Diabetology International
https://doi.org/10.1007/s13340-023-00624-2

MINI-REVIEW

Heterogeneity of adipose tissue-resident macrophages-beyond M1/M2 paradigm

Allah Nawaz[1,2] · Shiho Fujisaka[1] · Tomonobu Kado[1] · Ishtiaq Jeelani[3] · Kazuyuki Tobe[1]

Received: 11 January 2023 / Accepted: 17 March 2023

Abstract
Adipose tissue-resident macrophages (ATMs) are reported to be important for maintaining adipose tissue remodeling and homeostasis. ATMs were classified for the first time in 2007 into the M1 and M2 types. This theory suggests that in the non-obese adipose tissue, the anti-inflammatory, alternatively activated macrophages (AAMs) predominate, and regulate tissue homeostasis, remodeling, and insulin sensitivity. On the other hand, classically activated M1-type macrophages increase rapidly in obesity, secrete inflammatory cytokines, such as TNFα and IL-6, and induce insulin resistance. In recent years, experimental findings that cannot be explained by this theory have been clarified one after another and the theory is being reconsidered. In this review, based on recent findings, we summarize reports on the novel metabolic regulatory functions of ATMs beyond the M1/M2 paradigm.

Keywords M2 macrophage · Preadipocytes · Adipose tissue · Insulin resistance

What is the M1/M2-type macrophage theory?

Macrophages are present throughout the body and play integral roles in homeostasis and metabolic adaptation. Fifteen years have elapsed since Lumeng et al. proposed the "phenotypic switching theory" [1]. They categorized macrophages into the M1 and M2 types according to their surface markers. In the lean state, the majority of macrophages in the adipose tissue are of the anti-inflammatory M2-type (Fig. 1), while in the obese state, the majority of macrophages in the adipose tissue are of the proinflammatory M1-type macrophages (Fig. 2). M2-type macrophages are important for maintaining tissue homeostasis and remodeling (Fig. 1). M2-type macrophages are also involved in communication and crosstalk with other cells in the adipose tissue, including endothelial cells, preadipocytes, adipocyte progenitors, and eosinophils [2–5]. M2-type macrophages are also known to secrete Tgf-β and collagen-producing factors, thereby promoting fibrosis (Fig. 1d) [6]. Phenotypic transition of the M2-type to M1-type macrophages in the obese state contributes to insulin resistance through increased secretion of inflammatory cytokines such as TNFα and IL-6 (Fig. 2). Consistent with the above theory, other studies have shown that not only in the adipose tissue but also in other tissues, macrophages can be classified into the M1 and M2 types [7, 8]. In recent years, experimental findings that cannot be explained by this theory have been reported one after another, and the theory is being reconsidered.

Do M2-ATMs play anti-inflammatory roles?

Do all M2-ATMs, as good ATMs, exert anti-inflammatory functions? No; for example, in a transgenic mouse model of conditional depletion of M2-ATMs, no significant alterations in the expressions of inflammatory cytokines were observed in the adipose tissue of lean mice, while in obese

✉ Allah Nawaz
allah.nawaz@joslin.harvard.edu

✉ Kazuyuki Tobe
tobe@med.u-toyama.ac.jp

[1] First Department of Internal Medicine, Faculty of Medicine, University of Toyama, Toyama-Shi, Toyama 930-0194, Japan

[2] Section of Integrative Physiology and Metabolism, Joslin Diabetes Center, Harvard Medical School, Boston, MA 02215, USA

[3] Department of Medicine, Division of Endocrinology and Metabolism, University of California, San Diego, CA, USA

