurgi L, Nguyen KY, Youm YH, Rothlin CV, et al. IGF1 Shapes Macrophage Activation in Response to Immunometabolic Challenge. *Cell Rep* (2017) 19(2):225–34. doi: 10.1016/j.celrep.2017.03.046
67. Lv Y, Zhang SY, Liang X, Zhang H, Xu Z, Liu B, et al. Adrenomedullin 2 Enhances Beiging in White Adipose Tissue Directly in an Adipocyte-Autonomous Manner and Indirectly Through Activation of M2 Macrophages. *J Biol Chem* (2016) 291(45):23390–402. doi: 10.1074/jbc.M116.735563
68. Reitman ML. How Does Fat Transition From White to Beige? *Cell Metab* (2017) 26(1):14–6. doi: 10.1016/j.cmet.2017.06.011
69. Ye L, Wu J, Cohen P, Kazak L, Khandekar MJ, Jedrychowski MP, et al. Fat Cells Directly Sense Temperature to Activate Thermogenesis. *Proc Natl Acad Sci USA* (2013) 110(30):12480–5. doi: 10.1073/pnas.1310261110
70. Pellegrinelli V, Peirce VJ, Howard L, Virtue S, Turei D, Senzacqua M, et al. Adipocyte-Secreted BMP8b Mediates Adrenergic-Induced Remodeling of the Neuro-Vascular Network in Adipose Tissue. *Nat Commun* (2018) 9 (1):4974. doi: 10.1038/s41467-018-07453-x
71. Zeng X, Ye M, Resch JM, Jedrychowski MP, Hu B, Lowell BB, et al. Innervation of Thermogenic Adipose Tissue *via* a Calsyntenin 3beta-S100b Axis. *Nature* (2019) 569(7755):229–35. doi: 10.1038/s41586-019-1156-9
72. Asano A, Morimatsu M, Nikami H, Yoshida T, Saito M. Adrenergic Activation of Vascular Endothelial Growth Factor mRNA Expression in Rat Brown Adipose Tissue: Implication in Cold-Induced Angiogenesis. *Biochem J* (1997) 328(Pt 1):179–83. doi: 10.1042/bj3280179
73. Nisoli E, Tonello C, Benarese M, Liberini P, Carruba MO. Expression of Nerve Growth Factor in Brown Adipose Tissue: Implications for Thermogenesis and Obesity. *Endocrinology* (1996) 137(2):495–503. doi: 10.1210/endo.137.2.8593794
74. Mahdaviani K, Chess D, Wu Y, Shirihai O, Aprahamian TR. Autocrine Effect of Vascular Endothelial Growth Factor-A Is Essential for Mitochondrial Function in Brown Adipocytes. *Metabolism* (2016) 65 (1):26–35. doi: 10.1016/j.metabol.2015.09.012
75. Hu B, Jin C, Zeng X, Resch JM, Jedrychowski MP, Yang Z, et al. Gammadelta T Cells and Adipocyte IL-17RC Control Fat Innervation and Thermogenesis. *Nature* (2020) 578(7796):610–4. doi: 10.1038/s41586-020-2028-z
76. Meng X, Qian X, Ding X, Wang W, Yin X, Zhuang G, et al. Eosinophils Regulate Intra-Adipose Axonal Plasticity. *Proc Natl Acad Sci USA* (2022) 119 (3):e2112281119. doi: 10.1073/pnas.2112281119
77. Guy J, Hendrich B, Holmes M, Martin JE, Bird A. A Mouse Mecp2-Null Mutation Causes Neurological Symptoms That Mimic Rett Syndrome. *Nat Genet* (2001) 27(3):322–6. doi: 10.1038/85899
78. Yona S, Kim KW, Wolf Y, Mildner A, Varol D, Breker M, et al. Fate Mapping Reveals Origins and Dynamics of Monocytes and Tissue Macrophages Under Homeostasis. *Immunity* (2013) 38(1):79–91. doi: 10.1016/j.immuni.2012.12.001
79. Czimmerer Z, Varga T, Poliska S, Nemet I, Szanto A, Nagy L. Identification of Novel Markers of Alternative Activation and Potential Endogenous PPARgamma Ligand Production Mechanisms in Human IL-4 Stimulated Differentiating Macrophages. *Immunobiology* (2012) 217(12):1301–14. doi: 10.1016/j.imbio.2012.08.270
80. Balter NJ, Schwartz SL. Accumulation of Norepinephrine by Macrophages and Relationships to Known Uptake Processes. *J Pharmacol Exp Ther* (1977) 201(3):636–43.
81. Song W, Luo Q, Zhang Y, Zhou L, Liu Y, Ma Z, et al. Organic Cation Transporter 3 (Oct3) Is a Distinct Catecholamines Clearance Route in Adipocytes Mediating the Beiging of White Adipose Tissue. *PloS Biol* (2019) 17(1):e2006571. doi: 10.1371/journal.pbio.2006571
82. Camell CD, Sander J, Spadaro O, Lee A, Nguyen KY, Wing A, et al. Inflammasome-Driven Catecholamine Catabolism in Macrophages Blunts Lipolysis During Ageing. *Nature* (2017) 550(7674):119–23. doi: 10.1038/nature24022
83. Felten DL, O'Banion MK, Maida ME. *Netter's Atlas of Neuroscience. 3rd ed*. Philadelphia, PA, United States: Elsevier (2016). p. 153–231.
84. Wessler I, Kirkpatrick CJ. Acetylcholine Beyond Neurons: The Non-Neuronal Cholinergic System in Humans. *Br J Pharmacol* (2008) 154 (8):1558–71. doi: 10.1038/bjp.2008.185
85. Beckmann J, Lips KS. The Non-Neuronal Cholinergic System in Health and Disease. *Pharmacology* (2013) 92(5-6):286–302. doi: 10.1159/000355835
86. Reardon C, Duncan GS, Brustle A, Brenner D, Tusche MW, Olofsson PS, et al. Lymphocyte-Derived ACh Regulates Local Innate But Not Adaptive Immunity. *Proc Natl Acad Sci USA* (2013) 110(4):1410–5. doi: 10.1073/pnas.1221655110
87. Olofsson PS, Steinberg BE, Sobbi R, Cox MA, Ahmed MN, Oswald M, et al. Blood Pressure Regulation by CD4(+) Lymphocytes Expressing Choline Acetyltransferase. *Nat Biotechnol* (2016) 34(10):1066–71. doi: 10.1038/nbt.3663
88. Cox MA, Duncan GS, Lin GHY, Steinberg BE, Yu LX, Brenner D, et al. Choline Acetyltransferase-Expressing T Cells Are Required to Control Chronic Viral Infection. *Science* (2019) 363(6427):639–44. doi: 10.1126/science.aau9072
89. Wessler IK, Kirkpatrick CJ. The Non-Neuronal Cholinergic System: An Emerging Drug Target in the Airways. *Pulm Pharmacol Ther* (2001) 14 (6):423–34. doi: 10.1006/pupt.2001.0313
90. Hecker A, Mikulski Z, Lips KS, Pfeil U, Zakrzewicz A, Wilker S, et al. Pivotal Advance: Up-Regulation of Acetylcholine Synthesis and Paracrine Cholinergic Signaling in Intravascular Transplant Leukocytes During Rejection of Rat Renal Allografts. *J Leukoc Biol* (2009) 86(1):13–22. doi: 10.1189/jlb.1107722
91. Koarai A, Traves SL, Fenwick PS, Brown SM, Chana KK, Russell RE, et al. Expression of Muscarinic Receptors by Human Macrophages. *Eur Respir J* (2012) 39(3):698–704. doi: 10.1183/09031936.00136710
92. Jun H, Yu H, Gong J, Jiang J, Qiao X, Perkey E, et al. An Immune-Beige Adipocyte Communication *via* Nicotinic Acetylcholine Receptor Signaling. *Nat Med* (2018) 24(6):814–22. doi: 10.1038/s41591-018-0032-8
93. Giordano A, Song CK, Bowers RR, Ehlen JC, Frontini A, Cinti S, et al. White Adipose Tissue Lacks Significant Vagal Innervation and Immunohistochemical Evidence of Parasympathetic Innervation. *Am J Physiol Regul Integr Comp Physiol* (2006) 291(5):R1243–55. doi: 10.1152/ajpregu.00679.2005
94. Jun H, Ma Y, Chen Y, Gong J, Liu S, Wang J, et al. Adrenergic-Independent Signaling *via* CHRNA2 Regulates Beige Fat Activation. *Dev Cell* (2020) 54 (1):106–16.e5. doi: 10.1016/j.devcel.2020.05.017
95. Bonnot E. The Interscapular Gland. *J Anat Physiol* (1908) 43(Pt 1):43–58.
96. Lee D, Kim DW, Yoon S, Nam AR, Lee KH, Nam KH, et al. CXCL5 Secreted From Macrophages During Cold Exposure Mediates White Adipose Tissue Browning. *J Lipid Res* (2021) 62:100117. doi: 10.1016/j.jlr.2021.100117
97. Meng W, Xiao T, Liang X, Wen J, Peng X, Wang J, et al. The miR-182-5p/FGF21/acetylcholine Axis Mediates the Crosstalk Between Adipocytes and Macrophages to Promote Beige Fat Thermogenesis. *JCI Insight* (2021) 6(17): e150249. doi: 10.1172/jci.insight.150249
98. Xing J, Weng L, Yuan B, Wang Z, Jia L, Jin R, et al. Identification of a Role for TRIM29 in the Control of Innate Immunity in the Respiratory Tract. *Nat Immunol* (2016) 17(12):1373–80. doi: 10.1038/ni.3580
99. Kane H, Lynch L. Innate Immune Control of Adipose Tissue Homeostasis. *Trends Immunol* (2019) 40(9):857–72. doi: 10.1016/j.it.2019.07.006
100. Li Q, Lin L, Tong Y, Liu Y, Mou J, Wang X, et al. TRIM29 Negatively Controls Antiviral Immune Response Through Targeting STING for Degradation. *Cell Discov* (2018) 4:13. doi: 10.1038/s41421-018-0010-9
101. Adeyinka A, Kondamudi NP. *Cholinergic Crisis*. Treasure Island (FL: StatPearls (2022).

102. Mathar I, Vennekens R, Meissner M, Kees F, van der Mieren G, Camacho Londono JE, et al. Increased Catecholamine Secretion Contributes to Hypertension in TRPM4-Deficient Mice. *J Clin Invest* (2010) 120(9):3267–79. doi: 10.1172/JCI41348
103. Goldstein DS. Plasma Catecholamines and Essential Hypertension. An Analytical Review. *Hypertension* (1983) 5(1):86–99. doi: 10.1161/01.HYP.5.1.86

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C1QTNF3 is Upregulated During Subcutaneous Adipose Tissue Remodeling and Stimulates Macrophage Chemotaxis and M1-Like Polarization

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The adipose tissue undergoes substantial tissue remodeling during weight gain-induced expansion as well as in response to the mechanical and immunological stresses from a growing tumor. We identified the C1q/TNF-related protein family member *C1qtnf3* as one of the most upregulated genes that encode secreted proteins in tumor-associated inguinal adipose tissue - especially in high fat diet-induced obese mice that displayed 3-fold larger tumors than their lean controls. Interestingly, inguinal adipose tissue *C1qtnf3* was co-regulated with several macrophage markers and chemokines and was primarily expressed in fibroblasts while only low levels were detected in adipocytes and macrophages. Administration of C1QTNF3 neutralizing antibodies inhibited macrophage accumulation in tumor-associated inguinal adipose tissue while tumor growth was unaffected. In line with this finding, C1QTNF3 exerted chemotactic actions on both M1- and M2-polarized macrophages *in vitro*. Moreover, C1QTNF3 treatment of M2-type macrophages stimulated the ERK and Akt pathway associated with increased M1-like polarization as judged by increased expression of M1-macrophage markers, increased production of nitric oxide, reduced oxygen consumption and increased glycolysis. Based on these results, we propose that macrophages are recruited to adipose tissue sites with increased C1QTNF3 production. However, the impact of the immunomodulatory effects of C1QTNF3 in adipose tissue remodeling warrants future investigations.

Keywords: adipose tissue, breast cancer, obesity, C1QTNF3, CTRP3, macrophage

1 INTRODUCTION

Adipose tissue can dynamically alter its size, cellular composition, and metabolic function in response to hormonal and nutritional changes. This plasticity requires effective tissue remodeling processes in which macrophages are thought to play a key role. For instance, anti-inflammatory M2-type adipose tissue macrophages increase in response to fasting, weight loss or cold temperature, and are suggested to buffer excess lipids and stimulate thermogenesis (1–3). Interestingly, a recent study shows that resident macrophages protect against pathological adipose tissue remodeling in obesity (4). In contrast, increased accumulation of pro-inflammatory M1-type macrophages in obese adipose tissue is considered to contribute to chronic inflammation that causes insulin resistance (5, 6). Macrophages are also well known to infiltrate tumors. A high density of tumor-associated macrophages is correlated with worse prognosis for most cancers and unlike obese adipose tissue most macrophages in late-stage tumors are of the M2-type, which have been shown to stimulate tumor progression and metastasis (7–9). Clearly, macrophage subtypes exist through a continuum and the dichotomous M1-M2 model is often too simplistic to explain all the functional aspects (10–12).

Paracrine crosstalk between adipose tissue and cancer cells has been shown to enhance tumor growth (13–18), and the mechanical pressure from a growing tumor will also trigger substantial tissue remodeling in adjacent adipose tissue. To identify new adipose tissue-derived factors potentially important in subcutaneous adipose tissue remodeling and/or tumor progression, we compared the global gene expression profiles of breast cancer-associated and control inguinal/mammary white adipose tissue (IWAT) from lean and high fat diet (HFD)-induced obese female *C57Bl/6* mice. We chose to focus on upregulated secreted factors in breast cancer-associated IWAT that, to our knowledge, have not previously been studied in the context of adipose tissue remodeling and/or breast cancer progression. This approach led us to the cytokine C1Q and TNF related 3 (C1QTNF3, also called CTRP3, CORS26, cartducin and cartonectin) that belongs to the C1q/TNF-related protein-family. C1QTNF3 shares sequence homology with the insulin-sensitizing and anti-inflammatory adipokine adiponectin and is highly expressed in adipose tissue (19, 20). Since its discovery in 2001 (20), a number of experimental *in vitro* studies have been conducted and C1QTNF3 has been suggested to e.g. stimulate proliferation (21), inhibit LPS-induced inflammatory responses in fibroblasts, adipocytes and macrophages (22–25) and increase the secretion of adiponectin from adipocytes (21). Moreover, C1QTNF3 has been shown to exert beneficial effects on metabolism and inflammation *in vivo*; administration or transgenic overexpression of C1QTNF3 attenuated diet-induced hepatic steatosis and lowered glucose levels in Ob/Ob mice (26, 27) and C1QTNF3 knockout mice are more susceptible to collagen-induced arthritis in mice (28). Patients with type-2 diabetes have lower circulating C1QTNF3 levels (29) and visceral adipose tissue *C1qtnf3* levels are reduced in obesity/insulin resistant conditions (30–32), but less is known about *C1qtnf3* regulation in subcutaneous adipose tissue. Here, we found that IWAT *C1qtnf3* expression increases in response to both a growing a tumor and to HFD-induced obesity, and this *C1qtnf3* expression correlated with the expression of several macrophage markers and chemokines. We thus hypothesized that C1QTNF3 plays a role in macrophage regulation in breast cancer and/or subcutaneous adipose tissue remodeling. In line with this hypothesis, our data show that C1QTNF3 contributes to breast cancer-induced macrophage infiltration in IWAT. We found however no effect of C1QTNF3 neutralization on the macrophages within the tumor. Thus, IWAT C1QTNF3 affects macrophages locally in IWAT, but not in the adjacent tumor. *In vitro*, we demonstrate that C1QTNF3 is chemotactic for M1- and M2-macrophages and pushes M2 macrophages towards an M1-like phenotype. Thus, we propose that C1QTNF3 exerts immunomodulatory functions in a cell- and physiological state dependent manner.

2 MATERIALS AND METHODS

2.1 Animals, Breast Cancer Model and IWAT Dissection

Female and male *C57Bl/6* mice, obtained from Charles River Laboratories (MA, U.S.A), were allowed to acclimatize for one week upon arrival. The mice were maintained on standard housing conditions of 12-hour light/dark cycle and temperature with *ad libitum* access to water and regular chow or high fat diet (HFD, 60% kcal from fat, D12492 from Research Diets, NJ, U.S.A.) as indicated.

At the age of about 20 weeks, IWAT of female mice were transplanted orthotopically with the breast cancer cell line E0771 (1×10^5 cells in 50 μL) derived from a *C57Bl/6* mouse (33). Cells were suspended in an equal volume Matrigel (Matrigel Basement Membrane Matrix, Corning Inc, NY, U.S.A) and phosphate buffered saline (PBS). Sham control IWAT received Matrigel alone. The mice were euthanized 2-3 weeks after tumor transplantation, and sham control- and tumor-associated IWAT, and tumors were harvested. In brief, the inguinal lymph node was carefully removed. Thereafter, the tumor was excised containing a 1-2mm-sheet of surrounding adipose tissue. Tumor-associated IWAT were dissected out, and the adipose tissue sheet covering the tumor was carefully removed. Thus, some border material was discarded to avoid contamination. To ensure enough cells in the flow cytometry analysis, most IWAT was included while the most peripheral IWAT was removed in the RNA-sequencing experiment (**Supplementary Figure 1**). All experiments were approved by the regional Animal Ethics Committee in Gothenburg, Sweden.

2.2 Cell Lines and Cell Culture

The pre-adipocyte cell line 3T3-L1 (ZenBio, Durham, NC, U.S.A.) was maintained in supplemented DMEM growth media containing high glucose (4500 mg L^{-1}), fetal bovine serum (10%, FBS Gold) and penicillin–streptomycin (1%, Thermo Fisher Scientific, MA, U.S.A) (34). The differentiation into adipocytes was carried out according to

standard procedures (35). In brief, upon reaching sufficient confluency, cells were treated with a differentiation cocktail (1 µm dexamethasone, 850 nM insulin, and 0.5 mM 3-isobutyl-1-methylxanthine) for 2 days. Thereafter the media was interchanged with fresh media containing a second differentiation cocktail (850 nM insulin) for additional 2 days upon the media was returned to regular supplemented DMEM. The 3T3-L1 adipocytes maturity was determined by the prevalence of lipid droplets and then the cells were assayed, usually between 8 to 10 days from start of differentiation.

The E0771 cell line was maintained in supplemented RPMI containing glucose (2000 mg L^{-1}), supplemented with fetal bovine serum (10%, FBS Gold), penicillin–streptomycin (1%), HEPES (10 mM) and sodium pyruvate (1 mM, Thermo Fisher Scientific, MA, U.S.A.).

2.3 L-929 Conditioned Media

The L-929 fibroblast cell line (ATCC, VA, U.S.A) was cultured in DMEM containing high glucose (4500 mg L^{-1}) supplemented with 10% FBS, 1% Non-essential amino acids (NEAA, Thermo Fisher Scientific, MA, U.S.A.), 1% penicillin–streptomycin during 4 days upon the resulting media was harvested, sterile filtered (0.2 µM) and stored at -80°C until use.

2.4 Isolation of Bone Marrow Derived Stem Cells, Macrophage Differentiation and Activation

Femur and tibia bones were dissected and rinsed in ethanol. The ends of the bones were then cut off using a bone scissor. The bone marrow was flushed out with RPMI using a syringe and needle (26G). Cells of the bone marrow were further dissociated using a wider gauged needle (18G) and then pelleted (1000 rpm, 4 minutes at room temperature). Red blood cells were then depleted by adding lysis buffer (155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA, pH 7.2, for 5 minutes at room temperature), thereafter the reaction was impeded by adding cell culture media, and bone marrow derived cells pelleted (1000 rpm, 4 minutes at room temperature). The cells were resuspended in DMEM growth media containing low glucose (1000 mg L^{-1}) supplemented with 30% L-929 conditioned media, 1% NEAA and allowed to differentiate for 6 days. At day 6, the cells were re-seeded into appropriate experimental cell culture plates and the differentiation continued for another two days upon the macrophages were activated: M1-type (5 ng/mL LPS and 12 ng/mL interferon (IFN) -γ, Thermo Fisher Scientific, MA, U.S.A.) and M2-type [10 ng/mL interleukin (IL)-4, Thermo Fisher Scientific] polarization was induced during respectively, 24 and 48 h.

2.5 Adipose Tissue *Ex Vivo* Culture to Measure C1QTNF3 Secretion

Whole adipose tissue was collected in PBS (5 mM glucose) at room temperature. Tissues were manually severed with scissors in petri dishes (5-10 mg fragments). Thereafter, samples were filtered (100 µm mesh) and washed with PBS to remove cellular debris and blood. Samples were then incubated in 12-well plate pre-filled with RPMI (1 mL, serum-free, 2.5 nM dexamethasone, HEPES 10 mM, 0.5 nM insulin, sodium pyruvate 1 mM) for 6 hours. Protease inhibitors were added during the incubation time to minimize protein degradation (Complete™, Mini, EDTA-free Protease Inhibitor Cocktail, Sigma-Aldrich, MO, U.S.A). The tissues were snap frozen in liquid nitrogen while the supernatants were transferred to Eppendorf tubes and centrifuged (1000 rpm, 4 minutes) to pellet cellular debris upon the pure samples were snap-frozen. All samples were stored at -80°C until analysis.

2.6 C1QTNF3 Recombinant Protein

Recombinant human embryonic kidney 293 cells-produced mouse complement C1q tumor necrosis factor-related protein (C1QTNF3) dissolved in sterile Tris-HCL (10 mM), EDTA (1 mM), glycerol (10%) at pH 8 (MyBioSource, CA, U.S.A.) was used in all C1QTNF3 treatment experiments (controls received only this buffer).

2.7 C1QTNF3 Antibody Treatment *In Vivo*

Obese mice were given goat anti-mCTRP3/C1QTNF3/CORS26 (AF2436, Research and Diagnostic Systems, Inc. MN, U.S.A.) intraperitoneally (0.5 mg/kg) by injection every second day for 14 days after tumor transplantation. An antibody of the same isotype (polyclonal goat IgG) served as control.

2.8 C1QTNF3 Measurement in Supernatant and Serum

C1QTNF3 levels in supernatant from adipose tissue culture and in serum were determined by a commercial ELISA according to the manufacturer's protocol (CUSABIO, Houston, TX, U.S.A.).

2.9 Mitochondrial Respiration

2.9.1 E0771 Cells, and 3T3-L1 Pre- and Adipocytes

Seahorse cell culture microplates (Agilent Technologies, CA, U.S.A.) were seeded with either E0771 cells (at 40 000/well) or 3T3-L1 cells (at 10 000/well, and adipogenesis was induced as described above). Upon reaching maturity, the cells were treated with C1QTNF3 (1µg/ml) for 24 h, upon the mitochondrial function was assayed with a Cell Mito Stress Test in a Seahorse XFe96 Analyser (Agilent Technologies). In brief, the test utilizes inhibitors of the mitochondrial oxidative phosphorylation: ATPase inhibitor (1 µM oligomycin), proton uncoupler (0.5 µM FCCP) and complex I and III inhibitor (0.5 µM rotenone and antimycin A), are added in the respective order. The obtained oxygen consumption rate (OCR) values in response to these different inhibitors were used to calculate basal, maximal, non-mitochondrial, ATP production- and proton leak-linked respiration, as well as spare respiratory capacity. All Seahorse experiments have been repeated three times and the average in each experiment is based on 5-10 replicates per group.

2.9.2 Bone Marrow Derived Macrophages

Seahorse cell culture microplates were seeded with bone marrow derived macrophages (BMDM) at day 6 of maturation (at 80 000/well). The Cell Mito Stress Test was conducted similarly as described for above for 3T3-L1 cells except for compound concentrations (1 µM oligomycin, 2 µM FCCP, 0.5 µM rotenone and 0.5 µM antimycin A).

2.10 Glycolytic Function

The glycolytic function, as judged by extracellular acidification rate (ECAR), was measured in cultured BMDM by the Glycolytic Stress Test (Agilent Technologies) in a Seahorse XFe96 Analyser (Agilent Technologies, CA, U.S.A.). In brief, this test utilizes the addition of glucose (10 mM), oligomycin (1 μM) and a competitive inhibitor of glucose (50 mM 2-Deoxy-D-glucose), in the respective order. The obtained ECAR values were used to calculate glycolysis, glycolytic capacity, glycolytic reserve, and non-glycolytic acidification.

2.11 RNA-Isolation, cDNA-Synthesis, and Quantitative Real-Time PCR

RNA from tissue and cell lysates were isolated and purified using ReliaPrep RNA Cell MiniPrep System (Promega Corporation, WI, U.S.A) according to manufacturer's protocol, except for processing adipose tissue, which requires removal of lipids prior the chloroform extraction. The concentration of the total RNA was determined by a NanoDrop (Thermo Fisher Scientific, MA, U.S.A.), and cDNA was generated through reverse transcription (500-1000 ng RNA) using a priming mixed strategy, according to manufacturer's protocol (qScript Flex cDNA Synthesis Kit, Quanta Biosciences, MA, U.S.A). The gene expression was quantified through quantitative real-time PCR using SYBR-green (Fast SYBR® Green Master Mix, Applied Biosystems, CA, U.S.A) and the relative $\Delta\Delta C_q$ method with either *Actb* or *Tbp* as endogenous control. The stability of the reference genes was determined from an array of housekeeping genes using NormFinder. Primers (**Supplementary Table 1**) were used at a concentration of 0.5 μM.

2.12 RNA-Sequencing and Analysis

RNA was isolated from adipose tissue (located as described in **Supplementary Figure 1**) and provided to the Bioinformatics Core Facility at the Sahlgrenska Academy, University of Gothenburg. The samples were enriched for RNA transcripts by depleting the ribosomal RNA (RiboMinus Technology, Thermo Fisher Scientific). The concentrated samples were then submitted to cDNA synthesis upon the adenylated 3' ends were allowed to ligate with adapters, amplified and clustered on a chip (bridged PCR amplification). The clusters were then extended with single fluorescent labeled nucleotides and sequenced by imaging (reversible terminator sequencing, Illumina, CA, U.S.A.).

Quality assessment of the sequence reads was performed by generating QC statistics with FastQC (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc). RNA-sequencing reads were filtered and trimmed with prinseq (0.20.3). Mapping towards the mouse genome (mm10 from UCSC) was performed with the STAR aligner (2.4.0f) with default parameters (36). Raw gene counts were generated with HTSeq (https://htseq.readthedocs.io/en/release_0.10.0/). DESeq2 was then used to find differentially expressed genes (37). Unsupervised clustering of gene expression profiles was calculated using k-means on genes that were expressed significantly different between chow-fed control adipose tissue and chow-fed tumor associated adipose tissue. RNA-sequencing data have been deposited in NCBI's Gene Expression Omnibus (38) and are accessible through GEO Series accession number GSE201316.

2.13 Primary Adipocyte and Stroma-Vascular Cell Isolation

Primary adipocytes and stroma-vascular cells (SVF) were isolated as in (39). Briefly, after collection, adipose tissue was minced and digested with 1 mg/ml collagenase type 2 (Sigma Aldrich, MO, U.S.A) in a buffer containing 123 mM NaCl, 5 mM KCl, 5 mM CaCl2 1.2 mM KH2PO4, 1.2 mM MgSO4, 25 mM HEPES, 2 mM glucose, 200 nM adenosine, 1.5% BSA. After digestion (30 min at 37°C), cells were filtered through 100 μm mesh and centrifuged (5min, 500g). Primary adipocytes were collected (floating fraction) and SVF (pelleted fraction) was further processed for red blood cell removal. Red blood cells were removed with RBC lysis buffer (Biolegend, CA, USA) treatment for 2 min at RT. After final wash, cells were resuspended in DMEM 10% FBS, counted on automatic cell counter (Countess 2, Thermo Fisher Scientific, MA, U.S.A.) and stained for flow cytometry analysis.

2.14 Magnetic Activated Cell Sorting

After IWAT and GWAT SVF isolation, cells were further separated into different subsets with magnetic activated cell sorting on OctoMACS™ separator (Miltenyi Biotec, Germany) per manufacturer's instructions. Briefly, cells were incubated with anti-F4/80 MicroBeads UltraPure (Miltenyi Biotec, Germany) for 15 min in dark and on ice. After two washes in MS buffer (PBS, 0.5% BSA, 2mM EDTA), cells were loaded onto pre-rinsed MS columns (Miltenyi Biotec, Germany) placed in the magnetic field. Columns were rinsed two more times with MS buffer, and entire flow-through containing unlabeled cells was collected. Then, column was removed from magnetic separator and placed on collection tube. After adding MS buffer, magnetically labelled cells were flushed out with plunger. For fibroblast separation, F4/80$^-$ cells were first depleted from leukocytes with anti-CD45 MicroBeads (Miltenyi Biotec, Germany). Negative fraction was subsequently incubated with anti-CD90.2 MicroBeads (Miltenyi Biotec, Germany). Cells were resuspended in Qiazol (Qiagen, Germany) for RNA isolation. Purity of all sorted cells (macrophages, F4/80$^+$), fibroblasts (F4/80$^-$CD45$^-$CD90.2$^+$) and all negative cells was confirmed with quantitative real-time PCR.

2.15 Flow Cytometry

Adipose tissue SVF cells were isolated as described above and cell suspensions from spleen and E0771 tumors were prepared by pushing tissue pieces though nylon mesh using a syringe plunger. Cells were resuspended in Fluorescence-Activated Cell Sorting (FACS) buffer and transferred to a round bottom 96-well plate. Samples were blocked with FC-block (4°C, 30 min, darkness) prior to staining with antibody panel (**Supplementary Table 2**). Samples were pelleted (1000 rpm, 3 min) and resuspended in FACS buffer to remove excess antibodies. Thereafter, samples were fixated and permeabilized prior to staining with intracellular

antibodies and then resuspended in FACS buffer to remove additional excess antibodies before analysis (FACSAria and FacsCanto II; BD Biosciences, Franklin Lakes, NJ, USA). Adipose tissue macrophages ($F4/80^+CD11b^+$) were subgrouped by their CD206 and NOS2 expression (**Supplementary Figure 2**). For *in vitro* studies, C1QTNF3 (1 μg/ml) was added to BMDM during polarization stimulus. Supernatants were collected for nitric oxide measurements and kept on -80°C until analysis. Cells were collected and stained with LIVE/DEAD™ Fixable Blue Dead Cell Stain Kit (Thermo Fisher Scientific, MA, U.S.A.) followed by staining with surface and intracellular antibodies as above. For proliferation studies, differentiated BMDM (M0, M1 and M2) were treated with C1QTNF3 (1 μg/ml) for 24h. EdU (5-ethynyl-2'-deoxyuridine, A1004, Invitrogen) in concentration of 10 μM was added for the last 3h of culture. Cells were collected, stained for surface antibodies, EdU with EdU-click it assay (Thermo Fisher Scientific, MA, U.S.A.), intracellular antibodies and analyzed with flow cytometry. Gates were set with Fluorescent Minus One controls. Flow cytometry data was analyzed using FlowJo software version 10.6.0 (FlowJo, LLC, OR, U.S.A.).

2.16 Migration Assay

M0, M1 and M2-polarized BMDMs were seeded (15000/well) on transwell 96-well permeable supports with 8 μm pore size (Corning) in serum-free media (DMEM 0.5% BSA). C1QTNF3 (1μg/ml), MCP1 (positive control, 100 ng/ml) or serum-free media (negative control) were added in the bottom part of the 96-well plate. After overnight incubation, inserts were washed twice with PBS, and the cells on the inside of the insert were gently removed with moistened cotton swabs. Cells on the lower surface of the membrane were stained with crystal violet solution (Sigma Aldrich) for 15 min. Excess dye was removed with extensive washing with dH_2O. After drying, bounded crystal violet was eluted with 33% acetic acid and the absorbance was measured at 590 nm on Spectramax i3x multiplate reader (Molecular Devices, CA, U.S.A.).

2.17 Western Blot

Cells were homogenized in lysis buffer (RIPA containing 150 mM sodium chloride, 1.0% Triton X-100, 0.5% sodium deoxycholate, 0.1% sodium dodecyl sulfate, 50 mM Tris, pH 8.0) and incubated on ice for 1 hour. Thereafter, the samples were centrifuged (12 000 rpm, 20 min, 4°C). The supernatants were collected, and the protein concentration was determined by the Pierce BCA Protein Assay (Thermo Fisher Scientific, MA, U.S.A.). The samples were then further processed by adding reducing agent (4x Laemmli Sample Buffer with β-mercaptoethanol, Bio-Rad Laboratories, CA, U.S.A.) and boiled to destruct the disulfide bonds (95°C, 5 min). Equal amounts of samples were separated on precast gels (Criterion TGX stain-free precast gel, Bio-Rad Laboratories, 100 V, 2 hours). The separated proteins were visualized using the stain free system and transferred to polyvinylidene difluoride (PVDF) membranes (Trans-Blot turbo Mini/Midi PVDF transfer pack, Bio-Rad Laboratories, CA, U.S.A.). To measure the activity of MEK/ERK1/2 and AKT signaling pathways the PVDF membrane was first blocked (TBS-Tween, BSA 5%, 30 min, room temperature). The primary antibodies (**Supplementary Table 3**) were added and allowed to incubate overnight at 4°C. Thereafter, a secondary antibody was added (room temperature, 60 min) upon the membranes were visualized using the ChemiDoc Imaging System (Bio-Rad Laboratories, CA, U.S.A.). The protein size was confirmed using a protein standard (Precision Plus Protein™ Dual Color Standards, Bio-Rad Laboratories, CA, U.S.A.). Background was subtracted prior data analysis, which was performed in Image Lab 6.0.1 (Bio-Rad Laboratories, CA, U.S.A.).

2.18 Nitric Oxide Measurements

Supernatants were transferred into Costar 96-well black plate with clear bottom (MERCK, Germany) in duplicates. 4,5-Diaminofluorescein (Sigma-Aldrich, MO, U.S.A.) was added in the final concentration of 10 μM. Fluorescence was measured on Spectramax i3x multiplate reader (Molecular Devices, CA, U.S.A.) with excitation wavelength of 495nm and emission wavelength of 525nm. Values were corrected with control sample (pure media).

2.19 Statistical Analysis

Student's t-test was used for comparisons between two groups, and 1-way and 2-way ANOVA were used for comparisons between several groups and repeated measurements. Dunnett multiple comparison test for one-way ANOVA and Šidák correction for two-way ANOVA were used when multiple comparisons were done within the same analysis. The square of the Pearson's correlation coefficient was used to measure the linear relation between variables. A p-value of <0.05 was considered significant and data are presented as mean ± SEM.

3 RESULTS

3.1 Inguinal/Mammary Adipose Tissue C1QTNF3 Levels Increase in Response to Breast Cancer

Female mice, fed either regular chow or high fat diet (HFD) for 16 weeks, were injected with E0771 cancer cells resuspended in PBS mixed with Matrigel into their left inguinal/mammary fat pad (tumor-associated IWAT) while their right inguinal fat pad (control IWAT) received PBS-Matrigel solution alone. Tumors, tumor-associated and control IWAT were collected two weeks after cancer cell injections (**Supplementary Figure 1**). As expected, the HFD-fed mice developed obesity and exhibited almost three times larger tumors than the chow-fed lean controls (**Figures 1A, B**). This difference in E0771 tumor progression between lean and obese mice agrees with previous studies (18, 40). The global gene expression profiles of cancer-associated and control IWAT were compared using RNA-sequencing. To identify similarly regulated genes, we performed unsupervised cluster analysis of genes that were significantly different between cancer-associated and control IWAT in chow-fed lean mice. This analysis resulted in the identification of five clusters where genes within each cluster were co-regulated across the four groups (**Figure 1C** and **Supplementary Table 4**). Thereafter, we chose to take a closer look on the cluster where the genes were upregulated by tumor presence and where this regulation was

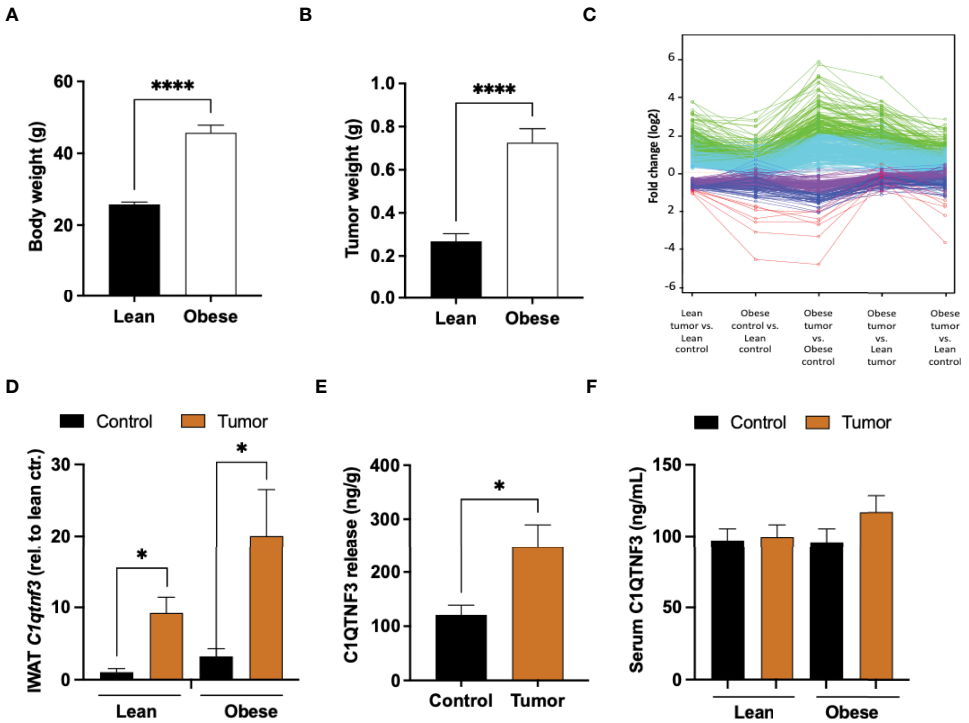

FIGURE 1 | Increased *C1qtnf3* levels in tumor-associated adipose tissue. **(A)** Body weight and **(B)** E0771 tumor weight of lean and HFD-induced obese C57BL/6 female mice (N = 18-20/group). **(C)** Unsupervised cluster analysis of genes (detected by RNA-sequencing) that were significantly different between cancer-associated and control (sham) adipose tissue in chow-fed lean mice: Green cluster includes genes that were most upregulated in tumor-associated adipose tissue and where this regulation was enhanced by obesity (**Table 1**). **(D)** *C1qtnf3* mRNA levels in control (sham) and tumor-associated lean and obese inguinal white adipose tissue (IWAT) as judged by qPCR (N = 4-6/group), **(E)** C1QTNF3 protein release from control and tumor-associated obese female IWAT (N = 9/group), and **(F)** serum C1QTNF3 levels in lean and obese female mice with or with E0771 breast tumor (N = 5-10/group). *p < 0.05, ****p < 0.0001 for the indicated comparisons.

enhanced by obesity/HFD (green-colored cluster in **Figure 1C**). Within this cluster, we found several genes that are known to be involved in tumor progression e.g., the acute phase reactant *Saa3*, several chemokines (*Ccl2, Ccl7, Ccl8, Ccl12*) as well as several macrophage markers such as *Emr1* (*F4/80*), *Ccr5*, *Itgam* (*Cd11b*) and *CD68*. One of the most regulated genes (as judged by fold change between control and tumor-associated adipose tissue) within this cluster was however *C1qtnf3* (**Table 1** and **Figure 1D**), an adipokine that to our knowledge has not previously been studied in the context of breast cancer progression. Importantly, the increased *C1qtnf3* expression in tumor-associated IWAT was associated with increased release of C1QTNF3 protein (**Figure 1E**). The circulating levels of the C1QTNF3 were however unaffected by both diet-induced obesity and experimental breast cancer (**Figure 1F).**

3.2 *C1qtnf3* Is Primarily Expressed in the Stromal Vascular Fraction of Adipose Tissue and Displays an Adipose Depot-Specific Response to HFD-Induced Obesity

To further characterize the regulation of *C1qtnf3*, we quantified *C1qtnf3* levels in different fat depots and cell types. In unchallenged male mice, *C1qtnf3* mRNA levels were higher in gonadal (GWAT) and mesenteric white adipose tissue (MWAT) than in IWAT, and *C1qtnf3* was much more abundant in the stromal vascular fraction (SVF) than in the adipocyte fraction (**Figure 2A**). In line with a previous study (41), cultured 3T3-L1 preadipocytes (fibroblasts) displayed relatively high expression of *C1qtnf3* and this expression was substantially reduced in mature 3T3-L1 adipocytes (**Figure 2B**). The *C1qtnf3* expression was even lower in peritoneal macrophages than in 3T3-L1 adipocytes and undetectable in E0771 breast cancer cells (**Figure 2B**). To further clarify the *C1qtnf3* expression pattern, we analyzed the expression in sorted adipose tissue resident macrophages ($F4/80^+$cells) and fibroblasts ($F4/80^-CD45^-CD90.2^+$ cells) from IWAT and GWAT. This confirmed relatively low expression in macrophages compared to fibroblasts, but there was also relatively high expression in cells negative for all markers (**Figures 2C, D** and **Supplementary Figures 3A, B**). Moreover, the IWAT SVF expression of *C1qtnf3* was increased in HFD-induced obese female and male mice (**Figure 2E** and **Supplementary Figure 3C**), while the adipocyte *C1qtnf3* expression remained low and unaltered by HFD/obesity. To further elucidate the regulation of adipose tissue *C1qtnf3*, we analyzed the *C1qtnf3* levels in IWAT, MWAT and GWAT after 8- and 16-week-chow versus HFD feeding in male mice. After 8 weeks of HFD-feeding, the *C1qtnf3* expression was increased in IWAT, GWAT and MWAT and the expression remained elevated in IWAT and MWAT also after 16 weeks of HFD-feeding.

TABLE 1 | Green cluster: Genes most upregulated in tumor-associated compared to control inguinal/mammary adipose tissue. Red numbers indicate significant log2-fold changes.

ADIPOSE TISSUE GENE ID	LEAN Tumor vs. Control	OBESE Tumor vs. Control	CONTROL Obese vs.Lean	TUMOR Obese vs. Lean
Slfn4	3.75	5.02	0.67	1.94
Ccl7	3.72	3.09	1.93	1.30
Has1	3.31	3.76	1.34	1.79
Ccl8	3.17	2.79	0.08	-0.31
C1qtnf3	3.15	3.51	1.22	1.57
Ly6c2	3.12	2.22	1.65	0.75
Chi3l3	3.07	3.08	2.79	2.79
Oas3	3.05	3.23	0.53	0.70
Ms4a4c	2.81	1.93	2.43	1.55
Ccl2	2.80	3.14	1.98	2.32
Saa3	2.79	2.07	2.72	2.00
Clec4d	2.76	2.57	2.46	2.27
Ccl12	2.72	3.29	0.17	0.73
Cilp	2.71	1.90	1.64	0.83
Clec4n	2.71	1.89	1.17	0.36
Oas1g	2.57	2.80	0.19	0.43
Slfn1	2.53	1.99	0.99	0.46
Fcgr1	2.47	3.32	0.26	1.10
Bst1	2.36	1.98	1.72	1.33
Nxpe5	2.34	2.77	0.17	0.60

In contrast, 16-week HFD-feeding was associated with reduced GWAT *C1qtnf3* expression (**Figure 2F**). As expected from the cluster analysis (**Figure 1C**, **Table 1**), the expression of *C1qtnf3* and the pan-macrophage marker *Emr1* were positively correlated in IWAT (R^2 = 0.53, p<0.0001) and MWAT (R^2 = 0.58, p<0.0001). However, no such correlation was found in GWAT (R^2 = 0.02, p=0.54). There was no impact of 8- or 16-week-HFD-feeding on circulating C1QTNF3 levels (**Supplementary Figure 3D**).

3.3 Antibody-Mediated Blockage of C1QTNF3 Reduces the Infiltration of Macrophages Into Tumor-Associated Adipose Tissue

To study the potential role of C1QTNF3 in tumor immunity and/or progression, we first characterized the macrophage infiltration in E0771 tumors, tumor-associated and control IWAT and spleen of untreated lean and HFD-induced obese female mice (body weight: 23.9±0.5 vs. 36.0±2.1 g, p<0.0001). The macrophage infiltration (% $CD11b^+F4/80^+$ of viable cells) was similar between tumors from lean and obese mice, but obesity was associated with an increased proportion of mixed M1-M2-type (% $CD206^+NOS2^+$ of $CD11b^+F4/80^+$ cells, **Supplementary Figures 4A, B**). However, neither M2-type-($CD206^+NOS2^-$) nor M1-type-($CD206^+NOS2^-$) tumor associated macrophages were altered by obesity (**Supplementary Figure 4B**). In line with the global gene expression data (**Supplementary Table 4**), there was an increased accumulation of macrophages in tumor-associated IWAT compared to control IWAT in obese mice, but this increase was not seen in chow-fed lean mice (**Figure 3A**). It is possible that we fail to detect tumor-induced IWAT macrophage accumulation in the lean setting as the flow cytometry analysis includes also adipose tissue that is more distant to the tumor (**Supplementary Figure 1**). 2-way ANOVAs show that HFD/obesity had no effect on IWAT macrophage abundance or polarization (**Figures 3A, B**), while tumor presence reduced the proportion of M2-type macrophages [F_{tumor} (1, 6)=11.8, p=0.01] and increased the proportion of mixed M1-M2-type macrophages [F_{tumor} (1, 6)=16.5, p=0.007] (**Figure 3B**). In addition, HFD/obesity increased the percentage of total splenic macrophages, but tumor presence had no effect on neither the macrophage abundance nor the subset distribution (**Supplementary Figures 4C, D**). Led by these data, we chose to study the role of C1QTNF3 on macrophage accumulation and tumor growth in the HFD-induced obese setting. To this aim, we treated HFD-induced obese E0771 tumor-bearing female mice with either C1QTNF3 or isotype control antibody. The mice weighed similar prior to the implantation of tumor and antibody treatment (control IgG: 44.4±0.7 g; C1QTNF3 IgG: 44.7±0.8 g) and at termination (control IgG: 42.7±0.8 g; C1QTNF3 IgG: 44.7±0.9 g). We found that mice treated with isotype control antibody displayed the expected increase in total macrophages (as % $CD11b^+F4/80^+$ of viable cells) in tumor-associated IWAT compared to control IWAT, but this increase was absent in C1QTNF3 IgG treated mice (**Figure 3C**). According to 2-way ANOVAs, tumor presence reduced the proportion of M2-type macrophages [F_{tumor} (1, 18)=123.4, p<0.0001], increased the proportion of both mixed M1-M2-type [F_{tumor} (1, 18)=40.8, p<0.0001] and M1-type macrophages [F_{tumor} (1, 18)=16.0, p=0.0008] (**Figure 3D**). However, C1QTNF3 IgG treatment had no effect on IWAT macrophage polarization (**Figure 3D**), and we saw no difference between treatment groups in total, M1- or M2-type macrophages in tumor and spleen (data not shown). The overall higher macrophage infiltration in IWAT in this experiment compared to the HFD-fed group in **Figure 3A** may be due to the difference in weight gain between these experiments. Moreover, glucose levels changed similarly between groups after tumor implant and antibody treatment (Δglucose: -0.52±0.7 vs. -0.13±0.6 mM, p=0.7) supporting the notion that C1QTNF3, under this experimental condition, acts locally rather than acting as a classical hormone.

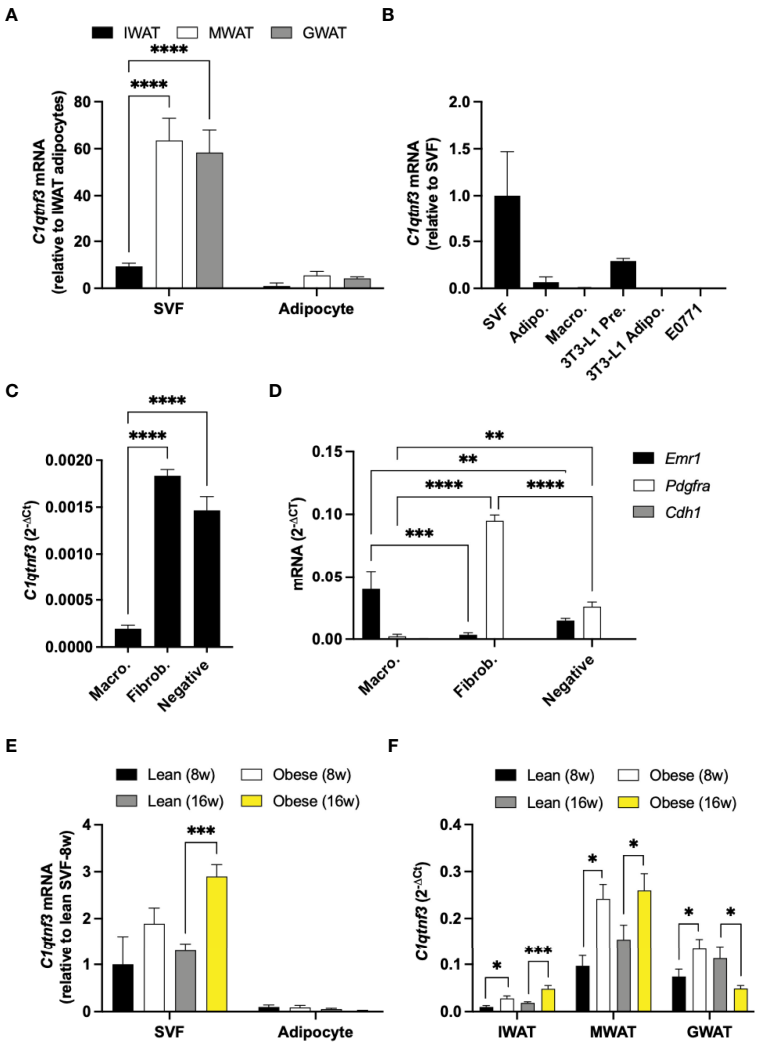

FIGURE 2 | *C1qtnf3* is primarily expressed in the stromal vascular fraction of adipose tissue and displays an adipose depot-specific response to HFD-feeding. **(A)** *C1qtnf3* expression in the stromal vascular (SVF) and adipocyte fraction from inguinal (IWAT), mesenteric (MWAT) and gonadal white adipose tissue (GWAT) from unchallenged male mice on chow (N = 4/group, 2-way ANOVA: $F_{cell\ type}$ (1, 18) = 77.11, $p < 0.0001$). **(B)** *C1qtnf3* expression in IWAT SVF, primary IWAT adipocytes, peritoneal macrophages, 3T3-L1 preadipocytes, 3T3-L1 adipocytes and E0771 breast cancer cells (N = 4-6/group). **(C)** *C1qtnf3* expression in IWAT macrophages ($F4/80^+$), fibroblasts ($F4/80^-CD45^-CD90.2^+$) and in remaining $F4/80^-CD45^-CD90.2^-$ SVF cells, and **(D)** analysis of cell population purity by qPCR. **(E)** Female IWAT SVF and adipocytes in response to 8- and 16-week (w) high fat diet (HFD) feeding (N = 10/group, 2-way ANOVA: $F_{cell\ type}$ (1, 54) = 51.99, $p < 0.0001$). **(F)** IWAT, MWAT and GWAT *C1qtnf3* expression in response to 8- and 16-week (w) HFD feeding in male mice (N = 6-10/group). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$ for the indicated comparisons.

Finally, there was no difference in the average tumor weight between groups (**Supplementary Figure 4E**) and we detected no lung metastasis in any of the animals.

3.4 C1QTNF3 Increases Macrophage Chemotaxis and Alters Macrophage Proliferation in a Subtype-Dependent Manner

To elucidate the mechanism for the inhibitory action of C1QTNF3 neutralization on tumor-induced IWAT macrophage accumulation, we analyzed the effect of C1QTNF3 treatment on chemotaxis and proliferation in bone marrow derived macrophages. We found that C1QTNF3 treatment stimulated chemotaxis of both M1- and M2-type macrophages and a similar trend was seen also for naïve (M0) macrophages (**Figure 3E**). The effect of C1QTNF3 on proliferation depended on polarization; C1QTNF3 treatment for 24h inhibited the proliferation in M0, whilst not affecting the proliferation of M1- and M2-type macrophages (**Figure 3F**).

3.5 C1QTNF3 Enhances M1-Like Polarization in Cultured Macrophages

Based on the subtype-dependent effect of C1QTNF3 on macrophage proliferation, we hypothesized that C1QTNF3 also regulates other functional aspects in a subtype-dependent

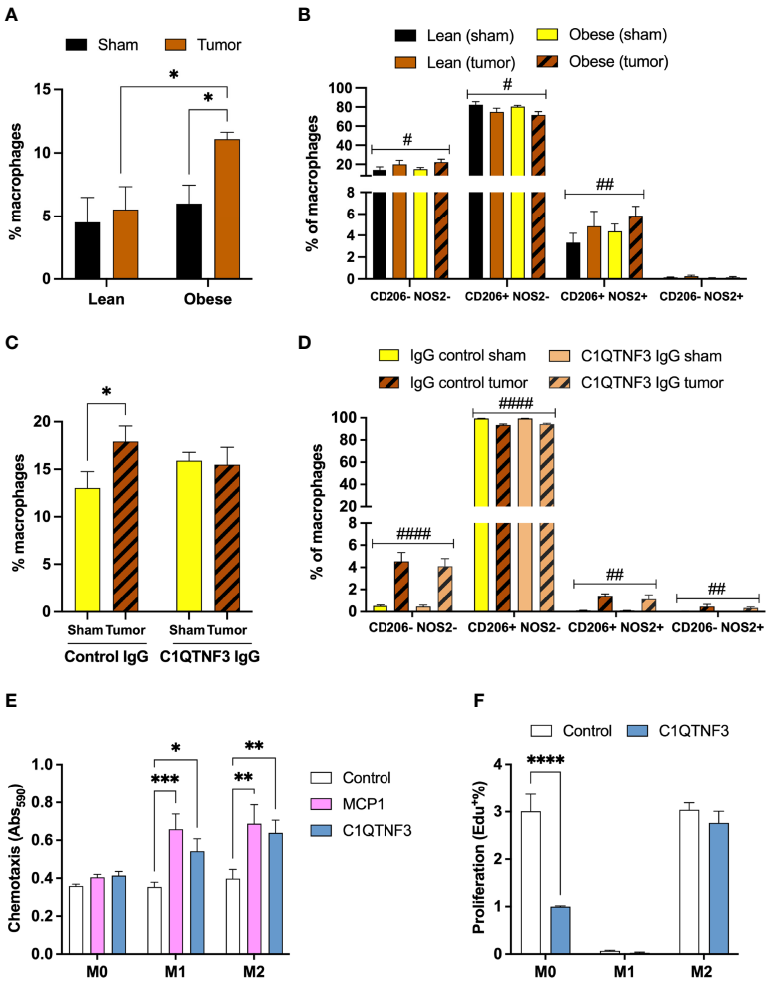

FIGURE 3 | C1QTNF3 neutralization inhibits tumor-induced macrophage infiltration in adjacent inguinal adipose tissue in obese mice. **(A)** Total macrophages (% F4/80$^+$CD11b$^+$ of viable cells) and **(B)** % CD206$^-$NOS2$^-$, % CD206$^+$NOS2$^-$ (M2-type), % CD206$^+$NOS2$^+$ (mixed M1-M2-type), % CD206$^-$NOS2$^+$ (M1-type) of total macrophages in control and tumor-associated IWAT from lean and HFD-induced obese female mice (N = 4/group, each IWAT sample was pooled from 2-3 mice, $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ for the effect of tumor in 2-way ANOVA) **(C)** Total macrophages in control and tumor-associated IWAT from isotype control and C1QTNF3-IgG treated HFD-induced obese female mice (N = 5-6/group, 2-way ANOVA: $F_{treatment \times tumor}$ (1, 9) = 9.222, $p < 0.05$). **(D)** % CD206$^-$NOS$^-$, M2-type, mixed M1-M2-type, and % M1-type of total macrophages in control and tumor-associated IWAT (N = 5-6/group, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$ and $^{\#\#\#\#}p < 0.0001$ for the effect of tumor in 2-way ANOVA). **(E)** MCP1 (100 ng/ml) and C1QTNF3 (1 µg/ml)-induced chemotaxis (N = 5-10/group) and **(F)** proliferation, measured as % of EdU incorporation, with or without C1QTNF3 (1 µg/ml) of M0-, M1- and M2-polarized bone marrow derived macrophages (N = 3-4/group). $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ for the indicated comparisons.

manner. To test this hypothesis, we treated bone marrow derived M0-, M1- and M2-type macrophages with mammalian-produced C1QTNF3 and analyzed oxygen consumption rate (OCR). Non-polarized macrophages were treated with C1QTNF3 during the entire 48h-period of their M1- and M2-type induction (M0-macrophages received either vehicle or C1QTNF3 alone). We found a rather dramatic effect of C1QTNF3 treatment on mitochondrial function in M2-type macrophages; C1QTNF3-treated M2-type macrophages displayed a reduction in most measured respiration parameters (**Figure 4A**). Similar results, albeit to a lesser extent, were seen in M0 and M1-type macrophages (**Figure 4B** and **Supplementary Figure 5A**). The smaller effect on respiration in M1-type macrophages is expected since these cells rely mostly on glycolysis. These data indicate that C1QTNF3 induces a metabolic shift from oxidative phosphorylation towards increased reliance on glycolysis for ATP-production in M2-type macrophages. Indeed, C1QTNF3-treated M2-type macrophages displayed increased basal glycolysis as judged by extracellular acidification rate (ECAR) (**Figure 4C**). Moreover, the glycolytic capacity and reserve were increased in both M0- and M2-type macrophages indicating that C1QTNF3 induces a macrophage phenotype that can meet an increased demand for ATP production (e.g. during an acute immune response) and/or can sustain in a competitive tumor microenvironment where nutrients are scarce (**Figures 4C, D**). This metabolic shift was

echoed by a phenotype switch; C1QTNF3 treatment led to reduced % of $CD206^{+}NOS2^{-}$ cells in M0- and M2-polarized macrophages and an increased % of $CD206^{+}NOS2^{+}$ cells in M2-polarized macrophages (**Figures 5A, B**). Moreover, C1QTNF3-treated M2-type macrophages showed an increased production of nitric oxide (**Figure 5C**). An M1-like shift in polarization of M0 and M2-macrophages was also supported by an increased expression of M1-type polarization markers, such as Inducible nitric oxide synthase (*Nos2)* and Interleukin-1b (*Il1b*) (**Figure 5D**). Arginase-1 (*Arg1)*, an enzyme that typically is associated with M2-type macrophages, was also increased in C1QTNF3-treated M2-type macrophages (**Figure 5E**). While this may appear surprising, increased *Arg1* expression is seen in LPS-stimulated murine macrophages, possibly as a mean to attenuate NO-mediated inflammation (42, 43). Increased *Arg1* may thus in some contexts reflect increased pro-inflammatory signaling. In further support of the increased reliance of glycolysis in C1QTNF3-treated M2-type macrophages, the expression of several genes involved in glycolysis such as Aldolase A (*Aldoa*), Glucose transporter 1 (*Glut1*) and Hypoxia-inducible factor 1-alpha (*Hif1a*) was increased (**Figure 5F** and **Supplementary Figure 5B**).

Given the observed rather potent effect of C1QTNF3 on macrophage metabolism, we asked whether C1QTNF3 exerts

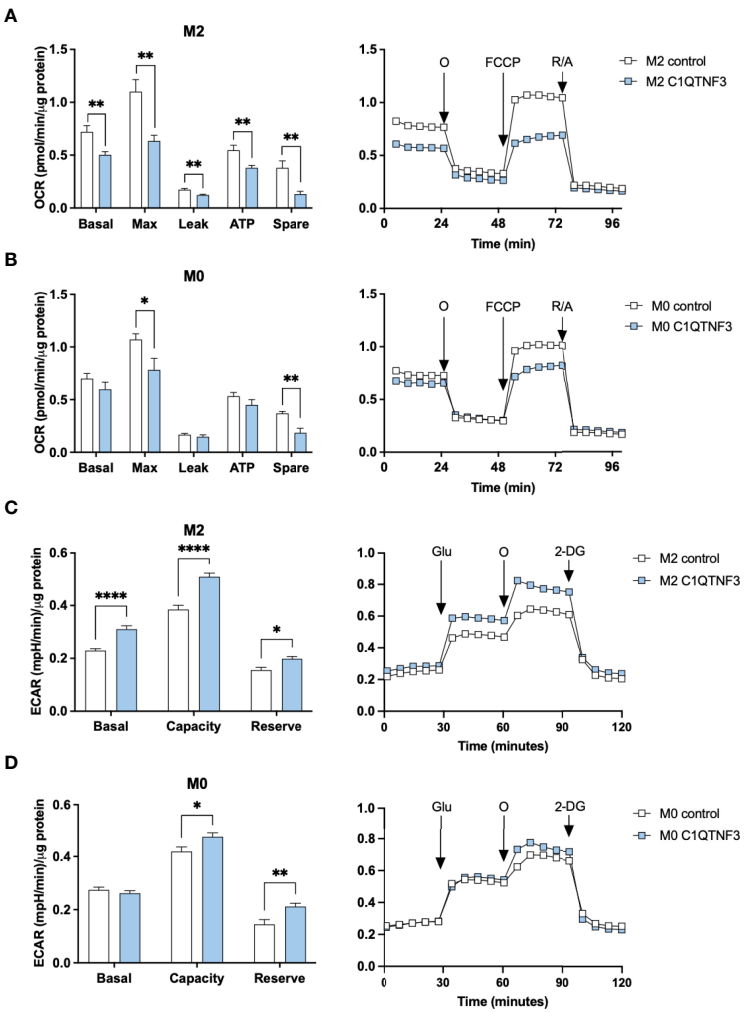

FIGURE 4 | C1QTNF3 reduces respiration and increases glycolysis in bone marrow derived macrophages. Mitochondrial function, as determined by changes in basal respiration (Basal), maximal respiration (Max), proton leak-related respiration (Leak), ATP production-linked respiration (ATP) and spare respiratory capacity (Spare), was estimated from the oxygen consumption rate (OCR) of cultured bone marrow derived **(A)** M2- (N = 13-15/group) and **(B)** M0-type (N = 6-8/group) macrophages in response to subsequent addition of oligomycin (O), FCCP, and rotenone (R)/antimycin A as indicated in the right panels. Data are presented as OCR normalized to total protein levels. **(C)** Glycolytic function, as determined by basal glycolysis rate (Basal), glycolytic capacity (Capacity), and glycolytic reserve (Reserve), was estimated from the extracellular acidification rate (ECAR) in cultured bone marrow derived **(C)** M2- and **(D)** M0-type macrophages in response to subsequent addition of glucose (Glu), oligomycin (O) and 2-Deoxy-D-glucose (2-DG). Data are presented as ECAR normalized to total protein levels (N = 6-12/group). Prior to these OCR and ECAR measurements, the macrophages were treated with or without C1QTNF3 (1 µg/ml) for 48 h along with control (M0) or IL-4 to induce M2-polarization. *p < 0.05, **p < 0.01, ****p < 0.0001 for the indicated comparisons.

acute effect on respiration or whether the observed effects on respiration rely on long-term treatment during the activation process. To answer this question, we treated M0- and M2-type macrophages with C1QTNF3 for only 1h and assessed the effect on respiration. Although long-term treatment was more effective in this regard, we found that 1h C1QTNF3 treatment reduced the maximal respiration and spare respiratory capacity in M2-type macrophages (**Figure 6A).**

The negative effect of C1QTNF3 on macrophage respiration was to us unexpected findings and prompted us to also test the effect of C1QTNF3 on mitochondrial function in cultured pre-adipocytes, adipocytes, and E0771 breast cancer cells. In sharp contrast to the observed effect in macrophages, C1QTNF3 slightly increased the ATP production-coupled respiration in pre-adipocytes while there was no effect of C1QTNF3 on respiration either in adipocytes or in E0771 breast cancer cells (**Supplementary Figures 5C-E**). These data suggest that C1QTNF3 treatment affects the mitochondrial function in a cell-specific manner.

Previous research shows that C1QTNF3 can activate the MEK/ERK1/2 and PI3K/Akt pathways (26, 44), which can affect a wide a range of processes in macrophages including their viability, development, immunological and metabolic function. We therefore tested whether these pathways are regulated by C1QTNF3 also in our experimental settings. Indeed, we found that 15 minutes C1QTNF3 treatment increased the ERK-activation of cultured M2-type macrophages about 2-fold more than vehicle control treatment (**Figure 6B**), while there was no effect of C1QTNF3 on Akt-activation at that time point (**Figure 6C**). However, 60-minute C1QTNF3 treatment led to increased Akt-activation (**Figure 6C**).

4 DISCUSSION

Here, we show that mRNA and protein levels of C1QTNF3 are upregulated in IWAT in response to breast cancer and that C1QTNF3-neutralizing antibody treatment inhibits the breast

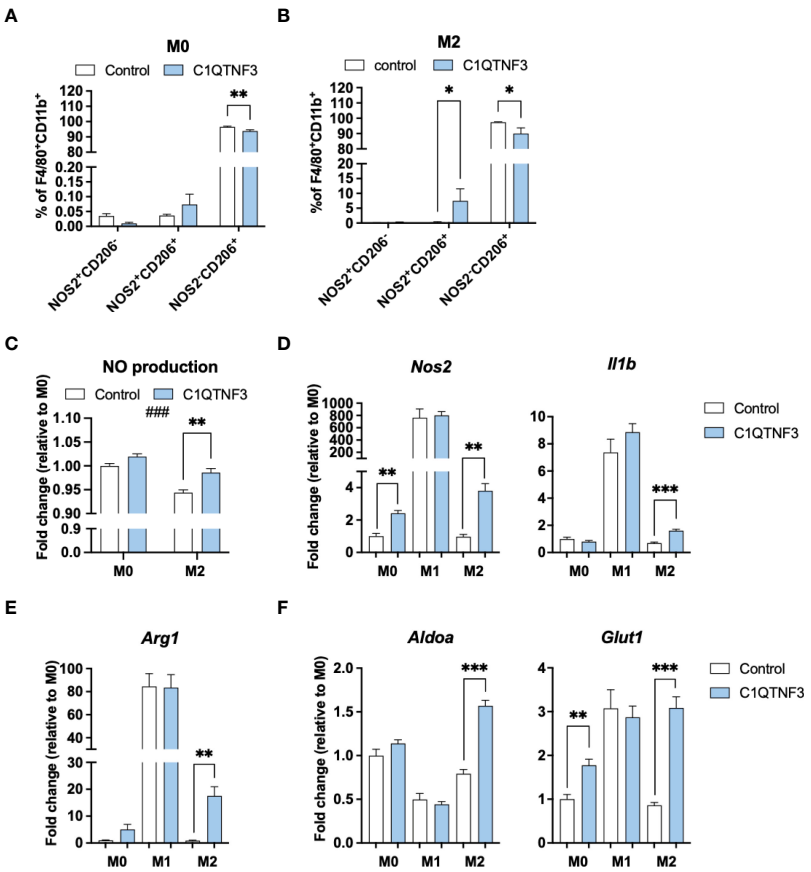

FIGURE 5 | C1QTNF3 enhances M1-polarization and the expression of glycolysis markers in M0- and M2-type bone marrow derived macrophages. **(A, B)** Flow cytometry analysis of NOS2 and CD206 and **(C)** nitric oxide (NO) production in cultured bone marrow derived M0-, and M2-polarized macrophages (N = 3-5/group, $^{###}p < 0.001$ for the effect of treatment on NO production in 2-way ANOVA). The expression of the **(D)** M1-markers *Nos2* and *IL1b*, the **(E)** M2 marker *Arg1*, **(F)** the glycolysis-related genes *Aldoa*, and *Glut1* in cultured bone marrow derived M0-, M1- and M2-type macrophages (N = 6/group). NO production and gene expression data are presented as fold change relative to the M0 control. The macrophages were treated with or without or C1QTNF3 (1 μg/ml) for 48 h along with control (M0), IL-4 to induce M2-polarization, or LPS & IFNγ to induce M1- polarization. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ for the indicated comparisons.

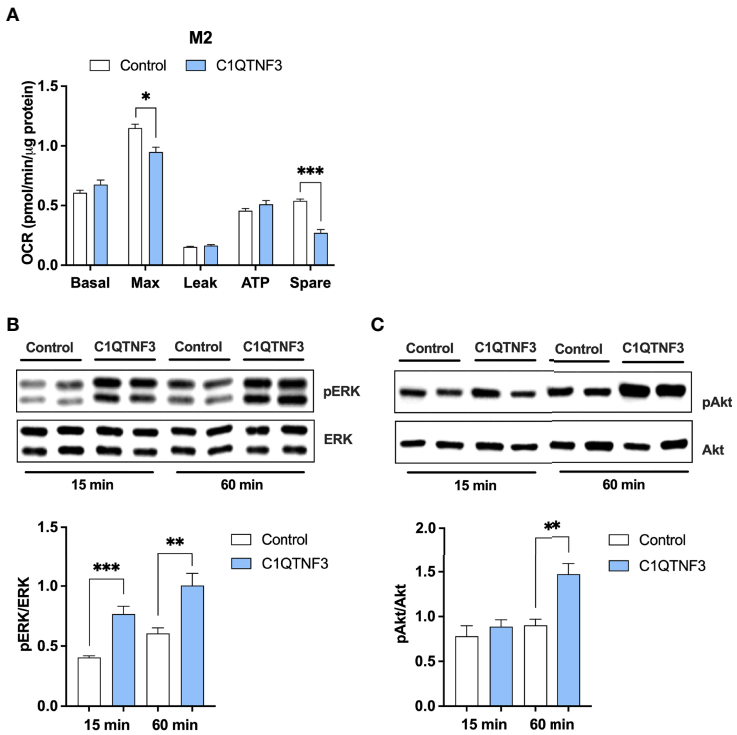

FIGURE 6 | Short-term C1QTNF3 treatment activates the ERK- and Akt-pathways associated with reduced maximal respiration and reduced spare respiratory capacity in M2-type bone marrow derived macrophages. M2-type bone marrow-derived macrophages were treated with C1QTNF3 (1 µg/ml) for 1 h prior the **(A)** mitochondrial function analysis (N = 6-8/group). Representative blots and quantification of **(B)** phosphorylated (p)-ERK and total ERK and **(C)** p-Akt and total Akt in M2-type bone marrow-derived macrophages in response to 15- and 60-minute C1QTNF3 or vehicle treatment (N = 5/group). ERK and Akt activation are presented as a ratio of phosphorylated over total levels (N = 5/group). *p < 0.05, **p < 0.01, ***p < 0.001 for the indicated comparisons.

cancer-induced macrophage recruitment to IWAT in HFD-induced obese mice. Based on recombinant C1QTNF3 treatment experiments *in vitro*, we demonstrate that C1QTNF3 stimulates macrophage chemotaxis, and has profound inhibitory effects on M2-type macrophage respiration associated with increased glycolysis and increased M1-like polarization.

4.1 Orthotopic E0771 Breast Cancer – A Mouse Model That Can Reveal New Factors Involved in Subcutaneous Adipose Tissue Remodeling

We found that many macrophage-related genes that were up-regulated in tumor-associated compared to control adipose tissue in lean mice showed an even stronger tumor-induced upregulation in obese adipose tissue indicating that they may be involved in the enhanced tumor progression in obesity. However, contrary to this idea, we did not detect major changes in the percentage or polarization of tumor-associated macrophages between lean and HFD-induced obese mice. Moreover, our recent work shows that interactions between tumor and adjacent adipose tissue stimulate tumor growth to a similar degree in lean and obese mice (18), suggesting that the increased E0771 tumor growth in the obese mice primarily is due to systemic metabolic and/or endocrine alteration. Thus, we interpret the enhanced macrophage accumulation along with many of the enhanced transcriptional changes in obese tumor-associated IWAT rather as a consequence from the faster growing tumors than the cause. While this was disappointing from a breast cancer perspective, the orthotopic E0771 breast cancer model led us to C1QTNF3, thus showing its potential for identifying factors that are involved in subcutaneous adipose tissue remodeling.

4.2 Source of Adipose Tissue C1QTNF3

C1QTNF3 is considered an adipokine as it is highly expressed in adipose tissue. In line with some previous studies (19, 41), we found however, relatively low *C1qtnf3* expression in mature (primary and cultured) adipocytes, while the SVF of adipose tissue and cultured pre-adipocytes displayed higher levels. Moreover, our data suggest that fibroblasts, but not macrophages, constitute a major source for adipose tissue C1QTNF3. In support of this, publicly available single cell transcriptomics data show that the highest *C1qtnf3* expression within adipose tissue SVF is found in the mesenchymal stem cell cluster while other cell types such as lymphocytes and myeloid and endothelial cells express very modest levels (45). Thus, the *C1qtnf3* expression that we detect in $CD45^-F4/80^-CD90.2^-$ cells may originate from mesenchymal cells expressing no or low levels of CD90.2.

4.3 Regulation of Adipose Tissue C1QTNF3 Levels

We found that the elevated *C1qtnf3* mRNA levels in tumor-associated IWAT was associated with a ~70% increase in released C1QTNF3 protein. However, serum levels of C1QTNF3 in tumor-bearing mice did not change, indicating that changes in local C1QTNF3 levels are more important than systemic in this context. Also, chronic HFD-feeding/obesity upregulated the IWAT *C1qtnf3* expression in both males and females, but to a lesser extent than in tumor-bearing mice. There was also no effect of chronic HFD-feeding on IWAT macrophage accumulation in females as judged by flow cytometry. In an additional HFD/obesity study in males, we found that *C1qtnf3* is regulated in an adipose depot-specific manner. 8- and 16-week HFD feeding were associated with upregulated *C1qtnf3* expression in both IWAT and MWAT. In contrast, 16-week HFD feeding led to reduced *C1qtnf3* levels in GWAT. To our knowledge, previous studies that have reported a downregulation of *C1qtnf3* in obese rodent adipose tissue have analyzed GWAT, and our data are thus in line with those observations (30–32). Long-term HFD feeding in male *C57Bl/6* mice is typically associated with increased adipocyte death and a dramatic increase in macrophage infiltration in GWAT (46) that is not seen in other fat depots. It is thus possible that the reduction in *C1qtnf3* in GWAT (but not in IWAT and MWAT) reflects this pathological tissue remodeling. A potent acute pro-inflammatory response within adipose tissue is essential for healthy adipose tissue expansion (47). Hence, the HFD-induced increase in IWAT *C1qtnf3* may play a role in this physiological acute pro-inflammatory response that must occur to effectively remodel the extracellular matrix and vasculature to accommodate the growing adipocytes in this depot. Overall, the immunological responses to HFD/obesity have been studied in detail in the GWAT in male mice. Much less is known about the immunological responses in other fat depots (and in females) – our data suggest that immune responses in subcutaneous adipose tissue may be much more dynamic than previously anticipated, and this warrants further investigations.

4.4 Metabolic and Immunological Effects of C1QTNF3

Pharmacological treatment with C1QTNF3, associated with a 3-fold elevation of circulating C1QTNF3 levels, lowers glucose levels in normal and insulin-resistant *ob/ob* mice (26). In this study, we were however unable to detect a significant effect C1QTNF3-neutralizing antibody treatment on glucose levels in HFD-fed obese breast cancer-bearing mice. We believe that this lack of glucose-regulatory effect of C1QTNF3 neutralizing antibodies is not surprising; firstly, breast cancer bearing mice did not display altered serum C1QTNF3 levels (this study) and mice lacking C1QTNF3 have unaltered glucose metabolism (48). Moreover, we found no effect on respiration in either cultured E0771 cancer cells or 3T3-L1 adipocytes although there was a small positive effect on respiration in cultured 3T3-L1 preadipocytes in line with a previous study (49). In contrast, we noticed a rather potent negative effect of C1QTNF3 on respiration in cultured bone marrow derived macrophages associated with increased M1-like polarization. The effector function of macrophages is linked to their metabolic function, where M1-type polarization parades with increased aerobic glycolysis, while M2-type polarization is associated with increased oxidative phosphorylation (10, 16). Our data is thus well in line with this concept; C1QTNF3 reduces the capacity for respiration in macrophages and thereby suppresses full polarization towards the oxidative M2-type phenotype (50, 51). The different response in different cell types may depend on the physiological context, whether the cells express a C1QTNF3 receptor or not, and what signaling pathways that dominates in respective cell type.

Previous research suggests that C1QTNF3 exerts anti-inflammatory effects (22, 23, 25, 28), and we were therefore initially surprised to find that antibody-mediated blockage of C1QTNF3 reduced the tumor-induced macrophage accumulation along with the chemotactic and pro-inflammatory effects of C1QTNF3 on bone marrow derived macrophages *in vitro*. On the other hand, our study shows that C1QTNF3 expression is regulated in a similar manner as many pro-inflammatory chemotactic factors in breast cancer-associated IWAT. Our results are also in line with a recent clinical study where adipose tissue *C1qtnf3* levels correlated positively with levels of *Tnfa*, *Il6* and *Ccl2* (52). Moreover, neither genetic overexpression nor knockout of C1QTNF3 alters the GWAT and the systemic inflammatory response to a sublethal dose of LPS *in vivo*, and mice overexpressing C1QTNF3 display *increased* levels of several chemokines, although this difference between genotype disappears when animals are challenged with HFD/obesity (53). In the obese state, C1QTNF3 overexpression is instead associated with reduced serum levels of IL-5 and TNF-α, while soluble gp130 levels increased (53). Thus, C1QTNF3 may both enhance and reduce pro-inflammatory responses dependent on the immunological setting. Nevertheless, our results may be particularly difficult to reconcile with a study by Lin and colleagues where wheat germ-produced C1QTNF3 dose-dependently inhibited TNF-α, IL-6, MCP1, MMP9 and IL-1β release in LPS-stimulated THP-1 macrophages and mouse peritoneal macrophages (24). In our hands, we saw only small effects of C1QTNF3 treatment on LPS/IFNγ-stimulated bone marrow derived macrophages, while a M1-like phenotype was induced in IL-4-stimulated bone marrow derived macrophages. One possible reason underlying this discrepancy may be that bone marrow derived macrophages display a different phenotype in the context of C1QTNF3 treatment than other macrophage *in vitro*/*ex vivo* models (54). Also, we did not detect an effect of C1QTNF3 neutralization on macrophage polarization *in vivo*. It is possible that we fail to detect such an effect as C1QTNF3 attracts both M1- and M2-type macrophages. Alternatively, the effect of C1QTNF3 on macrophage polarization is outruled by more potent macrophage polarizing factors in tumor-associated HFD-induced obese IWAT. Besides differences in macrophage types and experimental settings, the dose, and the source of the

recombinant C1QTNF3 protein may play a role. In this study, we have used a relatively high dose (1 μg/ml) of mammalian cell-produced mouse C1QTNF3 resulting in mammalian-type posttranslational modifications such as glycosylation that allows for correct folding and subsequent multimerization into higher-order oligomers (presumably trimeric, hexameric and high molecular weight oligomers) (19). Although the significance of posttranslational modifications and the different C1QTNF3 oligomeric forms is yet to be determined, we believe it is likely to affect the biological function of the protein.

Our current knowledge about potential C1QTNF3-receptors and downstream signaling pathways is very limited. To date, Lysosomal-associated membrane protein 1, Lysosome membrane protein 2 (55) and Adiponectin receptor 2 (56) have been suggested as potential receptors for C1QTNF3 but the significance of these finding are yet largely unknown. However, several studies show that C1QTNF3 activates the MEK/ERK and the PI3K/Akt pathway and its beneficial metabolic and anti-inflammatory effects are thought to primarily depend on the PI3K/Akt pathway (26, 41, 57–59). In this study, we found that C1QTNF3 activates both these pathways in M2-type macrophages. However, the increase in Akt-phosphorylation was not seen until after 60 minutes of C1QTNF3-stimulation indicating that the PI3K/Akt pathway is not an immediate target of C1QTNF3 in this cell type. Moreover, Akt exists in three different isoforms and Akt1 and Akt2 can have opposing effects on macrophage polarization (60). The MEK/ERK1/2 pathway can also lead to opposing effects on macrophage polarization; it can be both a negative regulator of murine and human macrophage M2-type polarization (61) and an enhancer of M2-type polarization (62). While our experimental setting *in vitro* clearly reveals the pro-inflammatory action of C1QTNF3 as judged by the reduced proliferation of M0-type macrophages and the M1-polarizing effect, we also anticipate that the timing and the concentration of C1QTNF3, as well as the state and the type of the targeted macrophages, will determine whether C1QTNF3 primarily push M1- or M2-type polarization. For instance, the ERK1/2 activation that occur within minutes in response to growth factors leads to proliferation while the slower ERK1/2 activation in response to LPS leads to growth arrest and pro-inflammatory activation in bone marrow-derived macrophages (63). Notably, C1QTNF3 can also activate AMPK, the PKC signaling pathway and the cAMP/PKA pathway (64–68). Thus, we cannot rule out that additional mechanisms besides the ERK- and Akt-pathways are at play.

In conclusion, our data strengthen the notion that C1QTNF3 modulates signaling pathways in a cell- and (patho-)physiological-state-dependent manner as suggested by Petersen and colleagues (53). We propose that locally increased C1QTNF3 levels contribute to increased IWAT macrophage accumulation in response to a growing tumor. While this C1QTNF3-stimulated macrophage population appears not to be involved in tumor progression/rejection, its role in subcutaneous adipose tissue remodeling warrants further investigations.

DATA AVAILABILITY STATEMENT

The datasets for this study are included within the article and its supplementary material. RNA-sequencing data have been deposited in NCBI's Gene Expression Omnibus (38) and are accessible through GEO Series accession number GSE201316.

ETHICS STATEMENT

The animal study was reviewed and approved by The Regional Animal Ethics Committee in Gothenburg, Sweden.

AUTHOR CONTRIBUTIONS

IWA conceived the idea, supervised this work, interpreted data, made figures, and wrote the first draft of the manuscript. MV contributed conceptionally to this work, designed and performed experiments, interpreted data, made figures, wrote parts of the manuscript, and critically revised this manuscript. PM designed and performed experiments, interpreted data, made figures, and wrote parts of the manuscript. YWu, EP, YWa, and BC performed experiments and assisted in data interpretation. SC and AS gave valuable feedback to this work and assisted in data interpretation. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.914956/full#supplementary-material.

REFERENCES

1. Kosteli A, Sugaru E, Haemmerle G, Martin JF, Lei J, Zechner R, et al. Weight Loss and Lipolysis Promote a Dynamic Immune Response in Murine Adipose Tissue. *J Clin Invest* (2010) 120(10):3466–79. doi: 10.1172/JCI42845
2. Qiu Y, Nguyen KD, Odegaard JI, Cui X, Tian X, Locksley RM, et al. Eosinophils and Type 2 Cytokine Signaling in Macrophages Orchestrate Development of Functional Beige Fat. *Cell* (2014) 157(6):1292–308. doi: 10.1016/j.cell.2014.03.066
3. Asterholm IW, McDonald J, Blanchard PG, Sinha M, Xiao Q, Mistry J, et al. Lack of "Immunological Fitness" During Fasting in Metabolically Challenged Animals. *J Lipid Res* (2012) 53(7):1254–67. doi: 10.1194/jlr.M021725
4. Chen Q, Lai SM, Xu S, Tan Y, Leong K, Liu D, et al. Resident Macrophages Restrain Pathological Adipose Tissue Remodeling and Protect Vascular Integrity in Obese Mice. *EMBO Rep* (2021) 22(8):e52835. doi: 10.15252/embr.202152835
5. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic Inflammation in Fat Plays a Crucial Role in the Development of Obesity-Related Insulin Resistance. *J Clin Invest* (2003) 112(12):1821–30. doi: 10.1172/JCI19451
6. Lumeng CN, Bodzin JL, Saltiel AR. Obesity Induces a Phenotypic Switch in Adipose Tissue Macrophage Polarization. *J Clin Invest* (2007) 117(1):175–84. doi: 10.1172/JCI29881
7. Obeid E, Nanda R, Fu Y-X, Olopade OI. The Role of Tumor-Associated Macrophages in Breast Cancer Progression (Review). *Int J Oncol* (2013) 43 (1):5–12. doi: 10.3892/ijo.2013.1938
8. Pollard JW. Tumour-Educated Macrophages Promote Tumour Progression and Metastasis. *Nat Rev Cancer* (2004) 4:71. doi: 10.1038/nrc1256
9. Qiu SQ, Waaijer SJH, Zwager MC, de Vries EGE, van der Vegt B, Schroder CP. Tumor-Associated Macrophages in Breast Cancer: Innocent Bystander or Important Player? *Cancer Treat Rev* (2018) 70:178–89. doi: 10.1016/j.ctrv.2018.08.010
10. Sun S, Ji Y, Kersten S, Qi L. Mechanisms of Inflammatory Responses in Obese Adipose Tissue. *Annu Rev Nutr* (2012) 32:261–86. doi: 10.1146/annurev-nutr-071811-150623
11. Russo L, Lumeng CN. Properties and Functions of Adipose Tissue Macrophages in Obesity. *Immunology* (2018) 155(4):407–17. doi: 10.1111/imm.13002
12. Kratz M, Coats BR, Hisert KB, Hagman D, Mutskov V, Peris E, et al. Metabolic Dysfunction Drives a Mechanistically Distinct Proinflammatory Phenotype in Adipose Tissue Macrophages. *Cell Metab* (2014) 20(4):614–25. doi: 10.1016/j.cmet.2014.08.010
13. Dirat B, Bochet L, Dabek M, Daviaud D, Dauvillier S, Majed B, et al. Cancer-Associated Adipocytes Exhibit an Activated Phenotype and Contribute to Breast Cancer Invasion. *Cancer Res* (2011) 71(7):2455–65. doi: 10.1158/0008-5472.CAN-10-3323
14. Nieman KM, Kenny HA, Penicka CV, Ladanyi A, Buell-Gutbrod R, Zillhardt MR, et al. Adipocytes Promote Ovarian Cancer Metastasis and Provide Energy for Rapid Tumor Growth. *Nat Med* (2011) 17(11):1498–503. doi: 10.1038/nm.2492
15. Nieman KM, Romero IL, Van Houten B, Lengyel E. Adipose Tissue and Adipocytes Support Tumorigenesis and Metastasis. *Biochim Biophys Acta* (2013) 1831(10):1533–41. doi: 10.1016/j.bbalip.2013.02.010
16. Iyengar P, Combs TP, Shah SJ, Gouon-Evans V, Pollard JW, Albanese C, et al. Adipocyte-Secreted Factors Synergistically Promote Mammary Tumorigenesis Through Induction of Anti-Apoptotic Transcriptional Programs and Proto-Oncogene Stabilization. *Oncogene* (2003) 22(41):6408–23. doi: 10.1038/sj.onc.1206737
17. Wang YY, Attane C, Milhas D, Dirat B, Dauvillier S, Guerard A, et al. Mammary Adipocytes Stimulate Breast Cancer Invasion Through Metabolic Remodeling of Tumor Cells. *JCI Insight* (2017) 2(4):e87489. doi: 10.1172/jci.insight.87489
18. Micallef P, Wu Y, Bauza-Thorbrugge M, Chanclon B, Vujicic M, Peris E, et al. Adipose Tissue-Breast Cancer Crosstalk Leads to Increased Tumor Lipogenesis Associated With Enhanced Tumor Growth. *Int J Mol Sci* (2021) 22(21). doi: 10.3390/ijms222111881
19. Wong GW, Krawczyk SA, Kitidis-Mitrokostas C, Revett T, Gimeno R, Lodish HF. Molecular, Biochemical and Functional Characterizations of C1q/Tnf Family Members: Adipose-Tissue-Selective Expression Patterns, Regulation by Ppar-Gamma Agonist, Cysteine-Mediated Oligomerizations, Combinatorial Associations and Metabolic Functions. *Biochem J* (2008) 416 (2):161–77. doi: 10.1042/BJ20081240
20. Maeda T, Abe M, Kurisu K, Jikko A, Furukawa S. Molecular Cloning and Characterization of a Novel Gene, Cors26, Encoding a Putative Secretory Protein and Its Possible Involvement in Skeletal Development. *J Biol Chem* (2001) 276(5):3628–34. doi: 10.1074/jbc.M007898200
21. Li Y, Wright GL, Peterson JM. C1q/Tnf-Related Protein 3 (Ctrp3) Function and Regulation. *Compr Physiol* (2017) 7(3):863–78. doi: 10.1002/cphy.c160044
22. Hofmann C, Chen N, Obermeier F, Paul G, Büchler C, Kopp A, et al. C1q/Tnf-Related Protein-3 (Ctrp-3) Is Secreted by Visceral Adipose Tissue and Exerts Antiinflammatory and Antifibrotic Effects in Primary Human Colonic Fibroblasts. *Inflamm Bowel Dis* (2011) 17(12):2462–71. doi: 10.1002/ibd.21647
23. Kopp A, Bala M, Buechler C, Falk W, Gross P, Neumeier M, et al. C1q/Tnf-Related Protein-3 Represents a Novel and Endogenous Lipopolysaccharide Antagonist of the Adipose Tissue. *Endocrinology* (2010) 151(11):5267–78. doi: 10.1210/en.2010-0571
24. Lin J, Liu Q, Zhang H, Huang X, Zhang R, Chen S, et al. C1q/Tumor Necrosis Factor-Related Protein-3 Protects Macrophages Against Lps-Induced Lipid Accumulation, Inflammation and Phenotype Transition *Via* Ppargamma and Tlr4-Mediated Pathways. *Oncotarget* (2017) 8(47):82541–57. doi: 10.18632/oncotarget.19657
25. Weigert J, Neumeier M, Schäffler A, Fleck M, Schölmerich J, Schütz C, et al. The Adiponectin Paralog Cors-26 Has Anti-Inflammatory Properties and Is Produced by Human Monocytic Cells. *FEBS Lett* (2005) 579(25):5565–70. doi: 10.1016/j.febslet.2005.09.022
26. Peterson JM, Wei Z, Wong GW. C1q/Tnf-Related Protein-3 (Ctrp3), A Novel Adipokine That Regulates Hepatic Glucose Output. *J Biol Chem* (2010) 285 (51):39691–701. doi: 10.1074/jbc.M110.180695
27. Peterson JM, Seldin MM, Wei Z, Aja S, Wong GW. Ctrp3 Attenuates Diet-Induced Hepatic Steatosis by Regulating Triglyceride Metabolism. *Am J Physiol Gastrointest Liver Physiol* (2013) 305(3):G214–24. doi: 10.1152/ajpgi.00102.2013
28. Murayama MA, Kakuta S, Maruhashi T, Shimizu K, Seno A, Kubo S, et al. Ctrp3 Plays an Important Role in the Development of Collagen-Induced Arthritis in Mice. *Biochem Biophys Res Commun* (2014) 443(1):42–8. doi: 10.1016/j.bbrc.2013.11.040
29. Moradi N, Najafi M, Sharma T, Fallah S, Koushki M, Peterson JM, et al. Circulating Levels of Ctrp3 in Patients With Type 2 Diabetes Mellitus Compared to Controls: A Systematic Review and Meta-Analysis. *Diabetes Res Clin Pract* (2020) 169:108453. doi: 10.1016/j.diabres.2020.108453
30. Li X, Jiang L, Yang M, Wu YW, Sun JZ. Impact of Weight Cycling on Ctrp3 Expression, Adipose Tissue Inflammation and Insulin Sensitivity in C57bl/6j Mice. *Exp Ther Med* (2018) 16(3):2052–9. doi: 10.3892/etm.2018.6399
31. Li X, Jiang L, Yang M, Wu YW, Sun SX, Sun JZ. Expression of Ctrp3, A Novel Adipokine, in Rats at Different Pathogenic Stages of Type 2 Diabetes Mellitus and the Impacts of Glp-1 Receptor Agonist on it. *J Diabetes Res* (2014) 2014:398518. doi: 10.1155/2014/398518
32. Tan BK, Chen J, Hu J, Amar O, Mattu HS, Adya R, et al. Metformin Increases the Novel Adipokine Cartonectin/Ctrp3 in Women With Polycystic Ovary Syndrome. *J Clin Endocrinol Metab* (2013) 98(12):E1891–900. doi: 10.1210/jc.2013-2227
33. Casey AE, Laster WRJr., Ross GL. Sustained Enhanced Growth of Carcinoma Eo771 in C57 Black Mice. *Proc Soc Exp Biol Med* (1951) 77(2):358–62. doi: 10.3181/00379727-77-18779
34. Green H, Kehinde O. Sublines of Mouse 3t3 Cells That Accumulate Lipid. *Cell* (1974) 1(3):113–6. doi: 10.1016/0092-8674(74)90126-3
35. Kohn AD, Summers SA, Birnbaum MJ, Roth RA. Expression of a Constitutively Active Akt Ser/Thr Kinase in 3t3-L1 Adipocytes Stimulates Glucose Uptake and Glucose Transporter 4 Translocation. *J Biol Chem* (1996) 271(49):31372–8. doi: 10.1074/jbc.271.49.31372
36. Dobin A, Davis CA, Schlesinger F, Drenkow J, Zaleski C, Jha S, et al. Star: Ultrafast Universal Rna-Seq Aligner. *Bioinformatics* (2013) 29(1):15–21. doi: 10.1093/bioinformatics/bts635
37. Anders S, Huber W. Differential Expression Analysis for Sequence Count Data. *Genome Biol* (2010) 11(10):R106. doi: 10.1186/gb-2010-11-10-r106

38. Edgar R, Domrachev M, Lash AE. Gene Expression Omnibus: Ncbi Gene Expression and Hybridization Array Data Repository. *Nucleic Acids Res* (2002) 30(1):207–10. doi: 10.1093/nar/30.1.207
39. Chanclon B, Wu Y, Vujicic M, Bauza-Thorbrugge M, Banke E, Micallef P, et al. Peripancreatic Adipose Tissue Protects Against High-Fat-Diet-Induced Hepatic Steatosis and Insulin Resistance in Mice. *Int J Obes (Lond)* (2020) 44 (11):2323–34. doi: 10.1038/s41366-020-00657-6
40. Gu J-W, Young E, Patterson SG, Makey KL, Wells J, Huang M, et al. Postmenopausal Obesity Promotes Tumor Angiogenesis and Breast Cancer Progression in Mice. *Cancer Biol Ther* (2011) 11(10):910–7. doi: 10.4161/cbt.11.10.15473
41. Nishimoto H, Yamamoto A, Furukawa S, Wakisaka S, Maeda T. C1q/Tnf-Related Protein 3 Expression and Effects on Adipocyte Differentiation of 3t3-L1 Cells. *Cell Biol Int* (2017) 41(2):197–203. doi: 10.1002/cbin.10674
42. Qualls JE, Subramanian C, Rafi W, Smith AM, Balouzian L, DeFreitas AA, et al. Sustained Generation of Nitric Oxide and Control of Mycobacterial Infection Requires Argininosuccinate Synthase 1. *Cell Host Microbe* (2012) 12 (3):313–23. doi: 10.1016/j.chom.2012.07.012
43. El Kasmi KC, Qualls JE, Pesce JT, Smith AM, Thompson RW, Henao-Tamayo M, et al. Toll-Like Receptor-Induced Arginase 1 in Macrophages Thwarts Effective Immunity Against Intracellular Pathogens. *Nat Immunol* (2008) 9 (12):1399–406. doi: 10.1038/ni.1671
44. Akiyama H, Furukawa S, Wakisaka S, Maeda T. Cartducin Stimulates Mesenchymal Chondroprogenitor Cell Proliferation Through Both Extracellular Signal-Regulated Kinase and Phosphatidylinositol 3-Kinase/Akt Pathways. *FEBS J* (2006) 273(10):2257–63. doi: 10.1111/j.1742-4658.2006.05240.x
45. Tabula Muris C. Overall C, Logistical C, Organ C, Processing, Library P, Et al. Single-Cell Transcriptomics of 20 Mouse Organs Creates a Tabula Muris. *Nature* (2018) 562(7727):367–72. doi: 10.1038/s41586-018-0590-4
46. Strissel KJ, Stancheva Z, Miyoshi H, Perfield JW2nd, DeFuria J, Jick Z, et al. Adipocyte Death, Adipose Tissue Remodeling, and Obesity Complications. *Diabetes* (2007) 56(12):2910–8. doi: 10.2337/db07-0767
47. Wernstedt Asterholm I, Tao C, Morley TS, Wang QA, Delgado-Lopez F, Wang ZV, et al. Adipocyte Inflammation Is Essential for Healthy Adipose Tissue Expansion and Remodeling. *Cell Metab* (2014) 20(1):103–18. doi: 10.1016/j.cmet.2014.05.005
48. Wolf RM, Lei X, Yang ZC, Nyandjo M, Tan SY, Wong GW. Ctrp3 Deficiency Reduces Liver Size and Alters Il-6 and Tgfbeta Levels in Obese Mice. *Am J Physiol Endocrinol Metab* (2016) 310(5):E332–45. doi: 10.1152/ajpendo.00248.2015
49. Feng H, Wang J-Y, Zheng M, Zhang C-L, An Y-M, Li L, et al. Ctrp3 Promotes Energy Production by Inducing Mitochondrial Ros and Up-Expression of Pgc-1α in Vascular Smooth Muscle Cells. *Exp Cell Res* (2016) 341(2):177–86. doi: 10.1016/j.yexcr.2016.02.001
50. Biswas SK, Mantovani A. Orchestration of Metabolism by Macrophages. *Cell Metab* (2012) 15(4):432–7. doi: 10.1016/j.cmet.2011.11.013
51. Kelly B, O'neill LA. Metabolic Reprogramming in Macrophages and Dendritic Cells in Innate Immunity. *Cell Res* (2015) 25(7):771–84. doi: 10.1038/cr.2015.68
52. Masoodian SM, Toolabi K, Omidifar A, Zabihi H, Rahimipour A, Shanaki M. Increased Mrna Expression of Ctrp3 and Ctrp9 in Adipose Tissue From Obese Women: Is It Linked to Obesity-Related Parameters and Mrna Expression of Inflammatory Cytokines? *Rep Biochem Mol Biol* (2020) 9(1):71–81. doi: 10.29252/rbmb.9.1.71
53. Petersen PS, Wolf RM, Lei X, Peterson JM, Wong GW. Immunomodulatory Roles of Ctrp3 in Endotoxemia and Metabolic Stress. *Physiol Rep* (2016) 4(5): e12735. doi: 10.14814/phy2.12735
54. Wang C, Yu X, Cao Q, Wang Y, Zheng G, Tan TK, et al. Characterization of Murine Macrophages From Bone Marrow, Spleen and Peritoneum. *BMC Immunol* (2013) 14:6. doi: 10.1186/1471-2172-14-6
55. Li Y, Ozment T, Wright GL, Peterson JM. Identification of Putative Receptors for the Novel Adipokine Ctrp3 Using Ligand-Receptor Capture Technology. *PloS One* (2016) 11(10):e0164593. doi: 10.1371/journal.pone.0164593
56. Murayama MA, Chi HH, Matsuoka M, Ono T, Iwakura Y. The Ctrp3-Adipor2 Axis Regulates the Development of Experimental Autoimmune Encephalomyelitis by Suppressing Th17 Cell Differentiation. *Front Immunol* (2021) 12:607346. doi: 10.3389/fimmu.2021.607346
57. Hou M, Liu J, Liu F, Liu K, Yu B. C1q Tumor Necrosis Factor-Related Protein-3 Protects Mesenchymal Stem Cells Against Hypoxia- and Serum Deprivation-Induced Apoptosis Through the Phosphoinositide 3-Kinase/Akt Pathway. *Int J Mol Med* (2014) 33(1):97–104. doi: 10.3892/ijmm.2013.1550
58. Huang Y, Wan G, Tao J. C1q/Tnf-Related Protein-3 Exerts the Chondroprotective Effects in Il-1beta-Treated Sw1353 Cells by Regulating the Fgfr1 Signaling. *BioMed Pharmacother* (2017) 85:41–6. doi: 10.1016/j.biopha.2016.11.128
59. Li X, Jiang L, Yang M, Wu YW, Sun JZ, Sun SX. Ctrp3 Improves the Insulin Sensitivity of 3t3-L1 Adipocytes by Inhibiting Inflammation and Ameliorating Insulin Signalling Transduction. *Endokrynol Pol* (2014) 65(4):252–8. doi: 10.5603/EP.2014.0034
60. Vergadi E, Ieronymaki E, Lyroni K, Vaporidi K, Tsatsanis C. Akt Signaling Pathway in Macrophage Activation and M1/M2 Polarization. *J Immunol* (2017) 198(3):1006–14. doi: 10.4049/jimmunol.1601515
61. Long ME, Eddy WE, Gong KQ, Lovelace-Macon LL, McMahan RS, Charron J, et al. Mek1/2 Inhibition Promotes Macrophage Reparative Properties. *J Immunol* (2017) 198(2):862–72. doi: 10.4049/jimmunol.1601059
62. He L, Jhong JH, Chen Q, Huang KY, Strittmatter K, Kreuzer J, et al. Global Characterization of Macrophage Polarization Mechanisms and Identification of M2-Type Polarization Inhibitors. *Cell Rep* (2021) 37(5):109955. doi: 10.1016/j.celrep.2021.109955
63. Valledor AF, Comalada M, Xaus J, Celada A. The Differential Time-Course of Extracellular-Regulated Kinase Activity Correlates With the Macrophage Response Toward Proliferation or Activation. *J Biol Chem* (2000) 275 (10):7403–9. doi: 10.1074/jbc.275.10.7403
64. Hou Q, Lin J, Huang W, Li M, Feng J, Mao X. Ctrp3 Stimulates Proliferation and Anti-Apoptosis of Prostate Cells Through Pkc Signaling Pathways. *PloS One* (2015) 10(7):e0134006. doi: 10.1371/journal.pone.0134006
65. Otani M, Kogo M, Furukawa S, Wakisaka S, Maeda T. The Adiponectin Paralog C1q/Tnf-Related Protein 3 (Ctrp3) Stimulates Testosterone Production Through the Camp/Pka Signaling Pathway. *Cytokine* (2012) 58 (2):238–44. doi: 10.1016/j.cyto.2012.01.018
66. Kim JY, Min JY, Baek JM, Ahn SJ, Jun HY, Yoon KH, et al. Ctrp3 Acts as a Negative Regulator of Osteoclastogenesis Through Ampk-C-Fos-Nfatc1 Signaling in Vitro and Rankl-Induced Calvarial Bone Destruction in Vivo. *Bone* (2015) 79:242–51. doi: 10.1016/j.bone.2015.06.011
67. Yan Z, Zhao J, Gan L, Zhang Y, Guo R, Cao X, et al. Ctrp3 Is a Novel Biomarker for Diabetic Retinopathy and Inhibits Hghl-Induced Vcam-1 Expression in an Ampk-Dependent Manner. *PloS One* (2017) 12(6): e0178253. doi: 10.1371/journal.pone.0178253
68. Wu D, Lei H, Wang JY, Zhang CL, Feng H, Fu FY, et al. Ctrp3 Attenuates Post-Infarct Cardiac Fibrosis by Targeting Smad3 Activation and Inhibiting Myofibroblast Differentiation. *J Mol Med (Berl)* (2015) 93(12):1311–25. doi: 10.1007/s00109-015-1309-8

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The Roles of Adipose Tissue Macrophages in Human Disease

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Macrophages are a population of immune cells functioning in antigen presentation and inflammatory response. Research has demonstrated that macrophages belong to a cell lineage with strong plasticity and heterogeneity and can be polarized into different phenotypes under different microenvironments or stimuli. Many macrophages can be recruited by various cytokines secreted by adipose tissue. The recruited macrophages further secrete various inflammatory factors to act on adipocytes, and the interaction between the two leads to chronic inflammation. Previous studies have indicated that adipose tissue macrophages (ATMs) are closely related to metabolic diseases like obesity and diabetes. Here, we will not only conclude the current progress of factors affecting the polarization of adipose tissue macrophages but also elucidate the relationship between ATMs and human diseases. Furthermore, we will highlight its potential in preventing and treating metabolic diseases as immunotherapy targets.

Keywords: Adipose tissue macrophages, inflammation, obesity, diabetes, insulin resistance (IR), insulin sensitivity (IS)

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INTRODUCTION

Obesity is caused by the excessive accumulation of lipids in adipose tissues. In recent years, obesity has become the causing factor of many chronic diseases, including type 2 diabetes mellitus (T2DM), hypertension, cardiovascular and cerebrovascular diseases, and breast cancer, thus posing a burden on not only patients' health and finance but also social, medical system (1–4). Apart from storing nutrients, adipose tissue is also an important immune organ containing many immune cells, among which macrophages function in maintaining immune levels. "Obesity is metabolic inflammation" was first proposed by Spiegelman in 1993 (5). It was not until 2003 that researchers discovered macrophage markers in the adipose tissue of obese animals, finding that the higher the macrophage content, the higher the obesity level of the animal (6). The traditional theory holds that macrophages in peripheral tissues are derived from monocytes in the blood (7). Visceral adipose tissue (VAT), a type of white adipose tissue (WAT), is the primary location of inflammatory response in obesity. Although many immune cells participate in the inflammatory response, adipose tissue macrophages (ATM) are considered the most important and characteristic immune cells (8). The proportion of

macrophages in total cells in normal adipose tissue is only 10%, but it can reach 50% in obese people (6). Based on the difference in function and activation markers, macrophages are divided into pro-inflammatory M1 and anti-inflammatory M2, with M1 macrophages contributing mostly to the increase in obesity (8–10).

T2DM poses a serious threat to human health, with 80% of its patients caused by overweight or obese. Insulin resistance (**Figure 1**), a common pathological feature of obesity, occurs when organs are insensitive to insulin stimulation, leading to high blood sugar levels, thus causing diabetes (11–13). Obesity and age-related factors are major risk factors for insulin resistance (14). Obesity stimulates NF-κB, JNK, and other signaling pathways to promote the expression of inflammatory factors, thus influencing the insulin signaling pathway and causing insulin resistance (15). Here, we will summarize the role of ATMs in human diseases and mainly focus on obesity and T2DM, thus providing new insight into the treatment of these diseases as therapeutic targets.

OVERVIEW OF ATMS

Macrophages are pivotal in the body's immune system, and they are distributed in various tissues and organs throughout the body, including adipose tissue. Hematopoietic progenitor cells (HPCs) in the bone marrow can differentiate into monocytes upon being stimulated by various cytokines, which will transfer to VAT through the bloodstream to form ATM, thus producing corresponding inflammatory mediators and promoting HPC differentiation (16). Previous studies have shown that ATM mostly appears during embryonic development and will polarize into different phenotypes based on environment, like body weight (17, 18). When an individual is obese, macrophages are often polarized to a pro-inflammatory type, the M1 type (19, 20). With the induction of lipopolysaccharide and saturated fatty acid, M1 macrophages can activate and secrete tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), interleukin-12 (IL-12), interleukin-1β (IL-1β) and other pro-inflammatory factors, leading to inflammation and insulin resistance (**Table 1**) (21). ATM manifests as an anti-inflammatory type when the individual is thin, namely the M2 type. Both M1 and M2 types have CD11b molecules on the surface. In addition, the M1 type expresses CD11c molecules, and the M2 type expresses CD206, CD301, and macrophage galactose type C-type lectin 1 specifically (29). Different from the M1 type, ATM undergoes the M2 type polarization with the induction of IL-4 and IL-13 and secretes anti-inflammatory mediators such as IL-10 and IL-1 receptor antagonists to play an anti-inflammatory role and maintain insulin sensitivity (21, 22).

MECHANISMS OF ATMS POLARIZATION AND RECRUITMENT

M1 Recruitment and Polarization

M1 macrophages are activated by helper T lymphocyte Th1 cytokines such as interferon, TNF, and LPS (lipopolysaccharide). The pathogenesis of obesity is closely related to the recruitment of ATMs polarized to the pro-inflammatory M1 phenotype (23). The proportion of CD11c-positive monocytes in obese patients

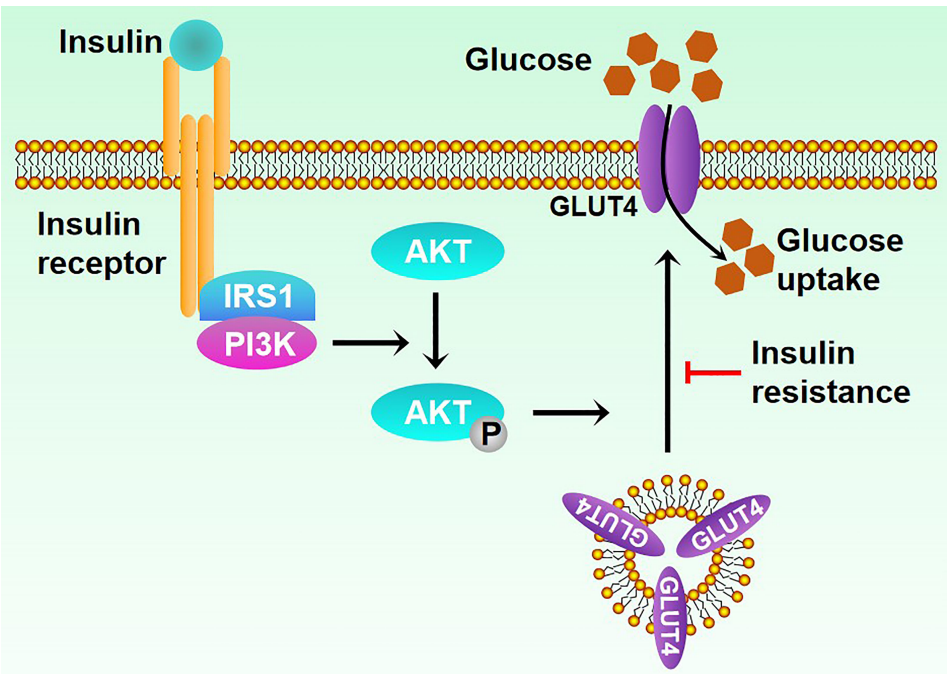

FIGURE 1 | Mechanisms of insulin resistance.

TABLE 1 | Properties of adipose tissue macrophages.

Properties	M1 macrophages	M2 macrophages	Refs
Inducer	TNF, LPS, Interferon	IL-4, IL-10, IL-13, IL-1β, TGFβ, LPS, Glucocorticoids	(21–28)
Secreted factors	TNF-α, IL-6, IL-12, IL-1β	Antagonists of IL-1 and IL-10 receptor	(21, 22)
Cell surface factor	CD11c	CD206, CD301	(29)

was higher than that of normal people, which would decrease after a low-fat diet (30). Therefore, identifying the factors that can polarize ATMs to M1 and recruit macrophages to peripheral tissues in the process of obesity is of great significance for the prevention and treatment of obesity. Accumulating studies have indicated that various signaling pathways contribute to the recruitment and polarization of M1 ATMs during the progression of obesity.

MAPK (Mitogen-activated protein kinase) is a family of serine-threonine protein kinases that can be activated by different extracellular stimuli and cell adhesion, including four subfamilies: ERK, P38, JNK, and ERK5, and it is significant in the pathophysiological process of obesity (31). An early study observed the overexpression of the genes involved in p38 and JNK signaling pathways in adipose tissue of obese people (32). An animal study in mice showed that the increase of M1 ATMs proportion is achieved by increasing mRNA transcription and protein expression levels of JNK (33). In a classical study, the researchers constructed a JNK KO mouse, then fed JNK knockout mice and WT mice with a normal diet and a high-fat diet, and found that high-fat feeding increased ATMs in WT mice had few effects on KO mice (34). Moreover, the increase of macrophages in WT mice was attributed to a significant increase of M1 macrophages, while the numbers of M1 and M2 macrophages in KO mice did not show significant changes. Furthermore, the expression of M1-related genes was down-regulated, and the expression of M2-related genes was up-regulated in KO mice. These data together suggest that the activation of the MAPK signaling pathway may be related to the polarization of ATMs towards the M1 type.

Toll-like receptors are a class of innate immune receptors that are widely expressed on the surface of monocytes, macrophages, and lymphocytes, among which TLR-4 contributes to the LPS response (35). A previous study found that the transcription level of TLR4 mRNA in obese patients was remarkably higher than that in normal people, suggesting that the activation of the TLR-4 receptor may be related to the infiltration of ATMs in the process of obesity (36). Results from different labs confirmed that TLR-4 receptor deficiency reduces inflammation in adipose tissue, and TLR-4 has a positive role in the polarization of ATMs towards M1 (37, 38).

The transcription factor NF-κB is the main regulator of immune homeostasis and inflammation, discovered 30 years ago (39). Studies have demonstrated that activation of NF-κB signaling could facilitate the M1 polarization of macrophages in 3T3-L1 cell lines (40, 41). Other studies have shown that inhibiting NF-κB signaling can promote the release of IL-10 and other anti-inflammatory factors from ATMs (42). In addition, Cao et al. also observed this phenomenon in the mouse model (43). These studies strongly demonstrate that NF-κB can mediate the polarization of ATMs towards M1.

In addition to the above signaling pathways, other factors can also lead to the polarization of ATMs towards M1, including lysosomes and the AMPK signaling pathway (44–47), indicating that the polarization of ATMs towards M1 is a complex process with the coordination of multiple pathways, which needs further investigation.

M2 Recruitment and Polarization

Th2 cytokines can activate anti-inflammatory M2 macrophage in three ways: M2a subtypes activated by IL-4 and IL-13; M2b subtype activated by immune complexes combined with IL-1β or bacterial lipopolysaccharide; M2c subtype induced by IL-10, TGFβ or glucocorticoids. During the process of inflammation resolution, M1 phenotype macrophages are polarized towards the M2 phenotype and are accompanied by the recruitment of M2 phenotype macrophages. Nuclear receptor transcription factors are significant in macrophage polarization, such as PPAR family members.