Published online: 03 April 2023

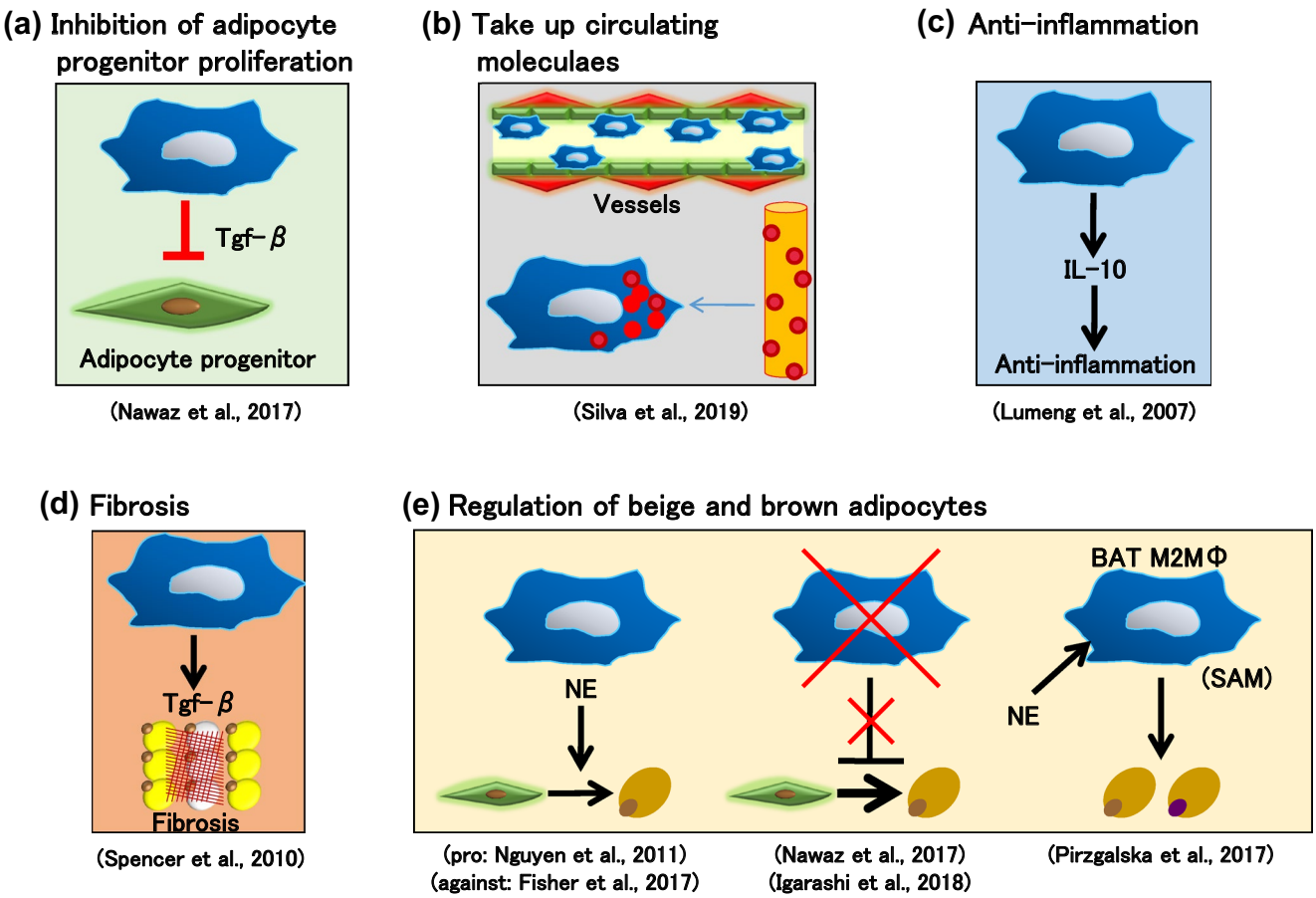

Fig. 1 M2 macrophages in lean adipose tissues. M2-ATMs inhibit adipocyte progenitor proliferation/differentiation through Tgf-β (**a**), take up circulating macromolecules (**b**), secrete anti-inflammatory cytokines, including IL-10 (**c**), and promote fibrosis (**d**). M2-ATMs are also reported to be associated with beiging and browning of white adipocytes (**e**), but their mechanisms are a matter of controversy

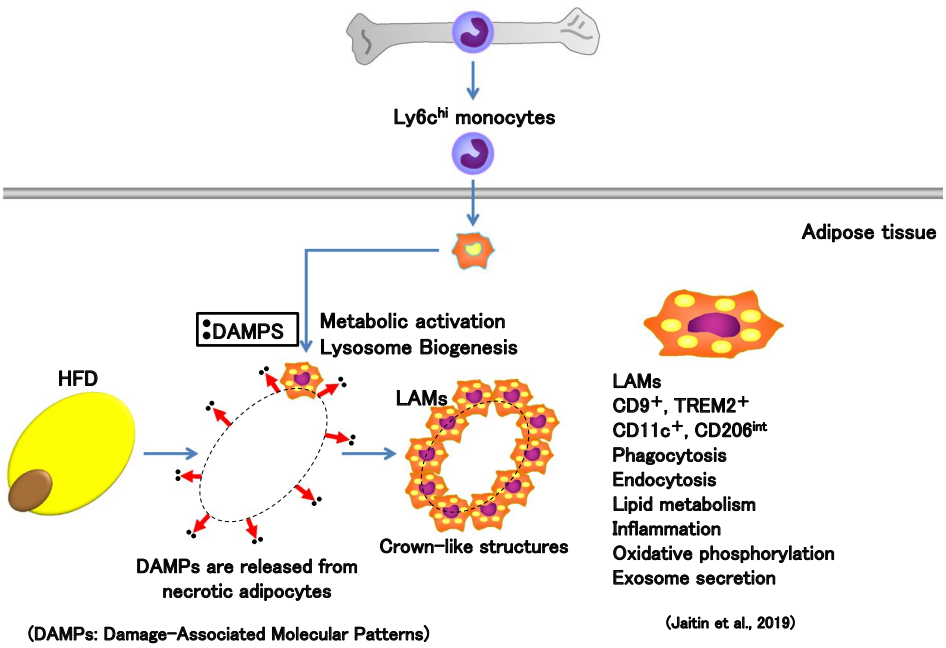

Fig. 2 M2 macrophages in obese adipose tissues. Obesity induces the recruitment of circulating Ly6c + monocytes to adipose tissue, and they differentiate into macrophages that regulate metabolism and lysosomal biogenesis. Adipocyte cell death that occurs in obese adipose tissue may result in the release of damage-associated molecular patterns (DAMPs) that induce proinflammatory responses in immune cells, and these ATMs that are present around dead adipocytes form crown-like structures (CLSs). Lipid-associated macrophages (LAMs) are also a major constituent of ATMs that are involved in disposing lipids released by dead adipocytes

mice, inflammation was attenuated [4]. Contrary to the findings reported by others, we and Lumeng group showed that depletion of M2-ATMs results in improved insulin sensitivity [4, 9]. At the same time, we also found that depletion of M2-ATMs results in increased proliferation of preadipocytes (Adipocyte Progenitors: APs) and increased numbers of small adipocytes (Fig. 3a, b). Furthermore, these effects were considered as being attributable to M2-ATM-derived Tgf-β1, as shown in CD206-CreER^{T2}; Tgf-β1 floxed mice, suggesting that M2-ATMs provide a microenvironment for preadipocytes/APs and regulate their number and quality via TGF-β signaling. Another report also showed that mice lacking in the IL-10 gene are less prone to obesity, and in fact, showed improved glucose tolerance under the high-fat diet (HFD) condition (Fig. 1c) [10]. These IL10$^{-/-}$ mice showed enhanced energy expenditure and thermogenesis, indicating

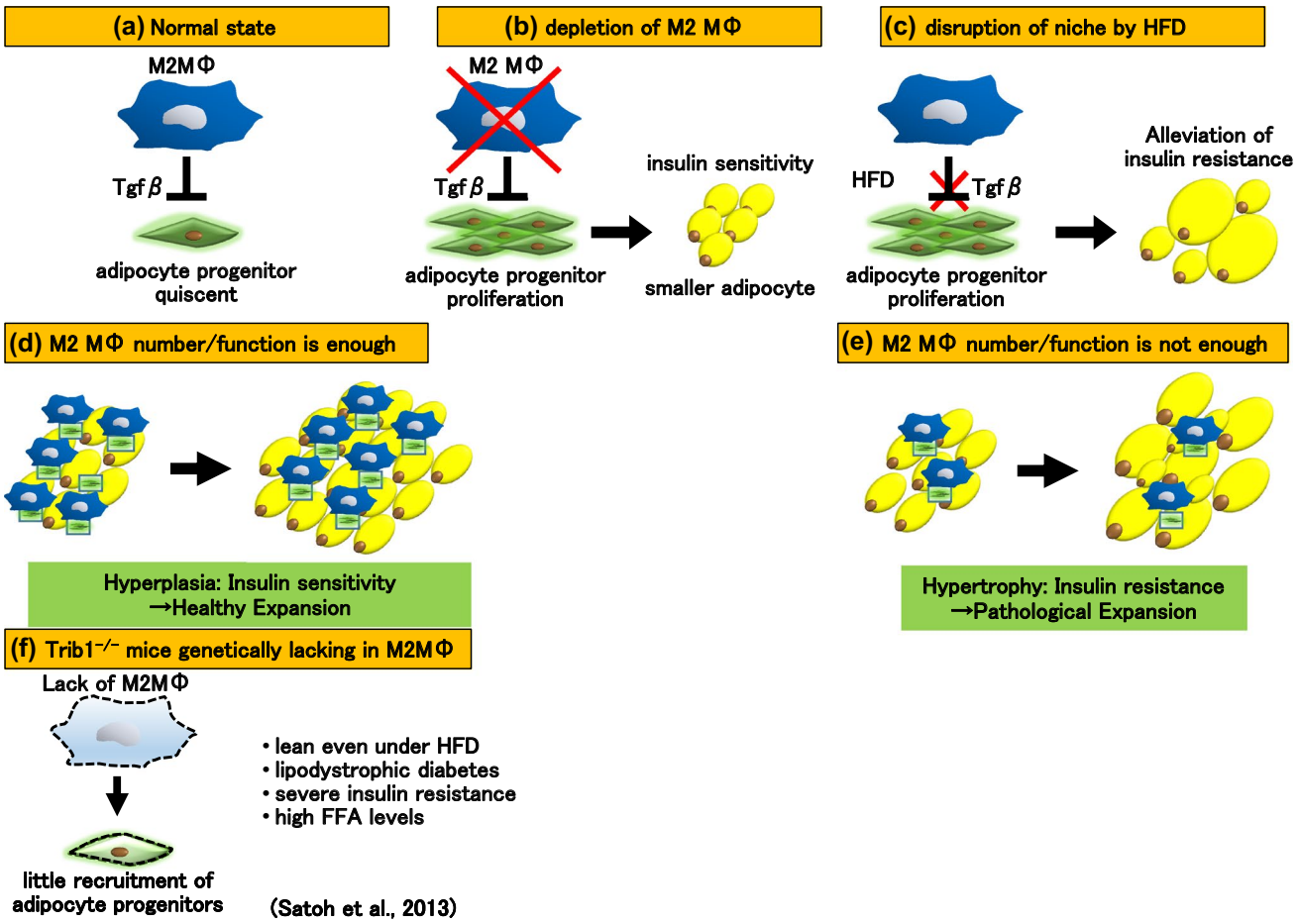

Fig. 3 M2 macrophages serve as a niche for preadipocytes that contributes to maintaining adipose tissue homeostasis. **a** On a normal chow diet, M2 macrophages form a niche with preadipocytes via TGFβ thus keeping preadipocytes in a quiescent state, avoiding unnecessary division and preventing cell senescence and their exhaustion to maintain their pool. **b** Depletion of M2 macrophages release their inhibitory effect of proliferation and differentiation of preadipocytes. As preadipocytes are constantly receiving growth and differentiation signals from blood circulation, they begin to proliferate and differentiate to generate small matured adipocytes, thus inducing insulin sensitivity. **c** When animals are put on a high-fat diet, signals of free fatty acids from the blood circulation might stimulate PPARγ in the preadipocytes and reduce the expression of molecules involved in M2 macrophage-preadipocyte interaction and the niche structure is disrupted. As a result, preadipocytes are released from the niche and begin to proliferate and differentiate to generate matured adipocytes and exacerbation of insulin resistance is at least in part alleviated. **d** The following explanation is based on the assumption that the number of M2 macrophages and that of preadipocytes are almost parallel. When the number of M2 macrophages is large, there may be more preadipocytes, and more adipocytes are generated even under high-fat diet, making it less likely to become insulin resistant (hyperplasia: healthy expansion). **e** On the other hand, when the number of M2 macrophages is not enough, there are less preadipocytes and less matured adipocytes generated under a high-fat diet, then existing adipocytes become larger, leading to inflammation and insulin resistance (hypertrophy: pathological expansion). Similarly, when the function of M2 macrophages is impaired, or the number of preadipocytes regulated by M2 macrophages is decreased, these result in reduced adipogenesis and insulin resistance under a high-fat diet. **f** The reason why Trib1-deficient mice lacking M2 macrophages in adipose tissue exhibit a lipodystrophic diabetes phenotype is that there may be only a small number of preadipocytes recruited in this mouse adipose tissue because of the lack of M2 macrophages, resulting in little adipogenesis occurred [28]