PPARγ is highly expressed in anti-inflammation macrophages and is important (48, 49). Previous research has found that activation of PPARγ can promote the conversion of M1 type macrophages to M2 type macrophages, improve insulin resistance caused by obesity, and reduce the expression of inflammatory factors (50, 51). After specific activation of PPARγ signaling in mice, it was found that the number of M1 macrophages in ATMs decreased along with the expression of M1-related genes, and the number of M2 macrophages increased, along with the expression of M2-related genes (52, 53). Furthermore, the ex vivo therapy model also demonstrated that activation of PPARγ signaling could induce the polarization of macrophages toward M2 macrophages and induce the recruitment of M2 macrophages (54). The above studies demonstrate that PPARγ is involved in ATM polarization towards M2 and M2 macrophage recruitment.

Previous studies also prove that adiponectin can promote the M2 polarization of macrophages (55). After adiponectin knockout in mice, the expression of M1-related genes was up-regulated, and the expression of M2-related genes was down-regulated. In addition, recombinant adiponectin can up-regulate the expression of M2-related genes as well (56). These results suggest that adiponectin can facilitate the polarization of adipose tissue macrophage towards M2.

IL-4 secreted by immune cells in adipose tissue can also mediate M2 polarization (24). Overexpression or knockout of IL-4 was shown to up-regulate or down-regulate the expression of M2-related genes, respectively (25). These studies demonstrate that IL-4 can mediate adipose tissue macrophage polarization

toward M2. Some subsequent studies also found that cytokines such as IL-10, IL-13, and IL-33 can also mediate the polarization of macrophages towards the M2 phenotype (26–28).

ATMS AND OBESITY

In the obese state, the adipose tissue is under low-intensity inflammation, and the infiltration of ATMs in it is significantly increased to a percentage of 41% compared to a normal state, accompanied by M1 polarization (6). The histological method shows that many M1 type ATMs gather around the dying adipocytes, and crown-like structures (CLSs) appear, associated with obesity-related insulin resistance (57). Further studies have shown that Mincle (macrophage-inducible C-type lectin) in ATMs is involved in the formation of CLSs, and its expression level is positively related to adipose tissue interstitial fibrosis, thus promoting liver fibrosis, progression of hepatic steatosis, and insulin resistance (58–60).

During obesity, ATM is stimulated by inflammatory factors such as IFN-γ, leukotriene B4 (LTB4), and monocyte chemoattractant protein-1 (MCP-1) released by fatty tissue, followed by M1 polarization (8, 61). Previous studies also found that the expression of IL-6, monocyte MCP-1, resistin, lipase (Adip-sin), leptin, and other factors in obese adipose tissue is up-regulated, which increases the expression of vascular endothelial cell adhesion molecules, thus recruiting monocytes in the blood, and promoting the infiltration of ATMs (**Figure 2**). Further studies confirmed that MCP-1 recruits ATMs through CCR2, while LTB4 recruits ATMs through its receptor BLT1 (62–64). The M1 type ATM secretes inflammatory factors such as IL-6, TNF-α, IL-1β, MCP-1, and PAI-1 (plasminogen activator inhibitor-1), which further increase ATM levels and maintain the M1 phenotype, thus forming a vicious circle. Studies have shown that the occurrence of various obesity-related chronic diseases, such as type 2 diabetes and atherosclerosis, are inseparable from inflammatory factors such as IL-6 and TNF-α (65, 66). In addition, in a previous study, Shimizu et al. verified that neuronal guidance molecules are also involved in the recruitment of ATMs, such as Sema3E, which can promote adipose tissue inflammation through its receptor PlexinD1 (67). Other molecules such as osteocalcin are also involved in the recruitment of ATMs and the progression of adipose tissue inflammation and may be targeted for intervention in metabolic-related diseases such as obesity (68). There is also a positive feedback loop between ATMs derived from blood monocytes and myeloid progenitors in bone marrow tissue. The NLRP3 inflammasome of ATMs is activated to stimulate myeloid progenitor cells to differentiate into monocytes and neutrophils by secreting IL-1β, and intervening in this circuit can reduce adipose tissue inflammation (16). Besides, Zhuang et al. found that miR-223 can inhibit the polarization of ATM to M1 type and ultimately inhibit the inflammatory response

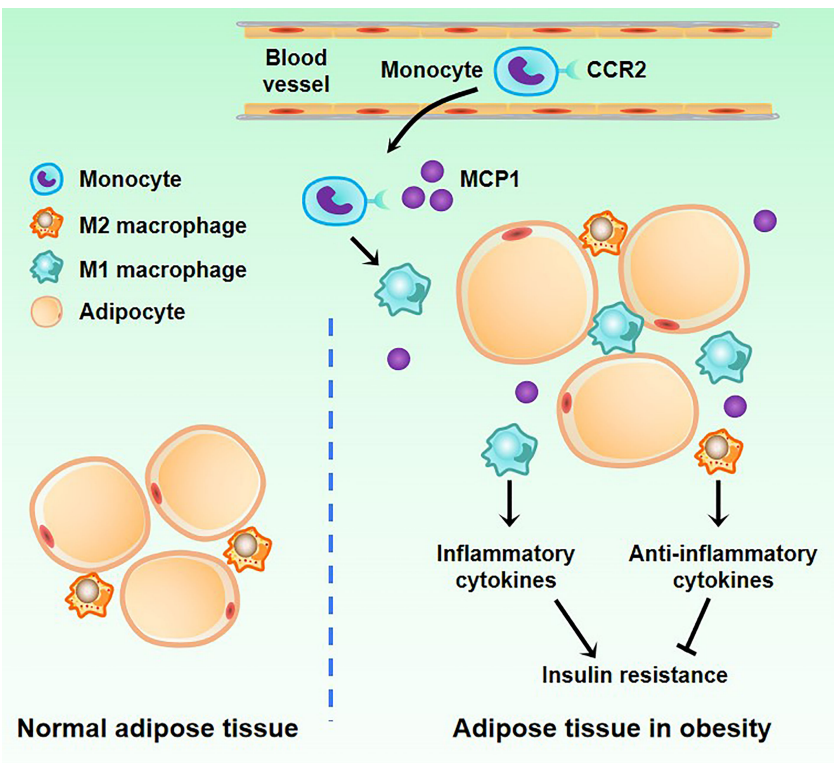

FIGURE 2 | Changes of macrophages in adipose tissue in obesity. There are a small number of M2 macrophages in normal adipose tissue. When obesity occurs, blood monocytes accumulate in adipose tissue. Under the induction of the MCP1 factor secreted by adipose tissue, monocytes differentiate into M1 macrophages.

of adipose tissue while knocking out the miR-223 gene can aggravate the inflammatory response and increase the proportion of M1 type in ATM (69). Other studies have shown that adipose tissue inflammatory response is closely related to the β1 subunit of AMPK, and the results suggest that these molecules and enzymes may provide new entry points for future obesity treatment (70). Another study showed that IL-6 could induce the IL-4 receptor expression of ATM. ATM was significantly polarized towards the M1 type in mice that did not express the IL-6R α chain, suggesting that IL-6 may affect ATM polarization, reducing inflammation in adipose tissue (71).

Growing evidence has indicated that macrophages have a greater impact on the remodeling process of adipose tissue. First, the adipose tissue of obese animals has a higher number of macrophages, which are an important component of adipose tissue. In addition, M1 macrophages can produce some inflammatory mediators and reactive oxygen that have a certain impact on the structure and function of adipocytes. These substances will affect the normal metabolism of adipocytes and increase the release of free fatty acids (FFA), leading to increased lipotoxicity and reduction in the synthesis and secretion of adiponectin (72). Compared with normal mice, adipocyte death was significantly increased in mice with higher fat content, and a similar situation occurred in obese people, indicating that an important pathological manifestation of obesity is adipocyte death (73). A study of adipose tissue of obese patients showed that after adipocyte apoptosis, ATM surrounded it with a coronal structure, forming huge multinucleated cells, but this phenomenon was not observed in the adipose tissue of non-obese people (20). Therefore, the infiltration and activation of ATM during obesity is a powerful mechanism of adipose tissue remodeling.

In addition to the abnormal recruitment and polarization of ATMs in the adipose tissue of obese animals, their emigration is also abnormal, which is mediated by signaling molecules such as chemokines and neural guidance molecules. One previous study reported that Netrin-1 was up-regulated in ATMs of obese patients and mouse models, thus inhibiting the migration of ATMs through its receptor Unc5b (74).

M1 type ATMs are considered pro-inflammatory phenotypes in adipose tissue, and M2 type ATMs are considered anti-inflammatory phenotypes, but ATMs cannot be mechanically recognized in practice. A growing number of studies have shown that ATMs have multiple origins, with their functions spanning pure pro- or anti-inflammatory effects, and they are highly plastic and can achieve phenotypic transformation under specific circumstances, which can be therapeutic targets in the future (75, 76).

ATMS AND IR, T2MD

More and more studies have demonstrated that ATMs are important in IR and T2MD. Next, we will clarify the relationship between ATMs and IR and T2MD (**Figure 3**).

In a previous study, scientists demonstrated that M1 macrophages could aggravate insulin resistance, and CD11c+ cell depletion led to decreased adipose tissue inflammation and rapid normalization of insulin sensitivity (77). In another study, scientists observed that $CD11c^+$ ATM ablation could reduce adipose tissue inflammatory gene expression and improve insulin resistance in the Ccr2 KO mice model (78). It can be seen that $CD11c^+$ ATM infiltration of adipose tissue is one of the reasons for insulin resistance, where increasing FFAs may aggravate insulin resistance. Fetuin-A is a glycoprotein secreted by the liver, and its plasma concentration increases in obesity (79). With the mediation of Fetuin-A, FFAs can indirectly activate TLR4 of CD11c+ ATM so that nuclear factor downstream of TLR4 inhibits the phosphorylation of protein kinase β/nuclear factor-B (IKKβ/NF-κB) and c-Jun N-terminal kinase-activator protein 1 (JNK/AP-1) inflammatory signaling pathway, enhancing inflammatory gene expression and secreting more inflammatory factors like TNF-α, IL-6, and MCP-1. Some studies have found that FFAs also activate TLR2 of ATM to participate in insulin resistance (80). Physiologically, insulin mediates the tyrosine phosphorylation of the insulin receptor substrate (IRS) through the insulin receptor, thus enhancing the downstream PI3K/Akt signaling pathway, promoting glucose uptake, and exerting the hypoglycemic effect. However, activated IKKβ and JNK can cause insulin resistance through IRS serine phosphorylation and blockage of IRS tyrosine phosphorylation and the downstream PI3K/Akt pathway (81). In addition, inflammatory factors secreted by macrophages, such as TNF-α, can further activate inflammatory pathways such as IKKβ/NF-kB, JNK/AP-1, and mTOR signaling pathway, forming a vicious circle (82).

Saturated fatty acids are also involved in insulin resistance. The researchers found that knockout of the CGI-58 (comparative gene identification-58) gene in obese mouse macrophages resulted in mitochondrial dysfunction and reactive oxygen species-mediated oxidative stress in ATM, resulting in the activation of NLRP3 inflammasome and downstream caspase-1, leading to the exacerbation of insulin resistance and hyperglycemia (83). NLRP3 inflammasome is a protein complex in the cell cytoplasm, a member of the NLRs family, and its expression is increased in the adipose tissue of obese diabetic patients (84). Activated NLRP3 inflammasome and downstream caspase-1 do not affect the ratio of M1/M2 in adipose tissue but promote the secretion of IL-1β and IL-18, leading to insulin resistance (84).

Unlike the M1 type, M2 type ATMs secrete the anti-inflammatory factor IL-10, thus inhibiting inflammation and enhancing insulin resistance (85). Therefore, activating factors of M2 macrophages also indirectly affect insulin sensitivity. PPARγ is a fatty acid sensor widely expressed in M2 ATMs, which mediates the activation of monocytes' polarization towards M2 macrophages (86, 87). With the PPARγ gene knocked out in obese mice, the expression levels of related genes in M2 type macrophages in adipose tissue decreased by 70% to 80%, while the expression levels of inflammatory genes in M1 type macrophages increased, accompanied by insulin resistance and exacerbated hyperglycemia, suggesting that PPARγ is vital in maintaining M2 macrophage phenotype and recovering insulin sensitivity (86). KLF4, as another M2-related cytokine, can

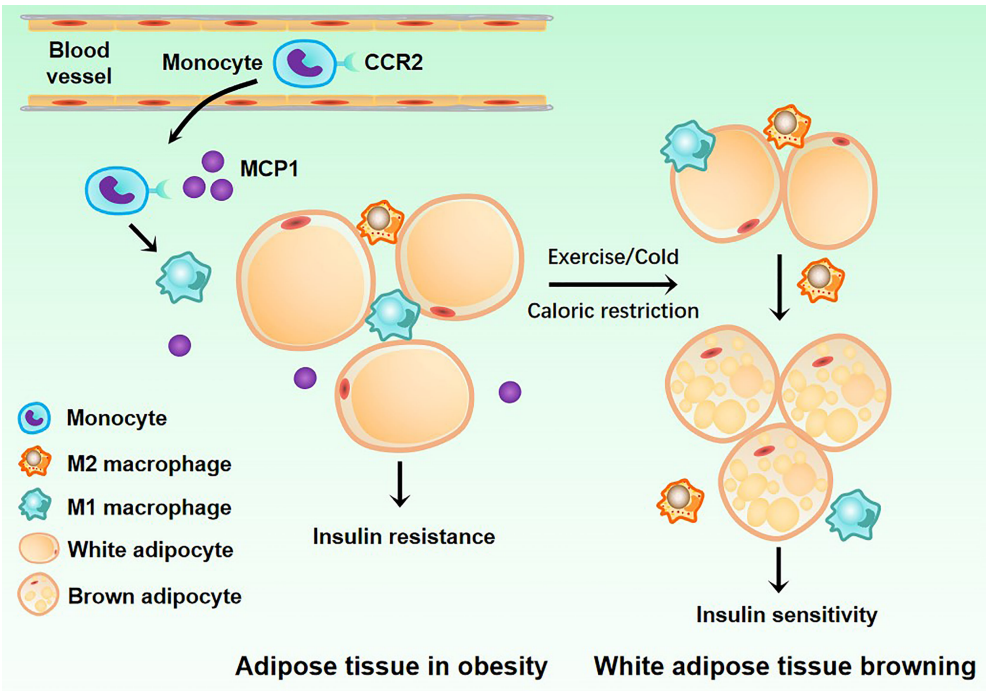

FIGURE 3 | Related mechanisms of adipose tissue macrophages and type2 diabetes mellitus. White adipose tissue acquires insulin resistance under the action of M1 macrophages. When the body is exercising or dieting, M2 macrophages can induce the transformation of white adipose tissue into brown adipose tissue, allowing it to regain insulin sensitivity.

synergize with IL-4 to activate STAT6 and inhibit the NF-kB signaling pathway, thus activating M2 type polarization and inhibiting M1 type polarization (88). Knockout of KLF4 in macrophages of obese mice would decrease the proportion of M2 type ATMs and worsen insulin resistance and hyperglycemia (88). In addition, compared with normal people, the expression level of KLF4 in subcutaneous adipose tissue of obese patients decreased by 50%, which may be one of the reasons for the increased M1/M2 ratio in adipose tissue.

In addition, ATM can secrete an exosome (Exos) containing microRNA (miRNA). Intravenous injection of ATM-secreted Exos (ATM–Exos) from obese mice into normal mice for 2 weeks resulted in impaired glucose tolerance and IS, suggesting the occurrence of T2DM. In contrast, when ATM-Exos from normal mice were injected into obese mice, their glucose tolerance and IS were significantly improved, and the overexpression of miR-155 in obese mice ATM-Exos inhibited the expression of its downstream IS-promoting target gene PPARγ, thereby impairing insulin signaling, leading to IR (89). Another study found that miR-29a was overexpressed in ATM-Exos of obese mice and transferred to adipocytes, cardiomyocytes, and hepatocytes, causing IR (90). ATM –Exos can be paracrine to insulin target cells, impacting intracellular insulin and glucose homeostasis. However, there are hundreds of miRNAs in ATM–Exos, and none of them affects IS alone, which may be that multiple miRNAs work together to affect adipose tissue metabolism. The above studies have shown that ATMs secrete exosomes carrying miRNAs, which can be transported to insulin target cells through paracrine or endocrine mechanisms, significantly enhancing the action of intracellular insulin, improving insulin sensitivity and overall glucose homeostasis.

Obesity is closely related to T2MD observed in clinical practice (91). Recent studies suggest that ATMs and the inflammatory response play a bridge role in this process (63, 69). The c-Jun N-terminal kinase (JNK) signaling pathway is significant in obesity-related metabolic responses. In a high-fat diet-induced obesity mouse model, although macrophage-specific JNK knockout did not affect the bodyweight of mice, it reduced ATMs infiltration and improved insulin sensitivity, and JNK knockout could inhibit the polarization of ATMs towards M1 (34). These suggest that ATMs-related inflammatory responses, rather than obesity itself, contribute to the development of obesity-related T2MD.

Further studies have shown that ATMs are involved in obesity-related T2MD by secreting cytokines such as upd3. Studies in Drosophila have shown that depletion of macrophages or macrophage-specific knockout of upd3 can inhibit the activation of the JAK-STAT signaling pathway, thus increasing insulin sensitivity without affecting body weight (92). Mincle in ATMs plays a role in the formation of CLSs and participates in obesity-related insulin resistance (58). Furthermore, the inflammatory cytokines secreted by ATMs may be causative factors leading to insulin resistance and T2MD. In addition, PAI-1 blood levels were significantly increased in obese individuals, and further studies confirmed that it was derived from ATMs stimulated by free fatty acids (93). It is worth noting that breaking the link between ATMs and NK cells, $CD8^+$T cells, and myeloid progenitor cells can inhibit ATMs-mediated inflammatory response and ultimately

reduce insulin resistance, which may bring light to the treatment of T2MD (16, 94, 95).

ATMS AND CLINICAL THERAPY

Metformin is still the first-line T2MD drug especially caused by obesity. Since macrophages are involved in insulin resistance, they are likely to be ideal targets for treating metabolic diseases. The strategy is to regulate the inflammation-related signaling pathways in macrophages, thus inhibiting their polarization toward M1 and reducing macrophages' production of inflammatory factors. Some small interfering RNAs and small molecule drugs block the activity of M1 macrophages by inhibiting NF-κB, JNK, and other signaling pathways in macrophages, reducing their infiltration in adipose tissue, thereby improving the body's sensitivity to insulin (96, 97). Nevertheless, the models used in most studies are mice, which did not enter clinical trials.

However, most clinical research reduces the level of inflammatory factors secreted by macrophages through inflammatory factor inhibitors to treat insulin resistance. TNF-α is the first pro-inflammatory cytokine involved in insulin resistance, but limited data can show that TNF-α is involved in glucose regulation in humans. Early research suggested that short-term administration of a single TNF-α antagonist could not modulate blood glucose homeostasis (98, 99). However, 50 patients with obesity-related metabolic diseases were treated with TNF-α inhibitor etanercept for 6 months, which could significantly improve fasting blood glucose and increase adiponectin content in blood (100). The mechanism by which TNF-α inhibitors improve blood sugar still needs further investigation.

The interaction of CCR2 with its ligand MCP-1 affects monocyte migration into tissues and regulates monocyte-to-macrophage differentiation, producing pro-inflammatory cytokines and amplifying adipose tissue inflammation (20). Accumulating studies in mice have demonstrated that CCR2 selective inhibitors or CCR2/5 inhibitors can significantly improve type 2 diabetes (101–104). Combined with CCR2 inhibitors, metformin can treat diabetes by lowering blood sugar and inhibiting inflammation. A clinical trial involving 332 diabetic nephropathy patients showed that based on standard treatment, taking the CCR2 selective inhibitor, CCX140-B, could further reduce urinary protein and protect the kidneys. Compared with the placebo group, fasting blood glucose levels were significantly lower in the inhibitor group compared to the placebo group, although there was little change in HbA1C level (102). TRIM29 inhibits the secretion of IL6 and CCL2/5 in alveolar macrophages (105). CCL2/CCR2 is not the only pathway affecting the recruitment and differentiation of macrophages. The chemokine regulatory network is very complex, with CCR1-CCL3/4/5, CX3CR1-CX3CL1, and CXCR3-CXCL10 involved in macrophage differentiation. Therefore, utilizing CCR2 inhibitors to regulate macrophages to improve insulin resistance requires the support of more clinical trial data.

CONCLUDING REMARKS AND PERSPECTIVES

The infiltration of pro-inflammatory macrophages in adipose tissue increases in obesity, and many inflammatory factors are secreted, resulting in adipose tissue inflammation. Inflammatory responses inhibit adipocyte insulin signaling, leading to insulin resistance. An adipose tissue macrophage is a key factor in obesity-induced insulin resistance by regulating a series of insulin-related and inflammatory factor-related signaling pathways through paracrine interactions between adipocytes and macrophages. In recent years, adipose macrophages have become a research hotspot based on their important role in insulin resistance. The in-depth study of macrophages has added new insights to the pathogenesis of metabolic diseases. In different microenvironments or under different stimuli, macrophages can show different activation modes and polarize into subtypes with different functions. Each subtype is involved in obesity, insulin resistance, T2MD, and other diseases such as atherosclerosis and severe acute pancreatitis (SAP). Therefore, the polarization direction of macrophages can be induced by regulating various factors affecting the polarization of macrophages, thereby stabilizing the balance between M1/M2 types of macrophages *in vivo*, which will make macrophages a potential new target for the treatment of metabolic diseases and bring a boon to human health (**Figure 4**).

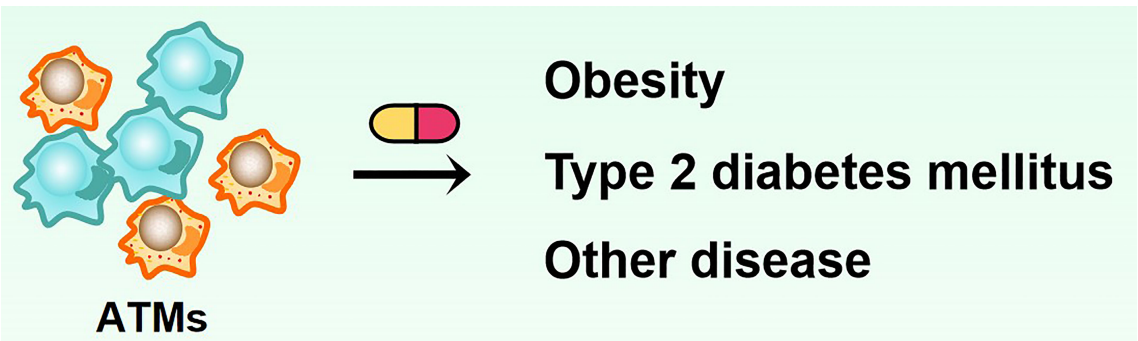

FIGURE 4 | Adipose tissue macrophages can be used as a potential therapeutic target for treating obesity and diabetes.

AUTHOR CONTRIBUTIONS

CZ contributed directly to this review. WL wrote the preliminary version of the manuscript. YQ and WL polished the manuscript's language and prepared figures. All authors were involved in the manuscript preparation, including figure modification, paper discussion, manuscript writing, and editing. All authors have read and approved the final manuscript.

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REFERENCES

1. Koliaki C, Liatis S, Kokkinos A. Obesity and Cardiovascular Disease: Revisiting an Old Relationship. *Metabolism* (2019) 92:98–107. doi: 10.1016/j.metabol.2018.10.011
2. Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, Esquivel-Soto J, Morales-González A, Esquivel-Chirino C, et al. Inflammation, Oxidative Stress, and Obesity. *Int J Mol Sci* (2011) 12(5):3117–32. doi: 10.3390/ijms12053117
3. Powell-Wiley TM, Poirier P, Burke LE, Després JP, Gordon-Larsen P, Lavie CJ, et al. Obesity and Cardiovascular Disease: A Scientific Statement From the American Heart Association. *Circulation* (2021) 143(21):e984–e1010. doi: 10.1161/CIR.0000000000000973
4. Khan S, Luck H, Winer S, Winer DA. Emerging Concepts in Intestinal Immune Control of Obesity-Related Metabolic Disease. *Nat Commun* (2021) 12(1):2598. doi: 10.1038/s41467-021-22727-7
5. Spiegelman BM, Hotamisligil GS. Through Thick and Thin: Wasting, Obesity, and TNF Alpha. *Cell* (1993) 73(4):625–7. doi: 10.1016/0092-8674(93)90243-J
6. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW, et al. Obesity is Associated With Macrophage Accumulation in Adipose Tissue. *J Clin Invest* (2003) 112(12):1796–808. doi: 10.1172/JCI200319246
7. Amano SU, Cohen JL, Vangala P, Tencerova M, Nicoloro SM, Yawe JC, et al. Local Proliferation of Macrophages Contributes to Obesity-Associated Adipose Tissue Inflammation. *Cell Metab* (2014) 19(1):162–71. doi: 10.1016/j.cmet.2013.11.017
8. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage Activation and Polarization: Nomenclature and Experimental Guidelines. *Immunity* (2014) 41(1):14–20. doi: 10.1016/j.immuni.2014.06.008
9. Lumeng CN, Bodzin JL, Saltiel AR. Obesity Induces a Phenotypic Switch in Adipose Tissue Macrophage Polarization. *J Clin Invest* (2007) 117(1):175–84. doi: 10.1172/JCI29881
10. Hill AA, Reid Bolus W, Hasty AH. A Decade of Progress in Adipose Tissue Macrophage Biology. *Immunol Rev* (2014) 262(1):134–52. doi: 10.1111/imr.12216
11. Meshkani R, Vakili S. Tissue Resident Macrophages: Key Players in the Pathogenesis of Type 2 Diabetes and its Complications. *Clin Chim Acta* (2016) 462:77–89. doi: 10.1016/j.cca.2016.08.015
12. Castoldi A, Naffah de Souza C, Câmara NO, Moraes-Vieira PM. The Macrophage Switch in Obesity Development. *Front Immunol* (2015) 6:637. doi: 10.3389/fimmu.2015.00637
13. Veit M, van Asten R, Olie A, Prinz P. The Role of Dietary Sugars, Overweight, and Obesity in Type 2 Diabetes Mellitus: A Narrative Review. *Eur J Clin Nutr* (2022). doi: 10.1038/s41430-022-01114-5
14. Ye J. Mechanism of Insulin Resistance in Obesity: A Role of ATP. *Front Med* (2021) 15(3):372–82. doi: 10.1007/s11684-021-0862-5
15. Ray I, Mahata SK, De RK. Obesity: An Immunometabolic Perspective. *Front Endocrinol (Lausanne)* (2016) 7:157. doi: 10.3389/fendo.2016.00157
16. Nagareddy PR, Kraakman M, Masters SL, Stirzaker RA, Gorman DJ, Grant RW, et al. Adipose Tissue Macrophages Promote Myelopoiesis and Monocytosis in Obesity. *Cell Metab* (2014) 19(5):821–35. doi: 10.1016/j.cmet.2014.03.029
17. Epelman S, Lavine KJ, Randolph GJ. Origin and Functions of Tissue Macrophages. *Immunity* (2014) 41(1):21–35. doi: 10.1016/j.immuni.2014.06.013
18. Wang N, Liang H, Zen K. Molecular Mechanisms That Influence the Macrophage M1-M2 Polarization Balance. *Front Immunol* (2014) 5:614. doi: 10.3389/fimmu.2014.00614
19. Chylikova J, Dvorackova J, Tauber Z, Kamarad V. M1/M2 Macrophage Polarization in Human Obese Adipose Tissue. *BioMed Pap Med Fac Univ Palacky Olomouc Czech Repub* (2018) 162(2):79–82. doi: 10.5507/bp.2018.015
20. Engin AB. Adipocyte-Macrophage Cross-Talk in Obesity. *Adv Exp Med Biol* (2017) 960:327–43. doi: 10.1007/978-3-319-48382-5_14
21. Thomas D, Apovian C. Macrophage Functions in Lean and Obese Adipose Tissue. *Metabolism* (2017) 72:120–43. doi: 10.1016/j.metabol.2017.04.005
22. Van den Bossche J, Baardman J, Otto NA, van der Velden S, Neele AE, van den Berg SM, et al. Mitochondrial Dysfunction Prevents Repolarization of Inflammatory Macrophages. *Cell Rep* (2016) 17(3):684–96. doi: 10.1016/j.celrep.2016.09.008
23. Makki K, Froguel P, Wolowczuk I. Adipose Tissue in Obesity-Related Inflammation and Insulin Resistance: Cells, Cytokines, and Chemokines. *ISRN Inflammation* (2013) 2013:139239. doi: 10.1155/2013/139239
24. Oeser K, Schwartz C, Voehringer D. Conditional IL-4/IL-13-Deficient Mice Reveal a Critical Role of Innate Immune Cells for Protective Immunity Against Gastrointestinal Helminths. *Mucosal Immunol* (2015) 8(3):672–82. doi: 10.1038/mi.2014.101
25. Xie L, Fu Q, Ortega TM, Zhou L, Rasmussen D, O'Keefe J, et al. Overexpression of IL-10 in C2D Macrophages Promotes a Macrophage Phenotypic Switch in Adipose Tissue Environments. *PLoS One* (2014) 9(1):e86541. doi: 10.1371/journal.pone.0086541
26. Yoshida S, Kobayashi Y, Nakama T, Zhou Y, Ishikawa K, Arita R, et al. Increased Expression of M-CSF and IL-13 in Vitreous of Patients With Proliferative Diabetic Retinopathy: Implications for M2 Macrophage-Involving Fibrovascular Membrane Formation. *Br J Ophthalmol* (2015) 99(5):629–34. doi: 10.1136/bjophthalmol-2014-305860
27. Zdrenghea MT, Makrinioti H, Muresan A, Johnston SL, Stanciu LA. The Role of Macrophage IL-10/Innate IFN Interplay During Virus-Induced Asthma. *Rev Med Virol* (2015) 25(1):33–49. doi: 10.1002/rmv.1817
28. Li D, Guabiraba R, Besnard AG, Komai-Koma M, Jabir MS, Zhang L, et al. IL-33 Promotes ST2-Dependent Lung Fibrosis by the Induction of Alternatively Activated Macrophages and Innate Lymphoid Cells in Mice. *J Allergy Clin Immunol* (2014) 134(6):1422–32.e11. doi: 10.1016/j.jaci.2014.05.011
29. Chmelar J, Chung KJ, Chavakis T. The Role of Innate Immune Cells in Obese Adipose Tissue Inflammation and Development of Insulin Resistance. *Thromb Haemost* (2013) 109(3):399–406. doi: 10.1160/TH12-09-0703
30. Wu H, Perrard XD, Wang Q, Perrard JL, Polsani VR, Jones PH. CD11c Expression in Adipose Tissue and Blood and its Role in Diet-Induced Obesity. *Arterioscler Thromb Vasc Biol* (2010) 30(2):186–92. doi: 10.1161/ATVBAHA.109.198044
31. Burotto M, Chiou VL, Lee JM, Kohn EC. The MAPK Pathway Across Different Malignancies: A New Perspective. *Cancer* (2014) 120(22):3446–56. doi: 10.1002/cncr.28864
32. Blüher M, Bashan N, Shai I, Harman-Boehm I, Tarnovscki T, Avinaoch E, et al. Activated Ask1-MKK4-P38mapk/JNK Stress Signaling Pathway in Human Omental Fat Tissue may Link Macrophage Infiltration to Whole-

Body Insulin Sensitivity. *J Clin Endocrinol Metab* (2009) 94(7):2507–15. doi: 10.1210/jc.2009-0002
33. Nakamitsu PZ, Compri CM, de Fraia Pinto L, Gotardo ÉM, de Oliveira CC, Ribeiro ML, et al. Thalidomide Controls Adipose Tissue Inflammation Associated With High-Fat Diet-Induced Obesity in Mice. *Endocr Metab Immune Disord Drug Targets* (2015) 15(2):151–8. doi: 10.2174/1871530314666141128115225
34. Han MS, Jung DY, Morel C, Lakhani SA, Kim JK, Flavell RA, et al. JNK Expression by Macrophages Promotes Obesity-Induced Insulin Resistance and Inflammation. *Science* (2013) 339(6116):218–22. doi: 10.1126/science.1227568
35. Fitzgerald KA, Kagan JC. Toll-Like Receptors and the Control of Immunity. *Cell* (2020) 180(6):1044–66. doi: 10.1016/j.cell.2020.02.041
36. Catalán V, Gómez-Ambrosi J, Rodríguez A, Ramírez B, Rotellar F, Valentí V, et al. Increased Tenascin C and Toll-Like Receptor 4 Levels in Visceral Adipose Tissue as a Link Between Inflammation and Extracellular Matrix Remodeling in Obesity. *J Clin Endocrinol Metab* (2012) 97(10):E1880–9. doi: 10.1210/jc.2012-1670
37. Poggi M, Bastelica D, Gual P, Iglesias MA, Gremeaux T, Knauf C, et al. C3H/HeJ Mice Carrying a Toll-Like Receptor 4 Mutation are Protected Against the Development of Insulin Resistance in White Adipose Tissue in Response to a High-Fat Diet. *Diabetologia* (2007) 50(6):1267–76. doi: 10.1007/s00125-007-0654-8
38. Ni W, Zhang Q, Liu G, Wang F, Yuan H, Guo Y, et al. Escherichia Coli Maltose-Binding Protein Activates Mouse Peritoneal Macrophages and Induces M1 Polarization via TLR2/4 In Vivo and In Vitro. *Int Immunopharmacol* (2014) 21(1):171–80. doi: 10.1016/j.intimp.2014.04.025
39. Barnabei L, Laplantine E, Mbongo W, Rieux-Laucat F, Weil R. NF-κb: At the Borders of Autoimmunity and Inflammation. *Front Immunol* (2021) 12:716469. doi: 10.3389/fimmu.2021.716469
40. Park KS. Aucubin, a Naturally Occurring Iridoid Glycoside Inhibits TNF-α-Induced Inflammatory Responses Through Suppression of NF-κb Activation in 3T3-L1 Adipocytes. *Cytokine* (2013) 62(3):407–12. doi: 10.1016/j.cyto.2013.04.005
41. Hsu CL, Lin YJ, Ho CT, Yen GC. The Inhibitory Effect of Pterostilbene on Inflammatory Responses During the Interaction of 3T3-L1 Adipocytes and RAW 264.7 Macrophages. *J Agric Food Chem* (2013) 61(3):602–10. doi: 10.1021/jf304487v
42. Turner JJ, Foxwell KM, Kanji R, Brenner C, Wood S, Foxwell BM, et al. Investigation of Nuclear Factor-κb Inhibitors and Interleukin-10 as Regulators of Inflammatory Signalling in Human Adipocytes. *Clin Exp Immunol* (2010) 162(3):487–93. doi: 10.1111/j.1365-2249.2010.04260.x
43. Cao C, Li L, Chen W, Zhu Y, Qi Y, Wang X, et al. Deficiency of Ikkε Inhibits Inflammation and Induces Cardiac Protection in High-Fat Diet-Induced Obesity in Mice. *Int J Mol Med* (2014) 34(1):244–52. doi: 10.3892/ijmm.2014.1746
44. Linden MA, Pincu Y, Martin SA, Woods JA, Baynard T. Moderate Exercise Training Provides Modest Protection Against Adipose Tissue Inflammatory Gene Expression in Response to High-Fat Feeding. *Physiol Rep* (2014) 2(7). doi: 10.14814/phy2.12071
45. Gabriel TL, Tol MJ, Ottenhof R, van Roomen C, Aten J, Claessen N, et al. Lysosomal Stress in Obese Adipose Tissue Macrophages Contributes to MITF-Dependent Gpnmb Induction. *Diabetes* (2014) 63(10):3310–23. doi: 10.2337/db13-1720
46. Shin E, Shin S, Kong H, Lee S, Do SG, Jo TH, et al. Dietary Aloe Reduces Adipogenesis via the Activation of AMPK and Suppresses Obesity-Related Inflammation in Obese Mice. *Immune Netw* (2011) 11(2):107–13. doi: 10.4110/in.2011.11.2.107
47. Weng SY, Schuppan D. AMPK Regulates Macrophage Polarization in Adipose Tissue Inflammation and NASH. *J Hepatol* (2013) 58(3):619–21. doi: 10.1016/j.jhep.2012.09.031
48. Kidani Y, Bensinger SJ. Liver X Receptor and Peroxisome Proliferator-Activated Receptor as Integrators of Lipid Homeostasis and Immunity. *Immunol Rev* (2012) 249(1):72–83. doi: 10.1111/j.1600-065X.2012.01153.x
49. Matsushita Y, Ogawa D, Wada J, Yamamoto N, Shikata K, Sato C, et al. Activation of Peroxisome Proliferator-Activated Receptor Delta Inhibits Streptozotocin-Induced Diabetic Nephropathy Through Anti-Inflammatory Mechanisms in Mice. *Diabetes* (2011) 60(3):960–8. doi: 10.2337/db10-1361
50. Hulsmans M, Geeraert B, Arnould T, Tsatsanis C, Holvoet P. PPAR Agonist-Induced Reduction of Mcp1 in Atherosclerotic Plaques of Obese, Insulin-Resistant Mice Depends on Adiponectin-Induced Irak3 Expression. *PLoS One* (2013) 8(4):e62253. doi: 10.1371/journal.pone.0062253
51. Ohno H, Shinoda K, Spiegelman BM, Kajimura S. Pparγ Agonists Induce a White-to-Brown Fat Conversion Through Stabilization of PRDM16 Protein. *Cell Metab* (2012) 15(3):395–404. doi: 10.1016/j.cmet.2012.01.019
52. Penna-de-Carvalho A, Graus-Nunes F, Rabelo-Andrade J, Mandarim-de-Lacerda CA, Souza-Mello V. Enhanced Pan-Peroxisome Proliferator-Activated Receptor Gene and Protein Expression in Adipose Tissue of Diet-Induced Obese Mice Treated With Telmisartan. *Exp Physiol* (2014) 99(12):1663–78. doi: 10.1113/expphysiol.2014.081596
53. Fujisaka S, Usui I, Kanatani Y, Ikutani M, Takasaki I, Tsuneyama K, et al. Telmisartan Improves Insulin Resistance and Modulates Adipose Tissue Macrophage Polarization in High-Fat-Fed Mice. *Endocrinology* (2011) 152(5):1789–99. doi: 10.1210/en.2010-1312
54. Bullers SJ, Baker SC, Ingham E, Southgate J. The Human Tissue-Biomaterial Interface: A Role for Pparγ-Dependent Glucocorticoid Receptor Activation in Regulating the CD163+ M2 Macrophage Phenotype. *Tissue Eng Part A* (2014) 20(17-18):2390–401. doi: 10.1089/ten.tea.2013.0628
55. Lovren F, Pan Y, Quan A, Szmitko PE, Singh KK, Shukla PC, et al. Adiponectin Primes Human Monocytes Into Alternative Anti-Inflammatory M2 Macrophages. *Am J Physiol Heart Circ Physiol* (2010) 299(3):H656–63. doi: 10.1152/ajpheart.00115.2010
56. Ohashi K, Parker JL, Ouchi N, Higuchi A, Vita JA, Gokce N, et al. Adiponectin Promotes Macrophage Polarization Toward an Anti-Inflammatory Phenotype. *J Biol Chem* (2010) 285(9):6153–60. doi: 10.1074/jbc.M109.088708
57. Lumeng CN, DelProposto JB, Westcott DJ, Saltiel AR. Phenotypic Switching of Adipose Tissue Macrophages With Obesity is Generated by Spatiotemporal Differences in Macrophage Subtypes. *Diabetes* (2008) 57(12):3239–46. doi: 10.2337/db08-0872
58. Tanaka M, Ikeda K, Suganami T, Komiya C, Ochi K, Shirakawa I, et al. Macrophage-Inducible C-Type Lectin Underlies Obesity-Induced Adipose Tissue Fibrosis. *Nat Commun* (2014) 5:4982. doi: 10.1038/ncomms5982
59. Tanaka M. Molecular Mechanism of Obesity-Induced Adipose Tissue Inflammation; the Role of Mincle in Adipose Tissue Fibrosis and Ectopic Lipid Accumulation. *Endocr J* (2020) 67(2):107–11. doi: 10.1507/endocrj.EJ19-0417
60. Ichioka M, Suganami T, Tsuda N, Shirakawa I, Hirata Y, Satoh-Asahara N, et al. Increased Expression of Macrophage-Inducible C-Type Lectin in Adipose Tissue of Obese Mice and Humans. *Diabetes* (2011) 60(3):819–26. doi: 10.2337/db10-0864
61. Maurizi G, Della Guardia L, Maurizi A, Poloni A. Adipocytes Properties and Crosstalk With Immune System in Obesity-Related Inflammation. *J Cell Physiol* (2018) 233(1):88–97. doi: 10.1002/jcp.25855
62. Gutierrez DA, Kennedy A, Orr JS, Anderson EK, Webb CD, Gerrald WK, et al. Aberrant Accumulation of Undifferentiated Myeloid Cells in the Adipose Tissue of CCR2-Deficient Mice Delays Improvements in Insulin Sensitivity. *Diabetes* (2011) 60(11):2820–9. doi: 10.2337/db11-0314
63. Li P, Oh DY, Bandyopadhyay G, Lagakos WS, Talukdar S, Osborn O, et al. LTB4 Promotes Insulin Resistance in Obese Mice by Acting on Macrophages, Hepatocytes and Myocytes. *Nat Med* (2015) 21(3):239–47. doi: 10.1038/nm.3800
64. Spite M, Hellmann J, Tang Y, Mathis SP, Kosuri M, Bhatnagar A, et al. Deficiency of the Leukotriene B4 Receptor, BLT-1, Protects Against Systemic Insulin Resistance in Diet-Induced Obesity. *J Immunol* (2011) 187(4):1942–9. doi: 10.4049/jimmunol.1100196
65. Arai S, Miyazaki T. Impacts of the Apoptosis Inhibitor of Macrophage (AIM) on Obesity-Associated Inflammatory Diseases. *Semin Immunopathol* (2014) 36(1):3–12. doi: 10.1007/s00281-013-0405-5
66. Komohara Y, Fujiwara Y, Ohnishi K, Shiraishi D, Takeya M. Contribution of Macrophage Polarization to Metabolic Diseases. *J Atheroscler Thromb* (2016) 23(1):10–7. doi: 10.5551/jat.32359
67. Shimizu I, Yoshida Y, Moriya J, Nojima A, Uemura A, Kobayashi Y, et al. Semaphorin3E-Induced Inflammation Contributes to Insulin Resistance in Dietary Obesity. *Cell Metab* (2013) 18(4):491–504. doi: 10.1016/j.cmet.2013.09.001

68. Nomiyama T, Perez-Tilve D, Ogawa D, Gizard F, Zhao Y, Heywood EB, et al. Osteopontin Mediates Obesity-Induced Adipose Tissue Macrophage Infiltration and Insulin Resistance in Mice. *J Clin Invest* (2007) 117 (10):2877–88. doi: 10.1172/JCI31986
69. Zhuang G, Meng C, Guo X, Cheruku PS, Shi L, Xu H, et al. A Novel Regulator of Macrophage Activation: miR-223 in Obesity-Associated Adipose Tissue Inflammation. *Circulation* (2012) 125(23):2892–903. doi: 10.1161/CIRCULATIONAHA.111.087817
70. Galic S, Fullerton MD, Schertzer JD, Sikkema S, Marcinko K, Walkley CR, et al. Hematopoietic AMPK β1 Reduces Mouse Adipose Tissue Macrophage Inflammation and Insulin Resistance in Obesity. *J Clin Invest* (2011) 121 (12):4903–15. doi: 10.1172/JCI58577
71. Mauer J, Chaurasia B, Goldau J, Vogt MC, Ruud J, Nguyen KD, et al. Signaling by IL-6 Promotes Alternative Activation of Macrophages to Limit Endotoxemia and Obesity-Associated Resistance to Insulin. *Nat Immunol* (2014) 15(5):423–30. doi: 10.1038/ni.2865
72. Martinez-Santibañez G, Lumeng CN. Macrophages and the Regulation of Adipose Tissue Remodeling. *Annu Rev Nutr* (2014) 34:57–76. doi: 10.1146/annurev-nutr-071812-161113
73. Kuroda M, Sakaue H. Adipocyte Death and Chronic Inflammation in Obesity. *J Med Invest* (2017) 64(3.4):193–6. doi: 10.2152/jmi.64.193
74. Ramkhelawon B, Hennessy EJ, Ménager M, Ray TD, Sheedy FJ, Hutchison S, et al. Netrin-1 Promotes Adipose Tissue Macrophage Retention and Insulin Resistance in Obesity. *Nat Med* (2014) 20(4):377–84. doi: 10.1038/nm.3467
75. Russo L, Lumeng CN. Properties and Functions of Adipose Tissue Macrophages in Obesity. *Immunology* (2018) 155(4):407–17. doi: 10.1111/imm.13002
76. Herrada AA, Olate-Briones A, Rojas A, Liu C, Escobedo N, Piesche M, et al. Adipose Tissue Macrophages as a Therapeutic Target in Obesity-Associated Diseases. *Obes Rev* (2021) 22(6):e13200. doi: 10.1111/obr.13200
77. Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG. Ablation of CD11c-Positive Cells Normalizes Insulin Sensitivity in Obese Insulin Resistant Animals. *Cell Metab* (2008) 8(4):301–9. doi: 10.1016/j.cmet.2008.08.015
78. Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, et al. CCR2 Modulates Inflammatory and Metabolic Effects of High-Fat Feeding. *J Clin Invest* (2006) 116(1):115–24. doi: 10.1172/JCI24335
79. Pal D, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, et al. Fetuin-A Acts as an Endogenous Ligand of TLR4 to Promote Lipid-Induced Insulin Resistance. *Nat Med* (2012) 18(8):1279–85. doi: 10.1038/nm.2851
80. Nguyen MT, Favelyukis S, Nguyen AK, Reichart D, Scott PA, Jenn A, et al. A Subpopulation of Macrophages Infiltrates Hypertrophic Adipose Tissue and is Activated by Free Fatty Acids via Toll-Like Receptors 2 and 4 and JNK-Dependent Pathways. *J Biol Chem* (2007) 282(48):35279–92. doi: 10.1074/jbc.M706762200
81. Solinas G, Karin M. JNK1 and IKKbeta: Molecular Links Between Obesity and Metabolic Dysfunction. *FASEB J* (2010) 24(8):2596–611. doi: 10.1096/fj.09-151340
82. Gao Z, Zuberi A, Quon MJ, Dong Z, Ye J. Aspirin Inhibits Serine Phosphorylation of Insulin Receptor Substrate 1 in Tumor Necrosis Factor-Treated Cells Through Targeting Multiple Serine Kinases. *J Biol Chem* (2003) 278(27):24944–50. doi: 10.1074/jbc.M300423200
83. Miao H, Ou J, Ma Y, Guo F, Yang Z, Wiggins M, et al. Macrophage CGI-58 Deficiency Activates ROS-Inflammasome Pathway to Promote Insulin Resistance in Mice. *Cell Rep* (2014) 7(1):223–35. doi: 10.1016/j.celrep.2014.02.047
84. Vandanmagsar B, Youm YH, Ravussin A, Galgani JE, Stadler K, Mynatt RL, et al. The NLRP3 Inflammasome Instigates Obesity-Induced Inflammation and Insulin Resistance. *Nat Med* (2011) 17(2):179–88. doi: 10.1038/nm.2279
85. Toita R, Kawano T, Murata M, Kang JH. Anti-Obesity and Anti-Inflammatory Effects of Macrophage-Targeted Interleukin-10-Conjugated Liposomes in Obese Mice. *Biomaterials* (2016) 110:81–8. doi: 10.1016/j.biomaterials.2016.09.018
86. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, et al. Macrophage-Specific PPARgamma Controls Alternative Activation and Improves Insulin Resistance. *Nature* (2007) 447(7148):1116–20. doi: 10.1038/nature05894
87. Bouhlel MA, Derudas B, Rigamonti E, Dièvart R, Brozek J, Haulon S, et al. PPARgamma Activation Primes Human Monocytes Into Alternative M2 Macrophages With Anti-Inflammatory Properties. *Cell Metab* (2007) 6 (2):137–43. doi: 10.1016/j.cmet.2007.06.010
88. Liao X, Sharma N, Kapadia F, Zhou G, Lu Y, Hong H, et al. Krüppel-Like Factor 4 Regulates Macrophage Polarization. *J Clin Invest* (2011) 121 (7):2736–49. doi: 10.1172/JCI45444
89. Ying W, Riopel M, Bandyopadhyay G, Dong Y, Birmingham A, Seo JB, et al. Adipose Tissue Macrophage-Derived Exosomal miRNAs Can Modulate In Vivo and In Vitro Insulin Sensitivity. *Cell* (2017) 171(2):372–384.e12. doi: 10.1016/j.cell.2017.08.035
90. Liu T, Sun YC, Cheng P, Shao HG. Adipose Tissue Macrophage-Derived Exosomal miR-29a Regulates Obesity-Associated Insulin Resistance. *Biochem Biophys Res Commun* (2019) 515(2):352–8. doi: 10.1016/j.bbrc.2019.05.113
91. Kell J. The Addition of Gemtuzumab Ozogamicin to Chemotherapy in Adult Patients With Acute Myeloid Leukemia. *Expert Rev Anticancer Ther* (2016) 16(4):377–82. doi: 10.1586/14737140.2016.1162099
92. Woodcock KJ, Kierdorf K, Pouchelon CA, Vivancos V, Dionne MS, Geissmann F, et al. Macrophage-Derived Upd3 Cytokine Causes Impaired Glucose Homeostasis and Reduced Lifespan in Drosophila Fed a Lipid-Rich Diet. *Immunity* (2015) 42(1):133–44. doi: 10.1016/j.immuni.2014.12.023
93. Kishore P, Li W, Tonelli J, Lee DE, Koppaka S, Zhang K, et al. Adipocyte-Derived Factors Potentiate Nutrient-Induced Production of Plasminogen Activator Inhibitor-1 by Macrophages. *Sci Transl Med* (2010) 2(20):20ra15. doi: 10.1126/scitranslmed.3000292
94. Nishimura S, Manabe I, Nagasaki M, Eto K, Yamashita H, Ohsugi M, et al. CD8+ Effector T Cells Contribute to Macrophage Recruitment and Adipose Tissue Inflammation in Obesity. *Nat Med* (2009) 15(8):914–20. doi: 10.1038/nm.1964
95. McNelis JC, Olefsky JM. Macrophages, Immunity, and Metabolic Disease. *Immunity* (2014) 41(1):36–48. doi: 10.1016/j.immuni.2014.05.010
96. Ye L, Liang S, Guo C, Yu X, Zhao J, Zhang H, et al. Inhibition of M1 Macrophage Activation in Adipose Tissue by Berberine Improves Insulin Resistance. *Life Sci* (2016) 166:82–91. doi: 10.1016/j.lfs.2016.09.025
97. Zhang C, Qian D, Zhao H, Lv N, Yu P, Sun Z, et al. MiR17 Improves Insulin Sensitivity Through Inhibiting Expression of ASK1 and Anti-Inflammation of Macrophages. *BioMed Pharmacother* (2018) 100:448–54. doi: 10.1016/j.biopha.2018.02.012
98. Paquot N, Castillo MJ, Lefèbvre PJ, Scheen AJ. No Increased Insulin Sensitivity After a Single Intravenous Administration of a Recombinant Human Tumor Necrosis Factor Receptor: Fc Fusion Protein in Obese Insulin-Resistant Patients. *J Clin Endocrinol Metab* (2000) 85(3):1316–9. doi: 10.1210/jcem.85.3.6417
99. Ofei F, Hurel S, Newkirk J, Sopwith M, Taylor R. Effects of an Engineered Human Anti-TNF-Alpha Antibody (CDP571) on Insulin Sensitivity and Glycemic Control in Patients With NIDDM. *Diabetes* (1996) 45(7):881–5. doi: 10.2337/diab.45.7.881
100. Stanley TL, Zanni MV, Johnsen S, Rasheed S, Makimura H, Lee H, et al. TNF-Alpha Antagonism With Etanercept Decreases Glucose and Increases the Proportion of High Molecular Weight Adiponectin in Obese Subjects With Features of the Metabolic Syndrome. *J Clin Endocrinol Metab* (2011) 96 (1):E146–50. doi: 10.1210/jc.2010-1170
101. Sullivan T, Miao Z, Dairaghi DJ, Krasinski A, Wang Y, Zhao BN, et al. CCR2 Antagonist CCX140-B Provides Renal and Glycemic Benefits in Diabetic Transgenic Human CCR2 Knockin Mice. *Am J Physiol Renal Physiol* (2013) 305(9):F1288–97. doi: 10.1152/ajprenal.00316.2013
102. de Zeeuw D, Bekker P, Henkel E, Hasslacher C, Gouni-Berthold I, Mehling H, et al. The Effect of CCR2 Inhibitor CCX140-B on Residual Albuminuria in Patients With Type 2 Diabetes and Nephropathy: A Randomised Trial. *Lancet Diabetes Endocrinol* (2015) 3(9):687–96. doi: 10.1016/S2213-8587(15)00261-2
103. Kang YS, Lee MH, Song HK, Ko GJ, Kwon OS, Lim TK, et al. CCR2 Antagonism Improves Insulin Resistance, Lipid Metabolism, and Diabetic Nephropathy in Type 2 Diabetic Mice. *Kidney Int* (2010) 78(9):883–94. doi: 10.1038/ki.2010.263
104. Sullivan TJ, Miao Z, Zhao BN, Ertl LS, Wang Y, Krasinski A, et al. Experimental Evidence for the Use of CCR2 Antagonists in the Treatment of Type 2 Diabetes. *Metabolism* (2013) 62(11):1623–32. doi: 10.1016/j.metabol.2013.06.008

105. Xing J, Weng L, Yuan B, Wang Z, Jia L, Jin R, et al. Identification of a Role for TRIM29 in the Control of Innate Immunity in the Respiratory Tract. *Nat Immunol* (2016) 17(12):1373–80. doi: 10.1038/ni.3580

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Adipose tissue macrophages in remote modulation of hepatic glucose production

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Hepatic glucose production (HGP) is fine-regulated *via* glycogenolysis or gluconeogenesis to maintain physiological concentration of blood glucose during fasting-feeding cycle. Aberrant HGP leads to hyperglycemia in obesity-associated diabetes. Adipose tissue cooperates with the liver to regulate glycolipid metabolism. During these processes, adipose tissue macrophages (ATMs) change their profiles with various physio-pathological settings, producing diverse effects on HGP. Here, we briefly review the distinct phenotypes of ATMs under different nutrition states including feeding, fasting or overnutrition, and detail their effects on HGP. We discuss several pathways by which ATMs regulate hepatic gluconeogenesis or glycogenolysis, leading to favorable or unfavorable metabolic consequences. Furthermore, we summarize emerging therapeutic targets to correct metabolic disorders in morbid obesity or diabetes based on ATM-HGP axis. This review puts forward the importance and flexibility of ATMs in regulating HGP, proposing ATM-based HGP modulation as a potential therapeutic approach for obesity-associated metabolic dysfunction.

KEYWORDS

hepatic glucose production, adipose tissue, macrophage, liver, gluconeogenesis

Introduction

Glucose is an essential energy source for most tissue cells in mammals. The circulation levels of glucose are strictly controlled within a stable range in healthy individuals. Abnormally low level of blood glucose, known as hypoglycemia, produces deleterious effects on physiological functions of various tissues and organs. Severe hypoglycemia substantially reduces glucose supply to the brain, resulting in central nervous system damage with consequent seizures, coma and death. In contrast, chronic hyperglycemia, a state of long-term high blood glucose usually in diabetes mellitus, may lead to dysfunction and/or failure of various organs (1). Thus, glucose homeostasis is critical for physiological fasting-feeding cycle particularly in overnutrition state.

During the fed state, dietary intake supplies exogenous glucose to provide energy; while under fasting conditions, endogenous glucose production becomes an indispensable way to maintain blood glucose levels. The liver produces endogenous glucose that accounts for the largest amount of glucose output. As such, the capacity for hepatic glucose production (HGP) plays a crucial role in maintaining blood glucose during fasting. Contrarily, aberrant elevations of HGP lead to hyperglycemia and associated metabolic disorders, particularly in individuals with morbid obesity and associated diabetes mellitus (2, 3). Thus, HGP modulation is a pivotal approach for the treatment of obesity-associated diabetes.

Adipose tissue closely interacts with the liver to regulate energy metabolism. In the fed state, triglycerides synthesized in hepatocytes are packed into VLDL and delivered into adipose tissue for storage (4). In the fasted state, the lipolysis of adipose tissue supplies the liver with non-esterified fatty acids (NEFAs) and glycerol that serve as the precursors of HGP (4–6). Besides metabolic products, adipose tissue communicates with the liver by exchanging diverse information including adipokines and extracellular vesicles (EVs) (5, 7). During these interactions, macrophages exhibit high heterogeneity in dynamic adipose tissue niches, playing flexible roles in metabolic and immune processes. Accumulating studies are unraveling the involvement of adipose tissue macrophages (ATMs) in hepatic glycolipid metabolism. Here, we review their emerging roles in modulating HGP in the context of physiological fasting-feeding cycle as well as obesity or diabetes. Related ATM profiles and regulatory mechanisms for hepatic gluconeogenesis are discussed.

HGP

Hepatocytes produce endogenous glucose relying on gluconeogenesis and glycogenolysis. The former generates glucose from non-carbohydrate substrates, while the latter produces glucose through glycogen breakdown. Both glycogenolysis and gluconeogenesis contribute to HGP during fasting period, whereas glycogenolysis only exists during early fasting stages due to limited glycogen storage (3, 4, 8, 9).