that M2-ATM-derived IL-10 suppresses the expressions of genes related to thermogenesis in mature adipocytes via adipocyte IL-10 receptor α, exacerbating obesity and glucose tolerance.

In contrast, alternatively activated macrophages/M2-ATMs, reported as a major source of catecholamine or norepinephrine (NE), contribute to non-shivering thermogenesis and to the browning of white adipose tissue (Fig. 1e) [11, 12]. In contrast, another report indicated that M2-ATMs do not produce sufficient NE and do not promote the browning of white adipose tissue [13]; rather, depletion of M2-ATMs promotes the browning of white adipose tissue [3, 4]. Pirzgalska et al. found a subset of ATMs that was closely associated with sympathetic neurons, which contain catecholamine, not by biosynthesis but via uptake. They called this subset of ATMs "sympathetic neuron-associated macrophages (SAMs)" (Fig. 1e) [14]. They and others also showed that the SAMs are involved in the functions of brown adipose tissue or in the beiging of white adipocytes [14–17]. ATMs are required for the metabolic adaptation of adipose tissue, which occurs in a complex manner, and greater caution is needed in defining the functions of macrophages under different stimuli or in different experimental settings. These reports raise the question of whether to revisit this classification or the "Phenotypic switch theory" of macrophages in the adipose tissue.

What are the types of ATMs that exist in lean adipose tissue?

Adipose tissue of lean mice contains a large population of macrophages (MΦs) called M2-ATMs that express genes encoding *CD206, Lyve1, Mgl1, IL-10, CD163*, etc. Recent reports have referred to this population as vascular-associated macrophages (VAMs) [18], and perivascular macrophages (PVM) (Fig. 1b) [19]. Silva et al. categorized ATMs into four subtypes according to the expression of CD206, namely, VAM1, VAM2, PreVAMs, and $CD11c^{+}CD64^{+}$ double-positive (DP) ATMs [18]. They called the $CD206^{hi}$ subpopulation as VAM, because this population was found to surround blood vessels. VAM was further classified into VAM1 ($MHCII^{hi}Tim4^{int}$) and VAM2 ($Tim4^{hi}MHCII^{int}$) according to the expression of the gene encoding Tim4 (T cell immunoglobulin- and mucin-domain containing molecule-4). $Tim4^{+}$ macrophages correspond to previously known long-lived embryonic macrophages [20]. VAM, especially VAM2, shows abundant expressions of genes involved in anti-inflammatory functions and pathways related to insulin sensitivity. They also express genes involved in tissue repair, detoxifying functions, endocytosis, and micropinocytosis. In fact, they have a rapid endocytic capacity of diverse macromolecules present in the bloodstream. VAM, especially VAM2, do not originate from the bone marrow (BM), but rather represents self-renewed non-bone marrow, embryonically derived macrophages in the adipose tissue. On the other hand, subtypes with intermediate/low CD206 expression levels are classified into $CD64^{+}CD11c^{-}$ preVAM and $CD11c^{+}CD64^{+}$ DP macrophages. RNA-seq analyses further revealed that the VAM1, VAM2, and preVAM clusters are located near each other and share several transcriptional factors. On the other hand, the $CD11c^{+}CD64^{+}$ DP macrophages share transcriptional factors with monocytes.

Ontogeny

For a long time, macrophages were thought to be derived from circulating monocytes [21]. Recent studies have revealed that resident macrophages in most tissues are not derived from circulating monocytes, but are of embryonic origin and are maintained by self-renewal [22–25]. In some tissues, blood monocytes are recruited and differentiate into macrophages as the tissue grows after birth. As in other tissues, adipose tissue also contains a mixed population of macrophages, namely, both embryonically-derived and BM-derived macrophages. In adipose tissue, Félix et al. used single-cell proteomics to show that the ATMs gradually transition from fetal-derived macrophages to monocyte-derived macrophages after birth [26]. At 5 weeks of age, $CD206^{+}Tim4^{+}$ cells are predominant, while at 23 weeks of age, this subpopulation is significantly decreased and instead, the population of monocyte-derived $CD206^{+}$ $Tim4^{-}$ cells is increased. On the other hand, $CD206^{-}CD11c^{+}$ macrophages, which are only seen in small numbers at 5 weeks of age, increase in number by 23 weeks of age. To confirm the proliferation of $F4/80^{hi}Tim4^{+}$ ATMs in the eWAT of obese mice, the results of a proliferation assay showed that $F4/80^{hi}Tim4^{+}$ ATMs had higher proliferating potential than $F4/80^{hi}Tim4^{+}$ ATMs from lean mice, suggesting that obesity induces the proliferation of resident ATMs.

Single-cell RNA-sequencing (scRNA-seq) analyses of white adipose tissue allowed further characterization of ATMs into different clusters based on the expression of *Tim4* [27]. Both $F4/80^{hi}Tim4^{+}$ and $F4/80^{hi}Tim4^{-}$ populations show high expression levels of CD206. The former was considered as being embryonically derived ATMs that survived by self-renewal, and the latter are thought to represent a subpopulation derived from circulating monocytes, and differentiated into M2-ATMs. HFD stimulation significantly increases the number and proliferation of $F4/80^{hi}Tim4^{+}$ populations, indicating that these ATMs undergo self-renewal in the obese state.

Role of M2-ATMs in maintaining insulin sensitivity in the lean and obese states

Decrease in the number of M2-ATMs through deletion of the *Trib1* gene whose product is involved in proteolysis, causes lipodystrophic diabetes with increased lipolysis [28]. In these mice, M2-ATMs were markedly decreased in the adipose tissue. This report suggests that M2-ATMs somehow regulate adipocyte differentiation and lipogenesis pathways. We recently reported that M2-ATMs in the lean state provide a niche for adipocyte progenitors in a Tgf-β-dependent way, which helps to maintain the stemness of the progenitors by avoiding unnecessary division and cell senescence, and preventing exhaustion of adipocyte progenitors (Fig. 1a, and Fig. 3a) [4, 29]. From these two reports, we presumed that M2-ATMs may be involved in the recruitment of adipocyte progenitors and/or maintenance of their pool in a healthy state. This might be the reason for lipodystrophic phenotype in *Trib1* gene-deficient mice lacking in M2-ATMs. Thus, the population size of M2-ATMs is closely associated with the size of the adipocyte progenitor pool, which might be the reason why a larger number and higher quality of M2-ATMs are essential for the maintenance of adipose tissue health even under the HFD condition.

How do M2-ATMs maintain insulin sensitivity under the HFD condition?

Several reports show that the M2-ATMs found in adipose tissue are necessary for maintaining the health of the adipose tissue and also for maintaining insulin sensitivity (Figs. 1 and 3) [30–32]. In fact, mice lacking in transcriptional factors, signaling molecules that are necessary for differentiation of M2-ATMs, including PPARγ, PPARδ, KLF4, Irs2, and IL-4, are prone to obesity and insulin resistance under the HFD condition [2, 31–37]. These reports also support our notion that M2-ATMs are involved in the healthy expansion of adipose tissue under an energy excess state by recruiting adipocyte progenitors and maintaining their pool enough to counteract the energy excess to keep adipocyte smaller and less inflammatory, thus making the body insulin sensitive. Lack of eosinophils, the predominant IL-4-secreting cells in the WAT, is associated with reduced differentiation of ATMs into M2-ATMs and promotion of obesity-induced insulin resistance [2, 33]. Cox et al. highlighted the importance of embryonically-derived macrophages in protecting against obesity [38]. When they deleted the PDGFcc gene in macrophage progenitors in the embryonic stage in mice, they found that the mice had less brown adipose tissue thermogenesis and were more prone to obesity and insulin resistance. These data are consistent with our data that M2-ATMs provide a niche not only for white adipocyte progenitors but also for beige adipocyte progenitors (Fig. 1e).

How are ATMs remodeled in obesity?