Hepatic gluconeogenesis

Gluconeogenesis is a biological process of glucose generation from non-carbohydrate precursors such as glycerol, lactate, pyruvate and glucogenic amino acids (8, 10). As part of the reverse reaction of glycolysis, gluconeogenesis requires four critical enzymes to bypass the irreversible steps. Pyruvate carboxylase (PC) carboxylates pyruvate derived from glycolysis into oxaloacetate, which is decarboxylated by phosphoenolpyruvate carboxykinase (PEPCK) to form phosphoenolpyruvate. Fructose-1,6-bisphosphatase catalyzes fructose 1,6-bisphosphate into fructose 6-phosphate; while glucose 6-phosphatase (G6Pase) dephosphorylates glucose-6-phosphate (G6P) into glucose (8, 11, 12). Hepatic gluconeogenesis is strictly regulated by hormones in different nutrition states. These hormones regulate gluconeogenic gene expression by promoting or suppressing the binding of specific transcription factors with their promoters. To date, multiple transcription factors including CREB, PGC-1α, FOXO1 have been identified to promote PEPCK and/or G6Pase transactivation (13). In fasting conditions, glucagon secretion is increased whilst insulin levels decreased. Upon binding to their receptors on hepatocytes, glucagon activates cAMP-PKA signaling to mediate CREB activation, thereby promoting PEPCK and G6Pase transcriptions. CREB-mediated hepatic gluconeogenesis can be further enhanced by coactivator p300/CBP and CRTC2. In addition, CREB stimulates PGC-1α expression, another coactivator to cooperate with FOXO1 in promoting PEPCK and G6Pase transcriptions. During the fed state, insulin secretion is stimulated whilst glucagon production is inhibited. Insulin signaling mediates downstream AKT activation that phosphorylates FOXO1 for degradation, thereby reducing PEPCK and G6Pase transactivation (4, 9, 14–16). Fasting-induced glucocorticoid also contributes to hepatic gluconeogenesis through direct transactivation of PEPCK or G6Pase (17, 18). In case of hormone dysregulation, for instance, insulin resistance in human or animals with type 2 diabetes leads to the failure of FOXO1 degradation, causing hyperglycemia *via* abnormal gluconeogenesis (16, 19).

Hepatic glycogenolysis

Glycogenolysis is a process of breaking down glycogen into glucose. Glycogen phosphorylase breaks down glycogen into glucos-1-phosphate, and the latter is converted into G6P by phosphoglucomutase, which is hydrolyzed into glucose by G6Pase. Glycogenolysis also indirectly contributes to hepatic gluconeogenesis by supplying glycolytic intermediates. A very recent study on mouse models provides evidence that glycogen in liver and muscle tissue supplies glycolytic intermediate lactic acid, hence contributing to gluconeogenesis even in the fed state (20).

ATMs and their influences on HGP

There are two kinds of typical adipose tissue in mammals, white adipose tissue (WAT) responsible for energy storge and brown adipose tissue (BAT) responsible for energy dissipation (21). WAT has a close crosstalk with the liver to regulate metabolism and immune reactions. Under different pathophysiological conditions, heterogenous ATMs constitute special immune microenvironments, producing broad impacts

on both adipose tissues and liver. Based on phenotypic characteristics, ATMs can be classified into M1 macrophages expressing high levels of proinflammatory cytokines like TNF-α, IL-1, IL-6, iNOS, and M2 macrophages with high levels of anti-inflammatory IL-10 and arginase 1. M2 macrophages are predominant in lean WAT to maintain immune hemostasis in healthy individuals, whereas M1 macrophages are accumulated in obese WAT and mediate adipose tissue inflammation and insulin resistance in metabolically unhealthy individuals. However, during metabolic switch from homeostasis to disorders, some phenotype markers may be overlapped between M1 and M2 macrophages and some potential markers have not been identified or included. There are still some difficulties to depict full and precise ATM profiles based on this simple classification, we therefore specify the phenotypic characteristics of ATMs related to HGP modulation here.

Cytokines from ATMs on HGP Modulation

IL-10 in concert with insulin action: Physiological control of HGP

Insulin is considered as the primary contributor to HGP suppression in the fed state. A recent study adds ATM-derived IL-10 to the list of HGP suppression, providing an elaborate explanation for feeding-induced HGP reduction. An earlier study showed that *in vitro* treatment with IL-10 plus IL-1β inhibited glucose production in primary rat hepatocytes through reducing PEPCK expression, but the underlying mechanisms remained unclear (22). Using different myeloid-specific gene knockout mouse models, Toda et al. demonstrated that macrophages, in response to postprandial signals LPS and insulin, produced IL-10 to suppress gluconeogenic gene expression and hepatocyte glucose production. This inhibitory effect was mediated by IL-10-stimulated Stat3 activation. Feeding induced IL-10 from ATMs, whereas obesity markedly decreased IL-10-producing macrophages in epididymal WAT. Thus, ATM-derived IL-10 contributes to HGP suppression in cooperation with insulin in fed state (Figure 1). Upon obesity, ATMs with low IL-10 production failed to inhibit hepatic gluconeogenesis, resulting in high HGP and hyperglycemia. Importantly, IL-10 restoration successfully lowered the plasma glucose in obese mice, accompanied by the suppression of gluconeogenic genes in the liver (23, 24). Therefore, ATM-derived IL-10 may be a promising indicator to monitor HGP homeostasis during fasting-feeding cycle and a potential therapeutic target for obesity-induced metabolic dysfunction.

TNF-α in association with insulin resistance: Pathological stimulation of HGP

Insulin resistance is the major cause and typical feature of type 2 diabetes, which is closely associated with abnormal hepatocyte gluconeogenesis that contributes to enhanced HGP (16, 25, 26). An early study provided direct evidence that mice received constant infusion of TNF-α showed significant elevation in HGP together with decrease in insulin action and increase in glucagon secretion (27). Later, it was verified that TNF-α expression was significantly upregulated in WAT from rodents and human with obesity or diabetes, and closely

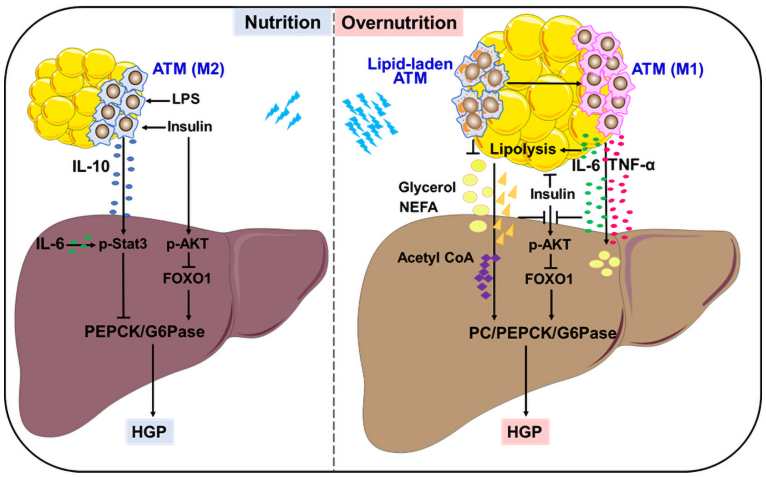

FIGURE 1
Remote modulation on HGP by ATMs. In the fed state, ATMs in response to insulin and LPS produce IL-10 to control gluconeogenesis-mediated HGP. In overnutrition state, ATMs secret large amounts of IL-6 and TNF-α, which link WAT inflammation with lipolysis, resulting in exacerbated HGP through interfering with insulin action or supplying precursors to stimulate aberrant gluconeogenesis. Lipid-associated ATMs play either protective or detrimental roles in controlling aberrant HGP.

associated with systemic insulin resistance. Importantly, TNF-α deficiency provided protection from obesity-induced insulin resistance in mice (28–30). ATMs are remarkably increased in obese WAT, which produce large amounts of TNF-α to drive insulin resistance and interfere with hepatic glycolipid metabolism (31, 32). TNF-α impairs insulin signaling by blocking tyrosine phosphorylation of insulin receptor substrate-1, the failure of AKT activation may facilitate HGP *via* glycogenolysis and gluconeogenesis. On the other hand, TNF-α may induce lipid overload in the liver, either from *de novo* lipogenesis through insulin-independent SREBP1 activation, or from excessive lipolysis of WAT elicited by insulin resistance (31, 33–35). Excessive lipolysis supplies lots of gluconeogenetic precursors like glycerol, NEFAs to promote aberrant HGP. Notably, more lipotoxic intermediators such as saturated palmitic acid and diacylglycerol are generated in these processes, which induce hepatic endoplasmic reticulum stress, inflammation and ensuing insulin resistance (5, 36–39). Therefore, TNF-α from obese ATMs may serve as a strong stimulator for abnormal HGP in obesity and associated diabetes mellitus (Figure 1).

IL-6 in cooperation with lipolysis: Indirect regulation of HGP

Besides TNF-α, IL-6 is another critical cytokine secreted from ATMs in obese condition. But differently, IL-6 regulates HGP in a more complex and flexible manner. To a large extent, the diversity of IL-6 modulation depends on its distinct target organs in different settings, which is more likely influenced by its sources and amounts. In fact, when directly acting on liver or primary hepatocytes, IL-6 at physiological concentrations elicited a suppression in glucagon-stimulated HGP and gluconeogenic genes (40, 41). In line with this, hepatic IL-6 that was induced by insulin action in the brain showed an inhibition on HGP through promoting Stat3 activation and reducing hepatocyte gluconeogenesis *via* PEPCK and G6pase suppression (42–44). Upon targeting adipocytes, IL-6 plays an indirect role in driving HGP process. In response to exercise or early fasting, skeletal muscle-derived IL-6 promotes lipolysis in WAT, thereby providing materials to support HGP (45–47). Of note, in case of morbid obesity, ATMs produce large amounts of IL-6 to stimulate excess WAT lipolysis, which abrogates insulin action on HGP suppression, resulting in hyperglycemia in rodents or adolescents (48, 49). Using *in vivo* metabolomics approach in combination with myeloid-specific JNK-deficient mice, and IL-6 neutralization or infusion treatment, Perry et al. provided evidence that insulin failed to suppress hepatic acetyl CoA, PC activity/flux and subsequent HGP in morbidly obese condition. During this process, IL-6 from ATMs played a key role in potentiating abnormal hepatic gluconeogenesis by increasing WAT lipolysis, leading to uncontrolled increases in hepatic acetyl CoA content and PC activity (Figure 1). These findings provide an alternative explanation for ATM-regulated, lipolysis-dependent HGP in diet-induced obesity, and propose a novel therapeutic target based on ATM-derived IL-6 (49). Moreover, the role of IL-6 in suppressing insulin signaling *via* SOCS3 induction in the liver should also be considered (48, 50, 51), which will not be detailed here.

ATMs on the bridge linking WAT inflammation with hepatic inflammation

As noted above, ATMs make pivotal contribution to hepatic insulin resistance through releasing proinflammatory cytokines. More interestingly, WAT inflammation also triggers hepatic inflammation, producing an overlapped interference on glycolipid homeostasis including HGP process. Through intraperitoneally transplanting visceral WAT from obese mice, Bijnen et al. established evident hepatic inflammation characterized by neutrophil and macrophage accumulation in receipt mice, which contributed to the development of non-alcoholic steatohepatitis (52). When ATMs were deleted from WAT prior to transplantation, this effect could be reversed. This study reveals the key position of ATMs in linking WAT inflammation and hepatic inflammation, in which $CD11c^+$ ATMs that highly express neutrophil chemotactic genes are considered to recruit neutrophils followed by macrophages into the liver (52). In support of this, two earlier studies demonstrated an association of omental ATMs with hepatic inflammation and steatosis in insulin-resistant subjects, as well as the WAT inflammation prior to hepatic inflammation in mice during obesity development (53, 54).

Lipid-laden ATMs on HGP Modulation

Besides the anti- or pro-inflammatory ATMs in lean or obese conditions, a peculiar population of lipid-laden ATMs intersecting metabolism and immunity is significantly upregulated by obesity in human and mouse WAT, particularly visceral WAT (55, 56). These lipid-laden ATMs not only showed an association with human body mass index, but also had an inhibitory effect on insulin signaling of fat explants from lean mice (56). Through analyzing the lipid profiles of ATMs in genetically obese (*ob/ob*) mice that were treated with or without rosiglitazone, Prieur et al. proposed that the cytotoxic lipid species including free cholesterol and saturated triglyceride could induce the switch of ATMs from M2 toward M1 phenotype, thereby resulting in obesity-induced inflammation and insulin resistance (55). In agreement with this, obese mice that received diet switching from high-fat diet to normal chow diet had a rapid normalization of metabolic parameters including reduced hepatic steatosis and gluconeogenesis-based HPG, accompanied by an obvious reduction in lipid-laden ATMs (57). These findings imply a close linkage of lipid-laden ATMs with liver metabolism. Notably, however, accumulating evidences demonstrated that

some lipid-associated ATMs could improve glucose metabolic homeostasis by taking up and sequestering excessive lipids to avoid their damage on both adipose tissue and liver. Silence or deficiency of lipoprotein lipase or Trem2 associated with lipid metabolism abrogated the lipid uptake and storage in ATMs, resulting in insulin resistance and aggravating abnormal HGP *via* gluconeogenesis in obese mice (58, 59). As such, the lipid-associated ATMs may undergo dynamic changes with the development of obesity and have bidirectional regulation on HGP. It is very likely that ATMs assist in handling lipid during the metabolic adaptation to energy surplus, achieving a fine control of HGP through reducing lipid overload and toxicity on adipocytes and hepatocytes. By contrast, in case of lipid overload, toxic lipid species may stimulate ATMs to induce inflammation, insulin resistance and aberrant HPG, thereby producing deleterious effects on metabolic health (60, 61) (Figure 1). In agreement with this, an exosome-dependent transfer of neutral lipid from adipocytes to macrophages has recently been revealed (62, 63), further elucidation on lipid composition and amounts will help to elucidate their protective or detrimental effects on HGP modulation and consequent metabolic health.

Potential ATM-based Modulators for HGP

Given the diversity of ATMs in different nutrition states, emerging ATM-associated modulators for HGP have been identified, and several potential targets are briefly summarized here.

Osteopontin (OPN)

OPN is a secreted matrix glycoprotein significantly upregulated in human and mouse adipose tissue upon obesity, and ATMs are its important source. OPN contributes to ATM accumulation *via* recruitment or proliferation in obese WAT, which is therefore closely associated with obesity-induced WAT inflammation, insulin resistance and HGP. Genetic deletion or antibody neutralization of OPN successfully alleviated ATM infiltration, WAT and hepatic inflammation in obese mice, thus improving insulin sensitivity accompanied by normalization of HGP markers. Of note, FOXO1 decrease mediated by AKT activation and Stat3 activation have been involved in HGP correction, confirming that OPN plays critical role in stimulating hepatic gluconeogenesis, though its direct or indirect effects remain to be carefully investigated (64–67). Interestingly, obese mice with selectively silenced OPN in epididymal ATMs showed an improvement in systemic glucose tolerance, further implying the contribution of ATM-derived OPN to the dysregulation of glucose metabolism. In addition, it should be noted that a population of recruited hepatic macrophages expressing OPN has recently been found to promote the development of non-alcohol fatty liver disease (68), which is warranted to be further studied regarding its regulation on HGP.

Dipeptidyl peptidase 4 (DPP4)

DPP4 is a glycoprotein ubiquitously expressed by many cell types. Given its multiple roles in immune activation and metabolic influence, DPP4 has been considered as a potential therapeutic target for type 2 diabetes. DPP4 has a direct role in enzymatic cleavage of incretin hormone glucagon-like peptide-1, thereby interfering with normal glucose homeostasis. In addition, DPP4 is elevated in obese WAT where both adipocytes and ATMs are counted. Factors related to metabolic stress such as glucocorticoids and oxidized LDL have been reported to induce DPP4 upregulation on macrophages, which can stimulate macrophage inflammation and T cell activation, hence potentiating WAT inflammation and insulin resistance (69–72). As DPP4 can function in either soluble or membrane form, and hepatocytes also express DPP4, detailed functions of DPP4 including HGP modulation are yet to be determined.

Retinol binding protein 4 (RBP4)

RBP4 is a serum retinol transporter secreted by the liver and fat. In obese mice, RBP4 stimulates ATM activation dependent on toll-like receptor 2/4, leading to production of proinflammatory cytokines like TNF-α, IL-12, IL-6, IL-1β, and subsequent T cell activation. This action plays a critical role in eliciting obesity-induced WAT inflammation and systemic insulin resistance (73–75). Interestingly, EVs from visceral WAT of obese mice were found to carry RBP4 that possessed the capacity to activate macrophages and elicit insulin resistance (76). More recently, a study on women with obesity linked RBP4 with HGP relying on its stimulation on adipocyte lipolysis. Direct stimulation on basal lipolysis as well as increased lipolysis by indirectly activating ATMs were both included (77). Therefore, RBP4 may act as another promising candidate for ATM-based HGP modulation, particularly in obesity-related glucose dysregulation.

Concluding remarks and perspectives

Fine-regulation of HGP is critical to maintain blood glucose homeostasis in various nutrition states. HGP dysregulation contributes to hyperglycemia in obesity-associated diabetes.

During fasting-feeding cycle or metabolic stress like overnutrition, ATMs change their cytokine or lipid profiles to remotely modulate HGP. Feeding induces IL-10 from ATMs, which controls HGP by inhibiting gluconeogenesis in concert with insulin action. While exacerbated HGP usually coincides with large amounts of IL-6 and TNF-α from ATMs, which link WAT inflammation with lipolysis, further interfering with insulin action or supplying precursors to stimulate gluconeogenesis. In response to overnutrition, lipid-associated ATMs play either protective or detrimental roles in controlling aberrant HGP (Figure 1). Therefore, ATM-based HGP modulation may be the promising strategies to treat obesity-associated diabetes.

Regarding ATM-based HGP modulation, more regulatory modes and targets remain to be unraveled, whilst some regulators of macrophages, such as a E3 ubiquitin ligase TRIM29 that inhibits macrophage activation and proinflammatory cytokine production (78), would be the promising candidates. Besides secretary molecules, EVs may be another indispensable remoter for fat-liver crosstalk. It is therefore interesting to explore whether and how EVs regulate the interactions between ATMs, adipocytes and hepatocytes, and their influences on HGP homeostasis. Upon challenged with energy surplus, WAT undergoes the process from metabolic adaptation to maladaptation, in which HGP-related ATM phenotypes and functions need to be carefully determined due to their complexity and flexibility. Given that gluconeogenesis is precisely regulated by circadian clocks, it is yet open but interesting to explore the changes of ATMs in this process. Furthermore, possible HGP regulation by BAT macrophages is also attractive, due to their involvement in energy metabolism (79, 80).

Author contributions

YT and QJ drafted the manuscript. YT designed and created the figure. QW wrote and revised the manuscript, designed the figure. All authors approved the submitted version of the manuscript.

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References

1. American Diabetes A. Diagnosis and classification of diabetes mellitus. *Diabetes Care* (2009) 32(Suppl 1):S62–7. doi: 10.2337/dc13-S067.

2. Gerich JE. Role of the kidney in normal glucose homeostasis and in the hyperglycaemia of diabetes mellitus: Therapeutic implications. *Diabetes Med* (2010) 27(2):136–42. doi: 10.1111/j.1464-5491.2009.02894.x

3. Petersen MC, Vatner DF, Shulman GI. Regulation of hepatic glucose metabolism in health and disease. *Nat Rev Endocrinol* (2017) 13(10):572–87. doi: 10.1038/nrendo.2017.80

4. Rui L. Energy metabolism in the liver. *Compr Physiol* (2014) 4(1):177–97. doi: 10.1002/cphy.c130024

5. Targher G, Corey KE, Byrne CD, Roden M. The complex link between NAFLD and type 2 diabetes mellitus - mechanisms and treatments. *Nat Rev Gastroenterol Hepatol* (2021) 18(9):599–612. doi: 10.1038/s41575-021-00448-y

6. Kranendonk ME, Visseren FL, van Herwaarden JA, Nolte-'t Hoen EN, de Jager W, Wauben MH, et al. Effect of extracellular vesicles of human adipose tissue on insulin signaling in liver and muscle cells. *Obes (Silver Spring)* (2014) 22 (10):2216–23. doi: 10.1002/oby.20847

7. Fuchs A, Samovski D, Smith GI, Cifarelli V, Farabi SS, Yoshino J, et al. Associations among adipose tissue immunology, inflammation, exosomes and insulin sensitivity in people with obesity and nonalcoholic fatty liver disease. *Gastroenterology* (2021) 161(3):968–81.e12. doi: 10.1053/j.gastro.2021.05.008

8. Nuttall FQ, Ngo A, Gannon MC. Regulation of hepatic glucose production and the role of gluconeogenesis in humans: Is the rate of gluconeogenesis constant? *Diabetes Metab Res Rev* (2008) 24(6):438–58. doi: 10.1002/dmrr.863

9. Cui A, Ding D, Li Y. Regulation of hepatic metabolism and cell growth by the atf/creb family of transcription factors. *Diabetes* (2021) 70(3):653–64. doi: 10.2337/dbi20-0006

10. McCommis KS, Finck BN. Mitochondrial pyruvate transport: A historical perspective and future research directions. *Biochem J* (2015) 466(3):443–54. doi: 10.1042/BJ20141171

11. Beale EG, Hammer RE, Antoine B, Forest C. Disregulated glyceroneogenesis: PCK1 as a candidate diabetes and obesity gene. *Trends Endocrinol Metab* (2004) 15(3):129–35. doi: 10.1016/j.tem.2004.02.006

12. Hutton JC, O'Brien RM. Glucose-6-phosphatase catalytic subunit gene family. *J Biol Chem* (2009) 284(43):29241–5. doi: 10.1074/jbc.R109.025544

13. Oh KJ, Han HS, Kim MJ, Koo SH. CREB and FoxO1: Two transcription factors for the regulation of hepatic gluconeogenesis. *BMB Rep* (2013) 46(12):567–74. doi: 10.5483/BMBRep.2013.46.12.248

14. Nakae J, Kitamura T, Silver DL, Accili D. The forkhead transcription factor Foxo1 (Fkhr) confers insulin sensitivity onto glucose-6-phosphatase expression. *J Clin Invest* (2001) 108(9):1359–67. doi: 10.1172/JCI200112876

15. Lu M, Wan M, Leavens KF, Chu Q, Monks BF, Fernandez S, et al. Insulin regulates liver metabolism *in vivo* in the absence of hepatic akt and Foxo1. *Nat Med* (2012) 18(3):388–95. doi: 10.1038/nm.2686

16. Hatting M, Tavares CDJ, Sharabi K, Rines AK, Puigserver P. Insulin regulation of gluconeogenesis. *Ann N Y Acad Sci* (2018) 1411(1):21–35. doi: 10.1111/nyas.13435

17. Korenfeld N, Finkel M, Buchshtab N, Bar-Shimon M, Charni-Natan M, Goldstein I. Fasting hormones synergistically induce amino acid catabolism genes to promote gluconeogenesis. *Cell Mol Gastroenterol Hepatol* (2021) 12(3):1021–36. doi: 10.1016/j.jcmgh.2021.04.017

18. Xu S, Liu Y, Hu R, Wang M, Stöhr O, Xiong Y, et al. TAZ inhibits glucocorticoid receptor and coordinates hepatic glucose homeostasis in normal physiological states. *Elife* (2021) 10:e57462. doi: 10.7554/eLife.57462.

19. Magnusson I, Rothman DL, Katz LD, Shulman RG, Shulman GI. Increased rate of gluconeogenesis in type-ii diabetes-mellitus - a c-13 nuclear-magnetic-resonance study. *J Clin Invest* (1992) 90(4):1323–7. doi: 10.1172/JCI115997

20. TeSlaa T, Bartman CR, Jankowski CSR, Zhang Z, Xu X, Xing X, et al. The source of glycolytic intermediates in mammalian tissues. *Cell Metab* (2021) 33 (2):367–78.e5. doi: 10.1016/j.cmet.2020.12.020

21. Peirce V, Carobbio S, Vidal-Puig A. The different shades of fat. *Nature* (2014) 510(7503):76–83. doi: 10.1038/nature13477

22. Yerkovich ST, Rigby PJ, Fournier PA, Olynyk JK, Yeoh GC. Kupffer cell cytokines interleukin-1beta and interleukin-10 combine to inhibit phosphoenolpyruvate carboxykinase and gluconeogenesis in cultured hepatocytes. *Int J Biochem Cell Biol* (2004) 36(8):1462–72. doi: 10.1016/j.biocel 2003.10.022.

23. Toda G, Soeda K, Okazaki Y, Kobayashi N, Masuda Y, Arakawa N, et al. Insulin- and lipopolysaccharide-mediated signaling in adipose tissue macrophages regulates postprandial glycemia through akt-mtor activation. *Mol Cell* (2020) 79 (1):43–53.e4. doi: 10.1016/j.molcel.2020.04.033

24. Sulen A, Aouadi M. Fed macrophages hit the liver's sweet spot with il-10. *Mol Cell* (2020) 79(1):1–3. doi: 10.1016/j.molcel.2020.06.016.

25. Kubota N, Kubota T, Kajiwara E, Iwamura T, Kumagai H, Watanabe t, et al. Differential hepatic distribution of insulin receptor substrates causes selective insulin resistance in diabetes and obesity. *Nat Commun* (2016) 7:12977. doi: 10.1038/ncomms12977

26. Yan H, Yang W, Zhou F, Li X, Pan Q, Shen Z, et al. Estrogen improves insulin sensitivity and suppresses gluconeogenesis *via* the transcription factor foxo1. *Diabetes* (2019) 68(2):291–304. doi: 10.2337/db18-0638

27. Lang CH, Dobrescu C, Bagby GJ. Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output. *Endocrinology* (1992) 130(1):43–52. doi: 10.1210/endo.130.1.1727716

28. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. *Science* (1993) 259(5091):87–91. doi: 10.1126/science.7678183

29. Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-alpha in human obesity and insulin resistance. *J Clin Invest* (1995) 95(5):2409–15. doi: 10.1172/JCI117936

30. Uysal KT, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* (1997) 389(6651):610–4. doi: 10.1038/39335

31. Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. *J Clin Invest* (2006) 116(7):1793–801. doi: 10.1172/JCI29069

32. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* (2003) 112(12):1796–808. doi: 10.1172/JCI200319246

33. Yu YH, Ginsberg HN. Adipocyte signaling and lipid homeostasis: sequelae of insulin-resistant adipose tissue. *Circ Res* (2005) 96(10):1042–52. doi: 10.1161/ 01.RES.0000165803.47776.38

34. Leclercq IA, Da Silva Morais A, Schroyen B, Van Hul N, Geerts A. Insulin resistance in hepatocytes and sinusoidal liver cells: mechanisms and consequences. *J Hepatol* (2007) 47(1):142–56. doi: 10.1016/j.jhep.2007.04.002

35. Rui L, Aguirre V, Kim JK, Shulman GI, Lee A, Corbould A, et al. Insulin/ IGF-1 and TNF-alpha stimulate phosphorylation of IRS-1 at inhibitory Ser307 *via* distinct pathways. *J Clin Invest* (2001) 107(2):181–9. doi: 10.1172/JCI10934

36. Stefan N, Cusi K. A global view of the interplay between non-alcoholic fatty liver disease and diabetes. *Lancet Diabetes Endocrinol* (2022) 10(4):284–96. doi: 10.1016/S2213-8587(22)00003-1

37. Stefan N. Causes, consequences, and treatment of metabolically unhealthy fat distribution. *Lancet Diabetes Endocrinol* (2020) 8(7):616–27. doi: 10.1016/ S2213-8587(20)30110-8

38. Steensels S, Qiao J, Zhang Y, Maner-Smith KM, Kika N, Holman CD, et al. Acyl-coenzyme a thioesterase 9 traffics mitochondrial short-chain fatty acids toward *de novo* lipogenesis and glucose production in the liver. *Hepatology* (2020) 72(3):857–72. doi: 10.1002/hep.31409

39. Mashek DG. Hepatic lipid droplets: A balancing act between energy storage and metabolic dysfunction in NAFLD. *Mol Metab* (2021) 50:101115. doi: 10.1016/ j.molmet.2020.101115

40. Dent JR, Chowdhury MK, Tchijov S, Dulson D, Smith G. Interleukin-6 is a negative regulator of hepatic glucose production in the isolated rat liver. *Arch Physiol Biochem* (2016) 122(2):103–9. doi: 10.3109/13813455.2016.1146773

41. Christ B, Yazici E, Nath A. Phosphatidylinositol 3-kinase and protein kinase c contribute to the inhibition by interleukin 6 of phosphoenolpyruvate carboxykinase gene expression in cultured rat hepatocytes. *Hepatology* (2000) 31 (2):461–8. doi: 10.1002/hep.510310228

42. Ramadoss P, Unger-Smith NE, Lam FS, Hollenberg AN. STAT3 targets the regulatory regions of gluconeogenic genes. *in vivo Mol Endocrinol* (2009) 23 (6):827–37. doi: 10.1210/me.2008-0264

43. Inoue H, Ogawa W, Ozaki M, Haga S, Matsumoto M, Furukawa K, et al. Role of STAT-3 in regulation of hepatic gluconeogenic genes and carbohydrate metabolism. *in vivo Nat Med* (2004) 10(2):168–74. doi: 10.1038/nm980

44. Inoue H, Ogawa W, Asakawa A, Okamoto Y, Nishizawa A, Matsumoto M, et al. Role of hepatic STAT3 in brain-insulin action on hepatic glucose production. *Cell Metab* (2006) 3(4):267–75. doi: 10.1016/j.cmet.2006.02.009

45. Wueest S, Item F, Boyle CN, Jirkof P, Cesarovic N, Ellingsgaard H, et al. Interleukin-6 contributes to early fasting-induced free fatty acid mobilization in mice. *Am J Physiol Regul Integr Comp Physiol* (2014) 306(11):R861–7. doi: 10.1152/ ajpregu.00533.2013

46. Kistner TM, Pedersen BK, Lieberman DE. Interleukin 6 as an energy allocator in muscle tissue. *Nat Metab* (2022) 4(2):170–9. doi: 10.1038/s42255-022-00538-4

47. Wedell-Neergaard AS, Lang Lehrskov L, Christensen RH, Legaard DE, Dorph E, Larsen MK, et al. Exercise-induced changes in visceral adipose tissue mass are regulated by il-6 signaling: a randomized controlled trial. *Cell Metab* (2019) 29(4):844–55.e3. doi: 10.1016/j.cmet.2018.12.007

48. Hoene M, Weigert C. The role of interleukin-6 in insulin resistance, body fat distribution and energy balance. *Obes Rev* (2008) 9(1):20–9.doi: 10.1111/j.1467-789X.2007.00410.x.

49. Perry RJ, Camporez JG, Kursawe R, Titchenell PM, Zhang D, et al. Hepatic acetyl CoA links adipose tissue inflammation to hepatic insulin resistance and type 2 diabetes. *Cell* (2015) 160(4):745–58. doi: 10.1016/j.cell.2015.01.012

50. Senn JJ, Klover PJ, Nowak JJ, Zimmers TA, Koniaris LG, Furlanetto RW, et al. Suppressor of cytokine signaling-3 (SOCS-3), a potential mediator of interleukin-6-dependent insulin resistance in hepatocytes. *J Biol Chem* (2003) 278(16):13740–6. doi: 10.1074/jbc.M210689200

51. Kim JH, Kim JE, Liu HY, Cao W, Chen J. Regulation of interleukin-6-induced hepatic insulin resistance by mammalian target of rapamycin through the STAT3-SOCS3 pathway. *J Biol Chem* (2008) 283(2):708–15. doi: 10.1074/ jbc.M708568200

52. Bijnen M, et al. Adipose tissue macrophages induce hepatic neutrophil recruitment and macrophage accumulation in mice. *Gut* (2018) 67(7):1317–27. doi: 10.1136/gutjnl-2016-313654

53. van der Heijden RA, Sheedfar F, Morrison MC, Hommelberg PP, Kor D, Kloosterhuis NJ, et al. High-fat diet induced obesity primes inflammation in adipose tissue prior to liver in C57BL/6j mice. *Aging (Albany NY)* (2015) 7(4):256–68. doi: 10.18632/aging.100738

54. Tordjman J, Poitou C, Hugol D, Bouillot JL, Basdevant A, Bedossa P, et al. Association between omental adipose tissue macrophages and liver histopathology in morbid obesity: Influence of glycemic status. *J Hepatol* (2009) 51(2):354–62. doi: 10.1016/j.jhep.2009.02.031

55. Prieur X, Mok CY, Velagapudi VR, Núñez V, Fuentes L, Montaner D, et al. Differential lipid partitioning between adipocytes and tissue macrophages modulates macrophage lipotoxicity and M2/M1 polarization in obese mice. *Diabetes* (2011) 60(3):797–809. doi: 10.2337/db10-0705

56. Shapiro H, Pecht T, Shaco-Levy R, Harman-Boehm I, Kirshtein B, Kuperman Y, et al. Adipose tissue foam cells are present in human obesity. *J Clin Endocrinol Metab* (2013) 98(3):1173–81. doi: 10.1210/jc.2012-2745

57. Vataresc M, Bechor S, Haim Y, Pecht T, Tarnovscki T, Slutsky N, et al. Adipose tissue supports normalization of macrophage and liver lipid handling in obesity reversal. *J Endocrinol* (2017) 233(3):293–305. doi: 10.1530/JOE-17-0007

58. Jaitin DA, Adlung L, Thaiss CA, Weiner A, Li B, Descamps H, et al. Lipid-associated macrophages control metabolic homeostasis in a trem2-dependent manner. *Cell* (2019) 178(3):686–98.e14. doi: 10.1016/j.cell.2019.05.054

59. Aouadi M, Vangala P, Yawe JC, Tencerova M, Nicoloro SM, Cohen JL, et al. Lipid storage by adipose tissue macrophages regulates systemic glucose tolerance. *Am J Physiol Endocrinol Metab* (2014) 307(4):E374–83. doi: 10.1152/ ajpendo.00187.2014

60. Ertunc ME, Hotamisligil GS. Lipid signaling and lipotoxicity in metaflammation: indications for metabolic disease pathogenesis and treatment. *J Lipid Res* (2016) 57(12):2099–114. doi: 10.1194/jlr.R066514

61. Caslin HL, Bhanot M, Bolus WR, Hasty AH. Adipose tissue macrophages: Unique polarization and bioenergetics in obesity. *Immunol Rev* (2020) 295(1):101–13. doi: 10.1111/imr.12853

62. Flaherty SE, Grijalva A, Xu X, Ables E, Nomani A, Ferrante AW Jr. A lipase-independent pathway of lipid release and immune modulation by adipocytes. *Science* (2019) 363(6430):989–93. doi: 10.1126/science.aaw2586

63. Antonyak MA, Lukey MJ, Cerione RA. Lipid-filled vesicles modulate macrophages. *Science* (2019) 363(6430):931–2. doi: 10.1126/science.aaw6765

64. Nomiyama T, Perez-Tilve D, Ogawa D, Gizard F, Zhao Y, Heywood EB, et al. Osteopontin mediates obesity-induced adipose tissue macrophage infiltration and insulin resistance in mice. *J Clin Invest* (2007) 117(10):2877–88. doi: 10.1172/JCI31986

65. Kiefer FW, Zeyda M, Gollinger K, Pfau B, Neuhofer A, Weichhart T, et al. Neutralization of osteopontin inhibits obesity-induced inflammation and insulin resistance. *Diabetes* (2010) 59(4):935–46. doi: 10.2337/db09-0404

66. Kiefer FW, Neschen S, Pfau B, Legerer B, Neuhofer A, Kahle M, et al. Osteopontin deficiency protects against obesity-induced hepatic steatosis and attenuates glucose production in mice. *Diabetologia* (2011) 54(8):2132–42. doi: 10.1007/s00125-011-2170-0

67. Tardelli M, Zeyda K, Moreno-Viedma V, Wanko B, Grün NG, Staffler G, et al. Osteopontin is a key player for local adipose tissue macrophage proliferation in obesity. *Mol Metab* (2016) 5(11):1131–7. doi: 10.1016/j.molmet.2016.09.003

68. Remmerie A, Martens L, Thoné T, Castoldi A, Seurinck R, Pavie B, et al. Osteopontin expression identifies a subset of recruited macrophages distinct from kupffer cells in the fatty liver. *Immunity* (2020) 53(3):641–57.e14. doi: 10.1016/j.immuni.2020.08.004

69. Diaz-Jimenez D, Petrillo MG, Busada JT, Hermoso MA, Cidlowski JA. Glucocorticoids mobilize macrophages by transcriptionally up-regulating the exopeptidase DPP4. *J Biol Chem* (2020) 295(10):3213–27. doi: 10.1074/jbc.RA119.010894

70. Deacon CF. Physiology and pharmacology of DPP-4 in glucose homeostasis and the treatment of type 2 diabetes. *Front Endocrinol (Lausanne)* (2019) 10:80. doi: 10.3389/fendo.2019.00080

71. Rao X, Zhao S, Braunstein Z, Mao H, Razavi M, Duan L, et al. Oxidized LDL upregulates macrophage DPP4 expression *via* TLR4/TRIF/CD36 pathways. *EBioMedicine* (2019) 41:50–61. doi: 10.1016/j.ebiom.2019.01.065

72. Zillessen P, Celner J, Kretschmann A, Pfeifer A, Racké K, Mayer P. Metabolic role of dipeptidyl peptidase 4 (DPP4) in primary human (pre) adipocytes. *Sci Rep* (2016) 6:23074. doi: 10.1038/srep23074

73. Moraes-Vieira PM, Yore MM, Sontheimer-Phelps A, Castoldi A, Norseen J, Aryal P, et al. Retinol binding protein 4 primes the NLRP3 inflammasome by signaling through toll-like receptors 2 and 4. *Proc Natl Acad Sci USA* (2020) 117(49):31309–18. doi: 10.1073/pnas.2013877117

74. Moraes-Vieira PM, Yore MM, Sontheimer-Phelps A, Castoldi A, Norseen J, Aryal P, et al. RBP4 activates antigen-presenting cells, leading to adipose tissue inflammation and systemic insulin resistance. *Cell Metab* (2014) 19(3):512–26. doi: 10.1016/j.cmet.2014.01.018

75. Moraes-Vieira PM, Castoldi A, Aryal P, Wellenstein K, Peroni OD, Kahn BB. Antigen presentation and t-cell activation are critical for rbp4-induced insulin resistance. *Diabetes* (2016) 65(5):1317–27. doi: 10.2337/db15-1696

76. Deng ZB, Poliakov A, Hardy RW, Clements R, Liu C, Liu Y, et al. Adipose tissue exosome-like vesicles mediate activation of macrophage-induced insulin resistance. *Diabetes* (2009) 58(11):2498–505. doi: 10.2337/db09-0216

77. Kilicarslan M, de Weijer BA, Simonyté Sjödin K, Aryal P, Ter Horst KW, Cakir H, et al. RBP4 increases lipolysis in human adipocytes and is associated with increased lipolysis and hepatic insulin resistance in obese women. *FASEB J* (2020) 34(5):6099–110. doi: 10.1096/fj.201901979RR

78. Xing J, Weng L, Yuan B, Wang Z, Jia L, Jin R, et al. Identification of a role for TRIM29 in the control of innate immunity in the respiratory tract. *Nat Immunol* (2016) 17(12):1373–80. doi: 10.1038/ni.3580

79. Wolf Y, Boura-Halfon S, Cortese N, Haimon Z, Sar Shalom H, Kuperman Y, et al. Brown-adipose-tissue macrophages control tissue innervation and homeostatic energy expenditure. *Nat Immunol* (2017) 18(6):665–74. doi: 10.1038/ni.3746

80. Rosina M, Ceci V, Turchi R, Chuan L, Borcherding N, Sciarretta F, et al. Ejection of damaged mitochondria and their removal by macrophages ensure efficient thermogenesis in brown adipose tissue. *Cell Metab* (2022) 34(4):533–48.e12. doi: 10.1016/j.cmet.2022.02.016

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Perspective on direction of control: Cellular metabolism and macrophage polarization

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Macrophages are innate immune cells with high phenotypic plasticity. Depending on the microenvironmental cues they receive, they polarize on a spectrum with extremes being pro- or anti-inflammatory. As well as responses to microenvironmental cues, cellular metabolism is increasingly recognized as a key factor influencing macrophage function. While pro-inflammatory macrophages mostly use glycolysis to meet their energetic needs, anti-inflammatory macrophages heavily rely on mitochondrial respiration. The relationship between macrophage phenotype and macrophage metabolism is well established, however its precise directionality is still under question. Indeed, whether cellular metabolism *per se* influences macrophage phenotype or whether macrophage polarization dictates metabolic activity is an area of active research. In this short perspective article, we sought to shed light on this area. By modulating several metabolic pathways in bone marrow-derived macrophages, we show that disruption of cellular metabolism does *per se* influence cytokine secretion profile and expression of key inflammatory genes. Only some pathways seem to be involved in these processes, highlighting the need for specific metabolic functions in the regulation of macrophage phenotype. We thus demonstrate that the intact nature of cellular metabolism influences macrophage phenotype and function, addressing the directionality between these two aspects of macrophage biology.

KEYWORDS

macrophage, metabolism, energetics, mitochondria, Inflammation

Introduction

Macrophages are innate immune cells that populate all tissues and have a number of homeostatic roles (e.g., removing dead cells and cellular debris, recycling iron). One of their main immune functions is to recognize and phagocytose pathogens (1). Following recognition, they also produce cytokines that can recruit and induce differentiation of

monocytes and T cells. Additionally, they play an important role in tissue repair once the immune response has been terminated (1).

Macrophages can respond to a variety of molecular cues through activation of Toll-Like Receptors (TLRs), and will secrete cytokines accordingly. Following activation, they will transition from a quiescent state to an activated one. Depending on the cues they receive, they can adopt different activated phenotypes. Based on *in vitro* experiments, activated macrophages have been historically divided into M1 pro-inflammatory or M2 anti-inflammatory macrophages (2). Stimulating macrophages with lipopolysaccharide (LPS) and interferon-γ (IFN-γ) triggers their differentiation into M1 macrophages whereas interleukin 4 (IL4) and IL13 promote an M2 phenotype (3, 4).

M1 and M2 macrophages display different metabolic properties. M1 macrophages have increased glucose uptake through the Hypoxia Inducible Factor 1α (HIF1α)-dependent upregulation of glucose transporters. This increased glucose uptake is essential to fuel glycolysis and thus produce ATP. M1 macrophages scarcely use mitochondrial respiration as they have targeted breaks in the tricarboxylic acid (TCA) cycle (Figure 1A) (5–7).

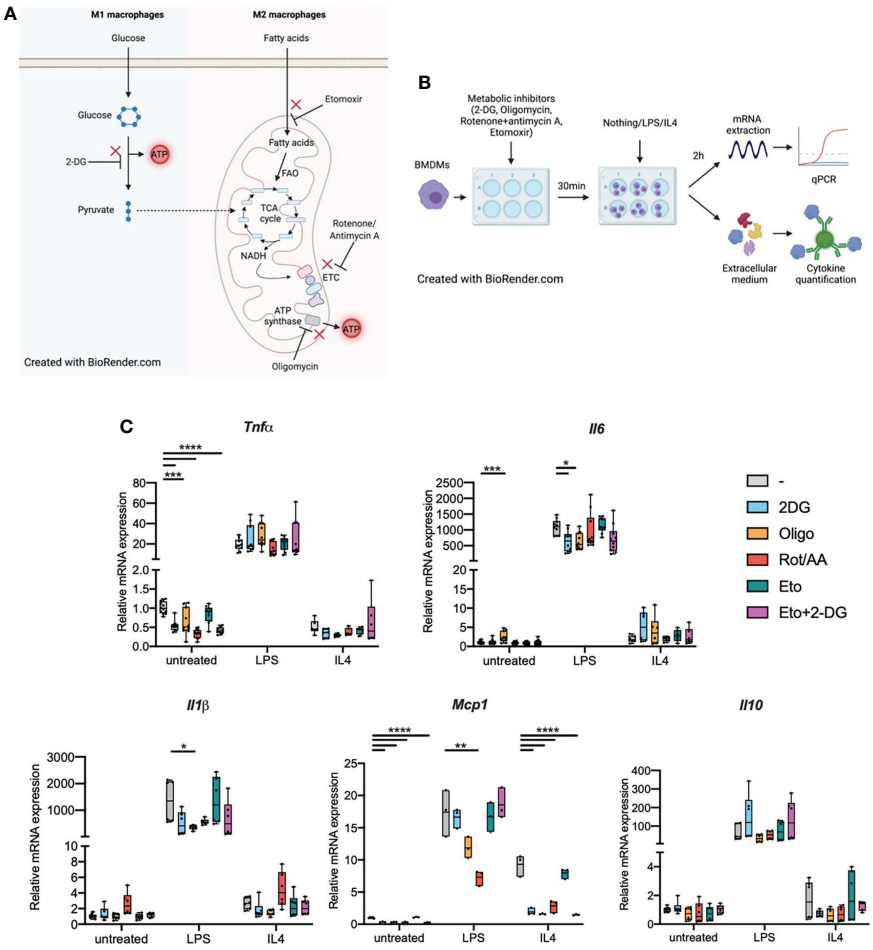

FIGURE 1
Metabolic inhibitors alter macrophage expression of inflammatory genes in response to LPS or IL4 **(A)**. M1 and M2 macrophage metabolism. FAO: fatty acid oxidation. ETC, Electron transport chain; TCA cycle, tricarboxylic acid cycle. **(B)** Experimental design: bone marrow-derived macrophages (BMDMs) were treated with metabolic inhibitors for 30 min. BMDMs were then either left untreated (M0 quiescent macrophages) or stimulated with LPS or IL4 to induce M1 and M2 polarizations respectively. Macrophage response was then assessed by quantifying cytokine concentration in the extracellular medium or through mRNA quantification by qPCR. **(C)** Relative mRNA expression for *Tnfa*, *Il6*, *Il1b*, *Mcp1* and *Il10* in quiescent BMDMs either left untreated or treated with 2-deoxyglucose (2-DG), oligomycin (Oligo), rotenone/antimycin A (Rot/AA), Etomoxir (Eto) or a combination of 2-DG and Etomoxir (Eto+2-DG). All points are shown in box plots with line at median. One-way ANOVA was used to determine statistical significance between groups within each stimulation condition (untreated, LPS, IL4) *p < 0.05, **p < 0.01, ***p < 0,001, ****p < 0,0001. Unless otherwise stated, differences between groups are not statistically significant. A and B Created with BioRender.com.

M2 macrophages, on the other hand, produce ATP mostly through mitochondrial respiration and have only limited glycolytic activity. Fueling the TCA cycle, M2 macrophages enhance their uptake of fatty acids. Fatty acid oxidation (FAO) provides Acetyl-CoA to a fully functional TCA cycle (8). TCA cycle-derived NADH is then used by the electron transport chain (ETC) to generate ATP through ATP synthase (Figure 1A) (7, 9).

In vivo, environmental factors that vary between different tissues or between homeostasis and disease are increasingly recognized as powerful dictators of macrophage metabolism. Macrophages in inflammatory conditions and inflammatory diseases are predominantly glycolytic. For example, macrophages in rheumatoid arthritis (RA) up-regulate the enolase enzyme, which stimulates production of pro-inflammatory cytokines, and show elevated succinate levels (10, 11). In diet-induced obesity, adipose tissue macrophages are hypermetabolic, up-regulating both glycolytic activity and mitochondrial respiration (12). Yet, these macrophages were long considered M1-like and are known to promote insulin resistance and metabolic syndrome (9). Such environmentally adapted profiles raise the question of precise directionality between macrophage metabolism and inflammatory profile.

Here, we use bone marrow-derived macrophages (BMDMs), with inhibitors of specific metabolic pathways to shed light on the direction of control between cellular metabolism and capacity to mount an inflammatory response. Using cytokine production as a functional read-out for macrophage phenotype, we show that impairing metabolic pathways drastically impacts macrophage phenotype. Some metabolic pathways have differential effects on cytokine secretion profile, suggesting that additional factors may directly influence macrophage phenotype in synergy with metabolic parameters.

Results

Biasing cellular metabolism alters inflammatory marker expression in quiescent macrophages

We first wondered whether biasing macrophage metabolism could per se influence macrophages in their quiescent state. Following treatment with inhibitors of glycolysis, mitochondrial respiration or both, we quantified transcription of inflammatory markers Tnfa, Il6, Il1b, Mcp1 and Il10 in M0 BMDMs or in BMDMs that were subsequently polarized towards a M1 or M2 phenotype (Figure 1B). When pre-treated with 2-deoxyglucose (2DG), a non-metabolizable glucose analog which inhibits glycolysis. Quiescent BMDMs showed decreased Tnfa and Mcp1 transcription compared to cells that were not pre-treated with 2DG, there was no change for Il6, Il1b or Il10 (Figure 1C). Inhibiting the mitochondrial ETC with Rotenone and antimycin A (Rot/AA) resulted in a similar decrease in Tnfa transcription. Oligomycin, an ATP synthase inhibitor, and etomoxir which inhibits carnitine palmitoyl transferase 1a (Cpt1a), FAO's rate-limiting enzyme, did not show any significant effect on cytokine transcription (Figure 1C). The combination of etomoxir and 2DG (Eto+2DG) did not display any additive effect compared with the single inhibitor setting. These results suggest that cellular metabolism, in particular intact activity of glycolysis and mitochondrial ETC, is important to maintain macrophages in a quiescent state. However, the lack of impact of the different aforementioned metabolic inhibitors on Il6 and Il1b transcription suggests that metabolism only influences specific signals at quiescence. Conversely, some metabolic pathways resulted in increased transcription of inflammatory markers. For example, oligomycin results in increased Il6 expression in quiescent BMDMs (Figure 1C).

Cellular metabolism influences macrophage transcriptional response to M1-like and M2-like polarizing stimuli

We next wondered about the impact of cellular metabolism on macrophage capacity to polarize or sensitivity to polarizing agents. BMDMs were pretreated with metabolic inhibitors then stimulated with bacterial LPS, the main TLR4 ligand known to induce M1-like polarization, or with IL4, a known stimulator of M2-like polarization (Figure 1B).

In cells pretreated with 2DG, LPS-induced expression of Il6 was attenuated. This effect was also observed in cells pretreated with oligomycin for both Il1b and Il6 (Figure 1C). Upon pretreatment with Rot/AA, LPS-induced expression of Mcp1 was decreased. Etomoxir pretreatment did not affect the response to LPS. These data suggest a limited contribution of FAO to the transcriptional response to LPS. (Figure 1C). These results indicate functional specificity in the requirement for glycolysis and ATP synthase activity in LPS-induced expression of Il6, Il1b and Mcp1 versus Tnfa and Il10 (i.e., Il6 expression, but not Tnfa, requires intact glycolytic flux and ATP synthase activity).

Additionally, we confirmed a relative state of quiescence in untreated BMDM through their increased expression of inflammatory markers in response to LPS and through surface expression of MHCII following long-term treatment with LPS (Figure S1).

Following IL4 stimulation, few cytokines were affected at the transcriptional level. Only Mcp1 expression was affected (Figure 1C). Inhibiting any metabolic pathway other than FAO alone strongly decreased Mcp1 transcription in response to IL4. This indicates that FAO does not significantly contribute to the Mcp1 transcriptional response and while glycolysis did not seem to play any role in Mcp1 up-regulation following LPS stimulation, it was pivotal in the response to IL4.

Cellular metabolism influences macrophage cytokine secretion in response to an inflammatory challenge

As a functional readout, focusing on proinflammatory signalling, we measured cytokine secretion into the extracellular medium (Figure 2A). Inhibiting any of the aforementioned metabolic pathways resulted in decreased IL6, TNFα, and MCP1 secretion in response to LPS. Metabolic inhibition, without supplementary stimulation, did not induce significant changes in IL1β secretion. We did however observe a significant increase in IL1β secretion during LPS stimulation in cells that were pre-treated with Eto+2-DG, compared to other pretreatments (Figure 2A). While LPS stimulation alone is not expected to induce significant inflammasome activation, it is possible that the Eto+2DG combination, with LPS, induces cellular stresses that contribute to IL1β secretion.

In response to LPS, inhibiting glycolysis had the most dramatic and consistent effect across measured cytokines. Inhibiting the mitochondrial ETC at multiple levels, NADH: ubiquinone oxidoreductase, coenzyme Q-cytochrome c reductase or ATP synthase (Complexes I, III or V), comparably attenuated cytokine secretion. Inhibiting FAO had the least pronounced effect on cytokine secretion, even if it still decreased IL6 and MCP1 secretion (Figure 2A).

In response to IL4 stimulation, most inflammatory cytokines were secreted at low concentrations that were close to, or below, the detection limit for most samples (Figure 2B). TNFα was the exception that increased with IL4 treatment and its expression was potentiated upon pretreatment with etomoxir or with Rot/AA (Figure 2B). MCP1 expression showed a tendency to a decrease by oligomycin, Rot/AA and Eto+2-DG that did not however reach statistical significance (Figure S2).

These results suggest that targeting metabolic pathways alters macrophage secretory capacity in response to LPS or to IL4. Glycolysis and the mitochondrial ETC are instrumental in cytokine secretion in response to TLR4 ligation, while FAO contributes to a more limited extent. Elements of FAO and OXPHOS do however contribute to regulating TNFα expression in response to IL4. Overall, these findings show that metabolism is important for macrophage secretory profile. However, only specific metabolic pathways are capable of influencing transcriptional profile. Indeed, macrophage cellular metabolism greatly impacts macrophage capacity to polarize and specific metabolic pathways have differential roles in supporting an M1 or M2 phenotype (Table 1).

Discussion

Metabolism is tightly linked to macrophage polarization as M1 macrophages are predominantly glycolytic while M2

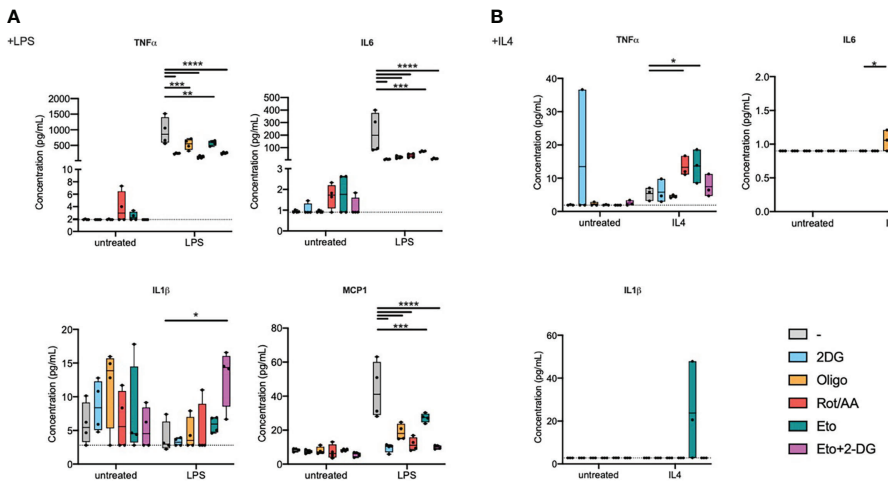

FIGURE 2
Metabolic inhibitors alter macrophage secretory profile in response to LPS or IL4. Bone marrow-derived macrophages (BMDMs) were pretreated with metabolic inhibitors for 30 min before being stimulated with M1 (LPS) or M2 (IL4) stimulus or left untreated for 2 h. Macrophage response was then assessed by quantifying cytokine concentration in the extracellular medium. **(A)** Concentration of TNFα, IL6, IL1β and MCP1 in cell culture media of BMDMs pretreated with 2-deoxyglucose (2-DG), oligomycin (Oligo), rotenone/antimycin A (Rot/AA), Etomoxir (Eto) or a combination of 2-DG and Etomoxir (Eto+2-DG) and then stimulated with LPS for 2h. **(B)** Concentration of TNFα and MCP1 in cell culture media of BMDMs pretreated with 2DG, Oligo, Rot/AA, Eto or Eto+2-DG and then stimulated with IL4 for 2h. All points are shown in box plots with line at median. The dashed line indicates detection limit of the assay. One-way ANOVA was used to determine statistical significance between groups within each stimulation condition (untreated, LPS, IL4) *p < 0.05, **p < 0.01, ***p < 0,001, ****p < 0,0001. Unless otherwise stated, differences between groups are not statistically significant.

TABLE 1 Summary of effects of metabolic inhibitor pretreatments on gene expression and cytokine concentration in media from macrophages.