Obesity induces a dramatic increase in the proportion of ATMs, which reaches around 40%-50% of stromal cells in both mice [39] and humans [40]. Although this increase is mainly attributable to monocyte-derived ATMs, local proliferation of tissue-resident M2-ATMs is also reported to contribute, at least in part, to this increase in the population of ATMs. Recent scRNA-seq studies further revealed that these ATMs are remodeled toward lipid-associated macrophages (LAMs) [41], rather than converting to classically activated M1 macrophages as originally proposed by Lumeng et al. [1]. Kratz et al., used the terminology metabolically activated macrophages (MMe), where they stimulated macrophages in vitro with insulin, glucose, and palmitate and found that these stimulated macrophages express PPARγ, ABCA1, CD36, and PLIN, but not M1 markers nor M2-markers (Fig. 2). They called these unique type macrophage population as MMe. These phenotypes of macrophage were mediated by two independent mechanisms: (1) palmitate binding to cell surface TLRs that drives pro-inflammatory cytokine production and (2) palmitate internalization by activating p62 and PPARγ, thereby promoting lipid metabolism and reducing inflammation. Metabolic stimuli (palmitate) promote inflammation independent of type I interferon response and this approach can be useful for disease-specific conditions to define diagnostic surface markers. Remodeling of macrophages by fatty acid/palmitate simulation plays an important role in obesity and other metabolic disorders. Their report provides evidence that the metabolic adaptation of macrophages to FFA uptake can be affected by factors that influences FFA amounts including WAT mass, adipocyte size, adipocyte progenitors, and insulin-resistant adipocytes, altogether contributing to metabolic dysfunction in patients with type 2 diabetes. Remodeling of ATMs towards LAM-like cells in obesity is essentially similar to the MMe [42] or lysosome-related lipid handling macrophages [43], as described previously, suggesting that LAMs have lipid handling properties distinct from conventional M1 or M2 macrophages.

In 2010, a subset of $CD11c^{+}CD206^{+}$ DP cells was identified in the subcutaneous adipose tissue of obese women [44]. This subset of macrophages was rich in intracellular lipids and resided around crown-like structures (CLS). They also reported the existence of a correlation between the ratio of

$CD11c^+CD206^+$ and $CD11c^-$ ATMs and insulin resistance. They further demonstrated that the $CD11c^+CD206^+$ DP subset of macrophages has both pro- and anti-inflammatory properties as well as high mitochondrial numbers. Shaul et al. reported that CLS-associated macrophages expressing MGL1 and CD11c constituted the majority of $CD11c^+$ macrophages in obese mice [45]. Consistent with this, Silva et al., also showed that the $CD206^{int/lo}CD11c^+CD64^+$ DP population of macrophages was markedly increased in mice with diet-induced obesity (DIO) [18]. $CD11c^+CD206^+$ DP has been considered to be a macrophage subpopulation corresponding to the inflammatory $CD11c^+$ macrophages reported so far [1, 46]. $CD11c^+CD206^+$ DP macrophages are derived from circulating monocytes, unlike VAM2 and VAM1. The $CD11c^+CD206^+$ DP macrophage population is a mixed phenotype that shows high expressions of genes encoding anti-inflammatory cytokine markers, including *CD206, IL-10, TGF-β1*, and *IL-1 receptor antagonist*, as well as of genes encoding pro-inflammatory cytokines, including *Tnf, Cxcl1,* and *IL-6*. Sárvári et al. reported that in obesity, the relative numbers of LAMs are increased and also, the expressions of genes involved in lipid handling (e.g., *Ppapγ, Lpl,* and *Dpep2*) are induced [19]. These LAMs have been demonstrated to be involved in the clearance of dead adipocytes and lipids [41, 43, 47, 48]. These data suggest that HFD-induced obesity leads to decreased expressions of canonical and subpopulation-specific macrophage genes and increased expressions of genes involved in lipid storage. These findings are consistent with the MMe theory [42], and do not support the notion that obesity induces the phenotypic transition of macrophages from the M2-type to M1-type macrophages. scRNA-seq recently identified at least three subsets of macrophages, including LAMs, in the adipose tissue in obesity [41, 47, 49–53] (Fig. 2). Hildreth et al. [51] analyzed the human adipose tissue and found that the macrophages in the tissue could be separated into three subsets: One subset was very similar to the LAMs, showing high expression levels of genes encoding *TREM2, CD9,* and *LPL*. Another subset was PVM, which showed high expression levels of genes encoding C1Q, LYVE1, and SELENOP. The last subset was IM, which showed high expression levels of genes encoding *CCL3L1, TNF,* and *CXCL3*. Both the LAMs and IM showed markedly elevated expressions of genes encoding IL-1β and TNF in proportion to the BMI. On the other hand, the PVM showed no expression of genes encoding IL-1β or TNF. Obesity was associated with increases in the populations of LAMs and IM, and a decrease in the population of PVM. Consistent with this, another study also identified three subsets of ATMs in human adipose tissue [53]. Hill et al. [46] also identified three macrophage populations such as $Ly6c^+$, $Ly6c^-CD9^+$, and $Ly6c^-CD9^-$, by flow cytometry and scRNA-seq in the eWAT of obese mice. $Ly6c^-CD9^+$ population was characterized by high expression levels of genes involved in lipid metabolism (*Cd9, Lpl and Plin2*), vesicle functions (*Vamp4*), lysosomal functions (*Cd63, Cts6, Ctss, Lamp1, and Lamp2*), and inflammation (*Ccl2, IL-1a, IL-18, and TNF*). On the other hand, $Ly6c^-CD9^-$ population showed high expressions of genes encoding typical M2 macrophage markers, such as *Mrc1* and *Mgl2*. Furthermore, they showed that the $Ly6c^-CD9^+$ ATMs were present in the CLS and stored lipids, and secreted exosomal vesicles.