	M0 macrophages (unstimulated)					M1 macrophages (LPS)					M2 macrophages (IL4)				
	2-DG	Oligo	Rot/AA	Eto	Eto+2-DG	2-DG	Oligo	Rot/AA	Eto	Eto+2-DG	2-DG	Oligo	Rot/AA	Eto	Eto+2-DG
Tnfa	down	down	down	=	down	=	=	=	=	=	=	=	=	=	=
Il6	=	up	=	=	=	down	down	=	=	=	=	=	=	=	=
Il1b	=	down	=	=	=	=	down	=	=	=	=	=	=	=	=
Mcp1	down	down	down	=	down	=	=	down	=	=	down	down	down	=	down
Il10	=	=	=	=	=	=	=	=	=	=	=	=	=	=	=
TNFα	=	=	=	=	=	down	down	down	=	down	=	=	=	=	=
IL6	=	=	=	=	=	down	down	down	down	down	=	=	=	=	=
IL1β	=	=	=	=	=	=	=	=	=	up	=	=	=	=	=
MCP1	=	=	=	=	=	down	down	down	down	down	=	=	=	=	=

Bone marrow-derived macrophages were pretreated with metabolic inhibitors for 30 min. Macrophages were either left untreated (M0 quiescent macrophages) or stimulated with LPS or IL4 to induce M1 and M2 polarizations respectively. Pretreatments were 2-deoxyglucose (2-DG), oligomycin (Oligo), rotenone/antimycin A (Rot/AA), Etomoxir (Eto) or a combination of 2-DG and Etomoxir (Eto+2-DG). Direction of change indicated is relative to the non-pretreated control in each polarization state. Gene expression results are indicated in lower case italics and proteins secreted are in upper case.

macrophages rely heavily on mitochondria. Here, we studied the impact of macrophage cellular metabolism on macrophage phenotype. We show that macrophage polarization depends on cellular metabolic activities and more particularly on some pivotal metabolic pathways like glycolysis. Interestingly, this dependency is functionally specific with respect to the cytokines affected and alters secretory capacity to a greater extent than transcription.

Additionally, we show that transcription of some cytokines was unexpectedly boosted by metabolic inhibition. It is possible that, following inhibition of a given pathway, metabolites used by this pathway are redirected towards other pathways in which activity is increased. This could be a mechanism of adaptation to the metabolically challenging environment. Such compensatory mechanisms have already been described in monocytes, in which IL6 production is quickly restored after depriving monocytes of ATP and glucose (13).

The impact of cellular metabolism on quiescent macrophage polarization differs between M1 and M2 macrophages. The impact of metabolic inhibition on M2 differentiation seems to be more limited than on M1 differentiation. This is of particular interest in some contexts like metabolic syndrome. In this disease, macrophages face changes in metabolic substrate availability, such as lipid overload. Moreover, in treated individuals, macrophages are also exposed to metabolically active therapeutic agents. Macrophages can accumulate in metabolic organs, adipose tissue and the liver, and exhibit an M1-like phenotype which is central to metabolic decline and the development of insulin resistance (9, 14, 15). Thus, our data suggest that changes in the metabolic environment of the cells could lead to changes in macrophage metabolic status and subsequently in macrophage phenotype. Alternatively, in therapeutic perspectives, altering availability of substrates or functioning of metabolic pathways can profoundly impact macrophage secretory profile. The question remains in defining at-risk groups and controlling cell-specific delivery of metabolically acting drugs. Future preclinical work will shed light on the feasibility of such approaches.

The M1/M2 classification has been challenged since it was first proposed. It poorly recapitulates the diversity of macrophage subpopulations found *in vivo*. Macrophages display great phenotypic and functional diversity depending on the tissues they populate, their ontogeny, or their states in health and disease (16). Such specificity could also arise in macrophage reliance on metabolic pathways or on adaptability of cellular metabolism. Additionally, recent studies have uncovered diversity in macrophage populations of a given tissue. For instance, 17 showed the existence of two subpopulations of macrophages that are conserved across several tissues and are localized in distinct tissular niches (17). Whether such subpopulations share common metabolic features or whether their different localizations also translate into different metabolic activities remains largely unknown. Differences in metabolic status between tissue-resident and monocyte-derived macrophages are still under investigation.

Future work could decipher the metabolic diversity of macrophages *in vivo*, adding mechanistic insight and rationale for redeployment of metabolically active therapeutics. Until recently, such investigations were considered technically challenging. However, techniques allowing single-cell resolution in analysis, e.g., SCENITH (18), are gaining accessibility and bring us closer to overcoming the technical challenges encountered when studying cellular heterogeneity across tissues, both in health and disease.

Macrophage polarization is known to play an active role in a number of diseases such as metabolic syndrome, as mentioned

above, auto-immune diseases or cancer. While M1-like macrophages are important drivers of the progression of metabolic diseases, a phenotypic shift from M1-like to M2-like macrophages is known to favor cancer progression and eventually metastasis by dampening the anti-tumor immune response (19). Macrophage metabolism could therefore become an interesting therapeutic target in such diseases. Indeed, targeting relevant metabolic pathways could allow reprogramming of macrophage polarization and thus improve the disease. In addition, macrophages themselves are more and more considered as potential therapeutic agents, for example as chimeric antigen receptor (CAR)-macrophages targeting and phagocytosing tumor cells (20, 21). Combinations of such therapeutic macrophages with metabolic drugs aimed at modulating their phenotype could be powerful tools in a wide range of diseases.

Methods

BMDMs production and stimulation

C57BL/6J mice were bred and housed at the "Centre d'Explorations Fonctionnelles" bdof Sorbonne University (UMS-28) on a 12h day/night cycle. All experiments were approved by the French ethical board (Paris-Sorbonne University, Charles Darwin N°5, 01026.02) and conducted in agreement with all French regulations and guidelines. Tibias and femurs from C57BL/6J mice were collected. Bone marrow cells were recovered by flushing the bones with PBS. After red blood cell lysis, cells were plated in culture-treated 12- or 24-well plates at a concentration of 1x106/mL in BMDM medium (DMEM with GlutaMAX supplemented with 10% FCS, 30% L929-conditioned medium and 100U/mL Penicilin, 100µg/mL streptomycin). L929 cell line produces high amounts of macrophage-colony stimulating factor and other proteins stimulating macrophage differentiation (22). L929-conditioned medium thus allows differentiation of hematopoietic stem cells into BMDMs. Culture medium was renewed every two days.

After 6 to 7 days of differentiation, BMDMs were pre-treated for 30 min with either 10mM 2-DG (D8375), 10µM rotenone (R8875), 5µM antimycin A (A8674), 10µM oligomycin (O4876), 10µM etomoxir (E1905) or a combination of several of these drugs for 30min. The cells were then stimulated with LPS (10ng/ml) (L2630) or IL4 (10ng/mL) (130-094-061, Miltenyi Biotech) for 2h in continued presence of metabolic inhibitors. Unless otherwise stated, all compounds were purchased from Sigma Aldrich.

Quantification of cytokine transcription

mRNAs were purified using either Qiagen RNeasy or Macherey-Nagel Nucleospin RNA kits, according to manufacturer's instruction.

mRNAs were retrotranscribed into cDNAs using M-MLV Reverse Transcriptase kit (Promega). Quantitative RT-PCRs were performed with MESA green mastermix (Eurogentec) and target-specific primers using QuantStudio 3 Real-Time PCR Systems (ThermoFisher Scientific). 18S RNA was used for normalization of mRNA levels. The DNA sequences of primers used for qPCR are listed in Table 2.

Quantification of cytokine secretion

Culture medium from stimulated BMDMs was recovered and cytokine quantification was performed using BioLegend LegendPlex kit according to manufacturer's instructions. Data were analyzed using Qognit software. Cytokines secreted by LPS-stimulated cells and cytokines secreted by IL4-stimulated cells were quantified using two lots of the LegendPlex kit.

Statistical analysis

Statistical analysis was performed with GraphPad Prism (La Jolla, CA, USA). Data are presented as mean ± SEM. Comparison between groups was performed with either one-way ANOVA followed by Tukey's test or two-way ANOVA followed by Dunnett's multiple comparison test.

TABLE 2 Sequences of DNA primers used in RT-qPCR reactions.

	Forward	Reverse
18S	GGGAGCCTGAGAAACGGC	GGGTCGGGAGTGGGTAATTT
Il6	TACCACTTCACAAGTCGGAGGC	CTGCAAGTGCATCATCGTTGTT
Tnfa	CCACCACGCTCTTCTGTCTA	CACTTGGTGGTTTGCTACGA
Mcp1	GGGCCTGCTGTTCACAGTT	CCAGCCTACTCATTGGGAT
Il10	GCTGGACAACATACTGCTAACC	ATTTCCGATAAGGCTTGGCAA
Il1b	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT

Data availability statement

Source data will be made available by the authors upon reasonable request.

Author contributions

RT, LO and FA designed the study. RT, LO and TE produced and analyzed experimental data. RT and FA wrote the manuscript. LO, TE and NV contributed in manuscript writing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fimmu.2022.918747/full#supplementary-material

SUPPLEMENTARY FIGURE 1
Macrophage expression profile at quiescence in response to metabolic inhibitors. Bone marrow-derived macrophages (BMDMs) were pretreated with metabolic inhibitors for 30 min and left untreated in clean cell culture media for 2 h after. Surface expression levels of MHCII by flow cytometry in BMDM that are unstimulated or stimulated with LPS for 2 h or 24 h. All points are shown in box plots with line at median. ns not significant, * p<0.05, *** p<0,001, **** p<0,0001. Unless otherwise stated, differences between groups are not statistically significant.

SUPPLEMENTARY FIGURE 2
MCP1 secretion by IL-4-stimulated BMDMs. Concentration of MCP1 in cell culture media of BMDMs pretreated with 2DG, Oligo, Rot/AA, Eto or Eto+2-DG and then stimulated with IL4 for 2h. All points are shown in box plots with line at median. One-way ANOVA was used to determine statistical significance between groups within each stimulation condition (untreated, LPS, IL4) * p<0.05, *** p<0,001, **** p<0,0001. Unless otherwise stated, differences between groups are not statistically significant.

References

1. Murray PJ, Wynn TA. Protective and pathogenic functions of macrophage subsets. *Nat Rev Immunol* (2011) 11:723–37. doi: 10.1038/nri3073

2. Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* (2002) 23:549–55. doi: 10.1016/s1471-4906(02)02302-5

3. Stein M, Keshav S, Harris N, Gordon S. Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. *J Exp Med* (1992) 176:287–92. doi: 10.1084/jem.176.1.287

4. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat Immunol* (2010) 11:889–96. doi: 10.1038/ni.1937

5. Tannahill GM, Curtis AM, Adamik J, Palsson-McDermott EM, McGettrick AF, Goel G, et al. Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature* (2013) 496:238–42. doi: 10.1038/nature11986

6. Meiser J, Krämer L, Sapcariu SC, Battello N, Ghelfi J, D'Herouel AF, et al. Pro-inflammatory macrophages sustain pyruvate oxidation through pyruvate dehydrogenase for the synthesis of itaconate and to enable cytokine expression. *J Biol Chem* (2016) 291:3932–46. doi: 10.1074/jbc.M115.676817

7. Wculek SK, Dunphy G, Heras-Murillo I, Mastrangelo A, Sancho D. Metabolism of tissue macrophages in homeostasis and pathology. *Cell Mol Immunol* (2022) 19:384–408. doi: 10.1038/s41423-021-00791-9

8. Huang SC-C, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, Smith AM, et al. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. *Nat Immunol* (2014) 15:846–55. doi: 10.1038/ni.2956

9. Orliaguet L, Dalmas E, Drareni K, Venteclef N, Alzaid F. Mechanisms of macrophage polarization in insulin signaling and sensitivity. *Front Endocrinol (Lausanne)* (2020) 11:62. doi: 10.3389/fendo.2020.00062

10. Kim S, Hwang J, Xuan J, Jung YH, Cha H-S, Kim KH. Global metabolite profiling of synovial fluid for the specific diagnosis of rheumatoid arthritis from other inflammatory arthritis. *PLoS One* (2014) 9:e97501. doi: 10.1371/journal.pone.0097501

11. Bae S, Kim H, Lee N, Won C, Kim H-R, Hwang Y, et al. α-enolase expressed on the surfaces of monocytes and macrophages induces robust synovial inflammation in rheumatoid arthritis. *J Immunol* (2012) 189:365–72. doi: 10.4049/jimmunol.1102073

12. Boutens L, Hooiveld GJ, Dhingra S, Cramer RA, Netea MG, Stienstra R. Unique metabolic activation of adipose tissue macrophages in obesity promotes inflammatory responses. *Diabetologia* (2018) 61:942–53. doi: 10.1007/s00125-017-4526-6

13. Krauss P-L, Pfeiffenberger M, Damerau A, Buttgereit T, Chen Y, Gaber T, et al. Production of IL-6 and phagocytosis are the most resilient immune functions in metabolically compromised human monocytes. *Front Immunol* (2021) 12:730672. doi: 10.3389/fimmu.2021.730672

14. Prieur X, Mok CYL, Velagapudi VR, Núñez V, Fuentes L, Montaner D, et al. Differential lipid partitioning between adipocytes and tissue macrophages

modulates macrophage lipotoxicity and M2/M1 polarization in obese mice. *Diabetes* (2011) 60:797–809. doi: 10.2337/db10-0705

15. Han MS, Jung DY, Morel C, Lakhani SA, Kim JK, Flavell RA, et al. JNK expression by macrophages promotes obesity-induced insulin resistance and inflammation. *Science* (2013) 339:218–22. doi: 10.1126/science.1227568

16. Blériot C, Chakarov S, Ginhoux F. Determinants of resident tissue macrophage identity and function. *Immunity* (2020) 52:957–70. doi: 10.1016/j.immuni.2020.05.014

17. Chakarov S, Lim HY, Tan L, Lim SY, See P, Lum J, et al. Two distinct interstitial macrophage populations coexist across tissues in specific subtissular niches. *Science* (2019) 363:eaau0964. doi: 10.1126/science.aau0964

18. Argüello RJ, Combes AJ, Char R, Gigan J-P, Baaziz AI, Bousiquot E, et al. SCENITH: A flow cytometry-based method to functionally profile energy metabolism with single-cell resolution. *Cell Metab* (2020) 32:1063–1075.e7. doi: 10.1016/j.cmet.2020.11.007

19. Pan Y, Yu Y, Wang X, Zhang T. Tumor-associated macrophages in tumor immunity. *Front Immunol* (2020) 11:583084. doi: 10.3389/fimmu.2020.583084

20. Abdin SM, Paasch D, Morgan M, Lachmann N. CARs and beyond: tailoring macrophage-based cell therapeutics to combat solid malignancies. *J Immunother Cancer* (2021) 9:e002741. doi: 10.1136/jitc-2021-002741

21. Klichinsky M, Ruella M, Shestova O, Lu XM, Best A, Zeeman M, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat Biotechnol* (2020) 38:947–53. doi: 10.1038/s41587-020-0462-y

22. Heap RE, Marín-Rubio JL, Peltier J, Heunis T, Dannoura A, Moore A, et al. Proteomics characterisation of the L929 cell supernatant and its role in BMDM differentiation. *Life Sci Alliance* (2021) 4:e202000957. doi: 10.26508/lsa.202000957

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Capturing the multifaceted function of adipose tissue macrophages

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Adipose tissue macrophages (ATMs) bolster obesity-induced metabolic dysfunction and represent a targetable population to lessen obesity-associated health risks. However, ATMs also facilitate adipose tissue function through multiple actions, including adipocyte clearance, lipid scavenging and metabolism, extracellular remodeling, and supporting angiogenesis and adipogenesis. Thus, high-resolution methods are needed to capture macrophages' dynamic and multifaceted functions in adipose tissue. Herein, we review current knowledge on regulatory networks critical to macrophage plasticity and their multifaceted response in the complex adipose tissue microenvironment.

KEYWORDS

obesity, adipose tissue, macrophage, immune response, single-cell RNA sequencing

1 Introduction

Obesity is a prevalent health risk to an expanding list of co-morbidities, increasing global rates of disability and mortality (1–3). Obesity induces white adipose tissue (WAT) dysfunction that significantly contributes to obesity-associated health risks through chronic, low-grade tissue inflammation, insulin resistance, hyperlipidemia, and hypertension (4). Studies have demonstrated that controlling obesity-associated WAT inflammation can improve tissue function and systemic health (5–9).

Within white adipose tissue, macrophages are the most abundant immune population. During obesity, the adipose tissue macrophage (ATM) population increases 10-fold in cell number and exacerbates local inflammation. ATMs originate from hematopoietic stem-cell-derived circulating monocytes and self-replicating tissue residents seeded during fetal development (10–12). Inhibiting macrophage expansion by limiting monocyte-derived macrophage recruitment during obesity lessens WAT inflammation (11, 13, 14). However, the ATM population is heterogeneous and critical to tissue function. Indeed, inhibition of specific ATM functions worsens systemic metabolic health (15). Thus, it is essential to understand the molecular signaling pathways that enrich beneficial ATM functions under obesity rather than eliminate them. Several ATM functions have been identified, including

dead adipocyte clearance, lipid scavenging and metabolism, extracellular remodeling, and supporting angiogenesis and adipogenesis. In addition, the macrophage population in brown adipose tissue is less defined, but several studies demonstrate their importance in maintaining thermogenesis (16–18). This review summarizes ATM functions important to white adipose tissue and relevant regulatory pathways that promote these actions. Herein, we aim to demonstrate the power of high-resolution investigations to characterize diverse macrophage populations and the need for function-based analysis to deconvolute targetable networks to lessen obesity-induced comorbidity and mortality.

2 Macrophage diversified responses

Macrophages can perform an array of functions to diverse stimuli, including pathogen- and damage-associated molecular patterns, cytokines, chemokines, metabolites, and extracellular vesicles (19, 20). Several models have been developed to classify macrophage features. One of the most widely utilized models is the M1/M2 paradigm, which delineates two central functional states: classically activated macrophages (M1) and alternatively activated macrophages (M2) (21). This and other *in vitro* models have allowed the characterization of regulatory mechanisms and signaling pathways crucial to several macrophage responses. It is now appreciated that these *in vitro*-based models do not recapitulate the complex stimuli experienced by tissue-resident macrophages but provide a basis to deconvolute the responses seen *in vivo*. Thus, mapped macrophage responses to stimuli experienced in adipose tissue and other physiologic and pathogenic states will be summarized in the following section (Figure 1A).

2.1 Cytokines and chemokines regulating macrophage actions

WAT is a source of adipokines, including immune modulatory cytokines, chemokines, and growth hormones. Many *in vitro* studies have detailed cytokines/chemokines that differentially contribute to the polarization of macrophages. Tumor necrosis factor alpha (TNF-α), interferon gamma (IFN-γ), C-C motif chemokine 2 (CCL2), CCL4, and interleukin 8 (CXCL8) can induce the classical activation of macrophages. M1-polarized macrophages provide acute pro-inflammatory effector functions by expressing reactive oxygen species, nitric oxide, and secretion of type-1 cytokines such as TNF-α, IFN-γ, and interleukin 1 beta (IL-1β). In contrast, macrophages are activated towards M2 polarization by IL-4, IL-13, IL-10, IL-17A, IL-25, and CCL5 to resolve acute inflammation and secrete type-2 cytokines, including IL-10 and arginase (21–25). The effect of cytokines on the polarization of macrophages has been investigated through *in vivo* studies in various contexts relevant to ATM functions (22, 26–28). Administration of IL-25 to obese mice mitigated weight gain through enhanced ATM M2-polarization, mitochondrial respiratory capacity, and lipolysis, demonstrating the therapeutic potential of targeting macrophage actions in obesity-associated disease (22).

2.2 Pattern recognition receptors relevant to ATMs

Macrophages are armed with an extensive repertoire of germline encoded pattern recognition receptors (PRRs) that recognize conserved molecular patterns that stimulate rapid innate immunity. Macrophages are also phagocytic and respond to particulate ligands from engulfed vesicles. Macrophage PRRs and ligands are summarized elsewhere (29); herein, we focus on those implicated in adipose tissue function. Receptor ligand interactions regulate macrophage responses *via* the release of stored mediators, transcription activation, and metabolic reprogramming. An important family of PRRs is Toll-like receptors (TLRs), which elicit pro-inflammatory responses to exogenous and endogenous molecular targets. TLR4 can be activated by lipopolysaccharide to initiate classical macrophage polarization (M1), as well as saturated fatty acids (30, 31), which are rich in obese AT. Inhibiting TLR4 signaling in models of obesity improves systemic metabolic function (32). Macrophages also express phosphatidylserine recognition receptors, scavenger receptors, type 3 complement receptors (CR3), β-glucan receptors, Fc receptors, and mannose receptors. Phosphatidylserine recognition receptors are a diverse group of proteins apt for recognizing apoptotic cells (33). Their dynamics in ATMs warrant further investigation, given their role in adipocyte clearance. Within scavenger receptors, CD36 is elevated in ATMs under obese stress (34). CD36 is a receptor for long-chain fatty acid transport (35) and binds various danger-associated molecular patterns (DAMPS) (31, 36, 37). Ligand-dependent activation of signaling cascades through CD36 relies on specific co-receptors and partners (33). Fatty acid binding to CD36 upregulates fatty acid oxidation (38), while DAMP interactions initiate a pro-inflammatory cascade in macrophages (39). CD36-mediated triacylglycerol uptake is necessary to support M2 activation through stimulating lipolysis and elevated oxidative phosphorylation (40). In the case of circulating macrophages, CD36 binding to oxidized low-density lipoproteins initiates a cellular metabolic shift to activate Mitogen-activated protein kinases (MAPK) signaling towards M1-polarization (39, 41, 42).

2.3 Key signaling pathways in macrophage function

Various stimuli elicit common downstream signaling pathways and metabolic programs, yielding similar macrophage responses. These signaling cascades' strength is tuned by small non-coding RNA molecules termed microRNA (miRNAs). The effect of miRNA on the polarization of

macrophages and its impact on ATM function has been extensively researched and is reviewed elsewhere (43). Notably, miR-155 and miR-223 are key regulators of macrophage polarization and profoundly impact systemic metabolism in obesity.

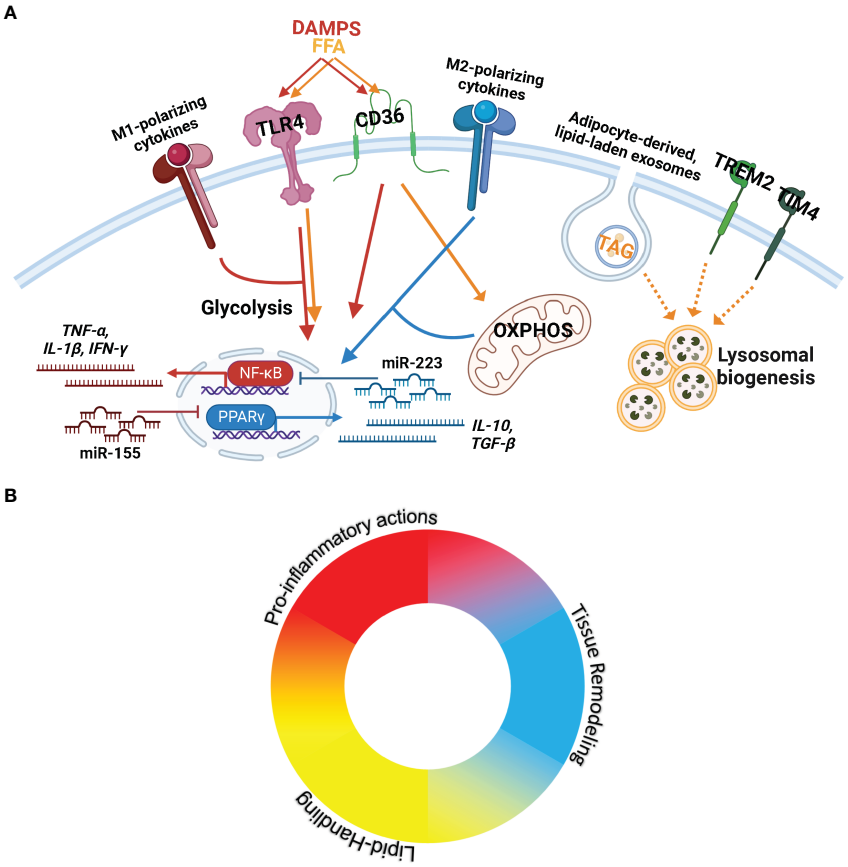

FIGURE 1
ATMs experience a complex microenvironment with opposing and compounding signals that results in a spectrum of activity. **(A)** Key stimuli, receptors, and signaling cascades known to control macrophage actions and implicated in ATM biology. (From Left to Right) M1-polarizing cytokines include TNF-α and IFN-γ. M1-polarizing chemokines are also present in AT and include CCL2, CCL4, and CXCL8. DAMPS= Danger-associated molecular patterns including exogenous (lipopolysaccharide) and endogenous signaling (e.g. apoptotic cells) are recognized through TLR4 to elicit pro-inflammatory signaling (JAK-STAT/NK-κB/MAPK) towards the production of type-1 cytokines (TNF-α, IL-1β, IFN-γ). miR-155 promotes M1-polarization through inhibition of signaling cascades towards PPARγ. FFA= Free Fatty Acids signaling through TLR4 elicits pro-inflammatory activation; CD36 transports FFAs, upregulating fatty acid oxidation and OXPHOS= Oxidative Phosphorylation. CD36 also recognizes DAMPs to activate pro-inflammatory activation. M2-polarizing cytokines (IL-4, IL-13, IL-10, IL-17A, IL-25) activate PPARγ to produce type-2 cytokines (IL-10, TGF-β). miR-223 promotes M2-polarization through the repression of pro-inflammatory signal cascade components. OXPHOS is required for M2 actions. Adipocyte-derived exosomes transport neutral lipids (including TAG=Triacylglycerols) into macrophages, which induce lysosomal biogenesis for lipid metabolism. Trem2 and Tim4 also elicit lysosomal biogenesis, but the ligands and downstream signaling cascades are unclear. **(B)** Macrophages are plastic, responding to diverse stimuli to provide appropriate action. Due to the plasticity of macrophages, their multifaceted capacity, and the complex microenvironment in AT, a single ATM can perform various actions. The ATM population is best represented as a spectrum of macrophage functions.

M1 polarization relies on the Janus kinase-signal transducer and activator of transcription (JAK-STAT) pathway *via* nuclear factor kappa B (NF-κB and MAPK signaling (44). The microRNA miR-155 supports pro-inflammatory activation of macrophages (45, 46) by repressing the translation of potent anti-inflammatory mediators including *Socs1*, *Ship1*, and *IL13Rα1* (47–50).

In contrast, M2 activation depends on the transcription factor Peroxisome proliferator-activated receptor γ (PPARγ), activated downstream of IL4-IL4R signaling. PPARγ stimulates miR-223 expression, supporting PPARγ in a positive feedback loop (51–53). miR-223 suppresses components of NF-κB signaling, including *Nfat5*, *Rasa1*, and *PKNOX1*, therein reducing pro-inflammatory cytokine production (51–53). Obese mice with systemic deletion of miR-223 had heightened WAT pro-inflammatory macrophage infiltration, inflammation, and worsened systemic metabolism (51).

2.4 Macrophage cellular metabolism

Macrophage cellular metabolism is integral to activation and function. For pro-inflammatory activation, macrophages rely on HIF1α-mediated aerobic glycolysis enrichment (40, 54, 55), increasing glucose and oxygen consumption likely to increase the production of reactive oxygen species (40). M2-polarization requires lipolysis and oxidative phosphorylation (56); inhibiting metabolic reprogramming severely weakens M2-mediated responses, including clearance of parasite helminth infections (56). Macrophage lipid metabolism is regulated through lipid uptake, synthesis, and clearance through mediators Sterol regulatory element-binding protein 1 (SREBP) and L-xylulose reductase (LXR). Inhibition of SREBP signaling in ATMs reduces cholesterol efflux, activating M1-polarization cascades and

increasing the proportion of pro-inflammatory ATMs (57). In addition, LXR-stimulated fatty acid synthesis represses pro-inflammatory cytokine production in ATMs and increases systemic insulin sensitivity in obesity (58). However, TLR4-mediated signaling inhibits LXR-mediated fatty acid synthesis and downstream Myeloid differentiation primary response protein (MyD88)- and TIR domain-containing adapter molecule (TRIF)-signaling pathways alters the lipid composition of macrophages to intensify inflammation (59). These opposing signaling cascades are likely responsible for the spectrum of ATMs activation states observed *in vivo* (Figure 1B).

3 Macrophage actions in white adipose tissue

ATM populations are heterogeneous, containing specialized subsets that can perform pro-inflammatory actions, lipid scavenging and metabolism, extracellular remodeling, and support angiogenesis and adipogenesis (Table 1). Functional characterization of tissue-resident macrophages is necessary to understand their regulation and identify modulatory pathways to promote metabolic health in obesity.

3.1 Pro-inflammatory actions

A primary function of recruited macrophages in WAT is immune activation for the removal of dead adipocytes, accomplished by pro-inflammatory ATMs. ATMs produce pro-inflammatory mediators that bolster tissue and systemic inflammation including TNF-α, IL-6, IL-1β, CCL2, Inducible nitric oxide synthase (iNOS), and others (65). Under obesity stress, WAT remodeling is continual, and the macrophage per adipocyte ratio increases significantly (10, 60, 66). The first characterization of ATM dynamics in lean and obese conditions found a shift from M2-like to M1-like cell predominance under obesity and secrete elevated TNF-α (60, 61). RNA-sequencing identified CD9-expressing, pro-inflammatory ATMs that surround dead adipocytes are abundant in obesity (15). ATMs also contribute to local tissue immune activation through antigen presentation to resident adaptive immune cells. During obesity, ATM express increased major histocompatibility complex II (MHCII) and co-stimulatory molecules to activate CD4+ T cells (67).

Notably, glucagon-like peptide-1 (GLP-1) agonists and GLP-1 analogs are a class of medication utilized in the treatment of obesity and type 2 diabetes (68, 69). Administration significantly reduces fat mass and macrophage per gram of fat in models of obesity (70). ATMs and peritoneal macrophages from treated mice also express less TNF-α and IL-6 (70), suggesting a possible direct role on macrophage activity. Given the impact of GLP-1 on obesity-induced health risk, further investigation into their impact on ATM actions is warranted.

3.2 Lipid-buffering

Macrophages support adipose tissue storage capacity by metabolizing lipids through lysosomal lipolysis. While all ATMs upregulate surface expression of the CD36 after a high-fat meal (34), subsets of specialized lipid-handling ATMs have been identified in both lean and obese WAT. In lean WAT, a subset of ATMs delineated by phospholipid-transporting ATPase ABCA1 (Abca1), T-cell immunoglobulin and mucin domain-containing protein 4 (Tim4), and lymphatic vessel endothelial hyaluronic acid receptor 1 (Lyve1) expression are self-replicating and most apt for lipid uptake and metabolism (34). After a meal, Tim4+ ATMs increase their lipid uptake, lysosomal content, and release HDL as part of the reverse cholesterol pathway (34).

During obesity, ATM transcriptomes demonstrate lysosome biogenesis is significantly enriched (66). Lipid-laden ATMs are more abundant and associated with elevated lysosome content (15, 62, 66). While Tim4+ ATMs persist in obesity, a novel lipid-laden ATM subset predominates. High-resolution investigations first delineated obesity-associated lipid-laden ATMs with CD9 expression (15), which were further demarcated by Triggering receptor expressed on myeloid cells 2 (Trem2) expression in both mice and humans (62). Recruited monocyte-derived macrophages are programmed by Trem2 signaling into lipid-laden ATMs (15). While inhibiting infiltration of monocyte-derived macrophages into obese WAT lessened overall tissue inflammation (11, 13, 14), specific inhibition of the Trem2 lipid-laden program of recruited macrophages exacerbates dyslipidemia and adipocyte hypertrophy, worsening overall metabolic health (15). Interestingly, ATMs uptake neutral lipids from adipocyte-derived exosomes, a distinct

TABLE 1 Macrophage functions in adipose tissue and identified markers/features of specialized cells.

Adipocyte Tissue Macrophage Functions	Key features	Citation
Pro-inflammatory functions (Dead adipocyte clearance, Immune activation)	CD9+, TNF-α expression	(15, 60, 61)
Lipid-Handling	Tim4+, Abca1+, Lyve-1+ (Lean) Trem2+, CD9+ (Obese)	(34, 62)
Extracellular Remodeling and Angiogenesis	Lyve-1+, MMP-9,-12 expression	(63)
Adipogenesis	Osteopontin expression	(61, 64)

While delineated features and markers have been identified for cells that are best equipped to perform these functions, macrophages are multifaceted and plastic in nature and likely perform multiple actions.

means of lipid handling for macrophages (71). Adipocyte-derived exosomes can induce ATM features from monocytes *in vitro* and, during obesity, are released at a significantly higher rate (71). Tim4 and Trem2 are relevant for ATM lysosome biogenesis. However, their ligand and downstream signaling cascades have not been elucidated. Tim4 and Trem2 are receptors for phosphatidylserine, a major component of exosomes (72, 73). Further research into the mechanism of adipocyte exosomes in developing lipid-laden ATMs could yield translatable discoveries for metabolic disease.

3.3 Extracellular remodeling and supporting angiogenesis and adipogenesis

WAT angiogenesis, adipogenesis, and extracellular remodeling are tightly linked processes to expand lipid storage capacity. ATMs regulate each process, but it is unclear if specialized subsets persist in WAT to perform these actions or if multifaceted macrophages contribute to these functions. While these cells do not recapitulate the M2 program, tissue remodeling and Transforming growth factor β (TGF-β) production have been described as an action of M2-polarized macrophages (74, 75). Whether important regulatory pathways towards M2 activation are responsible for ATM extracellular remodeling, angiogenesis, and adipogenesis remains unclear.

Angiogenesis is crucial to prevent hypoxia in expanding WAT. Increasing AT capillary density abrogates obesity-induced insulin resistance and metabolic dysfunction (76). A monocyte-derived ATMs expressing Lyve1 are recruited to hypoxic outgrowths of WAT in adolescent, lean mice. Lyve1+ ATMs express angiogenic matrix metalloproteinases (MMPs) and other angiogenic factors to form dense vascular networks that permit subsequent adipogenesis-mediated WAT development (63). MMPs degrade extracellular matrix components and are elevated in models of obesity. However, the extent of Lyve1+ ATM extracellular remodeling has yet to be explored. In addition, examples of macrophage-dependent angiogenesis in other contexts suggest M1 and M2 features are required for different stages of angiogenesis (77).

WAT requires extracellular remodeling to reduce stress on expanding and newly generated adipocytes and allow for proper vascularization. Unresolved remodeling and inflammation can lead to excessive extracellular matrix (ECM) component deposition, known as fibrosis. Obesity-induced WAT fibrosis has been linked to worsened metabolic dysfunction resistant to weight loss (78–80). In models of obesity-associated WAT fibrosis, macrophage depletion ameliorates fibrosis by reducing tissue inflammation and fibroblast activation (74). The primary mediator of macrophage-mediated activation of fibroblasts is TGF-β (63, 74, 81). In contrast, macrophages are also capable of ECM component uptake and degradation; however, these actions have not been explored in WAT.

Adipose tissue undergoes continual adipocyte turnover, refreshing the population through adipogenesis. In addition to adipogenesis within tissue outgrowths, ATMs initiate adipogenesis by expressing osteopontin to recruit pre-adipocytes towards a dying adipocyte (61, 64). In this way, the adipocyte is quickly replaced, and newly differentiated cells have space to develop; however, localization of the progenitor cell to the periphery of pro-inflammatory ATM clearance of a dead cell can be a double edge sword. Pro-inflammatory mediators such as TNF-α repress master adipogenic transcription factor PPARγ in pre-adipocytes (82, 83). During obesity, insufficient adipogenesis forces adipocytes to increase in size, or hypertrophy, and is correlated with increased inflammation, worsened metabolic health, and greater risk for co-morbidities (84–89).

4 Capturing dynamic ATM actions

Traditional methods to infer macrophage function rely on detecting a limited number of M1/M2 markers at the RNA or protein levels. Transcriptomic investigations, including microarrays and RNA-sequencing, have demonstrated the diversity of ATMs that cannot be neatly dichotomized into M1/M2 (15, 90) and revealed the importance of lipid-mediated reprogramming (15, 66). The advent of single-cell RNA-sequencing (scRNA-seq) has permitted unparalleled resolution to characterize the diversity in the immune compartment of adipose tissue.

4.1 Strategies in single-cell RNA-sequencing

ScRNA-seq generates highly dimensional data, and strategies to analyze begin with performing dimension reduction to facilitate downstream comparisons. Most strategies for dimension reduction utilize the whole transcriptome to establish similarities across cells, such as t-distributed stochastic neighbor embedding (tSNE) (91) and uniform manifold approximation and project (UMAP) (92). Following dimension reduction, cells are typically clustered into groups based on relative distances in the low-dimensional projection and overall transcriptomic similarity. These unsupervised algorithms delineate cells well based on major perturbations in the transcriptome to cluster cells based on lineage or for *de novo* subset identification. Researchers can then characterize clusters based on known cell markers and differential gene expression. However, due to frequent gene "dropouts" in scRNA-seq data, where expressed genes are not detected, and the variable turnover of marker proteins, traditional biomarker genes are not always reliable. To accommodate the disconnect observed between mRNA and protein levels, semi-supervised tools have been developed that use machine learning-based classifiers or manually curated lists of biomarkers to classify groups of cells based on their transcriptomic profiles (93–95). In addition, multiomic strategies combining scRNA-seq with targeted proteomic analysis (96) or spatial location (97) are becoming more popular. Application of scRNA-Seq in adipose tissue depicted the heterogeneity of ATMs, revealing the lipid-handling Tim4+ and obesity-associated Trem2+ population in mice (34, 62) and tissue immune cell dynamics across lineages in lean and obese humans (98).

Beyond identifying cell subsets, cell function is of great scientific interest in scRNA-seq studies. Several programs have been widely applied for the downstream cluster-based annotation of gene ontology from transcriptomics data, including DAVID (99, 100) and Qiagen Ingenuity Pathway Analysis (IPA) (101). However, these methods are not high-throughput and thus cannot represent the continuum of cell actions. In addition, while traditional whole-transcriptome dimension reduction and clustering techniques work well to distinguish cell lineages, differences in cell function, especially for cells such as macrophages, are often represented by much more subtle transcriptomic changes. Therefore, cell functional annotation in scRNA-seq data is a current technical challenge representing an area of active research.

4.2 Algorithms for function-guided cell annotation

Depicting the dynamic actions of macrophages in transcriptomics data has been challenging. Due to the whole-transcriptome input into unsupervised algorithms, functional distinctions are not intentionally utilized for clustering. Further, cells respond to diverse stimuli that induce divergent transcriptomes for multifaceted functions (102). Thus, capturing complex stimulation-induced signaling network changes towards multifaceted actions poses an important technical challenge in bioinformatics (103). To this end, we have designed two programs, MacSpectrum (90) and AtheroSpectrum (104), to depict three actions of macrophages along a spectrum of action intensity: monocyte maturation, macrophage inflammatory polarization, and atherosclerosis-related foaming. Applications of MacSpectrum in ATMs depicted the enrichment of pro-inflammatory ATMs in obesity (90), permitting investigation into obesity-associated macrophage inflammatory programs. Further, MacSpectrum characterized an ATM CD206+CD11c- subset enriched in diabetic obese humans as phenotypically distinct, with terminal differentiation and less pro-inflammatory than lipid-laden cells (105). AtheroSpectrum is tailored to atherosclerotic macrophages. Utilization of AtheroSpectrum revealed two novel macrophage foaming programs: homeostasis foaming and pathogenic foaming, the latter associated with cardiovascular disease. Depicting these distinct programs allowed for a focused investigation into pathogenic foaming that enabled leveraging program-specific genes to improve cardiovascular risk prediction models (104). These programs demonstrate the importance of depicting macrophage plasticity to parse out nuanced regulatory networks driving diversified macrophage function.

5 Discussion

Obesity is a major health risk in part due to adipose tissue dysfunction. In the adipose tissue, macrophages potentiate local inflammation through pro-inflammatory cytokine production and immune activation. However, ATMs represent a heterogeneous population that support tissue function through dead adipocyte clearance, lipid-buffering, extracellular remodeling, and supporting angiogenesis and adipogenesis. High-resolution techniques have allowed the identification of ATM subsets best suited to these functions. Leveraging scRNA-seq techniques to capture the spectrum of multifaceted macrophage actions allows for important macrophage programs to emerge that are not evident using traditional macrophage categorization models or low-resolution techniques. Function-guided macrophage annotations are important to understanding tissue heterogeneity and investigating ATM programs correlated with metabolic health.

Author contributions

AM wrote and revised the manuscript. LQ, KK, AV and BZ contributed to manuscript design and revisions. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Field AE, Coakley EH, Must A, Spadano JL, Laird N, Dietz WH, et al. Impact of overweight on the risk of developing common chronic diseases during a 10-year period. *Arch Intern Med* (2001) 161:1581–6. doi: 10.1001/archinte.161.13.1581

2. GBD 2015 Obesity Collaborators, Afshin A, Forouzanfar MH, Reitsma MB, Sur P, Estep K, et al. Health effects of overweight and obesity in 195 countries over 25 years. *N Engl J Med* (2017) 377:13–27. doi: 10.1056/NEJMoa1614362

3. Juonala M, Magnussen CG, Berenson GS, Venn A, Burns TL, Sabin MA, et al. Childhood adiposity, adult adiposity, and cardiovascular risk factors. *N Engl J Med* (2011) 365:1876–85. doi: 10.1056/NEJMoa1010112

4. Fruhbeck G. Overview of adipose tissue and its role in obesity and metabolic disorders. *Methods Mol Biol* (2008) 456:1–22. doi: 10.1007/978-1-59745-245-8_1

5. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* (2003) 112:1821–30. doi: 10.1172/JCI19451

6. Fleischman A, Shoelson SE, Bernier R, Goldfine AB. Salsalate improves glycemia and inflammatory parameters in obese young adults. *Diabetes Care* (2008) 31:289–94. doi: 10.2337/dc07-1338

7. Stanley TL, Zanni MV, Johnsen S, Rasheed S, Makimura H, Lee H, et al. TNF-alpha antagonism with etanercept decreases glucose and increases the proportion of high molecular weight adiponectin in obese subjects with features of the metabolic syndrome. *J Clin Endocrinol Metab* (2011) 96:146. doi: 10.1210/jc.2010-1170

8. Hauser TH, Salastekar N, Schaefer EJ, Desai T, Goldfine HL, Fowler KM, et al. Effect of targeting inflammation with salsalate: The TINSAL-CVD randomized clinical trial on progression of coronary plaque in overweight and obese patients using statins. *JAMA Cardiol* (2016) 1:413–23. doi: 10.1001/jamacardio.2016.0605

9. Oral EA, Reilly SM, Gomez AV, Meral R, Butz L, Ajluni N, et al. Inhibition of IKKvarepsilon and TBK1 improves glucose control in a subset of patients with type 2 diabetes. *Cell Metab* (2017) 26:157–170.e7. doi: 10.1016/j.cmet.2017.06.006

10. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AWJr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* (2003) 112:1796–808. doi: 10.1172/JCI200319246

11. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* (2006) 116:1494–505. doi: 10.1172/JCI26498

12. Amano SU, Cohen JL, Vangala P, Tencerova M, Nicoloro SM, Yawe JC, et al. Local proliferation of macrophages contributes to obesity-associated adipose tissue inflammation. *Cell Metab* (2014) 19:162–71. doi: 10.1016/j.cmet.2013.11.017

13. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, et al. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. *Nature* (2007) 447:1116–20. doi: 10.1038/nature05894

14. Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG. Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. *Cell Metab* (2008) 8:301–9. doi: 10.1016/j.cmet.2008.08.015

15. Hill DA, Lim HW, Kim YH, Ho WY, Foong YH, Nelson VL, et al. Distinct macrophage populations direct inflammatory versus physiological changes in adipose tissue. *Proc Natl Acad Sci U.S.A.* (2018) 115:E5096–105. doi: 10.1073/pnas.1802611115

16. Rosina M, Ceci V, Turchi R, Chuan L, Borcherding N, Sciarretta F, et al. Ejection of damaged mitochondria and their removal by macrophages ensure efficient thermogenesis in brown adipose tissue. *Cell Metab* (2022) 34:533–548.e12. doi: 10.1016/j.cmet.2022.02.016

17. Adachi Y, Ueda K, Nomura S, Ito K, Katoh M, Katagiri M, et al. Beiging of perivascular adipose tissue regulates its inflammation and vascular remodeling. *Nat Commun* (2022) 13:5117–6. doi: 10.1038/s41467-022-32658-6

18. Gallerand A, Stunault MI, Merlin J, Luehmann HP, Sultan DH, Firulyova MM, et al. Brown adipose tissue monocytes support tissue expansion. *Nat Commun* (2021) 12:5255–1. doi: 10.1038/s41467-021-25616-1

19. Locati M, Curtale G, Mantovani A. Diversity, mechanisms, and significance of macrophage plasticity. *Annu Rev Pathol* (2020) 15:123–47. doi: 10.1146/annurev-pathmechdis-012418-012718

20. Qu L, Matz AJ, Karlinsey K, Cao Z, Vella AT, Zhou B. Macrophages at the crossroad of meta-inflammation and inflammaging. *Genes (Basel)* (2022) 13:2074. doi: 10.3390/genes13112074

21. Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. *J Immunol* (2000) 164:6166–73. doi: 10.4049/jimmunol.164.12.6166

22. Feng J, Li L, Ou Z, Li Q, Gong B, Zhao Z, et al. IL-25 stimulates M2 macrophage polarization and thereby promotes mitochondrial respiratory capacity and lipolysis in adipose tissues against obesity. *Cell Mol Immunol* (2018) 15:493–505. doi: 10.1038/cmi.2016.71

23. Guo M, Härtlova A, Gierliński M, Prescott A, Castellvi J, Losa JH, et al. Triggering MSR1 promotes JNK-mediated inflammation in IL-4-activated macrophages. *EMBO J* (2019) 38:e100299. doi: 10.15252/embj.2018100299

24. Ruytinx P, Proost P, Van Damme J, Struyf S. Chemokine-induced macrophage polarization in inflammatory conditions. *Front Immunol* (2018) 9:1930. doi: 10.3389/fimmu.2018.01930

25. Czimmerer Z, Halasz L, Daniel B, Varga Z, Bene K, Domokos A, et al. The epigenetic state of IL-4-polarized macrophages enables inflammatory cistromic expansion and extended synergistic response to TLR ligands. *Immunity* (2022) 55:2006–2026.e6. doi: 10.1016/j.immuni.2022.10.004

26. Seki E, de Minicis S, Inokuchi S, Taura K, Miyai K, van Rooijen N, et al. CCR2 promotes hepatic fibrosis in mice. *Hepatology* (2009) 50:185–97. doi: 10.1002/hep.22952

27. Karlmark KR, Weiskirchen R, Zimmermann HW, Gassler N, Ginhoux F, Weber C, et al. Hepatic recruitment of the inflammatory Gr1+ monocyte subset upon liver injury promotes hepatic fibrosis. *Hepatology* (2009) 50:261–74. doi: 10.1002/hep.22950

28. Götze AM, Schubert C, Jung G, Dörr O, Liebetrau C, Hamm CW, et al. IL10 alters peri-collateral macrophage polarization and hind-limb reperfusion in mice after femoral artery ligation. *Int J Mol Sci* (2020) 21:2821. doi: 10.3390/ijms21082821

29. Gordon S. Pattern recognition receptors: doubling up for the innate immune response. *Cell* (2002) 111:927–30. doi: 10.1016/s0092-8674(02)01201-1

30. Shi H, Kokoeva MV, Inouye K, Tzameli I, Yin H, Flier JS. TLR4 links innate immunity and fatty acid-induced insulin resistance. *J Clin Invest* (2006) 116:3015–25. doi: 10.1172/JCI28898

31. Kopp A, Gross P, Falk W, Bala M, Weigert J, Buechler C, et al. Fatty acids as metabolic mediators in innate immunity. *Eur J Clin Invest* (2009) 39:924–33. doi: 10.1111/j.1365-2362.2009.02185.x

32. Vila IK, Badin P, Marques M, Monbrun L, Lefort C, Mir L, et al. Immune cell toll-like receptor 4 mediates the development of obesity- and endotoxemia-associated adipose tissue fibrosis. *Cell Rep* (2014) 7:1116–29. doi: 10.1016/j.celrep.2014.03.062

33. Penberthy KK, Ravichandran KS. Apoptotic cell recognition receptors and scavenger receptors. *Immunol Rev* (2016) 269:44–59. doi: 10.1111/imr.12376

34. Magalhaes MS, Smith P, Portman JR, Jackson-Jones LH, Bain CC, Ramachandran P, et al. Role of Tim4 in the regulation of ABCA1(+) adipose tissue macrophages and post-prandial cholesterol levels. *Nat Commun* (2021) 12:4434–7. doi: 10.1038/s41467-021-24684-7

35. Harmon CM, Abumrad NA. Binding of sulfosuccinimidyl fatty acids to adipocyte membrane proteins: Isolation and amino-terminal sequence of an 88-kD protein implicated in transport of long-chain fatty acids. *J Membr Biol* (1993) 133:43–9. doi: 10.1007/BF00231876

36. Chen Y, Zhang J, Cui W, Silverstein RL. CD36, a signaling receptor and fatty acid transporter that regulates immune cell metabolism and fate. *J Exp Med* (2022) 219:e20211314. doi: 10.1084/jem.20211314

37. Greenberg ME, Sun M, Zhang R, Febbraio M, Silverstein R, Hazen SL. Oxidized phosphatidylserine-CD36 interactions play an essential role in macrophage-dependent phagocytosis of apoptotic cells. *J Exp Med* (2006) 203:2613–25. doi: 10.1084/jem.20060370

38. Samovski D, Sun J, Pietka T, Gross RW, Eckel RH, Su X, et al. Regulation of AMPK activation by CD36 links fatty acid uptake to β-oxidation. *Diabetes* (2015) 64:353–9. doi: 10.2337/db14-0582

39. Chen Y, Yang M, Huang W, Chen W, Zhao Y, Schulte ML, et al. Mitochondrial metabolic reprogramming by CD36 signaling drives macrophage inflammatory responses. *Circ Res* (2019) 125:1087–102. doi: 10.1161/CIRCRESAHA.119.315833

40. Liu L, Lu Y, Martinez J, Bi Y, Lian G, Wang T, et al. Proinflammatory signal suppresses proliferation and shifts macrophage metabolism from myc-dependent to HIF1α-dependent. *Proc Natl Acad Sci USA* (2016) 113:1564–9. doi: 10.1073/pnas.1518000113

41. Rahaman SO, Lennon DJ, Febbraio M, Podrez EA, Hazen SL, Silverstein RL. A CD36-dependent signaling cascade is necessary for macrophage foam cell formation. *Cell Metab* (2006) 4:211–21. doi: 10.1016/j.cmet.2006.06.007

42. Moore KJ, El Khoury J, Medeiros LA, Terada K, Geula C, Luster AD, et al. A CD36-initiated signaling cascade mediates inflammatory effects of beta-amyloid. *J Biol Chem* (2002) 277:47373–9. doi: 10.1074/jbc.M208788200

43. Matz A, Qu L, Karlinsey K, Zhou B. Impact of microRNA regulated macrophage actions on adipose tissue function in obesity. *Cells* (2022) 11:1336. doi: 10.3390/cells11081336

44. Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. *Nat Rev Immunol* (2008) 8:958–69. doi: 10.1038/nri2448

45. Taganov KD, Boldin MP, Chang KJ, Baltimore D. NF-kappaB-dependent induction of microRNA miR-146, an inhibitor targeted to signaling proteins of innate immune responses. *Proc Natl Acad Sci USA* (2006) 103:12481–6. doi: 10.1073/pnas.0605298103

46. O'Connell RM, Taganov KD, Boldin MP, Cheng G, Baltimore D. MicroRNA-155 is induced during the macrophage inflammatory response. *Proc Natl Acad Sci USA* (2007) 104:1604–9. doi: 10.1073/pnas.0610731104

47. Androulidaki A, Iliopoulos D, Arranz A, Doxaki C, Schworer S, Zacharioudaki V, et al. The kinase Akt1 controls macrophage response to lipopolysaccharide by regulating microRNAs. *Immunity* (2009) 31:220–31. doi: 10.1016/j.immuni.2009.06.024

48. O'Connell RM, Chaudhuri AA, Rao DS, Baltimore D. Inositol phosphatase SHIP1 is a primary target of miR-155. *Proc Natl Acad Sci USA* (2009) 106:7113–8. doi: 10.1073/pnas.0902636106

49. Rajaram MV, Ni B, Morris JD, Brooks MN, Carlson TK, Bakthavachalu B, et al. Mycobacterium tuberculosis lipomannan blocks TNF biosynthesis by regulating macrophage MAPK-activated protein kinase 2 (MK2) and microRNA miR-125b. *Proc Natl Acad Sci USA* (2011) 108:17408–13. doi: 10.1073/pnas.1112660108

50. Martinez-Nunez RT, Louafi F, Sanchez-Elsner T. The interleukin 13 (IL-13) pathway in human macrophages is modulated by microRNA-155 *via* direct targeting of interleukin 13 receptor alpha1 (IL13Ralpha1). *J Biol Chem* (2011) 286:1786–94. doi: 10.1074/jbc.M110.169367

51. Zhuang G, Meng C, Guo X, Cheruku PS, Shi L, Xu H, et al. A novel regulator of macrophage activation: miR-223 in obesity-associated adipose tissue inflammation. *Circulation* (2012) 125:2892–903. doi: 10.1161/CIRCULATIONAHA.111.087817

52. Ying W, Tseng A, Chang RC, Morin A, Brehm T, Triff K, et al. MicroRNA-223 is a crucial mediator of PPARγ-regulated alternative macrophage activation. *J Clin Invest* (2015) 125:4149–59. doi: 10.1172/JCI81656

53. Zhou H, Xiao J, Wu N, Liu C, Xu J, Liu F, et al. MicroRNA-223 regulates the differentiation and function of intestinal dendritic cells and macrophages by targeting C/EBPβ. *Cell Rep* (2015) 13:1149–60. doi: 10.1016/j.celrep.2015.09.073

54. El Kasmi KC, Stenmark KR. Contribution of metabolic reprogramming to macrophage plasticity and function. *Semin Immunol* (2015) 27:267–75. doi: 10.1016/j.smim.2015.09.001

55. Newsholme P, Curi R, Gordon S, Newsholme EA. Metabolism of glucose, glutamine, long-chain fatty acids and ketone bodies by murine macrophages. *Biochem J* (1986) 239:121–5. doi: 10.1042/bj2390121

56. Huang SC, Everts B, Ivanova Y, O'Sullivan D, Nascimento M, Smith AM, et al. Cell-intrinsic lysosomal lipolysis is essential for alternative activation of macrophages. *Nat Immunol* (2014) 15:846–55. doi: 10.1038/ni.2956

57. Lee J, Lee SH, Lee E, Cho J, Song D, Lee YJ, et al. SCAP deficiency facilitates obesity and insulin resistance through shifting adipose tissue macrophage polarization. *J Adv Res* (2022) S2090-1232(22)00124-2. doi: 10.1016/j.jare.2022.05.013

58. Li P, Spann NJ, Kaikkonen MU, Lu M, Oh DY, Fox JN, et al. NCoR repression of LXRs restricts macrophage biosynthesis of insulin-sensitizing omega 3 fatty acids. *Cell* (2013) 155:200–14. doi: 10.1016/j.cell.2013.08.054

59. Hsieh W, Zhou QD, York AG, Williams KJ, Scumpia PO, Kronenberger EB, et al. Toll-like receptors induce signal-specific reprogramming of the macrophage lipidome. *Cell Metab* (2020) 32:128–143.e5. doi: 10.1016/j.cmet.2020.05.003

60. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* (2007) 117:175–84. doi: 10.1172/JCI29881

61. Lumeng CN, DelProposto JB, Westcott DJ, Saltiel AR. Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes. *Diabetes* (2008) 57:3239–46. doi: 10.2337/db08-0872

62. Jaitin DA, Adlung L, Thaiss CA, Weiner A, Li B, Descamps H, et al. Lipid-associated macrophages control metabolic homeostasis in a Trem2-dependent manner. *Cell* (2019) 178:686–98.e14. doi: 10.1016/j.cell.2019.05.054

63. Cho CH, Koh YJ, Han J, Sung HK, Jong Lee H, Morisada T, et al. Angiogenic role of LYVE-1-positive macrophages in adipose tissue. *Circ Res* (2007) 100:47. doi: 10.1161/01.RES.0000259564.92792.93

64. Lee YH, Petkova AP, Granneman JG. Identification of an adipogenic niche for adipose tissue remodeling and restoration. *Cell Metab* (2013) 18:355–67. doi: 10.1016/j.cmet.2013.08.003

65. Varol C, Mildner A, Jung S. Macrophages: development and tissue specialization. *Annu Rev Immunol* (2015) 33:643–75. doi: 10.1146/annurev-immunol-032414-112220

66. Xu X, Grijalva A, Skowronski A, van Eijk M, Serlie MJ, Ferrante AWJr. Obesity activates a program of lysosomal-dependent lipid metabolism in adipose tissue macrophages independently of classic activation. *Cell Metab* (2013) 18:816–30. doi: 10.1016/j.cmet.2013.11.001

67. Morris DL, Cho KW, Delproposto JL, Oatmen KE, Geletka LM, Martinez-Santibanez G, et al. Adipose tissue macrophages function as antigen-presenting cells and regulate adipose tissue CD4+ T cells in mice. *Diabetes* (2013) 62:2762–72. doi: 10.2337/db12-1404

68. Urva S, Coskun T, Loh MT, Du Y, Thomas MK, Gurbuz S, et al. LY3437943, a novel triple GIP, GLP-1, and glucagon receptor agonist in people with type 2 diabetes: a phase 1b, multicentre, double-blind, placebo-controlled, randomised, multiple-ascending dose trial. *Lancet* (2022) 400:1869–81. doi: 10.1016/S0140-6736(22)02033-5

69. Jastreboff AM, Aronne LJ, Ahmad NN, Wharton S, Connery L, Alves B, et al. Tirzepatide once weekly for the treatment of obesity. *N Engl J Med* (2022) 387:205–16. doi: 10.1056/NEJMoa2206038

70. Lee Y, Park M, Choung J, Kim S, Oh H, Choi C, et al. Glucagon-like peptide-1 inhibits adipose tissue macrophage infiltration and inflammation in an obese mouse model of diabetes. *Diabetologia* (2012) 55:2456–68. doi: 10.1007/s00125-012-2592-3

71. Flaherty SE, Grijalva A, Xu X, Ables E, Nomani A, Ferrante AWJ. A lipase-independent pathway of lipid release and immune modulation by adipocytes. *Science* (2019) 363:989–93. doi: 10.1126/science.aaw2586

72. Yoshida T, Hanayama R. TIM4-affinity methods targeting phosphatidylserine for isolation or detection of extracellular vesicles. *Methods Mol Biol* (2022) 2466:23–36. doi: 10.1007/978-1-0716-2176-9_2

73. Sudom A, Talreja S, Danao J, Bragg E, Kegel R, Min X, et al. Molecular basis for the loss-of-function effects of the alzheimer's disease-associated R47H variant of the immune receptor TREM2. *J Biol Chem* (2018) 293:12634–46. doi: 10.1074/jbc.RA118.002352

74. Martinez-Santibanez G, Singer K, Cho KW, DelProposto JL, Mergian T, Lumeng CN. Obesity-induced remodeling of the adipose tissue elastin network is independent of the metalloelastase MMP-12. *Adipocyte* (2015) 4:264–72. doi: 10.1080/21623945.2015.1027848

75. Rao L, Wang Y, Zhang L, Wu G, Zhang L, Wang F, et al. IL-24 deficiency protects mice against bleomycin-induced pulmonary fibrosis by repressing IL-4-induced M2 program in macrophages. *Cell Death Differ* (2021) 28:1270–83. doi: 10.1038/s41418-020-00650-6

76. Cao Y. Angiogenesis and vascular functions in modulation of obesity, adipose metabolism, and insulin sensitivity. *Cell Metab* (2013) 18:478–89. doi: 10.1016/j.cmet.2013.08.008

77. Spiller KL, Anfang RR, Spiller KJ, Ng J, Nakazawa KR, Daulton JW, et al. The role of macrophage phenotype in vascularization of tissue engineering scaffolds. *Biomaterials* (2014) 35:4477–88. doi: 10.1016/j.biomaterials.2014.02.012

78. Divoux A, Tordjman J, Lacasa D, Veyrie N, Hugol D, Aissat A, et al. Fibrosis in human adipose tissue: Composition, distribution, and link with lipid metabolism and fat mass loss. *Diabetes* (2010) 59:2817–25. doi: 10.2337/db10-0585

79. Lackey DE, Burk DH, Ali MR, Mostaedi R, Smith WH, Park J, et al. Contributions of adipose tissue architectural and tensile properties toward defining healthy and unhealthy obesity. *Am J Physiol Endocrinol Metab* (2014) 306:233. doi: 10.1152/ajpendo.00476.2013

80. Hasegawa Y, Ikeda K, Chen Y, Alba DL, Stifler D, Shinoda K, et al. Repression of adipose tissue fibrosis through a PRDM16-GTF2IRD1 complex improves systemic glucose homeostasis. *Cell Metab* (2018) 27:180–194.e6. doi: 10.1016/j.cmet.2017.12.005

81. Shen H, Huang X, Zhao Y, Wu D, Xue K, Yao J, et al. The hippo pathway links adipocyte plasticity to adipose tissue fibrosis. *Nat Commun* (2022) 13:6030–0. doi: 10.1038/s41467-022-33800-0

82. Meng L, Zhou J, Sasano H, Suzuki T, Zeitoun KM, Bulun SE. Tumor necrosis factor alpha and interleukin 11 secreted by malignant breast epithelial cells inhibit adipocyte differentiation by selectively down-regulating CCAAT/enhancer binding protein alpha and peroxisome proliferator-activated receptor gamma: Mechanism of desmoplastic reaction. *Cancer Res* (2001) 61:2250–5.

83. Cawthorn WP, Heyd F, Hegyi K, Sethi JK. Tumour necrosis factor-alpha inhibits adipogenesis *via* a beta-catenin/TCF4(TCF7L2)-dependent pathway. *Cell Death Differ* (2007) 14:1361–73. doi: 10.1038/sj.cdd.4402127

84. Pischon T, Boeing H, Hoffmann K, Bergmann M, Schulze MB, Overvad K, et al. General and abdominal adiposity and risk of death in Europe. *N Engl J Med* (2008) 359:2105–20. doi: 10.1056/NEJMoa0801891

85. Shungin D, Winkler TW, Croteau-Chonka DC, Ferreira T, Locke AE, Mägi R, et al. New genetic loci link adipose and insulin biology to body fat distribution. *Nature* (2015) 518:187–96. doi: 10.1038/nature14132

86. Wang QA, Tao C, Gupta RK, Scherer PE. Tracking adipogenesis during white adipose tissue development, expansion and regeneration. *Nat Med* (2013) 19:1338–44. doi: 10.1038/nm.3324

87. Jeffery E, Church CD, Holtrup B, Colman L, Rodeheffer MS. Rapid depot-specific activation of adipocyte precursor cells at the onset of obesity. *Nat Cell Biol* (2015) 17:376–85. doi: 10.1038/ncb3122

88. Wueest S, Rapold RA, Rytka JM, Schoenle EJ, Konrad D. Basal lipolysis, not the degree of insulin resistance, differentiates large from small isolated adipocytes in high-fat fed mice. *Diabetologia* (2009) 52:541–6. doi: 10.1007/s00125-008-1223-5

89. Gao H, Mejhert N, Fretz JA, Arner E, Lorente-Cebrián S, Ehrlund A, et al. Early b cell factor 1 regulates adipocyte morphology and lipolysis in white adipose tissue. *Cell Metab* (2014) 19:981–92. doi: 10.1016/j.cmet.2014.03.032

90. Li C, Menoret A, Farragher C, Ouyang Z, Bonin C, Holvoet P, et al. Single cell transcriptomics based-MacSpectrum reveals novel macrophage activation signatures in diseases. *JCI Insight* (2019) 5:e126453. doi: 10.1172/jci.insight.126453

91. Van der Maaten L, Hinton G. Visualizing data using t-SNE. *J Mach Learn Res* (2008) 9:2579–605.

92. Becht E, McInnes L, Healy J, Dutertre C, Kwok IWH, Ng LG, et al. Dimensionality reduction for visualizing single-cell data using UMAP. *Nat Biotechnol* (2018) 37:38–44. doi: 10.1038/nbt.4314

93. Abdelaal T, Michielsen L, Cats D, Hoogduin D, Mei H, Reinders MJT, et al. A comparison of automatic cell identification methods for single-cell RNA sequencing data. *Genome Biol* (2019) 20:194–z. doi: 10.1186/s13059-019-1795-z

94. Crowell HL, Soneson C, Germain P, Calini D, Collin L, Raposo C, et al. Muscat Detects subpopulation-specific state transitions from multi-sample multi-condition single-cell transcriptomics data. *Nat Commun* (2020) 11:6077–4. doi: 10.1038/s41467-020-19894-4

95. Pliner HA, Shendure J, Trapnell C. Supervised classification enables rapid annotation of cell atlases. *Nat Methods* (2019) 16:983–6. doi: 10.1038/s41592-019-0535-3

96. Lacar B. Generation of centered log-ratio normalized antibody-derived tag counts from Large single-cell sequencing datasets. *Methods Mol Biol* (2022) 2386:203–17. doi: 10.1007/978-1-0716-1771-7_14

97. Moncada R, Barkley D, Wagner F, Chiodin M, Devlin JC, Baron M, et al. Integrating microarray-based spatial transcriptomics and single-cell RNA-seq reveals tissue architecture in pancreatic ductal adenocarcinomas. *Nat Biotechnol* (2020) 38:333–42. doi: 10.1038/s41587-019-0392-8

98. Hildreth AD, Ma F, Wong YY, Sun R, Pellegrini M, O'Sullivan TE. Single-cell sequencing of human white adipose tissue identifies new cell states in health and obesity. *Nat Immunol* (2021) 22:639–53. doi: 10.1038/s41590-021-00922-4

99. Huang DW, Sherman BT, Lempicki RA. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* (2009) 4:44–57. doi: 10.1038/nprot.2008.211

100. Huang DW, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res* (2009) 37:1–13. doi: 10.1093/nar/gkn923

101. Krämer A, Green J, Pollard JJ, Tugendreich S. Causal analysis approaches in ingenuity pathway analysis. *Bioinformatics* (2014) 30:523–30. doi: 10.1093/bioinformatics/btt703

102. Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goerdt S, et al. Macrophage activation and polarization: nomenclature and experimental guidelines. *Immunity* (2014) 41:14–20. doi: 10.1016/j.immuni.2014.06.008

103. Holland CH, Tanevski J, Perales-Patón J, Gleixner J, Kumar MP, Mereu E, et al. Robustness and applicability of transcription factor and pathway analysis tools on single-cell RNA-seq data. *Genome Biol* (2020) 21:36–z. doi: 10.1186/s13059-020-1949-z

104. Li C, Qu L, Matz AJ, Murphy PA, Liu Y, Manichaikul AW, et al. AtheroSpectrum reveals novel macrophage foam cell gene signatures associated with atherosclerotic cardiovascular disease risk. *Circulation* (2022) 145:206–18. doi: 10.1161/CIRCULATIONAHA.121.054285

105. Muir LA, Cho KW, Geletka LM, Baker NA, Flesher CG, Ehlers AP, et al. Human CD206+ macrophages associate with diabetes and adipose tissue lymphoid clusters. *JCI Insight* (2022) 7:e146563. doi: 10.1172/jci.insight.146563

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Adipose tissue macrophages as potential targets for obesity and metabolic diseases

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Macrophage infiltration into adipose tissue is a key pathological factor inducing adipose tissue dysfunction and contributing to obesity-induced inflammation and metabolic disorders. In this review, we aim to present the most recent research on macrophage heterogeneity in adipose tissue, with a focus on the molecular targets applied to macrophages as potential therapeutics for metabolic diseases. We begin by discussing the recruitment of macrophages and their roles in adipose tissue. While resident adipose tissue macrophages display an anti-inflammatory phenotype and promote the development of metabolically favorable beige adipose tissue, an increase in pro-inflammatory macrophages in adipose tissue has negative effects on adipose tissue function, including inhibition of adipogenesis, promotion of inflammation, insulin resistance, and fibrosis. Then, we presented the identities of the newly discovered adipose tissue macrophage subtypes (e.g. metabolically activated macrophages, $CD9^+$ macrophages, lipid-associated macrophages, $DARC^+$ macrophages, and MFe^{hi} macrophages), the majority of which are located in crown-like structures within adipose tissue during obesity. Finally, we discussed macrophage-targeting strategies to ameliorate obesity-related inflammation and metabolic abnormalities, with a focus on transcriptional factors such as PPARγ, KLF4, NFATc3, and HoxA5, which promote macrophage anti-inflammatory M2 polarization, as well as TLR4/NF-κB-mediated inflammatory pathways that activate pro-inflammatory M1 macrophages. In addition, a number of intracellular metabolic pathways closely associated with glucose metabolism, oxidative stress, nutrient sensing, and circadian clock regulation were examined. Understanding the complexities of macrophage plasticity and functionality may open up new avenues for the development of macrophage-based treatments for obesity and other metabolic diseases.

KEYWORDS

macrophages, adipose tissue, plasticity, obesity, metabolic diseases

1 Introduction

Obesity has become a global pandemic, and its prevalence is increasing at an alarming rate (1). The rise in the prevalence of obesity significantly increases the risk of chronic metabolic diseases, such as cardiovascular disease, diabetes, hypertension, and cancer, and have a detrimental impact on both health and quality of life. Clarifying the pathogenesis of obesity is crucial for the prevention, treatment, and management of chronic metabolic diseases associated with obesity.

Obesity is characterized by an increase in the accumulation of macrophages in adipose tissue, which is accompanied by adipose tissue dysfunction, such as reduced adipogenesis and lipid storage capacity, adipocyte necrosis, inflammation, insulin resistance, and fibrosis (2). Adipose tissue stores excess energy in two ways: adipocyte hypertrophy and proliferation. Adipocyte proliferation is the healthy development of adipose tissue driven by preadipocyte proliferation and differentiation, whereas adipocyte hypertrophy is a pathological expansion of existing adipocytes with increased lipid storage and is closely related to adipocyte dysfunction (3). Hypertrophic adipocytes secrete a large number of chemokines, recruit immune cells, particularly macrophages, and cause chronic low-grade inflammation, insulin resistance, and the release of a large amount of free fatty acids into the circulation, eventually leading to obesity-related metabolic disorders (4).

A growing body of studies have indicated that innate immune cells play an important role in modulating adipose tissue activities during obesity (5). Among these cells, macrophages were the first and most important immune cells discovered infiltrating adipose tissue during obesity (6, 7). Macrophage infiltration has a significant impact on adipose tissue function and is a major cause of obesity-related metabolic diseases. Therefore, understanding the molecular mechanisms governing adipose tissue macrophages is critical for the prevention and treatment of obesity and other related metabolic diseases. Here, we review the current literature on adipose tissue macrophages with a particular emphasis on the heterogeneity and polarization of these cells during obesity in adipose tissue. We discuss the fundamental roles of macrophages in adipose tissue, highlighting macrophage-targeting strategies and assessing their therapeutic potential for treating obesity and related metabolic diseases.

2 Adipose tissue macrophages

2.1 Increased macrophage recruitment to adipose tissue in obesity

The primary sources of adipose tissue macrophages are tissue-resident macrophages and monocyte-derived recruited macrophages. Unlike most tissue-resident macrophages, which are derived from yolk sac primitive precursors and function to regulate tissue remodeling and maintain tissue homeostasis (8), a recent fate mapping study revealed that adipose tissue resident macrophages are derived from definitive embryonic hematopoietic precursors (9). These resident ATMs are phenotypically F4/80^{hi}CD11b^{+}CD169^{+} cells that can be further subdivided into three subtypes: MHCII^{low}, MHCII^{+}CD11c-, and MHCII^{+}CD11c^{+}. In response to HFD, the MHCII^{+}CD11c^{+} ATMs were rapidly increased in adipose tissue and replenished by bone marrow-derived monocytes, implying that recruited monocytes are the major cells contributing to increased ATMs in obesity.

Infiltration of monocyte-derived macrophages into adipose tissue during obesity was firstly reported in mouse models obesity and humans in 2003 (6, 7). The infiltrated macrophages were derived from bone marrow (7) and were contributed by increased diapedesis of blood monocytes (10). In contrast, weight loss by surgery reduced macrophage infiltration in adipose tissue of patients with obesity (11). Chemokine and its receptor interaction play crucial roles in the recruitment of circulating monocytes into adipose tissue during obesity. For example, monocyte chemoattractant protein (MCP-1 or CCL2), a chemokine produced in both adipocytes and the stromal vascular (SV) portion of adipose tissue, is significantly elevated in both blood and adipose tissue in obesity (12–17). Mice lacking CCL2 (18) or its receptor, CC chemokine receptor 2 (CCR2) (19) or using CCR2 inhibitor (20, 21), have lower adipose tissue macrophage infiltration and improved metabolic function in *db/db* and HFD-induced obese mice. Conversely, mice overexpressing CCL2 in adipose tissue have enhanced macrophage infiltration into adipose tissue and an unfavorable metabolic profile (18, 22). Moreover, mice with CCR2 deficiency in bone marrow cells or macrophages had lower macrophage numbers in adipose tissue after high-fat diet (HFD) feeding, indicating that CCR2 plays a crucial role in macrophage recruitment into adipose tissue during obesity (23, 24).

In addition to CCL2/CCR2, other chemokines and their receptors may play a role in the increased macrophage accumulation in adipose tissue in obesity. For instance, CCL chemokines (such as CCL3, CCL4, CCL5, CCL7, CCL8, CCL11, CCL18) and its receptors (such as CCR1, CCR3 and CCR5) have been linked to increased adipose tissue in obese (25) and human individuals (26, 27). Indeed, a dual CCR2/5 antagonist significantly reduces M1 macrophage infiltration into adipose tissue in HFD-induced obese mice, as well as improving adipose tissue inflammation and insulin resistance (IR) (28). Furthermore, CXCL12 produced by adipocytes interacts with its receptor CXCR4 to mediate macrophage recruitment into adipose tissue during HFD-induced obesity (29). In addition, other chemokines such as haptoglobin and C3a have also been reported to mediate macrophage recruitment into adipose tissue during obesity (30, 31). These studies taken together have demonstrated the therapeutic potential of focusing on macrophage recruitment into adipose tissue.

2.2 Adipose tissue macrophages polarized to pro-inflammatory phenotype in obesity

Increased macrophage infiltration into adipose tissue forms a crown-like structure (CLS) around necrotic adipocytes (32, 33). The number of CLS is strongly correlated with the expression of inflammatory cytokines like TNF-α (32), indicating that infiltrating macrophages have a pro-inflammatory effect on

adipose tissue in obesity. Lumeng et al. used PKH26 dye to label resident macrophages in adipose tissue and found that newly recruited adipose tissue macrophages (ATMs) in HFD-induced obese mice had a pro-inflammatory M1 phenotype (F4/80$^+$CD11c$^+$), whereas resident macrophages had an alternative activated M2 phenotype (F4/80$^+$CD206$^+$) (34–36).

Further examination of CD11c$^+$ ATMs from epididymal WAT (eWAT) revealed a mixed M1/M2 profile that was divided into three subtypes: resident ATMs as MGL1$^+$CD11c$^-$ expressing cells, CLS-associated MGL1$^-$/CD11c$^+$ ATMs, and MGL1^{med}/CD11c$^+$ ATMs (37). Similar to this work, resident ATMs in human adipose tissue have been shown to display M2 markers like CD206 and CD163, but they are also able to produce inflammatory cytokines (38, 39), indicating that these ATMs are mixed M1- and M2-polarized. Additionally, the number of ATMs in subcutaneous and omental adipose tissue of patients with obesity is higher than in lean subjects (40, 41). These findings collectively indicate that adipose tissue remodeling in obesity is connected to both an M1 and M2 progression.

Moreover, macrophage infiltration into adipose tissue during obesity is preferentially located in visceral adipose tissue in humans (42–44) and mice (33, 45), implying that visceral adipose tissue is the major adipose depot harboring the pro-inflammatory macrophages in obesity. The pro-inflammatory ATMs are one of the key cell types responsible to produce pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6, which contribute to obesity-related adipose tissue inflammation. In addition to the recruitment of circulating monocytes into adipose tissue, a local proliferation of macrophages in CLS also contributes to the increased ATMs in adipose tissue during obesity (46–48). These proliferating macrophages express M2 macrophage markers including CD206 and CD301 and form resident ATMs in the interstitial space (49). Even though these proliferating macrophages are M2 phenotype, their presence maintained adipose tissue inflammation in obese mice even after weight loss (50).

The accumulation of macrophages in adipose tissue is not only a defining feature of obesity, but also a major cause of obesity-related metabolic diseases such as liver steatosis and IR (51–57). As a result, reducing the number of macrophages in adipose tissue slows the onset of obesity and improves insulin sensitivity and glucose metabolism (58, 59), indicating that macrophages in adipose tissue play crucial roles in the development of obesity and metabolic disorders.

3 Adipose tissue macrophage subtypes and functions in adipose tissue

3.1 Newly identified macrophage subtypes in adipose tissue

In addition to previously classified pro-inflammatory and alternatively activated macrophages using F4/80 and CD11c or CD206 markers, a new class of ATMs known as M3 ATMs (CD11c$^-$CD206$^-$MGL1$^-$) that also localize to the CLS and uniquely express chemokine receptor Ccr7 has been reported (60). The presence of M3-like ATM suggests that different pathways may contribute to macrophage inflammation in the context of obesity. Additionally, another new type of ATM known as metabolically activated macrophages (MMe) were reported, which is produced when exposed to high levels of glucose, insulin, and palmitate. Rather than expressing classical M1 markers, MMe overexpress ATP binding cassette transporter (ABCA1), cluster of differentiation 36 (CD36), and perilipin 2 (PLIN2), which are regulated by peroxisome proliferator activated receptor gamma (PPARγ) (61). Moreover, MMe macrophages accumulated in CLS showed both beneficial and detrimental effects in response to high-fed diet feeding (62). For example, during the early stages of HFD-induced obesity, MMe macrophages increased adipose tissue inflammation by upregulating inflammatory markers such as TNF-α, IL-6, and IL-1β, as well as genes involved in lipid metabolism. In contrast, despite strong expression of pro-inflammatory and lipid metabolism genes in MMe macrophages, they are more active in the clearance of dead adipocytes *via* lysosomal exocytosis, hence inhibiting ectopic fat accumulation and IR in late-onset HFD-induced obesity. Mechanistically, TLR2, NOX2 and MyD88 have been proposed to modulate the positive and negative impact of MMe macrophages in HFD-induced obesity. Subsequent research suggested that MMe aggregation in breast adipose tissue may play a role in the development of triple-negative breast cancer (63).

Recent research using single-cell sequencing has revealed a much broader range of ATM phenotypes (Figure 1; Table 1). For example, CD9$^+$ATM, which also localizes in CLS in both mice and humans, was discovered to contain large amounts of intracellular lipids in lysosomal-like structures and to express genes associated with lysosome-dependent lipid metabolism, may have the same capacity as MMe to clear dead adipocytes *via* the lysosomal pathway. However, CD9$^+$ATM is distinct from MMe because it contains traditional M1/M2 markers like CD206 and CD11b (64). Adoptive transfer of CD9$^+$ ATM to lean mice leads to the up-regulation of genes related with obesity, suggesting that CD9$^+$ ATM may promote the development obesity and metabolic diseases (64). Triggering receptor expressed on myeloid cells 2 (TREM2), a pathologically induced immune signaling in Alzheimer's disease, metabolic diseases, and cancer, has been found to express in ATMs (69). A new subtype of macrophages termed as lipid-associated macrophages (LAM) was discovered in both mouse and human adipose tissue characterized by TREM2 expression (65). Despite the fact that mice with TREM2 deficiency had fewer LAM macrophages in CLS, they exhibited accelerated obesity with massive adipocyte hypertrophy, insulin resistance, and hyperlipidemia upon HFD feeding (65). In addition, single-cell sequencing studies have shown that CD9$^+$TREM2$^+$ ATMs have more specific surface markers CD45$^+$CD11b$^+$CD11c$^+$CD9$^+$TREM2$^+$ for better identification (70). In addition, a new subset of ATMs expressing Duffy antigen receptors for chemokines (DARC$^+$ ATMs) was also discovered to be recruited to CLS in eWAT under obesity conditions (66). DARC$^+$ATMs were generated in response to IL-

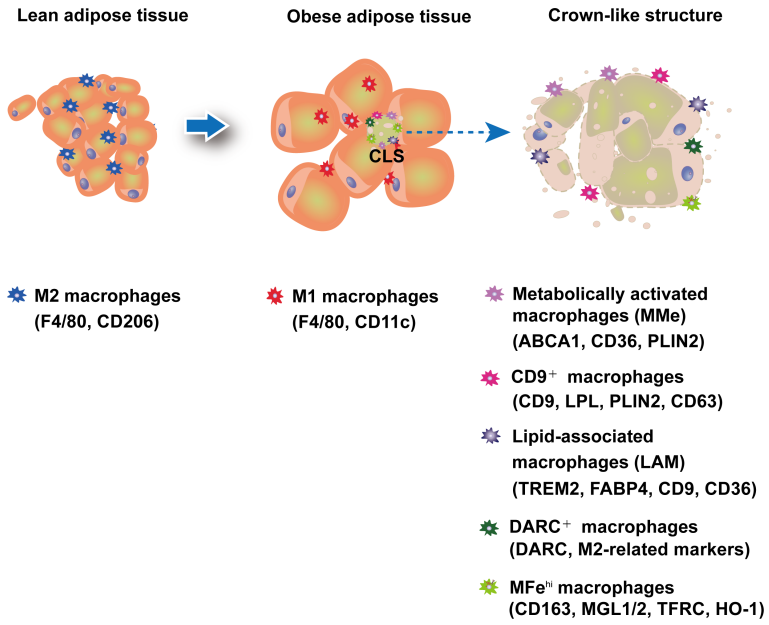

FIGURE 1
ATMs plasticity in adipose tissue. In lean adipose tissue, anti-inflammatory M2-like macrophages are predominant and maintain homeostasis. In obese adipose tissue, an increase in pro-inflammatory M1 ATMs forms a crown-like structure (CLS) surrounding dead adipocytes. Recent research has uncovered new macrophage subtypes, particularly in CLS.

22 stimulation and exhibited high levels of IL-22 receptor and M2-like anti-inflammatory properties to reduce adipose tissue inflammation in obesity (66).

Other than CLS, several distinct ATM phenotypes in adipose tissue have been reported. For instance, in the intercellular space of adipose tissue, a distinct ATMs population known as "MFe^{hi}" with higher cellular iron content and an iron-recycling gene expression profile was found (67). These "MFe^{hi}" ATMs displayed M2-like alternatively activation markers such as CD163 and MGL1/2 and decreased M1 markers (67). As a result, MFe^{hi} ATMs can manage high iron loads by storing iron, regulating iron-handling genes, and protecting adipocytes from iron overload (68). More research is needed to characterized these newly discovered macrophage subtypes and to determine the potential mechanisms that link these cells to obesity and related metabolic disorders.

3.2 Role of ATMs in adipose tissue function

The interactions between recruited pro-inflammatory macrophages and adipocytes are often harmful to the functions of adipocytes, including adipogenesis and lipid metabolism, inflammation, and related metabolic dysfunctions (Figure 2). In contrast, the resident macrophages in non-obese state are considered metabolically 'favorable' ATMs, which play important role in maintaining adipose tissue homeostasis *via* clearance of dead

TABLE 1 Newly identified adipose tissue subtypes.

Macrophages phenotype	Category	Location	Marker	Function	Reference
MMe (metabolically activated macrophages)	Recruited macrophages	CLS	ABCA1, CD36, PLIN2	Removing dead adipocytes through lysosomal exocytosis	(61) (62) (63)
CD9$^+$ macrophages	Recruited macrophages	CLS	CD9, LPL, PLIN2, CD63, LAMP2, CD16, CD206	Promotion of obesity	(64)
LAM (lipid-associated macrophages)	Recruited macrophages	CLS	TREM2, LIPA, LPL, CTSB, CTSL, FABP4, FABP5, LGALS1, LGALS3, CD9, CD36	Preventing metabolic disorders when adipocyte homeostasis is lost	(65)
DARC$^+$ macrophages	Recruited macrophages	CLS	DARC, Ly6C(low), M2-related marker(high)	Anti-inflammation and reducing immune cell infiltration.	(66)
MFe^{hi} macrophages	Resident macrophages	Intercellular space; CLS (a small number)	CD163, TFRC, HO-1, FTL1, FTH1, CP, SLC40A1, F4/80, CD11c(high), CD206(low)	Coping with iron metabolic disorders	(67) (68)

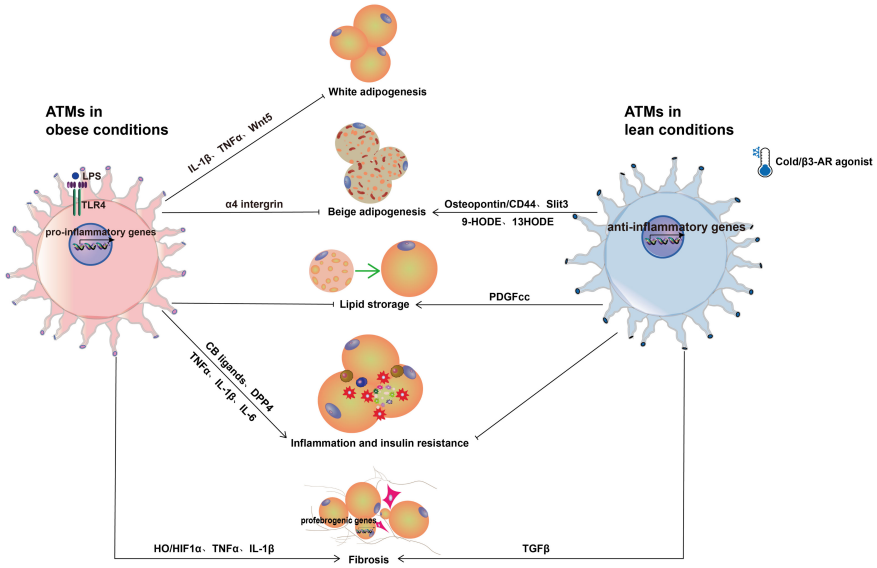

FIGURE 2
Role of ATMs in adipose tissue. In obese conditions, pro-inflammatory macrophages show detrimental effects on adipose tissue function such as inhibition of adipogenesis, promoting inflammation, insulin resistance, and fibrosis. The pro-inflammatory cytokines TNF-α and IL-1β and the protein factor Wnt5a inhibit preadipocyte differentiation when released by pro-inflammatory macrophages. In addition, TNF-α and IL-1β reduce the insulin sensitivity of adipocytes. Through LPS-TLR4 the LPS-induced CB ligands-CB1 signaling pathways, pro-inflammatory macrophages also aggravate adipose tissue inflammation. Moreover, macrophages secrete the enzyme DPP4, which causes both hyperglycemia and inflammation. In addition to inducing preadipocytes to produce abundant ECM, pro-inflammatory macrophages overproduce NO, which increases HIF-1α accumulation and promotes profibrogenic responses in preadipocytes, resulting in adipose tissue fibrosis. In lean conditions, ATMs are anti-inflammatory and play an important role in the formation and activation of beige adipocytes. In response to cold stimulation, ATMs polarize to an alternative activation state and promote the biogenesis of beige adipocytes *via* macrophage-secreted cytokine Slit3 and a sympathetic neuron-adipocyte signaling axis. Similar to cold stimulation, β3-AR agonists enhance the conversion of existing white adipocytes into beige adipocytes. Furthermore, β3-AR agonists induce alternative activation of macrophages to release osteopontin and the PPARγ ligands 9-HODE and 13-HODE, which stimulates beige adipocyte development.

adipocytes. They are also critical for beige adipogenesis and thermogenesis, which lead to improved metabolic functions (Figure 2). Here we focus on reviewing the recent literature on ATMs and major adipose tissue functions.

3.2.1 Role of ATMs in adipogenesis and lipid metabolism

The differentiation of preadipocytes to adipocytes is essential for the growth of adipose tissue in obesity. The expansion of white adipose tissue can dramatically enhance metabolic function and health. However, when immune cells, particularly pro-inflammatory macrophages, infiltrate adipose tissue, its potential to expand is inhibited. *In vitro* culture of preadipocytes with macrophage-conditioned medium elicits a pro-inflammatory response in both murine and human preadipocytes and impairs their differentiation to adipocytes (71–75), suggesting that macrophage-secreted factors contribute to its inhibitory effect on adipogenesis. Among the pro-inflammatory cytokines produced by macrophages, TNF-α and IL-1β have shown a direct inhibition on preadipocyte differentiation, however, neither TNF-α nor IL-1β neutralization reverses the anti-adipogenic effect of macrophage-conditioned medium (72, 76, 77), suggesting that other soluble factors could play a role. Wnt5a has been demonstrated to be expressed in human ATMs and circulating monocytes, and inhibition of Wnt5a activity in J774A.1 macrophage-conditioned medium improved mesenchymal precursor cells differentiation into adipocytes (78), suggesting that Wnt5a is a possible factor secreted by macrophages to suppress adipogenesis. Mechanistically, pro-inflammatory macrophages suppressed PPARγ activity in adipocytes by S-nitrosylation at cysteine 168, resulting in proteasome-dependent degradation of PPAR and decreased adipogenesis (79).

Beige adipose tissue is an inducible thermogenic type of adipose tissue that resides within white subcutaneous adipose tissue in mice and humans (80). Beige adipocytes can be induced by cold exposure, β3-adrenergic receptor (β3-AR) agonist, and PPAR ligands (81) *via* beige adipogenesis and white adipocytes conversion. Several studies have found that macrophages are critical players in the formation and activation of beige adipocytes (82–84). For instance, it has been demonstrated that pro-inflammatory macrophages directly interact with beige adipocytes *via* α4 integrin and VCAM-1, triggering a persistent inflammatory cycle in adipose tissue and inhibiting beige adipogenesis in obesity (85). In contrast, cold stimulation results in the production of the type 2 cytokines IL-4 and IL-13 by eosinophils, which activate macrophages and promotes the biogenesis of beige adipocytes (86). Furthermore, a recent study discovered the cytokine Slit3 secreted from anti-inflammatory macrophages promotes WAT beiging in response to cold *via* the sympathetic neuron-adipocyte signaling axis (87). In line with this discovery, subcutaneous WAT browning was significantly induced by injecting anti-inflammatory macrophages in obese mice induced by the HFD (88). However, a recent study found that conditionally and partially depleting

adipose tissue CD206$^+$ macrophages increased proliferation and differentiation of beige progenitors in normal and cold stimulated conditions (89, 90), suggesting that CD206$^+$ ATMs inhibit beige adipogenesis. This might be as a result of mixed populations of CD206$^+$CD11c$^+$ and CD206$^+$CD11c$^-$ ATMs present in CD206$^+$ macrophages. More research is needed to determine which subtype has the inhibitory effect on beige adipogenesis. Similar to cold stimulation, β3-AR agonist is a potent inducer of the conversion of existing white adipocytes into beige adipocytes (91). Recent data also point to a role for resident macrophages in promoting beige differentiation in response to β3-AR activation through the clearance of dead adipocytes, the secretion of the chemokine osteopontin to recruit PDGFRα$^+$CD44$^+$ beige progenitors into subcutaneous adipose depot, and the production of the PPARγ ligands 9-HODE and 13-HODE *via* ALOX15 activity (92, 93). Overall, resident ATMs support beige adipogenesis and offer a potential therapeutic strategy to enhance metabolic health in obesity. More research is necessary to test these findings in human settings.

The classical function of adipose tissue is to store surplus energy as triglyceride during food intake and release free fatty acids during fasting. Several early *in vitro* studies reported that LPS-stimulated macrophages activate the lipolysis of 3T3-L1 adipocytes (94), which is accompanied by an inhibition of lipoprotein lipase (95) and a decrease in fatty acids synthesis (96). Moreover, LPS/IFNγ-activated macrophages are related to increased mitochondrial activity in human adipocytes, indicating that macrophage activation state may influence adipocyte bioenergetics (97). A recent study discovered that adipose tissue resident macrophages, rather than recruited CCR2$^+$ macrophages, have an evolutionarily conserved role in lipid storage in adipocytes (98). In response to HFD feeding, these resident macrophages produce higher levels of PDGFcc, which promotes white adipocyte hypertrophy and hence prevents ectopic fat deposition in the liver and other tissues. Blocking PDGFcc reduces lipid accumulation in white adipocytes while increasing thermogenesis in brown adipocytes, indicating a vital role of PDGFcc in regulating lipid metabolism. Further study is needed to evaluate whether pharmacological inhibition of PDGFcc has therapeutic promise for obesity treatment.

3.2.2 Role of ATMs in inflammation and related metabolic disorders

Increased ATM accumulation in obesity is one of the key contributors contributing to obesity-induced inflammation both locally and systemically. Newly recruited pro-inflammatory macrophages release a considerable amount of pro-inflammatory cytokines such as TNF-α, IL-6 and IL-1β and impede insulin signaling transduction in adipocytes (99–101). Consistently, the infiltration of pro-inflammatory macrophages precedes the IR in obese mice *in vivo* (6, 102), suggesting a causal role for inflammation in the development of IR in obesity. Insulin-resistant adipocytes release more free fatty acids and activate ATMs, resulting in a vicious loop that exacerbates inflammation *via* TLR4 (103). Moreover, TLR2 and TLR9 deficiency promotes HFD-induced adiposity, visceral adipose inflammatory responses, and IR in mice (104, 105), indicating that TLRs play a significant role in adipose tissue inflammation and IR in obesity. LPS derived from gut microbiota is another potential factor for inducing inflammatory responses in adipose tissue. On the one hand, LPS activates ATMs *via* TLR4 and amplifies inflammation by adipocyte-macrophage interactions (106). On the other hand, LPS causes robust productions of endogenous ligands for cannabinoid (CB) receptors in ATMs (107), which contributes to chronic inflammation in visceral adipose tissue, hyperglycemia, and IR (108). Furthermore, CB1 receptor blockage reduced LPS-induced pro-inflammatory responses in macrophages, alleviated adipose tissue inflammation and glucose intolerance (108, 109). In addition, other inflammatory mediators or proteins also contribute to adipose inflammation in obesity. DPP4, an enzyme that effectively increases blood glucose levels by degrading incretin peptides, was found to be more abundant in F4/80$^+$ macrophages in CLS in adipose tissue than in adipocytes (110, 111). DPP4 inhibition dramatically reduced pro-inflammatory macrophage migration while producing an anti-inflammatory phenotype shift in adipose tissue macrophages, reducing obesity-induced inflammation and IR (112).

Additionally, pro-inflammatory macrophages play an important role in the development of adipose tissue fibrosis in obesity, which is another important pathogenic feature of obesity. Adipose tissue fibrosis is characterized by an increase in the expression and remodeling of extracellular matrix (ECM) proteins in WAT (113). The fibrotic deposition in adipose tissue has been observed as bundles of collagen fibers (Collagen I, III) in subcutaneous fat and thin fibrous lobule-like bands (Collagen VI) surrounding adipocytes in omental fat from subjects with obesity (114, 115). Collagens and fibronectin are expressed more abundantly in adipose tissue SV fractions than in adipocytes (114), indicating that SV fractions may be the primary cell types for fibrotic protein synthesis. Marcelin et al. have investigated the cellular origins of WAT fibrosis and discovered that pro-fibrotic cells originate from PDGFR$^+$CD9^{high} cells within adipose tissue SV fractions (116). Human preadipocytes cultured *in vitro* with LPS-activated macrophages had a pro-inflammatory phenotype and produced abundant ECM consisting of collagen 1, tenascin-C, and fibronectin (77, 117). Furthermore, macrophages dramatically increased the levels of ECM breakdown enzymes such as matrix metalloproteinases in both preadipocytes and adipocytes *via* the pro-inflammatory cytokines TNF-α and IL-1β (118, 119). In contrast to *in vitro* studies, anti-inflammatory macrophages have been linked to increased adipose tissue fibrosis in individuals with IR (115). Mechanistically, TGF-β has been shown to induce myofibroblast-like cells from adipose tissue progenitor cells (preadipocytes) treated with ATMs (120). Hypoxia is an additional essential component contributing to adipose fibrosis. The expansion of adipose tissue in obesity is associated with adipose tissue hypoxia, as has been demonstrated in adipose tissue of several obese mouse models (*ob/ob*, KKAy, diet-induced) (121–123) and human subjects with obesity (124). Mechanistically, adipose tissue hypoxia increases HIF-1α expression and stability, which triggers profibrogenic transcription in preadipocytes (125). Furthermore, pro-inflammatory macrophages overproduced NO, which elevated HIF-1α accumulation and promoted profibrogenic responses in

preadipocytes, resulting in adipose tissue fibrosis (126). Collectively, these findings suggest to the possibility of targeting pro-inflammatory macrophage-mediated inflammatory pathways to diminish obesity-induced inflammation, IR and fibrosis.

4 Targeting macrophages to improve metabolic health

Given that ATMs play critical roles in both the onset and progression of obesity-related metabolic disorders, strategies that target the phenotypic flexibility of macrophages to fulfill tissue environment needs have demonstrated great therapeutic promise. The following is a summary of the prospective treatment targets for obesity and related metabolic diseases that can be delivered to macrophages (Table 2; Figure 3).

4.1 Targeting macrophage polarization

ATMs have been shown to negatively modulate insulin action *via* $CD11c^+$ pro-inflammatory macrophages (157), indicating that pro-inflammatory macrophages are a target for the treatment of obesity-related insulin resistance. Fatty acids are one of the major factors controlling the activation of ATMs.For example, saturated free fatty acids/TLR signalling, TNF/TNF receptor signalling induce the classically activation of macrophages (158–163), while unsaturated fatty acids like oleic acid, linoleic acid, DHA, and n-3 PUFA induce alternatively activated phenotype. Furthermore, omega-3 PUFA can increase lipolysis and fatty acid re-esterification in alternatively activated macrophages (164). These findings indicate that consuming unsaturated fatty acids may polarize ATMs to alternatively activated phenotype, thereby regulating lipid metabolism or alleviating the symptoms of obesity-related diseases.

Rosiglitazone, a PPARγ activator, also encourages alternatively activated macrophage infiltration into adipose tissue in mice receiving HFD (165–168). PPARγ deficiency in macrophages promotes the predominance of pro-inflammatory macrophages and the decrease of alternatively activated macrophages in adipose tissue in obesity (148, 169), indicating that PPARγ is essential in controlling macrophage alternative activation. Moreover, an intact IL-4 and IL-13 signaling is required for maturation of alternatively activated ATMs and reducing diet-induced obesity and IR in mice (170, 171). However, myeloid cell-specific knockout of IL4R alpha decreased insulin sensitivity in lean mice while improving parameters of glucose homeostasis and partially protecting against adipose tissue inflammation in obese mice (172), indicating IL-4R signaling likely plays a significant role in maintaining the alternative activation of macrophage in lean conditions but not in obesity.

A number of transcription factors have been found to influence ATMs polarization. For instance, Krüppel-like factor 4 (KLF4) has been demonstrated to promote monocyte differentiation *in vivo* (173). Moreover, KLF4 is strongly induced in alternatively activated macrophages by STAT6 while being reduced in pro-inflammatory macrophages by NF-kB inhibition (150). Consistently, KLF4-deficient macrophages displayed increased pro-inflammatory cytokine expression, and myeloid-specific KLF4 deficiency predisposed mice to diet-induced obesity, glucose intolerance, and IR (150), indicating a crucial role for KLF4 in regulating macrophage polarization and maintenance of adipose tissue homeostasis. Similar metabolic problems were brought on by the knockdown of the protein known as glucocorticoid receptor-interacting protein 1 (GRIP1), which acts as a coactivator for KLF4 (151). Contrarily, nuclear factors of activated T cells (NFATc3) play a different role in controlling the transcription of various genes in immune cells. Nfatc3-/- mice showed adipose tissue macrophage polarization toward alternative activation, which significantly reduced hepatic steatosis and inflammation in HFD mice, indicating the potential role of NFATc3 in promoting adipose tissue inflammation (136). Homeobox A5 (HoxA5), a developmental transcription factor, has been demonstrated to support adipocyte differentiation by inhibiting the PKA/HSL pathway (174). HoxA5 has also been shown to reduce endoplasmic reticulum stress and inflammatory responses in adipocytes by blocking the eIF2/PERK signaling pathway (137). Additionally, Hoxa5 transcriptionally activated the PPARγ pathway to promote alternative activation of macrophage and WAT browning (137), which in turn alleviated obesity-induced chronic inflammation. These findings imply that Hoxa5 may represent a promising therapeutic target for the management of obesity.

Notably, some therapeutic options and drugs have been developed to treat obesity-related metabolic diseases by regulating macrophage polarization. For obese patients who have failed to respond to exercise and dietary changes, bariatric surgery is an option. Studies have shown that after bariatric surgery, ATMs is biased toward the alternative activation with an increase of CD163 expression (40). However, subsequent research expressed concern on this notion, claiming that modifications in CD163-positive cells do not precisely reflect metabolic improvements following weight loss (175). Further research into the mechanism of bariatric surgery is required. Metformin, the most popular anti-diabetic medication, is crucial for macrophage polarization. Metformin was shown to decrease pro-inflammatory markers like CD11c and MCP-1 in the adipose tissue of HFD mice (176). Additionally, *in vitro* metformin treatment to pro-inflammatory macrophages improved metabolic disorders in brown adipocytes (177). Dipeptidyl peptidase-4 (DDP4) inhibitors Linagliptin and Sitagliptin are both used primarily to control blood glucose levels in patients with type 2 diabetes. These two drugs have been shown to decrease obesity-induced inflammation and IR by inhibiting pro-inflammatory and promoting alternative activated macrophages because DDP4 is largely expressed in pro-inflammatory macrophages and its expression was significantly increased in obese mice (112, 178). Similar mechanisms are shared by a number of sodium-glucose cotransporter 2 inhibitors, including empagliflozin. Through the phenotypic switch of macrophages to alternative activation in the liver and WAT, empagliflozin can reduce body weight by inducing WAT browning and reducing inflammation associated with obesity (179, 180). In conclusion, targeting macrophage polarization is a feasible and worthwhile direction that may benefit the vast majority of patients suffering from metabolic diseases.

TABLE 2 Targeting macrophages for improving metabolic health.

Molecular targets	Approach	Phenotype
IKKβ	Myeloid cell specific IKKβ deletion (127)	↓ IR after HFD.
TLR4	Hematopoietic cell specific TLR4 deletion (128)	↓ IR, ↓adipose and liver inflammation
Fas	Myeloid/hematopoietic cell-specific Fas deletion (129)	↓ skeletal muscle IR, no effect on inflammation in liver and AT.
MyD88	Myeloid cell-specific MyD88 deletion (130)	↓ atherosclerosis, IR, and systemic inflammation after HFD.
TRAF3	Myeloid cell-specific TRAF3 deletion (131)	↓inflammation and IR in HFD-obese mice; ↑ inflammation in liver and adipose in lean mice.
ERV1	Myeloid cell-specific overexpression (132)	↓adiposity and inflammation after HFD.
NOX2	Myeloid cell-specific NOX2 deletion (133)	↓adiposity and adipose inflammation
HIF1α	Myeloid cell-specific HIF1α deletion (134)	↓systemic IR and inflammation after HFD
HO-1	HO-1+/- mice (135)	↓adiposity, adipose inflammation, and IR after HFD
NFATc3	NFATc3-/- mice (136)	↓hepatic steatosis and inflammation after HFD
HoxA5	HoxA5 overexpressed mice (137)	↓adiposity and inflammation after HFD
Insulin receptor (IR)	Mice with macrophage IR deletion (138)	↓IR after HFD
PTPB1	Macrophage PTPB1 deletion (139)	↓IR, liver damage and chronic inflammation
IRS2	Mice with macrophage IRS2 deletion (140)	↓adiposity and glucose intolerance after HFD
mTORC1	Myeloid cell-specific TSC1 deletion to constitutively activate mTROC1 (141)	↓obesity, glucose intolerance, and AT inflammation after HFD
SIRT1	Myeloid cell-specific SIRT1 deletion (142–145)	↓glucose tolerance, ↑ liver steatosis and AT inflammation
TREM2	TREM2 overexpressed mice (146)	↑AT inflammation, adiposity, IR after HFD.
Catalase	Global catalase deficiency (147)	↑oxidative stress, inflammation, and IR
PPARγ	Skeletal muscle and liver specific PPARγ depletion (148, 149)	↑IR in muscle and liver.
KLF4	Myeloid cell-specific KLF4 deletion (150)	↑adiposity, glucose intolerance, and IR after HFD
GRIP1	Myeloid cell-specific GRIP1 deletion (151)	↑ adipose inflammation, hyperglycemia, and IR
ATG7	Myeloid cell-specific ATG7 deletion (152)	↑adipose inflammation and hyperglycemia.
PDK1/FoxO1	Pdk1 deletion in macrophages; constitutive activation of nuclear Foxo1 (153)	↑ adipose inflammation and IR
Estrogen receptor α (ERα)	Macrophage ERα deletion (154)	↑adiposity, IR and atherosclerotic lesion area
SIRT6	Myeloid cell specific SIRT6 deletion (155)	↑ adipose and liver inflammation and IR
PER1/PER2	Myeloid cell-specific deletion of core clock genes Period1 (PER1) and Period2 (PER2) (156)	↑adipose inflammation and IR after HFD.

IR, insulin resistance; HFD, high-fat diet; ↑ increase; ↓ reduce.

4.2 Targeting macrophage inflammatory pathways

Adipose tissue inflammation is a major contributor to obesity-related metabolic diseases such as IR and hepatic steatosis. In adipose tissue, ATMs play dominant role in producing pro-inflammatory cytokines, which cause inflammation in obesity. NF-kB is one of the main masters of inflammatory responses. IKK is a crucial enzyme that activates NF-kB in myeloid cells. Mice with myeloid cell-specific IKKβ deletion preserved insulin sensitivity when fed with HFD (127). Furthermore, mice with hematopoietic cell-specific deletion of TLR4 demonstrated an improvement in peripheral insulin sensitivity after HFD feeding, which is associated with to a notable decrease in macrophage infiltration and inflammatory cytokines in both adipose tissue and the liver (128). MyD88, a TLR4 downstream signaling protein, is crucial in triggering inflammatory response. MyD88 deficiency in myeloid cells reduced macrophage infiltration to adipose tissue and their polarization to pro-inflammatory phenotype (130). Along with this, there is a considerable reduction in atherosclerosis, insulin resistance, and systemic inflammation induced by HFD feeding. Another typical intracellular signaling protein for TLRs is TNF receptor-associated factor 3 (TRAF3), which is anti-inflammatory in lean but pro-inflammatory in obese conditions.

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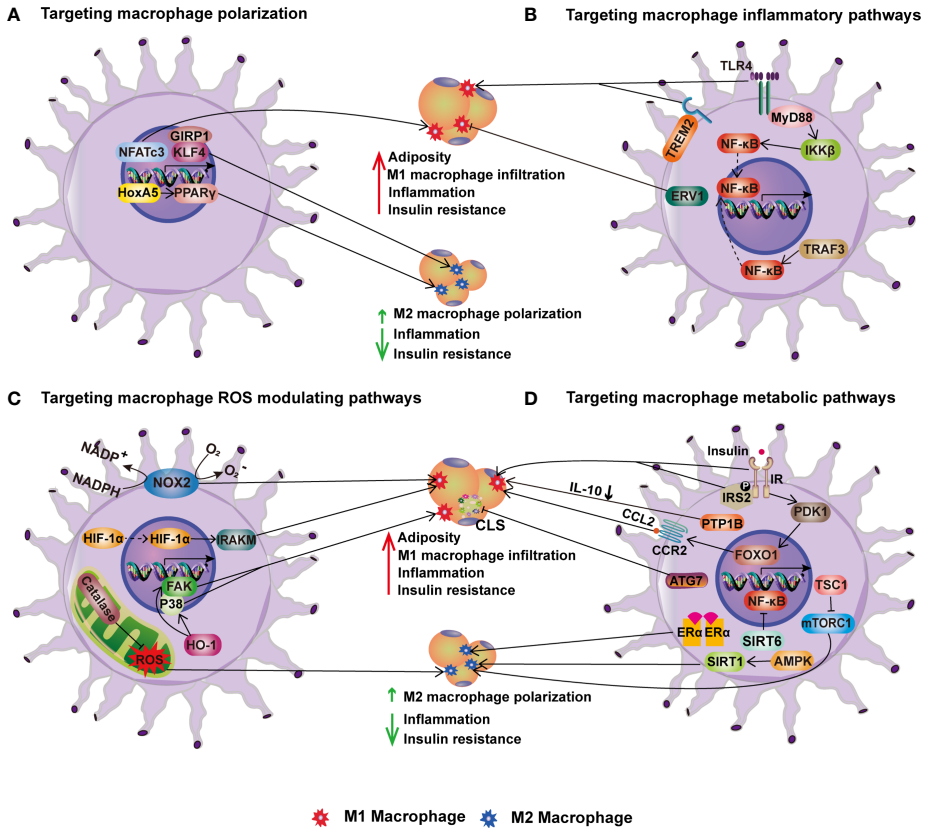

FIGURE 3
Targeting macrophages for improving metabolic health. **(A)** Targeting macrophage polarization. In HFD-induced obesity, transcription factors NFATc3, KLF4 and its coactivator GIRP1 enhance M1 macrophage polarization and infiltration into adipose tissue, inflammation, and insulin resistance. HoxA5 and PPAR, on the other hand, increase M2 macrophage polarization and thereby ameliorate obesity-induced inflammation and insulin resistance. **(B)** Targeting macrophage inflammatory pathways. TLR4-MyD88-IKK signaling and TRAF3 activation enhance adipose tissue M1 macrophage infiltration, inflammation, and IR in obesity *via* NF-B. On contrary, overexpression of ERV1 in macrophages reduces adiposity, hepatic and adipose inflammation, and hyperglycemia caused by HFD. **(C)** Targeting macrophage ROS modulating pathways. NOX2, HIF-1α, and HO-1 in macrophages increase obesity-induced adiposity, inflammation, and insulin resistance, whereas catalase inhibits inflammation *via* increasing M2 macrophage polarization. **(D)** Targeting macrophage metabolic pathways. The stimulation of macrophage insulin pathways such as IR-IRS2 and PDK1-FoxO1 signaling promotes HFD-induced obesity and insulin resistance. PTP1B, an insulin signaling negative regulator, induces IR by lowering IL-10. In contrast, mTORC1 activation improves M2 macrophage polarization and protects mice from HFD-induced obesity, inflammation, and insulin resistance. In addition, the ATG7-mediated autophagy pathway reduces CLS numbers and adipose tissue inflammation in obesity. Furthermore, other metabolic pathways regulated by ERα, SIRT1 and SIRT6 enhance M2 macrophage polarization, reducing inflammation and IR in obesity.

This is supported by research showing that myeloid cell-specific TRAF3 deletion reduced the number of macrophages in eWAT, as well as IR and the expression of pro-inflammatory cytokines in the liver and adipose tissue of obese mice (131). In contrast, TRAF3 deletion increased the expression of pro-inflammatory cytokines in the liver and adipose tissue of lean mice. Moreover, activation of the Fas signaling pathway may also be a crucial element of the inflammatory response. In HFD-induced obese mice, *ob/ob* mice, and mice acutely treated LPS, myeloid/hematopoietic cell-specific Fas-depletion preserved skeletal muscle insulin sensitivity, which was contributed by the decreased TNF-α levels in circulation (129). However, there was no difference in immune cell infiltration or local cytokine expression in adipose, liver, or skeletal muscle, indicating that the protective role of myeloid Fas depletion is more closely linked to a reduction of systemic inflammation.

Contrary to the inflammatory triggers listed above, it has been shown that TLR4 signaling from the triggering receptor expressed on myeloid cells 2 (TREM2) negatively modulates the inflammatory response in macrophages (181). A recent study has found that TREM2 may be involved in the inflammatory response in adipose tissues. Following HFD feeding, mice with TREM2 overexpression showed elevated macrophage and T cell recruitment into adipose tissue as well as increased adiposity, IR, and hepatic steatosis (146). These findings suggest that TREM2 acts as a novel regulator of adipogenesis and that inhibiting TREM2 signaling may be a therapeutic target for obesity and IR. To fully understand the underlying mechanisms of TREM2 in regulating the inflammatory response in adipose tissues, additional research on macrophage-specific deletion of TRME2 is required. Moreover, endogenous lipids known as specialized pro-resolving mediators (SPMs), which include resolvins, protectins, and maresins, mediate the resolution of inflammation (182). Mice overexpressing the human resolvin E1 receptor (ERV1) in myeloid cells displayed reduced adiposity, hepatic and adipose inflammation, and hyperglycemia induced by HFD (132). Resolvin E1, a natural ERV1 agonist, administration replicated the pro-resolving effects

obtained from ERV1 overexpression. This protective metabolic impact is in part explained by systemic activation of resolution programs leading to increased synthesis of specialized pro-resolving mediators. Taking together, targeting inflammatory pathways in macrophages offers a great potential for controlling adipose tissue inflammation and the ensuing metabolic disorders induced by obesity.

4.3 Targeting reactive oxygen species modulating pathways in macrophages

Oxidative stress and chronic inflammation are the important underlying factors for obesity-associated metabolic diseases. The imbalance between the oxidative and anti-oxidant systems of the cells and tissues results in the overproduction of oxygen free radicals and reactive oxygen species (ROS). Oxidative stress increases lipid peroxidation products, protein carbonylation which leads to cellular dysfunction. As the NADPH oxidase catalytic subunit, NOX2 has been demonstrated to be involved in obesity-induced IR, hyperlipidemia, and liver steatosis (183). Mice lacking myeloid-NOX2 showed reduced adiposity, adipose inflammation, and macrophage infiltration compared to controls when given a 16-week HFD diet (133). These results support the idea that NOX2 signaling in macrophages plays a role in the pathogenesis of obesity-induced metabolic disorders. Potentially, obesity may be reduced by targeted suppression of monocyte/macrophage NADPH oxidase in adipose tissue to maintain metabolic function.