Jaitin et al., also identified 3 subsets (Mac1, Mac2, Mac3) of ATMs by scRNA-seq analysis of eWAT in the lean and obese state [41] (Fig. 2). Mac1, the dominant subset of ATMs in the lean state, expressed *Retnla, Lyve1, Cd209f, Cd163*, etc., and are considered as the M2-ATMs. Obesity was associated with a dramatic increase in the populations of the Mac2 and Mac3 subsets of ATMs. The Mac3 subset, which they termed as LAMs, showed transcriptional signatures of *Trem2, Lgal3, Cd9, and Cd36*, which are known to be involved in lipid metabolism and phagocytosis. This subset of cells showed abundant expressions of genes involved in a pathway initiated by phago- and endocytosis, coupled with lipid metabolism and oxidative phosphorylation. The LAMs shared several properties with the previously described $CD11c^+CD206^+$ macrophages [44, 45], MMe [42], lysosomal-related lipid handling macrophages [43], and $Ly6c^-CD9^+$ ATMs[47] (Fig. 3).

Altogether, the LAMs or ATMs play critical roles in adipose tissue metabolism in both health and disease. However, careful attention needs to be paid to the molecular signatures of subsets of ATMs under different stimuli or in states of health and disease. If we look ATMs beyond the M1/M2 paradigm, they are central players not only in maintaining or preserving metabolic processes in the adipose tissue but are also essential for various cellular and molecular processes in other tissues [3–5, 29, 46, 53, 54]. As macrophages are present in all tissues, their functions or kinetics should be clearly defined. Macrophages or ATMs could serve as therapeutic targets for various diseases, and future research is warranted to clarify their local and systemic balance.

Macrophage-derived extracellular vesicles (sEV)/exosomes

ATMs play important roles in the regulation of adipose tissue metabolism in various ways. As discussed above, ATM-derived cytokines/transcriptional factors are essential for the maintenance of adipose tissue homeostasis, remodeling, tissue integrity, cellular metabolism, and systemic insulin sensitivity. Apart from the M1/M2 paradigm, recent reports show that ATMs also secrete sEV/exosomes and that these exosomes carry miRNAs that are also essential for adiposity and glucose metabolism [55–58]. These reports showed that

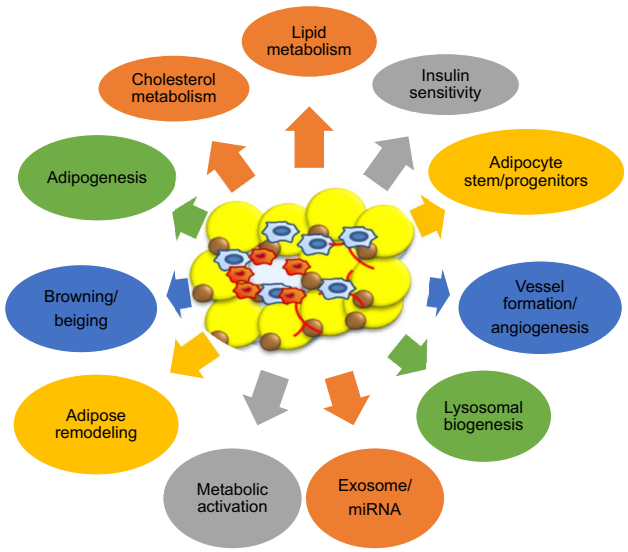

Fig. 4 Overview of the different functions of ATMs. ATMs not only contribute to maintaining the adipose tissue microenvironment, but also regulate several metabolic processes, including exosomal biogenesis, miRNA secretion, lipid metabolism, insulin sensitivity, lysosomal biogenesis, and energy expenditure

ATM-derived miR-155 and M2-ATM-derived miR-690 regulate inflammation and insulin sensitivity in the obese state. M2-ATMs communicate with other cells in adipose tissue or other tissues by secreting exosomes/miRNA, transcriptional factors, metabolites, cytokines, chemokines, and other mediators. Considering all facts, ATMs are important cells within adipose tissue that maintain adiposity, adipose tissue homeostasis, remodeling, and obesity-induced inflammation.

Summary

ATMs are a highly heterogeneous population in adipose tissue. While they are integral to maintaining the health of adipose tissue, they also play a major role in inducing metabolic stress or disease (Figs. 1, 2, 3). They can sense danger/stress and enable adipose tissue to adapt to changes in the environment and help in maintaining adipose tissue homeostasis through the secretion of cytokines, growth factors, transcriptional factors, sEV/exosomes, and miRNA, as shown in Fig. 4. They are also important for organ cross talk and play roles in regulating metabolism in other tissues, including the liver, muscle, etc. In addition, they acquire tissue-specific signals and their gene expression profiles show rich heterogeneity. Since this review does not focus on inflammatory aspects of M1 macrophages, please see the other excellent reviews on this topic [59, 60]. In our opinion, further research is warranted to clarify the roles of ATMs in the development of metabolic disorders and also for simplifying the classification of these cells into various types and subtypes. A stronger consensus is required to simplify the classification of ATMs.