Hypoxia is also a factor in the increased oxidative stress associated with obesity. The transcription factor hypoxia inducible factor-1 (HIF-1) regulates the expression of numerous hypoxic responsive genes by nuclear translocation and mediates adaptive responses to oxidative stress. HIF-1α has been demonstrated to contribute to oxidative stress and fibrosis in obese people (184). Additionally, macrophages in CLS and adipocytes are both hypoxic and inflammatory (185). In fact, mice with myeloid-specific HIF-1α deletion had enhanced adipose tissue vasculature development, which mitigated systemic IR and HFD-induced inflammation (134). Furthermore, a recent study identified interleukin-1 receptor-associated kinase M as the mechanism underlying HIF-1α-induced adipose tissue dysfunction in obesity (186), supporting the notion that HIF-1α in myeloid cells is crucial to obesity-related pathological growth of adipose tissue and systemic IR.

Additionally, heme oxygenase-1 (HO-1) is a stress-inducible enzyme that is crucial in several pathophysiological conditions, particularly inflammation and oxidative damage. Heme oxygenase (HO-1) expression was highly induced in the visceral adipose tissue, especially the SV fraction of HFD-fed mice. Myeloid HO-1 haploinsufficiency attenuated HFD-induced adiposity, adipose inflammation, and IR, due to impaired macrophage migration toward adipose tissue and reduced angiogenesis (135). Mechanistically, HO-1+/- macrophages displayed decreased chemoattractant-induced p38 phosphorylation and focal adhesion kinase expression (135). These findings point to a unique role of the myeloid cell HO-1 in adipose macrophage infiltration and IR development during obesity.

In contrast to the preceding factors, catalase, an important oxidative stress regulator, has been shown to control ATM polarization under both resting and metabolic stress conditions. Global catalase deficiency or use of the catalase inhibitor 3-aminotriazole causes oxidative stress, increased inflammation and IR in both lean and HFD-induced obese mice (147). Catalase inhibition increased pro-inflammatory macrophage accumulation but decreased alternatively activated macrophage accumulation in eWAT, indicating that endogenous catalase may be a critical regulator of obesity-related inflammation and IR.

4.4 Targeting macrophage metabolic pathways

Obesity-associated metabolic problems appear to be caused by a combination of metabolic endotoxemia and metabolic stress induced by chronic exposure to excessive amounts of nutrients. Because immune cell metabolism and function are inextricably connected, addressing the different metabolic pathways of macrophages could provide a unique opportunity to modify its phenotype and subsequent biological roles in obesity.

4.4.1 Insulin pathway as a target

Despite previous research, the main impact of macrophage insulin action on obesity and related metabolic disorders is still debated. Mice lacking macrophage insulin receptor were protected from the onset of obesity-related IR after HFD feeding (138). This protection was accompanied by lower macrophage counts in WAT and serum tumor TNF-α levels, which reflect a marked decrease in the local and systemic inflammation linked to obesity. These findings suggest that insulin action in myeloid cells plays an unexpectedly important role in regulating macrophage invasion into WAT and the development of obesity-associated IR. In line with this study, mice with macrophage insulin receptor substrate 2 (IRS2) deletion demonstrated protection from HFD-induced obesity and glucose intolerance due to increased energy expenditure *via* enhanced BAT activity and WAT beiging (140). Additionally, IRS2-deficient macrophages exhibited a transcriptional profile that was anti-inflammatory (140), indicating a crucial role for macrophage IRS2 signaling in ATM polarization and energy homeostasis. These findings may open therapeutic opportunities for the treatment of obesity. However, protein tyrosine phosphatase-1B (PTP1B), an intracellular protein that inhibits insulin and leptin signaling, has been shown to promote inflammation caused by obesity. Mice deficient in macrophage PTP1B displayed improved glucose and insulin tolerance, reduced liver damage and chronic inflammation after HFD feeding (139). The beneficial effect of PTP1B deletion in macrophages is due to increased IL-10 levels, which are inversely related to serum insulin and alanine transferase levels. These findings suggest that inhibiting myeloid PTP1B could be used to treat obesity-related inflammation and diabetes.

4.4.2 Nutrient sensing pathways as a target

Many studies have been conducted on the function of mTORC1 in obesity and associated inflammation. These studies have demonstrated the link between mTORC1 activation and obesity. Despite having no impact on the HFD-induced obesity, pharmacological mTORC1 inhibition by rapamycin worsened the inflammation and glucose intolerance, as shown by the rise in adipose tissue pro-inflammatory macrophages and elevated mRNA levels of pro-inflammatory cytokines such as TNF-α, IL-6, and MCP-1 (187). Additionally, macrophages derived from bone marrow exhibited pro-inflammatory phenotype as a result of *in vitro* mTORC1 inhibition (187). These results suggest that mTORC1 activity is a key regulator of macrophage plasticity and inflammation in adipose tissue. To further investigate the role of myeloid cell mTORC1 activation in obesity-induced inflammation, mice with myeloid cell specific TSC1 deletion and thus constitutive mTORC1 activation were generated. Mice lacking Tsc1 in macrophages exhibited protection from HFD-induced obesity, glucose intolerance, and adipose tissue inflammation (141). This protection was accompanied by mTORC1-dependent alternative activation of macrophages, indicating a protective role for mTORC1 activation in HFD-induced obesity and metabolic disorders. Unlike mTORC1, myeloid cell deficiency of mTORC2 obtained by Rictor deletion had no impact on HFD-induced obesity, adipose tissue inflammation, or systemic IR (188). However, mice lacking Rictor showed increased susceptibility to LPS-induced septic shock, indicating that mTORC2 is more important in diseases associated with severe inflammation than obesity-induced chronic low-grade inflammation.

Autophagy, a crucial cellular response pathway for sensing nutrient levels, is essential for cell survival and metabolism. When bred to *ob*/+ mice to induce metabolic stress, mice with myeloid cell-specific deletion of autophagy-related 7 (ATG7) displayed increased CLS numbers, activated NLRP3 inflammasome and IL-1β production in adipose tissue, as well as hyperglycemia (152). This was attributed to mitochondrial dysfunction in autophagy-deficient Macrophages, suggesting a critical role for macrophage autophagy in regulating adipose inflammation and insulin sensitivity in obesity.

As one of the key pathways regulating glucose and energy homeostasis, the 3-phosphoinositide-dependent protein kinase 1 (PDK1)/forkhead transcription factor (FoxO1) pathway has also been investigated in adipose tissue macrophages. PDK1 deletion in macrophages resulted in increased pro-inflammatory macrophages in adipose tissue and IR, which was reversed by inactivating nuclear FoxO1 (153). Furthermore, constitutively activating nuclear FoxO1 increased pro-inflammatory macrophages in adipose tissue *via* CCR2 and IR on HFD (153). Accordingly, PDK1 inhibits FoxO1 to regulate macrophage infiltration, and the PDK1/FoxO1 pathway in macrophages is essential for regulating macrophage polarization and insulin sensitivity in obesity.

Additionally, estrogen receptor alpha (ERα) plays a significant role in the control of glucose homeostasis (189). Even with a normal diet, mice with myeloid-specific ERα deletion displayed increased adiposity, IR, and atherosclerotic lesion area (154). Moreover, ERα deficiency reduced the response of isolated macrophages to IL-4-mediated alternative activation but promoted the inflammatory response to palmitate (154). This suggests that macrophage ER is important for suppressing inflammation and maintaining insulin sensitivity, making it a potential therapeutic target to combat obesity and IR.

4.4.3 Sirtuins as a target

Myeloid cell Sirtuin 1 (SIRT1) has been shown to play a protective role in studies of metabolic diseases caused by obesity. When given an HFD, mice with myeloid cell *Sirt1* deletion exhibited pro-inflammatory macrophage polarization in adipose tissue and increased adipose tissue macrophage hypoxia and inflammatory response (142–144), which impaired glucose tolerance and exacerbated liver steatosis (143, 145). In line with this, dietary quercetin has been demonstrated to reduce macrophage infiltration, control macrophage polarization, and regulate inflammation through the AMPK1/SIRT1 pathway, resulting in a reduction in HFD-induced IR and an increase in glucose uptake in adipose tissue (190). Similar to SIRT1, myeloid cell-specific SIRT6 knockout mice displayed increased pro-inflammatory macrophage infiltration in adipose and liver, as well as decreased insulin sensitivity *via* the NF-κB/STAT3 signaling pathway (155). These findings indicate that SIRT1 or SIRT6 in macrophages may be potential targets for combating obesity-induced tissue inflammation and IR.

4.4.4 Circadian pathways as a target

Numerous studies have linked metabolic disorders like obesity to circadian clocks. Circadian clock dysregulation induces pro-inflammatory macrophages and potentiates adipose tissue inflammation in mice with Period1 (PER1) and Period2 (PER2) deletion in macrophages, according to a previous study (156). High MCP-1 levels in mice with myeloid cell-specific PER1/PER2 disruption attracted pro-inflammatory macrophage infiltration and increased inflammation and IR in HFD-induced adipose tissue (156). Mechanistically, PPARγ2 levels were decreased in PER1/2-disrupted macrophages and restoration of PPARγ2 levels reduced the infiltration of pro-inflammatory macrophages in adipose tissue, suggesting that PPARγ may link the molecular clock genes and obesity-related inflammation.

5 Concluding remarks and perspectives

Increased ATMs are the major contributor to adipose tissue inflammation in obesity. Efforts have been made to target macrophage recruitment to improve metabolic health and have shown a great promise in obese mouse models. For instance, blocking CCL2-CCR2 has been shown to reduce macrophage recruitment in adipose tissue and mitigated the obesity-induced inflammation and IR. Moreover, a dual CCR2/CCR5 antagonist reduced macrophage-mediated inflammation and prevented IR, providing a therapeutic potential for metabolic diseases linked to obesity. Another promising strategy is to promote the polarization of ATMs toward alternative activation. Several transcription factors,

including PPARγ, KLF4, and HoxA5, have been shown to promote alternative activation of macrophages in adipose tissue and could be potential pharmacological targets. Additionally, strategies at targeting myeloid TLR4/NF-κB-mediated inflammatory pathways, ROS generating enzyme NOX2 and hypoxia adaptation factor HIF1α, and factors regulating glucose metabolism also appear to have a positive impact (Table 2; Figure 3). Further research is needed to validate the findings of mouse studies in humans.

The recent single cell RNA-sequencing studies have identified a broad spectrum of ATM subtypes, suggesting a heterogeneity and functional plasticity of ATMs in obesity. It remains to be determined the differences in the development, phenotype, and function of these newly discovered macrophages within adipose tissue. Also, understanding the regulatory factors and intracellular pathways that underpin functional differences between subtypes would provide new molecular targets. Finally, the development of new technologies that can target specific macrophage subtypes would considerably boost the translational potential of the aforementioned findings for the treatment of obesity and metabolic diseases.

Author contributions

Conceptualization: DG, HG and SL; literature search: XL, YR, KC, WW, and DG; writing: XL, YR, and DG; review and editing: XL, YR, KC, WW, DG, HG, and SL. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Chooi YC, Ding C, Magkos F. The epidemiology of obesity. *Metabolism* (2019) 92:6–10. doi: 10.1016/j.metabol.2018.09.005

2. Longo M, Zatterale F, Naderi J, Parrillo L, Formisano P, Raciti GA, et al. Adipose tissue dysfunction as determinant of obesity-associated metabolic complications. *Int J Mol Sci* (2019) 20(9):2358. doi: 10.3390/ijms20092358

3. Meister BM, Hong SG, Shin J, Rath M, Sayoc J, Park JY. Healthy versus unhealthy adipose tissue expansion: the role of exercise. *J Obes Metab Syndr* (2022) 31(1):37–50. doi: 10.7570/jomes21096

4. Hammarstedt A, Gogg S, Hedjazifar S, Nerstedt A, Smith U. Impaired adipogenesis and dysfunctional adipose tissue in human hypertrophic obesity. *Physiol Rev* (2018) 98(4):1911–41. doi: 10.1152/physrev.00034.2017

5. Michailidou Z, Gomez-Salazar M, Alexaki VI. Innate immune cells in the adipose tissue in health and metabolic disease. *J Innate Immun* (2022) 14(1):4–30. doi: 10.1159/000515117

6. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* (2003) 112(12):1821–30. doi: 10.1172/JCI200319451

7. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW Jr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* (2003) 112(12):1796–808. doi: 10.1172/JCI200319246

8. Gomez Perdiguero E, Klapproth K, Schulz C, Busch K, Azzoni E, Crozet L, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. *Nature* (2015) 518(7540):547–51. doi: 10.1038/nature13989

9. Chen Q, Ruedl C. Obesity retunes turnover kinetics of tissue-resident macrophages in fat. *J Leukoc Biol* (2020) 107(5):773–82. doi: 10.1002/JLB.1MA1219-275R

10. Curat CA, Miranville A, Sengenès C, Diehl M, Tonus C, Busse R, et al. From blood monocytes to adipose tissue-resident macrophages: induction of diapedesis by human mature adipocytes. *Diabetes* (2004) 53(5):1285–92. doi: 10.2337/diabetes.53.5.1285

11. Cancello R, Henegar C, Viguerie N, Taleb S, Poitou C, Rouault C, et al. Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. *Diabetes* (2005) 54(8):2277–86. doi: 10.2337/diabetes.54.8.2277

12. Takahashi K, Mizuarai S, Araki H, Mashiko S, Ishihara A, Kanatani A, et al. Adiposity elevates plasma MCP-1 levels leading to the increased CD11b-positive monocytes in mice. *J Biol Chem* (2003) 278(47):46654–60. doi: 10.1074/jbc.M309895200

13. Sartipy P, Loskutoff DJ. Monocyte chemoattractant protein 1 in obesity and insulin resistance. *Proc Natl Acad Sci U S A* (2003) 100(12):7265–70. doi: 10.1073/pnas.1133870100

14. Christiansen T, Richelsen B, Bruun JM. Monocyte chemoattractant protein-1 is produced in isolated adipocytes, associated with adiposity and reduced after weight loss in morbid obese subjects. *Int J Obes (Lond)* (2005) 29(1):146–50. doi: 10.1038/sj.ijo.0802839

15. Dahlman I, Kaaman M, Olsson T, Tan GD, Bickerton AS, Wåhlén K, et al. A unique role of monocyte chemoattractant protein 1 among chemokines in adipose tissue of obese subjects. *J Clin Endocrinol Metab* (2005) 90(10):5834–40. doi: 10.1210/jc.2005-0369

16. Di Gregorio GB, Yao-Borengasser A, Rasouli N, Varma V, Lu T, Miles LM, et al. Expression of CD68 and macrophage chemoattractant protein-1 genes in human adipose and muscle tissues: association with cytokine expression, insulin resistance, and reduction by pioglitazone. *Diabetes* (2005) 54(8):2305–13. doi: 10.2337/diabetes.54.8.2305

17. Chen A, Mumick S, Zhang C, Lamb J, Dai H, Weingarth D, et al. Diet induction of monocyte chemoattractant protein-1 and its impact on obesity. *Obes Res* (2005) 13(8):1311–20. doi: 10.1038/oby.2005.159

18. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* (2006) 116(6):1494–505. doi: 10.1172/JCI26498

19. Weisberg SP, Hunter D, Huber R, Lemieux J, Slaymaker S, Vaddi K, et al. CCR2 modulates inflammatory and metabolic effects of high-fat feeding. *J Clin Invest* (2006) 116(1):115–24. doi: 10.1172/JCI24335

20. Tamura Y, Sugimoto M, Murayama T, Ueda Y, Kanamori H, Ono K, et al. Inhibition of CCR2 ameliorates insulin resistance and hepatic steatosis in db/db mice. *Arterioscler Thromb Vasc Biol* (2008) 28(12):2195–201. doi: 10.1161/ATVBAHA.108.168633

21. Mulder P, van den Hoek AM, Kleemann R. The CCR2 inhibitor propagermanium attenuates diet-induced insulin resistance, adipose tissue inflammation and non-alcoholic steatohepatitis. *PLoS One* (2017) 12(1):e0169740. doi: 10.1371/journal.pone.0169740

22. Kamei N, Tobe K, Suzuki R, Ohsugi M, Watanabe T, Kubota N, et al. Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. *J Biol Chem* (2006) 281(36):26602–14. doi: 10.1074/jbc.M601284200

23. Ito A, Suganami T, Yamauchi A, Degawa-Yamauchi M, Tanaka M, Kouyama R, et al. Role of CC chemokine receptor 2 in bone marrow cells in the recruitment of macrophages into obese adipose tissue. *J Biol Chem* (2008) 283(51):35715–23. doi: 10.1074/jbc.M804220200

24. Kim J, Chung K, Choi C, Beloor J, Ullah I, Kim N, et al. Silencing CCR2 in macrophages alleviates adipose tissue inflammation and the associated metabolic syndrome in dietary obese mice. *Mol Ther Nucleic Acids* (2016) 5(1):e280. doi: 10.1038/mtna.2015.51

25. Jiao P, Chen Q, Shah S, Du J, Tao B, Tzameli I, et al. Obesity-related upregulation of monocyte chemotactic factors in adipocytes: involvement of nuclear factor-kappaB and c-jun NH2-terminal kinase pathways. *Diabetes* (2009) 58(1):104–15. doi: 10.2337/db07-1344

26. Huber J, Kiefer FW, Zeyda M, Ludvik B, Silberhumer GR, Prager G, et al. CC chemokine and CC chemokine receptor profiles in visceral and subcutaneous adipose tissue are altered in human obesity. *J Clin Endocrinol Metab* (2008) 93(8):3215–21. doi: 10.1210/jc.2007-2630

27. Eriksson Hogling D, Petrus P, Gao H, Bäckdahl J, Dahlman I, Laurencikiene J, et al. Adipose and circulating CCL18 levels associate with metabolic risk factors in women. *J Clin Endocrinol Metab* (2016) 101(11):4021–9. doi: 10.1210/jc.2016-2390

28. Huh JH, Kim HM, Lee ES, Kwon MH, Lee BR, Ko HJ, et al. Dual CCR2/5 antagonist attenuates obesity-induced insulin resistance by regulating macrophage recruitment and M1/M2 status. *Obes (Silver Spring)* (2018) 26(2):378–86. doi: 10.1002/oby.22103

29. Kim D, Kim J, Yoon JH, Ghim J, Yea K, Song P, et al. CXCL12 secreted from adipose tissue recruits macrophages and induces insulin resistance in mice. *Diabetologia* (2014) 57(7):1456–65. doi: 10.1007/s00125-014-3237-5

30. Maffei M, Funicello M, Vottari T, Gamucci O, Costa M, Lisi S, et al. The obesity and inflammatory marker haptoglobin attracts monocytes via interaction with chemokine (C-c motif) receptor 2 (CCR2). *BMC Biol* (2009) 7:87. doi: 10.1186/1741-7007-7-87

31. Mamane Y, Chung Chan C, Lavallee G, Morin N, Xu LJ, Huang J, et al. The C3a anaphylatoxin receptor is a key mediator of insulin resistance and functions by modulating adipose tissue macrophage infiltration and activation. *Diabetes* (2009) 58(9):2006–17. doi: 10.2337/db09-0323

32. Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J Lipid Res* (2005) 46(11):2347–55. doi: 10.1194/jlr.M500294-JLR200

33. Murano I, Barbatelli G, Parisani V, Latini C, Muzzonigro G, Castellucci M, et al. Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically obese mice. *J Lipid Res* (2008) 49(7):1562–8. doi: 10.1194/jlr.M800019-JLR200

34. Lumeng CN, Deyoung SM, Bodzin JL, Saltiel AR. Increased inflammatory properties of adipose tissue macrophages recruited during diet-induced obesity. *Diabetes* (2007) 56(1):16–23. doi: 10.2337/db06-1076

35. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* (2007) 117(1):175–84. doi: 10.1172/JCI29881

36. Lumeng CN, DelProposto JB, Westcott DJ, Saltiel AR. Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes. *Diabetes* (2008) 57(12):3239–46. doi: 10.2337/db08-0872

37. Shaul ME, Bennett G, Strissel KJ, Greenberg AS, Obin MS. Dynamic, M2-like remodeling phenotypes of CD11c+ adipose tissue macrophages during high-fat diet–induced obesity in mice. *Diabetes* (2010) 59(5):1171–81. doi: 10.2337/db09-1402

38. Zeyda M, Farmer D, Todoric J, Aszmann O, Speiser M, Györi G, et al. Human adipose tissue macrophages are of an anti-inflammatory phenotype but capable of excessive pro-inflammatory mediator production. *Int J Obes (Lond)* (2007) 31(9):1420–8. doi: 10.1038/sj.ijo.0803632

39. Bourlier V, Zakaroff-Girard A, Miranville A, De Barros S, Maumus M, Sengenes C, et al. Remodeling phenotype of human subcutaneous adipose tissue macrophages. *Circulation* (2008) 117(6):806–15. doi: 10.1161/CIRCULATIONAHA.107.724096

40. Aron-Wisnewsky J, Tordjman J, Poitou C, Darakhshan F, Hugol D, Basdevant A, et al. Human adipose tissue macrophages: m1 and m2 cell surface markers in subcutaneous and omental depots and after weight loss. *J Clin Endocrinol Metab* (2009) 94(11):4619–23. doi: 10.1210/jc.2009-0925

41. Fjeldborg K, Pedersen SB, Møller HJ, Christiansen T, Bennetzen M, Richelsen B. Human adipose tissue macrophages are enhanced but changed to an anti-inflammatory profile in obesity. *J Immunol Res* (2014) 2014:309548. doi: 10.1155/2014/309548

42. Yu R, Kim CS, Kwon BS, Kawada T. Mesenteric adipose tissue-derived monocyte chemoattractant protein-1 plays a crucial role in adipose tissue macrophage migration and activation in obese mice. *Obes (Silver Spring)* (2006) 14(8):1353–62. doi: 10.1038/oby.2006.153

43. Harman-Boehm I, Blüher M, Redel H, Sion-Vardy N, Ovadia S, Avinoach E, et al. Macrophage infiltration into omental versus subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity. *J Clin Endocrinol Metab* (2007) 92(6):2240–7. doi: 10.1210/jc.2006-1811

44. Michaud A, Drolet R, Noël S, Paris G, Tchernof A. Visceral fat accumulation is an indicator of adipose tissue macrophage infiltration in women. *Metabolism* (2012) 61(5):689–98. doi: 10.1016/j.metabol.2011.10.004

45. Okamoto Y, Higashiyama H, Rong JX, McVey MJ, Kinoshita M, Asano S, et al. Comparison of mitochondrial and macrophage content between subcutaneous and visceral fat in db/db mice. *Exp Mol Pathol* (2007) 83(1):73–83. doi: 10.1016/j.yexmp.2007.02.007

46. Amano SU, Cohen JL, Vangala P, Tencerova M, Nicoloro SM, Yawe JC, et al. Local proliferation of macrophages contributes to obesity-associated adipose tissue inflammation. *Cell Metab* (2014) 19(1):162–71. doi: 10.1016/j.cmet.2013.11.017

47. Zheng C, Yang Q, Cao J, Xie N, Liu K, Shou P, et al. Local proliferation initiates macrophage accumulation in adipose tissue during obesity. *Cell Death Dis* (2016) 7(3):e2167. doi: 10.1038/cddis.2016.54

48. Muir LA, Kiridena S, Griffin C, DelProposto JB, Geletka L, Martinez-Santibañez G, et al. Frontline science: rapid adipose tissue expansion triggers unique proliferation and lipid accumulation profiles in adipose tissue macrophages. *J Leukoc Biol* (2018) 103(4):615–28. doi: 10.1002/JLB.3HI1017-422R

49. Haase J, Weyer U, Immig K, Klöting N, Blüher M, Eilers J, et al. Local proliferation of macrophages in adipose tissue during obesity-induced inflammation. *Diabetologia* (2014) 57(3):562–71. doi: 10.1007/s00125-013-3139-y

50. Zamarron BF, Mergian TA, Cho KW, Martinez-Santibanez G, Luan D, Singer K, et al. Macrophage proliferation sustains adipose tissue inflammation in formerly obese mice. *Diabetes* (2017) 66(2):392–406. doi: 10.2337/db16-0500

51. Ahlin S, Sjöholm K, Jacobson P, Andersson-Assarsson JC, Walley A, Tordjman J, et al. Macrophage gene expression in adipose tissue is associated with insulin sensitivity and serum lipid levels independent of obesity. *Obes (Silver Spring)* (2013) 21(12):E571–6. doi: 10.1002/oby.20443

52. Cancello R, Tordjman J, Poitou C, Guilhem G, Bouillot JL, Hugol D, et al. Increased infiltration of macrophages in omental adipose tissue is associated with marked hepatic lesions in morbid human obesity. *Diabetes* (2006) 55(6):1554–61. doi: 10.2337/db06-0133

53. Makkonen J, Westerbacka J, Kolak M, Sutinen J, Cornér A, Hamsten A, et al. Increased expression of the macrophage markers and of 11beta-HSD-1 in subcutaneous adipose tissue, but not in cultured monocyte-derived macrophages, is associated with liver fat in human obesity. *Int J Obes (Lond)* (2007) 31(10):1617–25. doi: 10.1038/sj.ijo.0803635

54. Ortega Martinez de Victoria E, Xu X, Koska J, Francisco AM, Scalise M, Ferrante AW Jr, et al. Macrophage content in subcutaneous adipose tissue: associations with adiposity, age, inflammatory markers, and whole-body insulin action in healthy pima indians. *Diabetes* (2009) 58(2):385–93. doi: 10.2337/db08-0536

55. Wentworth JM, Naselli G, Brown WA, Doyle L, Phipson B, Smyth GK, et al. Pro-inflammatory CD11c+CD206+ adipose tissue macrophages are associated with insulin resistance in human obesity. *Diabetes* (2010) 59(7):1648–56. doi: 10.2337/db09-0287

56. Klimcakova E, Roussel B, Kovacova Z, Kovacikova M, Siklova-Vitkova M, Combes M, et al. Macrophage gene expression is related to obesity and the metabolic syndrome in human subcutaneous fat as well as in visceral fat. *Diabetologia* (2011) 54(4):876–87. doi: 10.1007/s00125-010-2014-3

57. Le KA, Mahurkar S, Alderete TL, Hasson RE, Adam TC, Kim JS, et al. Subcutaneous adipose tissue macrophage infiltration is associated with hepatic and visceral fat deposition, hyperinsulinemia, and stimulation of NF-kappaB stress pathway. *Diabetes* (2011) 60(11):2802–9. doi: 10.2337/db10-1263

58. Koppaka S, Kehlenbrink S, Carey M, Li W, Sanchez E, Lee DE, et al. Reduced adipose tissue macrophage content is associated with improved insulin sensitivity in thiazolidinedione-treated diabetic humans. *Diabetes* (2013) 62(6):1843–54. doi: 10.2337/db12-0868

59. Aouadi M, Tencerova M, Vangala P, Yawe JC, Nicoloro SM, Amano SU, et al. Gene silencing in adipose tissue macrophages regulates whole-body metabolism in obese mice. *Proc Natl Acad Sci U S A* (2013) 110(20):8278–83. doi: 10.1073/pnas.1300492110

60. Morris DL, Singer K, Lumeng CN. Adipose tissue macrophages: phenotypic plasticity and diversity in lean and obese states. *Curr Opin Clin Nutr Metab Care* (2011) 14(4):341–6. doi: 10.1097/MCO.0b013e328347970b

61. Kratz M, Coats BR, Hisert KB, Hagman D, Mutskov V, Peris E, et al. Metabolic dysfunction drives a mechanistically distinct proinflammatory phenotype in adipose tissue macrophages. *Cell Metab* (2014) 20(4):614–25. doi: 10.1016/j.cmet.2014.08.010

62. Coats BR, Schoenfelt KQ, Barbosa-Lorenzi VC, Peris E, Cui C, Hoffman A, et al. Metabolically activated adipose tissue macrophages perform detrimental and beneficial functions during diet-induced obesity. *Cell Rep* (2017) 20(13):3149–61. doi: 10.1016/j.celrep.2017.08.096

63. Tiwari P, Blank A, Cui C, Schoenfelt KQ, Zhou G, Xu Y, et al. Metabolically activated adipose tissue macrophages link obesity to triple-negative breast cancer. *J Exp Med* (2019) 216(6):1345–58. doi: 10.1084/jem.20181616

64. Hill DA, Lim HW, Kim YH, Ho WY, Foong YH, Nelson VL, et al. Distinct macrophage populations direct inflammatory versus physiological changes in adipose tissue. *Proc Natl Acad Sci USA* (2018) 115(22):E5096–105. doi: 10.1073/pnas.1802611115

65. Jaitin DA, Adlung L, Thaiss CA, Weiner A, Li B, Descamps H, et al. Lipid-associated macrophages control metabolic homeostasis in a Trem2-dependent manner. *Cell* (2019) 178(3):686–98.e14. doi: 10.1016/j.cell.2019.05.054

66. Kim EY, Noh HM, Choi B, Park JE, Kim JE, Jang Y, et al. Interleukin-22 induces the infiltration of visceral fat tissue by a discrete subset of Duffy antigen receptor for chemokine-positive M2-like macrophages in response to a high fat diet. *Cells* (2019) 8 (12):1587. doi: 10.3390/cells8121587

67. Orr JS, Kennedy A, Anderson-Baucum EK, Webb CD, Fordahl SC, Erikson KM, et al. Obesity alters adipose tissue macrophage iron content and tissue iron distribution. *Diabetes* (2014) 63(2):421–32. doi: 10.2337/db13-0213

68. Hubler MJ, Erikson KM, Kennedy AJ, Hasty AH. MFe(hi) adipose tissue macrophages compensate for tissue iron perturbations in mice. *Am J Physiol Cell Physiol* (2018) 315(3):C319–29. doi: 10.1152/ajpcell.00103.2018

69. Deczkowska A, Weiner A, Amit I. The physiology, pathology, and potential therapeutic applications of the TREM2 signaling pathway. *Cell* (2020) 181(6):1207–17. doi: 10.1016/j.cell.2020.05.003

70. Harasymowicz NS, Rashidi N, Savadipour A, Wu CL, Tang R, Bramley J, et al. Single-cell RNA sequencing reveals the induction of novel myeloid and myeloid-associated cell populations in visceral fat with long-term obesity. *FASEB J* (2021) 35(3): e21417. doi: 10.1096/fj.202001970R

71. Constant VA, Gagnon A, Landry A, Sorisky A. Macrophage-conditioned medium inhibits the differentiation of 3T3-L1 and human abdominal preadipocytes. *Diabetologia* (2006) 49(6):1402–11. doi: 10.1007/s00125-006-0253-0

72. Lacasa D, Taleb S, Keophiphath M, Miranville A, Clement K. Macrophage-secreted factors impair human adipogenesis: involvement of proinflammatory state in preadipocytes. *Endocrinology* (2007) 148(2):868–77. doi: 10.1210/en.2006-0687

73. Liu LF, Craig CM, Tolentino LL, Choi O, Morton J, Rivas H, et al. Adipose tissue macrophages impair preadipocyte differentiation in humans. *PLoS One* (2017) 12(2): e0170728. doi: 10.1371/journal.pone.0170728

74. Cao D, Ma F, Ouyang S, Liu Z, Li Y, Wu J. Effects of macrophages and CXCR2 on adipogenic differentiation of bone marrow mesenchymal stem cells. *J Cell Physiol* (2019) 234(6):9475–85. doi: 10.1002/jcp.27634

75. Moratal C, Raffort J, Arrighi N, Rekima S, Schaub S, Dechesne CA, et al. IL-1beta- and IL-4-polarized macrophages have opposite effects on adipogenesis of intramuscular fibro-adipogenic progenitors in humans. *Sci Rep* (2018) 8(1):17005. doi: 10.1038/s41598-018-35429-w

76. Isakson P, Hammarstedt A, Gustafson B, Smith U. Impaired preadipocyte differentiation in human abdominal obesity: role of wnt, tumor necrosis factor-alpha, and inflammation. *Diabetes* (2009) 58(7):1550–7. doi: 10.2337/db08-1770

77. Gagnon A, Yarmo MN, Landry A, Sorisky A. Macrophages alter the differentiation-dependent decreases in fibronectin and collagen I/III protein levels in human preadipocytes. *Lipids* (2012) 47(9):873–80. doi: 10.1007/s11745-012-3696-8

78. Bilkovski R, Schulte DM, Oberhauser F, Mauer J, Hampel B, Gutschow C, et al. Adipose tissue macrophages inhibit adipogenesis of mesenchymal precursor cells via wnt-5a in humans. *Int J Obes (Lond)* (2011) 35(11):1450–4. doi: 10.1038/ijo.2011.6

79. Yin R, Fang L, Li Y, Xue P, Li Y, Guan Y, et al. Pro-inflammatory macrophages suppress PPARgamma activity in adipocytes via s-nitrosylation. *Free Radic Biol Med* (2015) 89:895–905. doi: 10.1016/j.freeradbiomed.2015.10.406

80. Wu J, Boström P, Sparks LM, Ye L, Choi JH, Giang AH, et al. Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* (2012) 150(2):366–76. doi: 10.1016/j.cell.2012.05.016

81. Kaisanlahti A, Glumoff T. Browning of white fat: agents and implications for beige adipose tissue to type 2 diabetes. *J Physiol Biochem* (2019) 75(1):1–10. doi: 10.1007/s13105-018-0658-5

82. Hui X, Gu P, Zhang J, Nie T, Pan Y, Wu D, et al. Adiponectin enhances cold-induced browning of subcutaneous adipose tissue via promoting M2 macrophage proliferation. *Cell Metab* (2015) 22(2):279–90. doi: 10.1016/j.cmet.2015.06.004

83. Sakamoto T, Nitta T, Maruno K, Yeh YS, Kuwata H, Tomita K, et al. Macrophage infiltration into obese adipose tissues suppresses the induction of UCP1 level in mice. *Am J Physiol Endocrinol Metab* (2016) 310(8):E676–87. doi: 10.1152/ajpendo.00028.2015

84. Machida K, Okamatsu-Ogura Y, Shin W, Matsuoka S, Tsubota A, Kimura K. Role of macrophages in depot-dependent browning of white adipose tissue. *J Physiol Sci* (2018) 68(5):601–8. doi: 10.1007/s12576-017-0567-3

85. Chung KJ, Chatzigeorgiou A, Economopoulou M, Garcia-Martin R, Alexaki VI, Mitroulis I, et al. A self-sustained loop of inflammation-driven inhibition of beige adipogenesis in obesity. *Nat Immunol* (2017) 18(6):654–64. doi: 10.1038/ni.3728

86. Qiu Y, Nguyen KD, Odegaard JI, Cui X, Tian X, Locksley RM, et al. Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. *Cell* (2014) 157(6):1292–308. doi: 10.1016/j.cell.2014.03.066

87. Wang YN, Tang Y, He Z, Ma H, Wang L, Liu Y, et al. Slit3 secreted from M2-like macrophages increases sympathetic activity and thermogenesis in adipose tissue. *Nat Metab* (2021) 3(11):1536–51. doi: 10.1038/s42255-021-00482-9

88. Liu PS, Lin YW, Burton FH, Wei LN. Injecting engineered anti-inflammatory macrophages therapeutically induces white adipose tissue browning and improves diet-induced insulin resistance. *Adipocyte* (2015) 4(2):123–8. doi: 10.4161/21623945.2014.981438

89. Nawaz A, Aminuddin A, Kado T, Takikawa A, Yamamoto S, Tsuneyama K, et al. CD206(+) M2-like macrophages regulate systemic glucose metabolism by inhibiting proliferation of adipocyte progenitors. *Nat Commun* (2017) 8(1):286. doi: 10.1038/s41467-017-00231-1

90. Igarashi Y, Nawaz A, Kado T, Bilal M, Kuwano T, Yamamoto S, et al. Partial depletion of CD206-positive M2-like macrophages induces proliferation of beige progenitors and enhances browning after cold stimulation. *Sci Rep* (2018) 8 (1):14567. doi: 10.1038/s41598-018-32803-6

91. Jiang Y, Berry DC, Graff JM. Distinct cellular and molecular mechanisms for beta3 adrenergic receptor-induced beige adipocyte formation. *Elife* (2017) 6:e30329. doi: 10.7554/eLife.30329

92. Lee YH, Petkova AP, Granneman JG. Identification of an adipogenic niche for adipose tissue remodeling and restoration. *Cell Metab* (2013) 18(3):355–67. doi: 10.1016/j.cmet.2013.08.003

93. Lee YH, Kim SN, Kwon HJ, Maddipati KR, Granneman JG. Adipogenic role of alternatively activated macrophages in beta-adrenergic remodeling of white adipose tissue. *Am J Physiol Regul Integr Comp Physiol* (2016) 310(1):R55–65. doi: 10.1152/ajpregu.00355.2015

94. Pekala PH, Price SR, Horn CA, Hom BE, Moss J, Cerami A. Model for cachexia in chronic disease: secretory products of endotoxin-stimulated macrophages induce a catabolic state in 3T3-L1 adipocytes. *Trans Assoc Am Physicians* (1984) 97:251–9.

95. Mahoney JR Jr, Beutler BA, Le Trang N, Vine W, Ikeda Y, Kawakami M, et al. Lipopolysaccharide-treated RAW 264.7 cells produce a mediator that inhibits lipoprotein lipase in 3T3-L1 cells. *J Immunol* (1985) 134(3):1673–5.

96. Kurokawa J, Arai S, Nakashima K, Nagano H, Nishijima A, Miyata K, et al. Macrophage-derived AIM is endocytosed into adipocytes and decreases lipid droplets via inhibition of fatty acid synthase activity. *Cell Metab* (2010) 11(6):479–92. doi: 10.1016/j.cmet.2010.04.013

97. Keuper M, Sachs S, Walheim E, Berti L, Raedle B, Tews D, et al. Activated macrophages control human adipocyte mitochondrial bioenergetics via secreted factors. *Mol Metab* (2017) 6(10):1226–39. doi: 10.1016/j.molmet.2017.07.008

98. Cox N, Crozet L, Holtman IR, Loyher PL, Lazarov T, White JB, et al. Diet-regulated production of PDGFcc by macrophages controls energy storage. *Science* (2021) 373(6550):eabe9383. doi: 10.1126/science.abe9383

99. Permana PA, Menge C, Reaven PD. Macrophage-secreted factors induce adipocyte inflammation and insulin resistance. *Biochem Biophys Res Commun* (2006) 341(2):507–14. doi: 10.1016/j.bbrc.2006.01.012

100. Lumeng CN, Deyoung SM, Saltiel AR. Macrophages block insulin action in adipocytes by altering expression of signaling and glucose transport proteins. *Am J Physiol Endocrinol Metab* (2007) 292(1):E166–74. doi: 10.1152/ajpendo.00284.2006

101. Gao D, Madi M, Ding C, Fok M, Steele T, Ford C, et al. Interleukin-1beta mediates macrophage-induced impairment of insulin signaling in human primary adipocytes. *Am J Physiol Endocrinol Metab* (2014) 307(3):E289–304. doi: 10.1152/ajpendo.00430.2013

102. Maeda T, Noge I, Kagawa Y. Infiltration of M1 macrophages into adipose tissue of ddY-h mice preceding spontaneous appearances of insulin resistance. *Biol Pharm Bull* (2013) 36(5):825–32. doi: 10.1248/bpb.b12-01014

103. Suganami T, Nishida J, Ogawa Y. A paracrine loop between adipocytes and macrophages aggravates inflammatory changes: role of free fatty acids and tumor necrosis factor alpha. *Arterioscler Thromb Vasc Biol* (2005) 25(10):2062–8. doi: 10.1161/01.ATV.0000183883.72263.13

104. Davis JE, Braucher DR, Walker-Daniels J, Spurlock ME. Absence of Tlr2 protects against high-fat diet-induced inflammation and results in greater insulin-stimulated glucose transport in cultured adipocytes. *J Nutr Biochem* (2011) 22(2):136–41. doi: 10.1016/j.jnutbio.2009.12.008

105. Hong CP, Yun CH, Lee GW, Park A, Kim YM, Jang MH. TLR9 regulates adipose tissue inflammation and obesity-related metabolic disorders. *Obes (Silver Spring)* (2015) 23(11):2199–206. doi: 10.1002/oby.21215

106. Nakarai H, Yamashita A, Nagayasu S, Iwashita M, Kumamoto S, Ohyama H, et al. Adipocyte-macrophage interaction may mediate LPS-induced low-grade inflammation: potential link with metabolic complications. *Innate Immun* (2012) 18 (1):164–70. doi: 10.1177/1753425910393370

107. Liu J, Batkai S, Pacher P, Harvey-White J, Wagner JA, Cravatt BF, et al. Lipopolysaccharide induces anandamide synthesis in macrophages via CD14/MAPK/phosphoinositide 3-kinase/NF-kappaB independently of platelet-activating factor. *J Biol Chem* (2003) 278(45):45034–9. doi: 10.1074/jbc.M306062200

108. Mehrpouya-Bahrami P, Chitrala KN, Ganewatta MS, Tang C, Murphy EA, Enos RT, et al. Blockade of CB1 cannabinoid receptor alters gut microbiota and attenuates inflammation and diet-induced obesity. *Sci Rep* (2017) 7(1):15645. doi: 10.1038/s41598-017-15154-6

109. Miranville A, Herling AW, Biemer-Daub G, Voss MD. Reversal of inflammation-induced impairment of glucose uptake in adipocytes by direct effect of CB1 antagonism on adipose tissue macrophages. *Obes (Silver Spring)* (2010) 18 (12):2247–54. doi: 10.1038/oby.2010.81

110. Zhong J, Rao X, Deiuliis J, Braunstein Z, Narula V, Hazey J, et al. A potential role for dendritic cell/macrophage-expressing DPP4 in obesity-induced visceral inflammation. *Diabetes* (2013) 62(1):149–57. doi: 10.2337/db12-0230

111. Varin EM, Mulvihill EE, Beaudry JL, Pujadas G, Fuchs S, Tanti JF, et al. Circulating levels of soluble dipeptidyl peptidase-4 are dissociated from inflammation and induced by enzymatic DPP4 inhibition. *Cell Metab* (2019) 29(2):320–34.e5. doi: 10.1016/j.cmet.2018.10.001

112. Zhuge F, Ni Y, Nagashimada M, Nagata N, Xu L, Mukaida N, et al. DPP-4 inhibition by linagliptin attenuates obesity-related inflammation and insulin resistance by regulating M1/M2 macrophage polarization. *Diabetes* (2016) 65(10):2966–79. doi: 10.2337/db16-0317

113. Khan T, Muise ES, Iyengar P, Wang ZV, Chandalia M, Abate N, et al. Metabolic dysregulation and adipose tissue fibrosis: role of collagen VI. *Mol Cell Biol* (2009) 29(6):1575–91. doi: 10.1128/MCB.01300-08

114. Divoux A, Tordjman J, Lacasa D, Veyrie N, Hugol D, Aissat A, et al. Fibrosis in human adipose tissue: composition, distribution, and link with lipid metabolism and fat mass loss. *Diabetes* (2010) 59(11):2817–25. doi: 10.2337/db10-0585

115. Spencer M, Yao-Borengasser A, Unal R, Rasouli N, Gurley CM, Zhu B, et al. Adipose tissue macrophages in insulin-resistant subjects are associated with collagen VI and fibrosis and demonstrate alternative activation. *Am J Physiol Endocrinol Metab* (2010) 299(6):E1016–27. doi: 10.1152/ajpendo.00329.2010

116. Marcelin G, Ferreira A, Liu Y, Atlan M, Aron-Wisnewsky J, Pelloux V, et al. A PDGFRalpha-mediated switch toward CD9(high) adipocyte progenitors controls obesity-induced adipose tissue fibrosis. *Cell Metab* (2017) 25(3):673–85. doi: 10.1016/j.cmet.2017.01.010

117. Keophiphath M, Rouault C, Divoux A, Clément K, Lacasa D. CCL5 promotes macrophage recruitment and survival in human adipose tissue. *Arterioscler Thromb Vasc Biol* (2010) 30(1):39–45. doi: 10.1161/ATVBAHA.109.197442

118. O'Hara A, Lim FL, Mazzatti DJ, Trayhurn P. Microarray analysis identifies matrix metalloproteinases (MMPs) as key genes whose expression is up-regulated in human adipocytes by macrophage-conditioned medium. *Pflugers Arch* (2009) 458 (6):1103–14. doi: 10.1007/s00424-009-0693-8

119. Gao D, Bing C. Macrophage-induced expression and release of matrix metalloproteinase 1 and 3 by human preadipocytes is mediated by IL-1beta via activation of MAPK signaling. *J Cell Physiol* (2011) 226(11):2869–80. doi: 10.1002/jcp.22630

120. Bourlier V, Sengenès C, Zakaroff-Girard A, Decaunes P, Wdziekonski B, Galitzky J, et al. TGFbeta family members are key mediators in the induction of myofibroblast phenotype of human adipose tissue progenitor cells by macrophages. *PloS One* (2012) 7(2):e31274. doi: 10.1371/journal.pone.0031274

121. Ye J, Gao Z, Yin J, He Q. Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. *Am J Physiol Endocrinol Metab* (2007) 293(4):E1118–28. doi: 10.1152/ajpendo.00435.2007

122. Rausch ME, Weisberg S, Vardhana P, Tortoriello DV. Obesity in C57BL/6J mice is characterized by adipose tissue hypoxia and cytotoxic T-cell infiltration. *Int J Obes (Lond)* (2008) 32(3):451–63. doi: 10.1038/sj.ijo.0803744

123. Hosogai N, Fukuhara A, Oshima K, Miyata Y, Tanaka S, Segawa K, et al. Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes* (2007) 56(4):901–11. doi: 10.2337/db06-0911

124. Pasarica M, Rood J, Ravussin E, Schwarz JM, Smith SR, Redman LM. Reduced adipose tissue oxygenation in human obesity: evidence for rarefaction, macrophage chemotaxis, and inflammation without an angiogenic response. *Diabetes* (2009) 58 (3):718–25. doi: 10.2337/db08-1098

125. Sun K, Tordjman J, Clément K, Scherer PE. Fibrosis and adipose tissue dysfunction. *Cell Metab* (2013) 18(4):470–7. doi: 10.1016/j.cmet.2013.06.016

126. Jang JE, Ko MS, Yun JY, Kim MO, Kim JH, Park HS, et al. Nitric oxide produced by macrophages inhibits adipocyte differentiation and promotes profibrogenic responses in preadipocytes to induce adipose tissue fibrosis. *Diabetes* (2016) 65(9):2516–28. doi: 10.2337/db15-1624

127. Arkan MC, Hevener AL, Greten FR, Maeda S, Li ZW, Long JM, et al. IKK-beta links inflammation to obesity-induced insulin resistance. *Nat Med* (2005) 11(2):191–8. doi: 10.1038/nm1185

128. Saberi M, Woods NB, de Luca C, Schenk S, Lu JC, Bandyopadhyay G, et al. Hematopoietic cell-specific deletion of toll-like receptor 4 ameliorates hepatic and adipose tissue insulin resistance in high-fat-fed mice. *Cell Metab* (2009) 10(5):419–29. doi: 10.1016/j.cmet.2009.09.006

129. Wueest S, Mueller R, Blüher M, Item F, Chin AS, Wiedemann MS, et al. Fas (CD95) expression in myeloid cells promotes obesity-induced muscle insulin resistance. *EMBO Mol Med* (2014) 6(1):43–56. doi: 10.1002/emmm.201302962

130. Yu M, Zhou H, Zhao J, Xiao N, Roychowdhury S, Schmitt D, et al. MyD88-dependent interplay between myeloid and endothelial cells in the initiation and progression of obesity-associated inflammatory diseases. *J Exp Med* (2014) 211 (5):887–907. doi: 10.1084/jem.20131314

131. Chen Z, Shen H, Sun C, Yin L, Tang F, Zheng P, et al. Myeloid cell TRAF3 promotes metabolic inflammation, insulin resistance, and hepatic steatosis in obesity. *Am J Physiol Endocrinol Metab* (2015) 308(6):E460–9. doi: 10.1152/ajpendo.00470.2014

132. Sima C, Montero E, Nguyen D, Freire M, Norris P, Serhan CN, et al. ERV1 overexpression in myeloid cells protects against high fat diet induced obesity and glucose intolerance. *Sci Rep* (2017) 7(1):12848. doi: 10.1038/s41598-017-13185-7

133. Pepping JK, Vandanmagsar B, Fernandez-Kim SO, Zhang J, Mynatt RL, Bruce-Keller AJ. Myeloid-specific deletion of NOX2 prevents the metabolic and neurologic consequences of high fat diet. *PloS One* (2017) 12(8):e0181500. doi: 10.1371/journal.pone.0181500

134. Takikawa A, Mahmood A, Nawaz A, Kado T, Okabe K, Yamamoto S, et al. HIF-1alpha in myeloid cells promotes adipose tissue remodeling toward insulin resistance. *Diabetes* (2016) 65(12):3649–59. doi: 10.2337/db16-0012

135. Huang JY, Chiang MT, Yet SF, Chau LY. Myeloid heme oxygenase-1 haploinsufficiency reduces high fat diet-induced insulin resistance by affecting adipose macrophage infiltration in mice. *PloS One* (2012) 7(6):e38626. doi: 10.1371/journal.pone.0038626

136. Hu L, He F, Huang M, Peng M, Zhou Z, Liu F, et al. NFATc3 deficiency reduces the classical activation of adipose tissue macrophages. *J Mol Endocrinol* (2018) 61 (3):79–89. doi: 10.1530/JME-18-0070

137. Cao W, Zhang T, Feng R, Xia T, Huang H, Liu C, et al. Hoxa5 alleviates obesity-induced chronic inflammation by reducing ER stress and promoting M2 macrophage polarization in mouse adipose tissue. *J Cell Mol Med* (2019) 23 (10):7029–42. doi: 10.1111/jcmm.14600

138. Mauer J, Chaurasia B, Plum L, Quast T, Hampel B, Blüher M, et al. Myeloid cell-restricted insulin receptor deficiency protects against obesity-induced inflammation and systemic insulin resistance. *PloS Genet* (2010) 6(5):e1000938. doi: 10.1371/journal.pgen.1000938

139. Grant L, Shearer KD, Czopek A, Lees EK, Owen C, Agouni A, et al. Myeloid-cell protein tyrosine phosphatase-1B deficiency in mice protects against high-fat diet and lipopolysaccharide-induced inflammation, hyperinsulinemia, and endotoxemia through an IL-10 STAT3-dependent mechanism. *Diabetes* (2014) 63(2):456–70. doi: 10.2337/db13-0885

140. Rached MT, Millership SJ, Pedroni SMA, Choudhury AI, Costa ASH, Hardy DG, et al. Deletion of myeloid IRS2 enhances adipose tissue sympathetic nerve function and limits obesity. *Mol Metab* (2019) 20:38–50. doi: 10.1016/j.molmet.2018.11.010

141. Paschoal VA, Belchior T, Amano MT, Burgos-Silva M, Peixoto AS, Magdalon J, et al. Constitutive activation of the nutrient sensor mTORC1 in myeloid cells induced by Tsc1 deletion protects mice from diet-induced obesity. *Mol Nutr Food Res* (2018) 62 (17):e1800283. doi: 10.1002/mnfr.201800283

142. Schug TT, Xu Q, Gao H, Peres-da-Silva A, Draper DW, Fessler MB, et al. Myeloid deletion of SIRT1 induces inflammatory signaling in response to environmental stress. *Mol Cell Biol* (2010) 30(19):4712–21. doi: 10.1128/MCB.00657-10

143. Ka SO, Song MY, Bae EJ, Park BH. Myeloid SIRT1 regulates macrophage infiltration and insulin sensitivity in mice fed a high-fat diet. *J Endocrinol* (2015) 224 (2):109–18. doi: 10.1530/JOE-14-0527

144. Takikawa A, Usui I, Fujisaka S, Ikutani M, Senda S, Hattori S, et al. Deletion of SIRT1 in myeloid cells impairs glucose metabolism with enhancing inflammatory response to adipose tissue hypoxia. *Diabetol Int* (2016) 7(1):59–68. doi: 10.1007/s13340-015-0213-3

145. Jeon BT, Kim KE, Heo RW, Shin HJ, Yi CO, Hah YS, et al. Myeloid-specific deletion of SIRT1 increases hepatic steatosis and hypothalamic inflammation in mice fed a high-fat diet. *Metab Brain Dis* (2014) 29(3):635–43. doi: 10.1007/s11011-014-9542-3

146. Park M, Yi JW, Kim EM, Yoon IJ, Lee EH, Lee HY, et al. Triggering receptor expressed on myeloid cells 2 (TREM2) promotes adipogenesis and diet-induced obesity. *Diabetes* (2015) 64(1):117–27. doi: 10.2337/db13-1869

147. Park YS, Uddin MJ, Piao L, Hwang I, Lee JH, Ha H. Novel role of endogenous catalase in macrophage polarization in adipose tissue. *Mediators Inflammation* (2016) 2016:8675905. doi: 10.1155/2016/8675905

148. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, et al. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. *Nature* (2007) 447(7148):1116–20. doi: 10.1038/nature05894

149. Hevener AL, Olefsky JM, Reichart D, Nguyen MT, Bandyopadyhay G, Leung HY, et al. Macrophage PPAR gamma is required for normal skeletal muscle and hepatic insulin sensitivity and full antidiabetic effects of thiazolidinediones. *J Clin Invest* (2007) 117(6):1658–69. doi: 10.1172/JCI31561

150. Liao X, Sharma N, Kapadia F, Zhou G, Lu Y, Hong H, et al. Kruppel-like factor 4 regulates macrophage polarization. *J Clin Invest* (2011) 121(7):2736–49. doi: 10.1172/JCI45444

151. Coppo M, Chinenov Y, Sacta MA, Rogatsky I. The transcriptional coregulator GRIP1 controls macrophage polarization and metabolic homeostasis. *Nat Commun* (2016) 7:12254. doi: 10.1038/ncomms12254

152. Lee HY, Kim J, Quan W, Lee JC, Kim MS, Kim SH, et al. Autophagy deficiency in myeloid cells increases susceptibility to obesity-induced diabetes and experimental colitis. *Autophagy* (2016) 12(8):1390–403. doi: 10.1080/15548627.2016.1184799

153. Kawano Y, Nakae J, Watanabe N, Fujisaka S, Iskandar K, Sekioka R, et al. Loss of Pdk1-Foxo1 signaling in myeloid cells predisposes to adipose tissue inflammation and insulin resistance. *Diabetes* (2012) 61(8):1935–48. doi: 10.2337/db11-0770

154. Ribas V, Drew BG, Le JA, Soleymani T, Daraei P, Sitz D, et al. Myeloid-specific estrogen receptor alpha deficiency impairs metabolic homeostasis and accelerates atherosclerotic lesion development. *Proc Natl Acad Sci USA* (2011) 108(39):16457–62. doi: 10.1073/pnas.1104533108

155. Lee Y, Ka SO, Cha HN, Chae YN, Kim MK, Park SY, et al. Myeloid sirtuin 6 deficiency causes insulin resistance in high-fat diet-fed mice by eliciting macrophage polarization toward an M1 phenotype. *Diabetes* (2017) 66(10):2659–68. doi: 10.2337/db16-1446

156. Xu H, Li H, Woo SL, Kim SM, Shende VR, Neuendorff N, et al. Myeloid cell-specific disruption of Period1 and Period2 exacerbates diet-induced inflammation and insulin resistance. *J Biol Chem* (2014) 289(23):16374–88. doi: 10.1074/jbc.M113.539601

157. Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG. Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. *Cell Metab* (2008) 8(4):301–9. doi: 10.1016/j.cmet.2008.08.015

158. Pamir N, McMillen TS, Kaiyala KJ, Schwartz MW, LeBoeuf RC. Receptors for tumor necrosis factor-alpha play a protective role against obesity and alter adipose tissue macrophage status. *Endocrinology* (2009) 150(9):4124–34. doi: 10.1210/en.2009-0137

159. Kohlstedt K, Trouvain C, Namgaladze D, Fleming I. Adipocyte-derived lipids increase angiotensin-converting enzyme (ACE) expression and modulate macrophage phenotype. *Basic Res Cardiol* (2011) 106(2):205–15. doi: 10.1007/s00395-010-0137-9

160. Camell C, Smith CW. Dietary oleic acid increases m2 macrophages in the mesenteric adipose tissue. *PLoS One* (2013) 8(9):e75147. doi: 10.1371/journal.pone.0075147

161. Pini M, Touch S, Poirier H, Dalmas E, Niot I, Rouault C, et al. Adipose tissue adaptive response to trans-10,cis-12-conjugated linoleic acid engages alternatively activated M2 macrophages. *FASEB J* (2016) 30(1):241–51. doi: 10.1096/fj.15-276675

162. Talamonti E, Pauter AM, Asadi A, Fischer AW, Chiurchiù V, Jacobsson A. Impairment of systemic DHA synthesis affects macrophage plasticity and polarization: implications for DHA supplementation during inflammation. *Cell Mol Life Sci* (2017) 74(15):2815–26. doi: 10.1007/s00018-017-2498-9

163. Bashir S, Sharma Y, Elahi A, Khan F. Amelioration of obesity-associated inflammation and insulin resistance in c57bl/6 mice via macrophage polarization by fish oil supplementation. *J Nutr Biochem* (2016) 33:82–90. doi: 10.1016/j.jnutbio.2016.02.011

164. Rombaldova M, Janovska P, Kopecky J, Kuda O. Omega-3 fatty acids promote fatty acid utilization and production of pro-resolving lipid mediators in alternatively activated adipose tissue macrophages. *Biochem Biophys Res Commun* (2017) 490 (3):1080–5. doi: 10.1016/j.bbrc.2017.06.170

165. Stienstra R, Duval C, Keshtkar S, van der Laak J, Kersten S, Müller M. Peroxisome proliferator-activated receptor gamma activation promotes infiltration of alternatively activated macrophages into adipose tissue. *J Biol Chem* (2008) 283 (33):22620–7. doi: 10.1074/jbc.M710314200

166. Fujisaka S, Usui I, Bukhari A, Ikutani M, Oya T, Kanatani Y, et al. Regulatory mechanisms for adipose tissue M1 and M2 macrophages in diet-induced obese mice. *Diabetes* (2009) 58(11):2574–82. doi: 10.2337/db08-1475

167. Spencer M, Yang L, Adu A, Finlin BS, Zhu B, Shipp LR, et al. Pioglitazone treatment reduces adipose tissue inflammation through reduction of mast cell and macrophage number and by improving vascularity. *PLoS One* (2014) 9(7):e102190. doi: 10.1371/journal.pone.0102190

168. Kumar D, Goand UK, Gupta S, Shankar K, Varshney S, Rajan S, et al. Saroglitazar reduces obesity and associated inflammatory consequences in murine adipose tissue. *Eur J Pharmacol* (2018) 822:32–42. doi: 10.1016/j.ejphar.2018.01.002

169. Bassaganya-Riera J, Misyak S, Guri AJ, Hontecillas R. PPAR gamma is highly expressed in F4/80(hi) adipose tissue macrophages and dampens adipose-tissue inflammation. *Cell Immunol* (2009) 258(2):138–46. doi: 10.1016/j.cellimm.2009.04.003

170. Van Dyken SJ, Locksley RM. Interleukin-4- and interleukin-13-mediated alternatively activated macrophages: roles in homeostasis and disease. *Annu Rev Immunol* (2013) 31:317–43. doi: 10.1146/annurev-immunol-032712-095906

171. Lin SY, Yang CP, Wang YY, Hsiao CW, Chen WY, Liao SL, et al. Interleukin-4 improves metabolic abnormalities in leptin-deficient and high-fat diet mice. *Int J Mol Sci* (2020) 21(12):4451. doi: 10.3390/ijms21124451

172. Ackermann J, Arndt L, Kirstein M, Hobusch C, Brinker G, Klöting N, et al. Myeloid cell-specific IL-4 receptor knockout partially protects from adipose tissue inflammation. *J Immunol* (2021) 207(12):3081–9. doi: 10.4049/jimmunol.2100699

173. Alder JK, Georgantas RW 3rd, Hildreth RL, Kaplan IM, Morisot S, Yu X, et al. Kruppel-like factor 4 is essential for inflammatory monocyte differentiation *in vivo*. *J Immunol* (2008) 180(8):5645–52. doi: 10.4049/jimmunol.180.8.5645

174. Cao W, Xu Y, Luo D, Saeed M, Sun C. Hoxa5 promotes adipose differentiation via increasing DNA methylation level and inhibiting PKA/HSL signal pathway in mice. *Cell Physiol Biochem* (2018) 45(3):1023–33. doi: 10.1159/000487343

175. Cinkajzlova A, Lacinová Z, Kloučková J, Kaválková P, Trachta P, Kosák M, et al. An alternatively activated macrophage marker CD163 in severely obese patients: the influence of very low-calorie diet and bariatric surgery. *Physiol Res* (2017) 66 (4):641–52. doi: 10.33549/physiolres.933522

176. Jing Y, Wu F, Li D, Yang L, Li Q, Li R. Metformin improves obesity-associated inflammation by altering macrophages polarization. *Mol Cell Endocrinol* (2018) 461:256–64. doi: 10.1016/j.mce.2017.09.025

177. Pescador N, Francisco V, Vázquez P, Esquinas EM, González-Páramos C, Valdecantos MP, et al. Metformin reduces macrophage HIF1alpha-dependent proinflammatory signaling to restore brown adipocyte function in vitro. *Redox Biol* (2021) 48:102171. doi: 10.1016/j.redox.2021.102171

178. Zheng W, Zhou J, Song S, Kong W, Xia W, Chen L, et al. Dipeptidyl-peptidase 4 inhibitor sitagliptin ameliorates hepatic insulin resistance by modulating inflammation and autophagy in ob/ob mice. *Int J Endocrinol* (2018) 2018:8309723. doi: 10.1155/2018/8309723

179. Xu L, Nagata N, Nagashimada M, Zhuge F, Ni Y, Chen G, et al. SGLT2 inhibition by empagliflozin promotes fat utilization and browning and attenuates inflammation and insulin resistance by polarizing M2 macrophages in diet-induced obese mice. *EBioMedicine* (2017) 20:137–49. doi: 10.1016/j.ebiom.2017.05.028

180. Xu L, Nagata N, Chen G, Nagashimada M, Zhuge F, Ni Y, et al. Empagliflozin reverses obesity and insulin resistance through fat browning and alternative macrophage activation in mice fed a high-fat diet. *BMJ Open Diabetes Res Care* (2019) 7(1):e000783. doi: 10.1136/bmjdrc-2019-000783

181. Turnbull IR, Gilfillan S, Cella M, Aoshi T, Miller M, Piccio L, et al. Cutting edge: TREM-2 attenuates macrophage activation. *J Immunol* (2006) 177(6):3520–4. doi: 10.4049/jimmunol.177.6.3520

182. Spite M, Claria J, Serhan CN. Resolvins, specialized proresolving lipid mediators, and their potential roles in metabolic diseases. *Cell Metab* (2014) 19 (1):21–36. doi: 10.1016/j.cmet.2013.10.006

183. Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, et al. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* (2004) 114(12):1752–61. doi: 10.1172/JCI21625

184. Halberg N, Khan T, Trujillo ME, Wernstedt-Asterholm I, Attie AD, Sherwani S, et al. Hypoxia-inducible factor 1alpha induces fibrosis and insulin resistance in white adipose tissue. *Mol Cell Biol* (2009) 29(16):4467–83. doi: 10.1128/MCB.00192-09

185. Fujisaka S, Usui I, Ikutani M, Aminuddin A, Takikawa A, Tsuneyama K, et al. Adipose tissue hypoxia induces inflammatory M1 polarity of macrophages in an HIF-1alpha-dependent and HIF-1alpha-independent manner in obese mice. *Diabetologia* (2013) 56(6):1403–12. doi: 10.1007/s00125-013-2885-1

186. Poblete JMS, Ballinger MN, Bao S, Alghothani M, Nevado JB Jr, Eubank TD, et al. Macrophage HIF-1alpha mediates obesity-related adipose tissue dysfunction via interleukin-1 receptor-associated kinase m. *Am J Physiol Endocrinol Metab* (2020) 318 (5):E689–700. doi: 10.1152/ajpendo.00174.2019

187. Paschoal VA, Amano MT, Belchior T, Magdalon J, Chimin P, Andrade ML, et al. mTORC1 inhibition with rapamycin exacerbates adipose tissue inflammation in obese mice and dissociates macrophage phenotype from function. *Immunobiology* (2017) 222(2):261–71. doi: 10.1016/j.imbio.2016.09.014

188. Festuccia WT, Pouliot P, Bakan I, Sabatini DM, Laplante M. Myeloid-specific rictor deletion induces M1 macrophage polarization and potentiates in vivo pro-inflammatory response to lipopolysaccharide. *PLoS One* (2014) 9(4):e95432. doi: 10.1371/journal.pone.0095432

189. Bryzgalova G, Gao H, Ahren B, Zierath JR, Galuska D, Steiler TL, et al. Evidence that oestrogen receptor-alpha plays an important role in the regulation of glucose homeostasis in mice: insulin sensitivity in the liver. *Diabetologia* (2006) 49 (3):588–97. doi: 10.1007/s00125-005-0105-3

190. Dong J, Zhang X, Zhang L, Bian HX, Xu N, Bao B, et al. Quercetin reduces obesity-associated ATM infiltration and inflammation in mice: a mechanism including AMPKalpha1/SIRT1. *J Lipid Res* (2014) 55(3):363–74. doi: 10.1194/jlr.M038786

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Regulatory mechanisms of macrophage polarization in adipose tissue

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In adipose tissue, macrophages are the most abundant immune cells with high heterogeneity and plasticity. Depending on environmental cues and molecular mediators, adipose tissue macrophages (ATMs) can be polarized into pro- or anti-inflammatory cells. In the state of obesity, ATMs switch from the M2 polarized state to the M1 state, which contributes to chronic inflammation, thereby promoting the pathogenic progression of obesity and other metabolic diseases. Recent studies show that multiple ATM subpopulations cluster separately from the M1 or M2 polarized state. Various factors are related to ATM polarization, including cytokines, hormones, metabolites and transcription factors. Here, we discuss our current understanding of the potential regulatory mechanisms underlying ATM polarization induced by autocrine and paracrine factors. A better understanding of how ATMs polarize may provide new therapeutic strategies for obesity-related diseases.

KEYWORDS

adipose tissue macrophages, polarization, organokines, signaling pathway, obesity

1 Introduction

Obesity has become a major public health problem because it increases the risk of many diseases (e.g., type 2 diabetes mellitus, hypertension, osteoarthritis and several cancers) (1). Obesity is characterized by accumulation of adipose tissue (AT), which leads to infiltration of immune cells and chronic low-grade inflammation (2, 3). Being a major portion of AT immune cells, adipose tissue macrophages (ATMs) are key for healthy adipose homeostasis but can also contribute to the pathogenic progression of obesity and other metabolic diseases (4). ATMs can be polarized into distinct phenotypes under different physiological or pathological conditions. In addition to the pro-inflammatory M1 phenotype and anti-inflammatory M2 phenotype (5), several novel ATM subpopulations (e.g., MMe, Mox and LAMs) have been discovered in recent years (6–8). Polarization of ATMs are regulated by organokines and related signaling pathways. In this article, we summarize the characteristics and functions of different ATM subpopulations. In addition, we also

highlight the regulatory pathways and mechanisms of ATM polarization, providing novel insights for the treatment of obesity-diseases.

2 Polarization and function of ATMs

Many studies have classified macrophages according to the M1/M2 system, which is revealed by the secretory profile of cytokines and interleukins (9). Previous studies have described a "mixed" M1/M2 phenotype for ATMs that can be activated into a switching phenotype (10, 11). ATMs are mainly polarized towards the pro-inflammatory M1 phenotype in the obesity state, conversely, in the lean state, ATMs are polarized towards the anti-inflammatory M2 phenotype (5, 12). As technologies have advanced, accumulated evidence has suggested that ATM polarization is more variable than the M1 or M2 state. Distinct ATM subpopulations express specific markers and have unique transcriptional profiles and functions (as summarized in Table 1).

2.1 Classical activated macrophages (M1)

Obese AT is recognized as a low-grade, chronic inflammation condition accompanied by accumulation of ATMs and a phenotypic switch (2, 3). Obese adipocytes secrete various chemokines and adipokines (e.g., MCP-1, CXCL12 and Leptin), which recruit and switch macrophages from the M2 state to the M1 state (5). ATMs are recognized as cells that co-express F4/80 and CD11b in mice or CD68 and CD11b in human (2, 15). Moreover, CD11c is used as phenotypic markers of M1-ATMs, which produce pro-inflammatory mediators like IL-1β, TNF-α and nitric oxide (NO), acting as main effectors of inflammatory signals, impaired adipocyte function and insulin sensitivity (13, 14). Ablation of CD11c positive cells alleviated inflammation and improved insulin sensitivity in obese mice (16). The significance of M1-ATMs is also supported in human studies where CD11c positive ATMs has been associated with glucose intolerance and metabolic syndrome (15).

TABLE 1 Adipose tissue macrophages (ATMs) subpopulations.

	M1		M2		MMe	Mox	LAM
Markers	Human	Mouse	Human	Mouse	Human/mouse	Mouse	Human/mouse
	CD68, CD11c, CD86	F4/80, CD11b, CD11c	CD68, CD206, CD163	F4/80, CD11b, CD301, CD206	ABCA1, CD36, PLIN2	HO-1, Txnrd1, Srnx-1	CD9, CD36, TREM2
Functions	Production of reactive oxygen species for bacterial killing		Promotion of preadipocyte survival, tissue healing, resolution of inflammation		Eliminate dead adipocyte debris	Response to oxidized phospholipids (OxPLs) by upregulating Nrf2-dependent antioxidant enzymes	Counteract inflammation and adipocyte hypertrophy
Secreted factors	Galectin-3, resistin; IL-1; IL-18; IL-6; TNF-α		IL-10, IL-1RA, TGF-β, Protectin		IL-6, TNF-α, IL-1β (NOX2-dependent)		
Induced by	IFN-γ, TLR4, Saturated FFA, Aldosterone, LTB4, Ceramides, Type I interferons, PAMP/DAMP, LPS		Prostaglandin D2, IL-4, Meteorin-like, Adiponectin, IL-10, IL-13, T regulatory cells, Eosinophils, FAHFA, AMPK		High levels of glucose, insulin, and palmitate	Oxidized phospholipids, Nrf2	Trem2
Metabolic effects	Promote IR, Decrease UCP1		Promote insulin sensitivity, Increase UCP1, Promote mitochondrial health			Suppression of regular energy metabolism	Preventing adipocyte hypertrophy and loss of systemic lipid homeostasis under obese conditions
Dominant polarization	Obese adipose tissue		Lean adipose tissue		Obese adipose tissue	Lean adipose tissue	Obese adipose tissue
References	(13, 14)		(13, 14)		(6)	(7)	(12)

2.2 Alternatively activated macrophages (M2)

Unlike pro-inflammatory M1-ATMs, M2-ATMs attenuate inflammation to maintain adipose homeostasis (5). In the lean state, the dominant ATMs are considered as resident macrophages which express markers of M2 macrophages (e.g., CD206, CD301 and CD163) (2, 5). M2-ATMs are further divided into three major subtypes: M2a, M2b and M2c (17), which express specific markers and have unique functions. M2a-TAMs are characterized by high surface expression of IL-R and FIZZ1, and secrete TGF-?, IGF and fibronectin to contribute to tissue repair (18, 19). M2b-TAMs express high levels of IL-10, CCL1 and TNFSF14, and low levels of IL-12, exhibiting anti-inflammatory and immune-regulated effects (20). M2c-TAMs expressing multiple markers like CD14 and TLR1, have high expression of IL-10, TGF-? and Mer receptor tyrosine kinase, and are considered as anti-inflammatory and phagocytic macrophages (17).

2.3 Metabolically activated macrophages

ATMs have a particular metabolically activated phenotype called "MMe", which exhibit a mixture of M1 and M2 characteristics. The MMe phenotype, which can be identified by their surface markers CD36, ABCA1, and PLIN2, is stimulated by high levels of glucose, insulin, and palmitate (6). MMe macrophages not only promote insulin resistance (IR) *via* producing inflammatory cytokines, but also clear away dead adipocyte through lysosomal exocytosis, which protect AT from the deleterious effects of excess free fatty acids (FFAs) (21). Therefore, MMe macrophages perform both detrimental and beneficial functions during obesity.

2.4 Oxidized macrophages

Recently, a novel macrophage phenotype has been identified, known as Mox, mainly stimulated by oxidized lipids (7). High expression of Txnrd-1, Srnx-1 and HO-1 distinguishes Mox from the M1 or M2 phenotype. Compared with M1- and M2-TAMs, Mox macrophages exhibit restricted bioenergetics and more antioxidant production. Recent study has shown that Mox macrophages are the predominant ATMs in lean AT, while more energetic macrophages like M1- or M2-TAMs predominate during the development of obesity (7).

2.5 Lipid-associated macrophages

Recently, a novel ATM subpopulation defined as lipid-associated macrophages (LAMs) were discovered surrounding apoptotic adipocytes of obese AT (8). LAMs express highly conserved genes, including CD9, CD36, and the lipid receptor Trem2. LAMs utilize Trem2 as an extracellular lipid sensor and perform protective functions to combat adipocyte inflammation, hypertrophy, and metabolic dysfunction (22). In addition, LAMs express many immunosuppression-related genes such as Lgals1/3, suggesting that they may be involved in regulating inflammatory response induced by lipid accumulation (8).

3 Organokines: Integrators of ATM polarization

Accumulating evidences suggest that AT, liver, skeletal muscle and gut function as endocrine organs, producing various organokines (adipokines, hepatokines, myokines and gut cytokines) that are capable of recruiting macrophages or switching ATM phenotypes.

3.1 Adipokines

3.1.1 Pro-inflammatory adipokines

3.1.1.1 Leptin

AT secretes a variety of hormones/cytokines, which are called adipokines. Leptin, the first classical adipokine, is initially considered as a satiety signal that regulates body weight by reducing food intake and increasing energy expenditure (23, 24). Mice with leptin deficiency (ob/ob) or leptin receptor deficiency (db/db) develop morbid obesity due to hyperphagia (23, 25). However, due to leptin resistance, most forms of obesity of animals and human are associated with higher leptin levels rather than leptin deficiency (26).

In addition to its role in energy balance, leptin also functions as an immunomodulatory cytokine, inducing immunologic alterations in different cell types, including ATMs. The immunoregulatory effects of leptin were first assessed in ob/ob mice. Macrophages from ob/ob mice have impaired phagocytic activity and pro-inflammatory cytokine production, and exogenous leptin treatment improves the above defects (27). Leptin treatment in ob/ob mice also ameliorates IR while up-regulating the expression of M2 markers (e.g., Fizz-1, Arg-1 and Mgl-1) (28). Similarly, in vitro leptin treatment, CD14 human macrophages up-regulates typical M2 markers, while being able to increase the expression of M1 markers (e.g., IL-6, IL-1?, and MCP-1) (29). These studies suggest that leptin could be a contributor to the distinct ATMs phenotype. Besides, leptin induces the expression of vascular cell adhesion molecules, which can increase macrophage infiltration into AT (30). Leptin also stimulates macrophage proliferation in a dose-dependent manner (31), meaning obesity-associated hyperleptinemia can increase the proliferation of ATMs. Hence, increased leptin levels in the obese AT are, in part, responsible for accumulation and activation of ATMs.

3.1.1.2 Resistin

The name 'resistin' is coined from the original observation that it induces IR and has been proposed to link obesity and diabetes (32). Resistin is a 12.5-kDa peptide hormone, which is

predominantly expressed in white adipocytes in rodents whereas in humans its main source is peripheral blood mononuclear cells (PBMCs) and macrophages (33). In mice, circulating resistin levels are positively correlated to obesity and IR, and resistin-treated mice or resistin-overexpressing transgenic mice exhibit glucose intolerance and IR (34). Conversely, resistin knockout or resistin neutralization with antibodies improves insulin sensitivity in diet-induced obese mice (32). However, in humans, the link between increased resistin levels and obesity/IR remains under debate and needs more epidemiological studies (34).

In obese conditions, there is an increase in adipocytes and ATMs leading to increased resistin expression. Resistin induces the expression of chemokines (e.g., CCL2 and CXCL1) as well as adhesion molecules (e.g., ICAM-1 and VCAM-1) to promote monocytes infiltration into a variety of tissues and organs including AT (35). M0 macrophages originated from monocytes can further differentiate into distinct ATM subsets, including LAMs (8), M1 and M2 macrophages (36), under varying circumstances. Resistin originated from adipose resident macrophages stimulates the expression of pro-inflammatory cytokines like IL-6, IL-12 and TNF-α (37), indicating that resistin might promote an M1-like phenotype in ATMs. Thus, resistin works by autocrine, paracrine and endocrine modes, and affects the accumulation and polarization of macrophages in AT.

3.1.1.3 WISP1

Wingless-type (Wnt)-inducible signaling pathway protein-1 (WISP1), a matricellular protein, is a novel adipokine associated with inflammation in obesity (38–40). WISP1 levels in plasma and subcutaneous AT are elevated in obese subjects and are positively correlated with systemic inflammation and IR (38). WISP1 plays a pro-inflammatory role in AT inflammation. Stimulation of macrophages with WISP1 induces the secretion of pro-inflammatory cytokines (e.g., TNF-a and IL-6) and promotes M1 macrophage polarization (38). However, in adipocytes, WISP1 neither induces cytokine expression nor affects insulin signaling (38). This suggests that WISP1 contributes to AT inflammation and IR by regulating macrophages rather than adipocytes.

3.1.2 Anti-inflammatory adipokines

3.1.2.1 Adiponectin

As the best-known and most abundant adipokine in circulation, adiponectin is exclusively secreted from adipocytes (41). Unlike pro-inflammatory adipokines, which have a positive trend in conditions of obesity, adiponectin levels are reduced in obesity and are up-regulated after weight loss (42, 43). Many evidences proved that adiponectin shows protective activity in obesity and IR (44, 45).

Adiponectin acts as anti-inflammatory factor and plays important roles in the accumulation and polarization of ATMs. Adiponectin has been reported to promote calreticulin receptor-dependent clearance of apoptotic adipocytes (46). Since apoptosis of adipocytes is a key initial event that contributes to macrophage infiltration into AT (47), the inhibition of adipocyte apoptosis using adiponectin can suppress the infiltration of ATMs. Furthermore, adiponectin induces anti-inflammatory M2 macrophage proliferation in AT by the activation of AKT signaling (48), while suppresses pro-inflammatory M1 macrophage proliferation via inhibiting NF-κB signaling (49). Additionally, adiponectin induces the M1 to M2 macrophage polarization switch in AT. Macrophages from adiponectin deficient mice display increased M1 markers and decreased M2 markers, while systemic administration of adenovirus expressing adiponectin results in an increased expression of M2 markers in AT (50). Overall, adiponectin regulates the infiltration, proliferation, and polarization of ATMs, which accounts for its anti-inflammatory properties.

3.2 Hepatokines

3.2.1 Fetuin-A

Fetuin-A is a fatty acid-binding glycoprotein that is primarily expressed in the liver (51). Circulating Fetuin-A levels are higher in human subjects with obesity, type 2 diabetes mellitus (T2DM) and nonalcoholic fatty liver disease (NAFLD) (52). In obese AT, fatty acid-binding Fetuin-A acts as an endogenous ligand of TLR4 to promote inflammation and IR (53). By binding to the extracellular domain of TLR4, Fetuin-A activates NF-κB signaling to induce pro-inflammatory cytokines release and promote M1 macrophages polarization (53, 54). Moreover, Fetuin-A also serves as a chemoattractant, inducing the infiltration of macrophages into AT (55).

3.2.2 GDF-15

Growth differentiation factor 15 (GDF-15), a distant member of the TGF-β superfamily, is highly expressed in the liver (56) and AT (57). GDF-15 has been shown to be a stress responsive cytokine associated with obesity and diabetes (58). GDF-15 exerts its known anti-inflammatory properties against obesity by regulating at least in part the activation of ATMs. GDF-15 transgenic mice fed a HFD exhibit reduced NLRP3 inflammasome activity and lower levels of macrophage infiltration into AT (59).Recently, a study suggests that GDF-15 plays a role in the polarization of ATMs. GDF-15 expression in macrophages is induced by IL-4, which promotes M2 polarization of ATMs via the upregulation of oxidative metabolism (60).

3.3 Myokines

3.3.1 Irisin/FNDC5

Irisin, a 12 kDa peptide, is a novel myokine that is cleaved from fibronectin type III domain protein 5 (FNDC5) (61). In obese subjects, circulating irisin levels are reduced and are related with insulin sensitivity (62). Irisin has been reported to ameliorate adipose inflammation *via* up-regulating anti-inflammatory cytokine (e.g., adiponectin) and down-regulating pro-inflammatory cytokine (e.g., leptin and IL-6) (63). FNDC5 overexpression attenuates adipose tissue inflammation in HFD-induced obese mice by inhibiting macrophage recruitment and M1

phenotype polarization (64).Therefore, irisin's anti-inflammatory effect in AT includes reducing production of pro-inflammatory cytokines, suppressing macrophage proliferation and infiltration, and inducing M2 macrophage polarization.

3.3.2 Myostatin

Myostatin (MSTN), also termed as growth differentiation factor 8, is primarily produced by skeletal muscle, and negatively regulates skeletal muscle mass. A recent study has reported that serum myostatin levels are positively correlated with adipose inflammation, obesity and IR (65). Inhibition of myostatin in mice suppresses HFD-induced infiltration of macrophages and reduces the expression of pro-inflammatory cytokines in AT (66). In addition, myostatin inhibition increases irisin production and induces M2 macrophage polarization in AT, thus suppressing inflammation (66).

3.4 Gut cytokines

3.4.1 GLP-1

GLP-1 is a hormone secreted by intestinal L-cells and is associated with obesity-related inflammation. GLP-1 alleviates macrophage infiltration in AT of ob/ob mice and reduces M1-polarized specific mRNA expression (67). Another study has shown that GLP-1/GLP-1R signaling in macrophages suppresses M1 polarization and triggers M2 polarization (68).In light of these findings, we speculate that GLP-1 alleviates obesity-related inflammation *via* inhibiting macrophage recruitment and promoting M2 macrophage polarization in AT.

3.4.2 Ghrelin

Ghrelin is a gastrointestinal cytokine that increases appetite and promotes obesity (69). A number of studies have described that ghrelin has strong anti-inflammatory properties, however, the effects of ghrelin in macrophages are complex. *In vitro* model, ghrelin inhibits LPS-induced production of pro-inflammatory cytokines in macrophages (70). Ghrelin's function is mediated by its receptors GHSR. *In vivo* work has demonstrated that GHSR knockout or administration of Des-acyl ghrelin (functions as ghrelin antagonist), in obese mice, reduces macrophage infiltration, promotes macrophage polarization to M2 in AT, thus suppressing adipose inflammation (71, 72). These data suggest that Ghrelin/GHSR axis can act as a pro-inflammatory mediator in AT.

3.4.3 Other organokines

Studies have demonstrated that several other organokines, including adipokines (pro-inflammatory: Chemerin, PGRN and LCN2; anti-inflammatory: Omentin, Spexin, and Sfrp5), Hepatokines (pro-inflammatory: RBP4 and DPP4; anti-inflammatory: FGF21), Myokines (pro-inflammatory: IL-6; anti-inflammatory: DEL-1) and insulin are important players in regulation of the recruitment or polarization of macrophages in AT, and thus promote inflammation associated with obesity and metabolic disease (73, 74) (Figure 1).

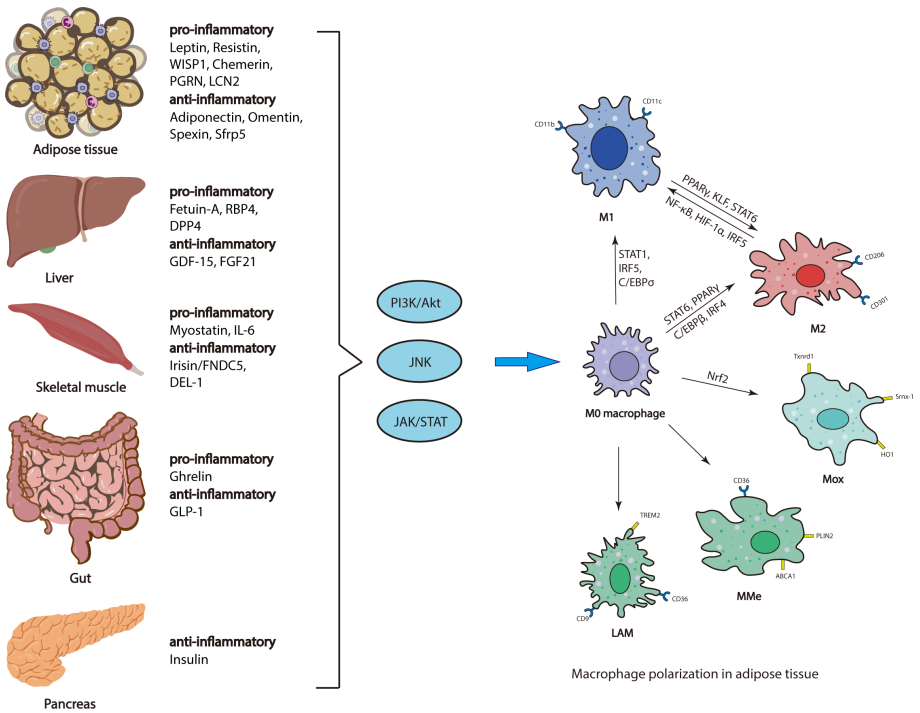

FIGURE 1
Regulatory mechanisms of ATM polarization. Adipose tissue, liver, skeletal muscle, gut and pancreas secrete various organokines, which are increased or decreased, initiating multiple downstream signaling pathways, thus affecting ATM polarization.

4 Regulatory pathways of ATM polarization

The past decades have revealed several key regulatory pathways of ATM polarization.

4.1 PI3K/Akt signaling pathway

The PI3K singling cascade is a central metabolic regulator, which is activated by metabolic stimuli including insulin, glucose, FFAs and various organokines. ATMs are exposed to increased levels of these stimuli in the obese state and adopts metabolism-dependent phenotypes. Numerous studies have implicated that PI3K/AKT singling plays an inhibitory role in TLRs-mediated inflammation and contributes to M2 macrophage polarization (75). Obesity is associated with increased circulating LPS, which initiates adipose inflammation and macrophage activation by activating TLR4 (76), suggesting PI3K/AKT singling can regulate ATM polarization. Indeed, a recent study has demonstrated that macrophage-intrinsic PI3K signaling promotes a beneficial ATM population characterized by lipid uptake (77). It is worth noting that Akt isoforms differentially contributes to macrophage polarization, with AKT1 ablation induing M1 activation and AKT2 ablation resulting in M2 phenotype activation (78). However, how individual Akt isoforms are activated by PI3Ks in the context of macrophages polarization remains to be elucidated.

4.2 C-Jun N-terminal kinase signaling pathway

Mixed-lineage kinase 3 (MLK3) deficiency attenuates JNK activation, reduces ATM accumulation and M1 activation in HFD-fed mice (79). In macrophages, PPARγ is efficiently phosphorylated by JNK (80). PPARγ knockout in murine myeloid cells induces M1 activation of ATMs, obesity and IR (81). Acute exercise improves insulin signaling in the White AT, at least in part by inducing macrophage polarization to M2 *via* down-regulating phospho-JNK (82). These studies have demonstrated that JNK signaling plays a pivotal role in both ATM accumulation and polarization.

4.3 JAK/STAT signaling pathway

IFNγ is the best known inducer of JAK-STAT signaling (83). In recent studies, deletion of either TRIM29 or TRIM18 increases the release of IFNγ that enhances inflammatory cytokine production and induces M1 macrophage polarization through activating STAT1 (84–86). SOCS proteins act as feedback inhibitors of the JAK/STAT signaling. Myeloid SOCS3 knockout mice exhibit prolonged activation of JAK/STAT signaling and increased expression of pro-inflammatory cytokines in macrophages (87). In contrast to STAT1, STAT6 promotes M2 macrophage polarization. Activation of STAT6 by IL-4 attenuates adipose inflammation by inducing proliferation of local ATMs and polarization of M2 phenotype (88).

5 Conclusion

The infiltration and polarization of macrophages in adipose tissue are beginning to be recognized as pivotal instigators of metabolic dysfunction and obesity. In response to over-nutrition, endocrine organs change metabolic phenotypes, release distinct secretome profiles, and shift adipose homeostasis into one that promotes macrophage invasion and polarization, and supports downstream chronic inflammation and IR.

Several endocrine organs are involved in regulation of ATM polarization, including adipose tissue, gut, liver, skeletal muscle and pancreas. Various organokines derived from these endocrine organs bind to their respective receptors to initiate multiple downstream signaling pathways (e.g., PI3K/AKT, JNK, and JAK/STAT), which regulate ATM polarization *via* different transcriptional regulators (e.g., PPARγ, STAT, C/EBP and IRFs) (Figure 1).

It's worth noting that the increase in the M1/M2 ratio of ATMs during obesity cannot be explained simply by the transformation from M2- to M1-phenotype macrophages but rather by the infiltration of circulating monocytes to AT followed by differentiating into M1 and M2 macrophages (36, 89). Mouse ATMs can be generated from circulating monocytes classified as $Ly6C^+$ and $Ly6C^-$ that are generally thought to preferentially differentiate into M1 and M2 macrophages, respectively (17). Human $CD14^+$ $CD16^-$ and $CD14^+$ $CD16^+$ monocytes are considered to resemble mouse $Ly6C^+$ inflammatory monocytes, while $CD14^{dim}$ $CD16^+$ monocytes resemble $Ly6C^-$ anti-inflammatory monocytes (17, 90). In human obese AT, pro-inflammatory macrophages have been described as $CD14^+CD16^+$ cells with high levels of M1 markers (91). Polarization of inflammatory monocytes has been implicated in the pathogenesis of obesity-related diseases including T2DM and atherosclerosis (92, 93), thus underlying mechanisms and approaches for resolving monocyte polarization conducive to disease regression need to be established.

Despite the great achievements made over the past few decades, a lot of questions on regulatory mechanisms of ATM polarization and its physiological and pathological functions are yet to be answered. Another limitation in the field is that current ATM-markers are ubiquitously expressed in macrophages in different tissues but not specifically in ATMs. More specific markers need to be identified, which will greatly facilitate our understanding on ATMs in health and metabolic diseases. Our understanding of the complexity of ATM subpopulations is inadequate since macrophages are highly plastic and heterogeneous cell populations. New technologies, including single-cell analysis, computational biology and bioinformatics, are being incorporated in this field and are expected to hopefully help address those challenges. With increasing understanding of regulatory mechanisms of ATM polarization, novel insights and

treatment strategies should emerge in the prevention of obesity-related diseases.

Author contributions

DP, GL and CJ drafted the original manuscript. DP and GL designed and created the figures and tables. JH and XH structured and revised the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

References

1. Saltiel AR, Olefsky JM. Inflammatory mechanisms linking obesity and metabolic disease. *J Clin Invest* (2017) 127(1):1–4. doi: 10.1172/JCI92035

2. Weisberg SP, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AWJr. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* (2003) 112(12):1796–808. doi: 10.1172/JCI200319246

3. Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, et al. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* (2003) 112(12):1821–30. doi: 10.1172/JCI200319451

4. Yao J, Wu D, Qiu Y. Adipose tissue macrophage in obesity-associated metabolic diseases. *Front Immunol* (2022) 13:977485. doi: 10.3389/fimmu.2022.977485

5. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J Clin Invest* (2007) 117(1):175–84. doi: 10.1172/JCI29881

6. Kratz M, Coats BR, Hisert KB, Hagman D, Mutskov V, Peris E, et al. Metabolic dysfunction drives a mechanistically distinct proinflammatory phenotype in adipose tissue macrophages. *Cell Metab* (2014) 20(4):614–25. doi: 10.1016/j.cmet.2014.08.010

7. Serbulea V, Upchurch CM, Schappe MS, Voigt P, DeWeese DE, Desai BN, et al. Macrophage phenotype and bioenergetics are controlled by oxidized phospholipids identified in lean and obese adipose tissue. *Proc Natl Acad Sci USA* (2018) 115(27):E6254–E6263. doi: 10.1073/pnas.1800544115

8. Jaitin DA, Adlung L, Thaiss CA, Weiner A, Li B, Descamps H, et al. Lipid-associated macrophages control metabolic homeostasis in a Trem2-dependent manner. *Cell.* (2019) 178(3):686–98.e14. doi: 10.1016/j.cell.2019.05.054

9. Gordon S, Martinez FO. Alternative activation of macrophages: mechanism and functions. *Immunity.* (2010) 32(5):593–604. doi: 10.1016/j.immuni.2010.05.007

10. Nakajima S, Koh V, Kua LF, So J, Davide L, Lim KS, et al. Accumulation of CD11c+CD163+ adipose tissue macrophages through upregulation of intracellular 11beta-HSD1 in human obesity. *J Immunol* (2016) 197(9):3735–45. doi: 10.4049/jimmunol.1600895

11. Sica A, Mantovani A. Macrophage plasticity and polarization: *in vivo* veritas. *J Clin Invest* (2012) 122(3):787–95. doi: 10.1172/JCI59643

12. Caslin HL, Bhanot M, Bolus WR, Hasty AH. Adipose tissue macrophages: unique polarization and bioenergetics in obesity. *Immunol Rev* (2020) 295(1):101–13. doi: 10.1111/imr.12853

13. Morris DL, Singer K, Lumeng CN. Adipose tissue macrophages: phenotypic plasticity and diversity in lean and obese states. *Curr Opin Clin Nutr Metab Care* (2011) 14(4):341–6. doi: 10.1097/MCO.0b013e328347970b

14. Thomas D, Apovian C. Macrophage functions in lean and obese adipose tissue. *Metabolism: Clin experimental.* (2017) 72:120–43. doi: 10.1016/j.metabol.2017.04.005

15. Wentworth JM, Naselli G, Brown WA, Doyle L, Phipson B, Smyth GK, et al. Pro-inflammatory CD11c+CD206+ adipose tissue macrophages are associated with insulin resistance in human obesity. *Diabetes.* (2010) 59(7):1648–56. doi: 10.2337/db09-0287

16. Patsouris D, Li PP, Thapar D, Chapman J, Olefsky JM, Neels JG. Ablation of CD11c-positive cells normalizes insulin sensitivity in obese insulin resistant animals. *Cell Metab* (2008) 8(4):301–9. doi: 10.1016/j.cmet.2008.08.015

17. Russo L, Lumeng CN. Properties and functions of adipose tissue macrophages in obesity. *Immunology.* (2018) 155(4):407–17. doi: 10.1111/imm.13002

18. Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. *Immunity.* (2016) 44(3):450–62. doi: 10.1016/j.immuni.2016.02.015

19. Lumeng CN, DelProposto JB, Westcott DJ, Saltiel AR. Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes. *Diabetes.* (2008) 57(12):3239–46. doi: 10.2337/db08-0872

20. Lefevre L, Gales A, Olagnier D, Bernad J, Perez L, Burcelin R, et al. PPARgamma ligands switched high fat diet-induced macrophage M2b polarization toward M2a thereby improving intestinal candida elimination. *PLoS One* (2010) 5(9):e12828. doi: 10.1371/journal.pone.0012828

21. Coats BR, Schoenfelt KQ, Barbosa-Lorenzi VC, Peris E, Cui C, Hoffman A, et al. Metabolically activated adipose tissue macrophages perform detrimental and beneficial functions during diet-induced obesity. *Cell Rep* (2017) 20(13):3149–61. doi: 10.1016/j.celrep.2017.08.096

22. Kim K, Park SE, Park JS, Choi JH. Characteristics of plaque lipid-associated macrophages and their possible roles in the pathogenesis of atherosclerosis. *Curr Opin lipidology.* (2022) 33(5):283–8. doi: 10.1097/MOL.0000000000000842

23. Ingalls AM, Dickie MM, Snell GD. Obese, a new mutation in the house mouse. *J Hered* (1950) 41(12):317–8. doi: 10.1093/oxfordjournals.jhered.a106073

24. Campfield LA, Smith FJ, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Sci (New York NY).* (1995) 269(5223):546–9. doi: 10.1126/science.7624778

25. Hummel KP, Dickie MM, Coleman DL. Diabetes, a new mutation in the mouse. *Sci (New York NY).* (1966) 153(3740):1127–8. doi: 10.1126/science.153.3740.1127

26. Friedman JM. Leptin and the endocrine control of energy balance. *Nat Metab* (2019) 1(8):754–64. doi: 10.1038/s42255-019-0095-y

27. Loffreda S, Yang SQ, Lin HZ, Karp CL, Brengman ML, Wang DJ, et al. Leptin regulates proinflammatory immune responses. *FASEB J Off Publ Fed Am Societies Exp Biol* (1998) 12(1):57–65. doi: 10.1096/fsb2fasebj.12.1.57

28. Hoffmann A, Kralisch S, Duhring S, Ebert T, Jeromin F, Kloting N, et al. Effects of leptin on macrophages in vivo. *Atherosclerosis.* (2014) 235(2):E194–E. doi: 10.1016/j.atherosclerosis.2014.05.572

29. Acedo SC, Gambero S, Cunha FG, Lorand-Metze I, Gambero A. Participation of leptin in the determination of the macrophage phenotype: an additional role in adipocyte and macrophage crosstalk. *In Vitro Cell Dev Biol Anim* (2013) 49(6):473–8. doi: 10.1007/s11626-013-9629-x

30. Wellen KE, Hotamisligil GS. Obesity-induced inflammatory changes in adipose tissue. *J Clin Invest* (2003) 112(12):1785–8. doi: 10.1172/JCI20514

31. Monteiro L, Pereira J, Palhinha L, Moraes-Vieira PMM. Leptin in the regulation of the immunometabolism of adipose tissue-macrophages. *J leukocyte Biol* (2019) 106 (3):703–16. doi: 10.1002/JLB.MR1218-478R

32. Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. *Nature.* (2001) 409(6818):307–12. doi: 10.1038/35053000

33. Patel L, Buckels AC, Kinghorn IJ, Murdock PR, Holbrook JD, Plumpton C, et al. Resistin is expressed in human macrophages and directly regulated by PPAR gamma activators. *Biochem Biophys Res Commun* (2003) 300(2):472–6. doi: 10.1016/S0006-291X(02)02841-3

34. Taouis M, Benomar Y. Is resistin the master link between inflammation and inflammation-related chronic diseases? *Mol Cell Endocrinol* (2021) 533:111341. doi: 10.1016/j.mce.2021.111341

35. Acquarone E, Monacelli F, Borghi R, Nencioni A, Odetti P. Resistin: a reappraisal. *Mech Ageing Dev* (2019) 178:46–63. doi: 10.1016/j.mad.2019.01.004

36. Fujisaka S, Usui I, Bukhari A, Ikutani M, Oya T, Kanatani Y, et al. Regulatory mechanisms for adipose tissue M1 and M2 macrophages in diet-induced obese mice. *Diabetes.* (2009) 58(11):2574–82. doi: 10.2337/db08-1475

37. Jiang CY, Wang W, Tang JX, Yuan ZR. The adipocytokine resistin stimulates the production of proinflammatory cytokines TNF-alpha and IL-6 in pancreatic acinar cells *via* NF-kappaB activation. *J Endocrinol Invest.* (2013) 36(11):986–92. doi: 10.3275/9002

38. Murahovschi V, Pivovarova O, Ilkavets I, Dmitrieva RM, Docke S, Keyhani-Nejad F, et al. WISP1 is a novel adipokine linked to inflammation in obesity. *Diabetes.* (2015) 64(3):856–66. doi: 10.2337/db14-0444

39. Tacke C, Aleksandrova K, Rehfeldt M, Murahovschi V, Markova M, Kemper M, et al. Assessment of circulating Wnt1 inducible signalling pathway protein 1 (WISP-1)/CCN4 as a novel biomarker of obesity. *J Cell Commun Signal* (2018) 12(3):539–48. doi: 10.1007/s12079-017-0427-1

40. Barchetta I, Cimini FA, Capoccia D, De Gioannis R, Porzia A, Mainiero F, et al. WISP1 is a marker of systemic and adipose tissue inflammation in dysmetabolic subjects with or without type 2 diabetes. *J Endocr Soc* (2017) 1(6):660–70. doi: 10.1210/js.2017-00108

41. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem* (1995) 270 (45):26746–9. doi: 10.1074/jbc.270.45.26746

42. Hoffstedt J, Arvidsson E, Sjolin E, Wahlen K, Arner P. Adipose tissue adiponectin production and adiponectin serum concentration in human obesity and insulin resistance. *J Clin Endocrinol Metab* (2004) 89(3):1391–6. doi: 10.1210/jc.2003-031458

43. Yang WS, Lee WJ, Funahashi T, Tanaka S, Matsuzawa Y, Chao CL, et al. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. *J Clin Endocrinol Metab* (2001) 86(8):3815–9. doi: 10.1210/jcem.86.8.7741

44. Adiyaman SC, Ozer M, Saydam BO, Akinci B. The role of adiponectin in maintaining metabolic homeostasis. *Curr Diabetes Rev* (2020) 16(2):95–103. doi: 10.2174/1573399815666190702155733

45. Kita S, Maeda N, Shimomura I. Interorgan communication by exosomes, adipose tissue, and adiponectin in metabolic syndrome. *J Clin Invest* (2019) 129 (10):4041–9. doi: 10.1172/JCI129193

46. Takemura Y, Ouchi N, Shibata R, Aprahamian T, Kirber MT, Summer RS, et al. Adiponectin modulates inflammatory reactions *via* calreticulin receptor-dependent clearance of early apoptotic bodies. *J Clin Invest* (2007) 117(2):375–86. doi: 10.1172/JCI29709

47. Alkhouri N, Gornicka A, Berk MP, Thapaliya S, Dixon LJ, Kashyap S, et al. Adipocyte apoptosis, a link between obesity, insulin resistance, and hepatic steatosis. *J Biol Chem* (2010) 285(5):3428–38. doi: 10.1074/jbc.M109.074252

48. Hui X, Gu P, Zhang J, Nie T, Pan Y, Wu D, et al. Adiponectin enhances cold-induced browning of subcutaneous adipose tissue *via* promoting M2 macrophage proliferation. *Cell Metab* (2015) 22(2):279–90. doi: 10.1016/j.cmet.2015.06.004

49. Luo Y, Liu M. Adiponectin: a versatile player of innate immunity. *J Mol Cell Biol* (2016) 8(2):120–8. doi: 10.1093/jmcb/mjw012

50. Ohashi K, Parker JL, Ouchi N, Higuchi A, Vita JA, Gokce N, et al. Adiponectin promotes macrophage polarization toward an anti-inflammatory phenotype. *J Biol Chem* (2010) 285(9):6153–60. doi: 10.1074/jbc.M109.088708

51. Denecke B, Gräber S, Schäfer C, Heiss A, Wöltje M, Jahnen-Dechent W. Tissue distribution and activity testing suggest a similar but not identical function of fetuin-b and fetuin-a. *Biochem J* (2003) 376(Pt 1):135–45. doi: 10.1042/bj20030676

52. Chung HS, Choi KM. Organokines in disease. *Adv Clin Chem* (2020) 94:261–321. doi: 10.1016/bs.acc.2019.07.012

53. Pal D, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, et al. Fetuin-a acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. *Nat Med* (2012) 18(8):1279–85. doi: 10.1038/nm.2851

54. Chattopadhyay D, Das S, Guria S, Basu S, Mukherjee S. Fetuin-a regulates adipose tissue macrophage content and activation in insulin resistant mice through MCP-1 and iNOS: involvement of IFNgamma-JAK2-STAT1 pathway. *Biochem J* (2021) 478(22):4027–43. doi: 10.1042/BCJ20210442

55. Chatterjee P, Seal S, Mukherjee S, Kundu R, Mukherjee S, Ray S, et al. Adipocyte fetuin-a contributes to macrophage migration into adipose tissue and polarization of macrophages. *J Biol Chem* (2013) 288(39):28324–30. doi: 10.1074/jbc.C113.495473

56. Adolph TE, Grabherr F, Mayr L, Grander C, Enrich B, Moschen AR, et al. Weight loss induced by bariatric surgery restricts hepatic GDF15 expression. *J Obes* (2018) 2018:7108075. doi: 10.1155/2018/7108075

57. Imbert A, Vialaneix N, Marquis J, Vion J, Charpagne A, Metairon S, et al. Network analyses reveal negative link between changes in adipose tissue GDF15 and BMI during dietary-induced weight loss. *J Clin Endocrinol Metab* (2022) 107(1):e130–e42. doi: 10.1210/clinem/dgab621

58. Keipert S, Ost M. Stress-induced FGF21 and GDF15 in obesity and obesity resistance. *Trends Endocrinol Metab* (2021) 32(11):904–15. doi: 10.1016/j.tem.2021.08.008

59. Wang X, Chrysovergis K, Kosak J, Eling TE. Lower NLRP3 inflammasome activity in NAG-1 transgenic mice is linked to a resistance to obesity and increased insulin sensitivity. *Obesity.* (2014) 22(5):1256–63. doi: 10.1002/oby.20638

60. Jung SB, Choi MJ, Ryu D, Yi HS, Lee SE, Chang JY, et al. Reduced oxidative capacity in macrophages results in systemic insulin resistance. *Nat Commun* (2018) 9 (1):1551. doi: 10.1038/s41467-018-03998-z

61. Bostrom P, Wu J, Jedrychowski MP, Korde A, Ye L, Lo JC, et al. A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature.* (2012) 481(7382):463–8. doi: 10.1038/nature10777

62. Moreno-Navarrete JM, Ortega F, Serrano M, Guerra E, Pardo G, Tinahones F, et al. Irisin is expressed and produced by human muscle and adipose tissue in association with obesity and insulin resistance. *J Clin Endocrinol Metab* (2013) 98(4): E769–78. doi: 10.1210/jc.2012-2749

63. Mazur-Bialy AI, Bilski J, Pochec E, Brzozowski T. New insight into the direct anti-inflammatory activity of a myokine irisin against proinflammatory activation of adipocytes. implication for exercise in obesity. *J Physiol Pharmacol* (2017) 68(2):243–51.

64. Xiong XQ, Geng Z, Zhou B, Zhang F, Han Y, Zhou YB, et al. FNDC5 attenuates adipose tissue inflammation and insulin resistance *via* AMPK-mediated macrophage polarization in obesity. *Metabolism: Clin experimental.* (2018) 83:31–41. doi: 10.1016/j.metabol.2018.01.013

65. Amor M, Itariu BK, Moreno-Viedma V, Keindl M, Jurets A, Prager G, et al. Serum myostatin is upregulated in obesity and correlates with insulin resistance in humans. *Exp Clin Endocrinol Diabetes.* (2019) 127(8):550–6. doi: 10.1055/a-0641-5546

66. Dong J, Dong Y, Dong Y, Chen F, Mitch WE, Zhang L. Inhibition of myostatin in mice improves insulin sensitivity *via* irisin-mediated cross talk between muscle and adipose tissues. *Int J Obes (2005).* (2016) 40(3):434–42. doi: 10.1038/ijo.2015.200

67. Lee YS, Park MS, Choung JS, Kim SS, Oh HH, Choi CS, et al. Glucagon-like peptide-1 inhibits adipose tissue macrophage infiltration and inflammation in an obese mouse model of diabetes. *Diabetologia.* (2012) 55(9):2456–68. doi: 10.1007/s00125-012-2592-3

68. Wan S, Sun H. Glucagon-like peptide-1 modulates RAW264.7 macrophage polarization by interfering with the JNK/STAT3 signaling pathway. *Exp Ther Med* (2019) 17(5):3573–9. doi: 10.3892/etm.2019.7347

69. Tschöp M, Smiley DL, Heiman ML. Ghrelin induces adiposity in rodents. *Nature.* (2000) 407(6806):908–13. doi: 10.1038/35038090

70. Waseem T, Duxbury M, Ito H, Ashley SW, Robinson MK. Exogenous ghrelin modulates release of pro-inflammatory and anti-inflammatory cytokines in LPS-stimulated macrophages through distinct signaling pathways. *Surgery.* (2008) 143 (3):334–42. doi: 10.1016/j.surg.2007.09.039

71. Yuan F, Ma J, Xiang X, Lan H, Xu Y, Zhao J, et al. Improvement of adipose macrophage polarization in high fat diet-induced obese GHSR knockout mice. *BioMed Res Int* (2018) 2018:4924325. doi: 10.1155/2018/4924325

72. Yuan F, Zhang Q, Dong H, Xiang X, Zhang W, Zhang Y, et al. Effects of des-acyl ghrelin on insulin sensitivity and macrophage polarization in adipose tissue. *J Transl Int Med* (2021) 9(2):84–97. doi: 10.2478/jtim-2021-0025

73. Mancuso P. The role of adipokines in chronic inflammation. *Immunotargets Ther* (2016) 5:47–56. doi: 10.2147/ITT.S73223

74. Ren Y, Zhao H, Yin C, Lan X, Wu L, Du X, et al. Adipokines, hepatokines and myokines: focus on their role and molecular mechanisms in adipose tissue inflammation. *Front endocrinology.* (2022) 13:873699. doi: 10.3389/fendo.2022.873699

75. Stark AK, Sriskantharajah S, Hessel EM, Okkenhaug K. PI3K inhibitors in inflammation, autoimmunity and cancer. *Curr Opin Pharmacol* (2015) 23:82–91. doi: 10.1016/j.coph.2015.05.017

76. Caesar R, Tremaroli V, Kovatcheva-Datchary P, Cani PD, Backhed F. Crosstalk between gut microbiota and dietary lipids aggravates WAT inflammation through TLR signaling. *Cell Metab* (2015) 22(4):658–68. doi: 10.1016/j.cmet.2015.07.026

77. Brunner JS, Vogel A, Lercher A, Caldera M, Korosec A, Puhringer M, et al. The PI3K pathway preserves metabolic health through MARCO-dependent lipid uptake by adipose tissue macrophages. *Nat Metab* (2020) 2(12):1427–42. doi: 10.1038/s42255-020-00311-5

78. Vergadi E, Ieronymaki E, Lyroni K, Vaporidi K, Tsatsanis C. Akt signaling pathway in macrophage activation and M1/M2 polarization. *J Immunol* (2017) 198 (3):1006–14. doi: 10.4049/jimmunol.1601515

79. Gadang V, Kohli R, Myronovych A, Hui DY, Perez-Tilve D, Jaeschke A. MLK3 promotes metabolic dysfunction induced by saturated fatty acid-enriched diet. *Am J Physiol Endocrinol Metab* (2013) 305(4):E549–56. doi: 10.1152/ajpendo.00197.2013

80. Yin R, Dong YG, Li HL. PPARgamma phosphorylation mediated by JNK MAPK: a potential role in macrophage-derived foam cell formation. *Acta Pharmacol Sin* (2006) 27(9):1146–52. doi: 10.1111/j.1745-7254.2006.00359.x

81. Odegaard JI, Ricardo-Gonzalez RR, Goforth MH, Morel CR, Subramanian V, Mukundan L, et al. Macrophage-specific PPARgamma controls alternative activation and improves insulin resistance. *Nature*. (2007) 447(7148):1116–20. doi: 10.1038/nature05894

82. Oliveira AG, Araujo TG, Carvalho BM, Guadagnini D, Rocha GZ, Bagarolli RA, et al. Acute exercise induces a phenotypic switch in adipose tissue macrophage polarization in diet-induced obese rats. *Obesity*. (2013) 21(12):2545–56. doi: 10.1002/oby.20402

83. Stark GR, Darnell JEJr. The JAK-STAT pathway at twenty. *Immunity*. (2012) 36 (4):503–14. doi: 10.1016/j.immuni.2012.03.013

84. Xing J, Weng L, Yuan B, Wang Z, Jia L, Jin R, et al. Identification of a role for TRIM29 in the control of innate immunity in the respiratory tract. *Nat Immunol* (2016) 17(12):1373–80. doi: 10.1038/ni.3580

85. Fang M, Zhang A, Du Y, Lu W, Wang J, Minze LJ, et al. TRIM18 is a critical regulator of viral myocarditis and organ inflammation. *J BioMed Sci* (2022) 29(1):55. doi: 10.1186/s12929-022-00840-z

86. Hu X, Chen J, Wang L, Ivashkiv LB. Crosstalk among jak-STAT, toll-like receptor, and ITAM-dependent pathways in macrophage activation. *J leukocyte Biol* (2007) 82(2):237–43. doi: 10.1189/jlb.1206763

87. Qin H, Holdbrooks AT, Liu Y, Reynolds SL, Yanagisawa LL, Benveniste EN. SOCS3 deficiency promotes M1 macrophage polarization and inflammation. *J Immunol* (2012) 189(7):3439–48. doi: 10.4049/jimmunol.1201168

88. Zheng C, Yang Q, Xu C, Shou P, Cao J, Jiang M, et al. CD11b regulates obesity-induced insulin resistance *via* limiting alternative activation and proliferation of adipose tissue macrophages. *Proc Natl Acad Sci USA* (2015) 112(52):E7239–48. doi: 10.1073/pnas.1500396113

89. Kanda H, Tateya S, Tamori Y, Kotani K, Hiasa K, Kitazawa R, et al. MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. *J Clin Invest* (2006) 116(6):1494–505. doi: 10.1172/JCI26498

90. Geng S, Zhang Y, Yi Z, Lu R, Li L. Resolving monocytes generated through TRAM deletion attenuate atherosclerosis. *JCI Insight* (2021) 6(20). doi: 10.1172/jci.insight.149651

91. Poledne R, Malinska H, Kubatova H, Fronek J, Thieme F, Kauerova S, et al. Polarization of macrophages in human adipose tissue is related to the fatty acid spectrum in membrane phospholipids. *Nutrients* (2019) 12(1). doi: 10.3390/nu12010008

92. Liang W, Qi Y, Yi H, Mao C, Meng Q, Wang H, et al. The roles of adipose tissue macrophages in human disease. *Front Immunol* (2022) 13:908749. doi: 10.3389/fimmu.2022.908749

93. Geng S, Chen K, Yuan R, Peng L, Maitra U, Diao N, et al. The persistence of low-grade inflammatory monocytes contributes to aggravated atherosclerosis. *Nat Commun* (2016) 7:13436. doi: 10.1038/ncomms13436

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