Acknowledgements This work was supported by Japan Society for the Promotion of Science (JSPS) KAKENHI (20K08882 to S.F., 19K17978 and 22K08647 to T.K, 20K22733 and 22K16408 to A. N., 20H03730 to K.T.), Grants-in-Aid from the Japan Society for the Promotion of Science (JSPS) Fellow (to A. N.), Tamura Science and Technology Foundation (to K.T. and S.F.), Japan Agency for Medical Research and Development Frontier Outstanding Research for Clinical Empowerment (AMED-FORCE) (22gm4010014h0002 to K.T.), Japan Diabetes Foundation (to S.F.), the Japan Foundation for Applied Enzymology (to T.K. and A.N.), Young Research Grant from the Japan Diabetes Society (to A. N.), Cell Science Research Foundation (to A.N.) and Manpei Suzuki Diabetes Foundation fellowship (to A.N).

Author contributions AN and KT wrote the manuscript. SF, IJ and TK helped in the literature search and manuscript writing. All the authors approved the final version of the paper for publication.

Data availability This article is an overview of macrophages and their metabolic functions, no data supports the contents of this article.

Declarations

Conflict of interest K.T. received honoraria for lectures from MSD K.K. and scholarship grants from Novo Nordisk Pharma Ltd., Takeda Pharmaceutical Company., Daiichi Sankyo Company, Ltd., Mitsubishi Tanabe Pharma Corporation, Asahi Kasei Pharma Corporation, Taisho Pharmaceutical Co., Ltd., Japan Diabetes Foundation and Japan Association for Diabetes Education and Care.

References

1. Lumeng CN, Bodzin JL, Saltiel AR. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. J Clin Invest. 2007;117(1):175–84. https://doi.org/10.1172/JCI29881.
2. Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, et al. Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis. Science. 2011;332(6026):243–7. https://doi.org/10.1126/science.1201475.
3. 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. https://doi.org/10.1038/s41598-018-32803-6.
4. 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. https://doi.org/10.1038/s41467-017-00231-1.
5. Fujisaka S, Usui I, Nawaz A, Takikawa A, Kado T, Igarashi Y, et al. M2 macrophages in metabolism. Diabetol Int. 2016;7(4):342–51. https://doi.org/10.1007/s13340-016-0290-y.
6. 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. https://doi.org/10.1152/ajpendo.00329.2010.

7. Martin E, Boucher C, Fontaine B, Delarasse C. Distinct inflammatory phenotypes of microglia and monocyte-derived macrophages in Alzheimer's disease models: effects of aging and amyloid pathology. Aging Cell. 2017;16(1):27–38. https://doi.org/10.1111/acel.12522.
8. Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep. 2014;6:13. doi: https://doi.org/10.12703/P6-13.
9. Westcott DJ, Delproposto JB, Geletka LM, Wang T, Singer K, Saltiel AR, et al. MGL1 promotes adipose tissue inflammation and insulin resistance by regulating 7/4hi monocytes in obesity. J Exp Med. 2009;206(13):3143–56. https://doi.org/10.1084/jem.20091333.
10. Rajbhandari P, Thomas BJ, Feng AC, Hong C, Wang J, Vergnes L, et al. IL-10 Signaling Remodels Adipose Chromatin Architecture to Limit Thermogenesis and Energy Expenditure. Cell. 2018;172(1–2):218–33 e17. doi: https://doi.org/10.1016/j.cell.2017.11.019.
11. 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. https://doi.org/10.1016/j.cell.2014.03.066.
12. 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. https://doi.org/10.1038/nature10653.
13. 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. https://doi.org/10.1038/nm.4316.
14. 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. https://doi.org/10.1038/nm.4422.
15. 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: https://doi.org/10.1126/science.aau0964.
16. Rahman MS, Jun H. The Adipose Tissue Macrophages Central to Adaptive Thermoregulation. Front Immunol. 2022;13:884126. doi: https://doi.org/10.3389/fimmu.2022.884126.
17. 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. https://doi.org/10.1038/ni.3746.
18. Silva HM, Bafica A, Rodrigues-Luiz GF, Chi J, Santos PDA, Reis BS, et al. Vasculature-associated fat macrophages readily adapt to inflammatory and metabolic challenges. J Exp Med. 2019;216(4):786–806. https://doi.org/10.1084/jem.20181049.
19. Sarvari AK, Van Hauwaert EL, Markussen LK, Gammelmark E, Marcher AB, Ebbesen MF, et al. Plasticity of Epididymal Adipose Tissue in Response to Diet-Induced Obesity at Single-Nucleus Resolution. Cell Metab. 2021;33(2):437-53 e5. https://doi.org/10.1016/j.cmet.2020.12.004.
20. Scott CL, Zheng F, De Baetselier P, Martens L, Saeys Y, De Prijck S, et al. Bone marrow-derived monocytes give rise to self-renewing and fully differentiated Kupffer cells. Nat Commun. 2016;7:10321. https://doi.org/10.1038/ncomms10321.
21. van Furth R, Cohn ZA, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL. The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. Bull World Health Organ. 1972;46(6):845–52.
22. 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. https://doi.org/10.1016/j.cmet.2013.11.017.
23. 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(6077):86–90. https://doi.org/10.1126/science.1219179.
24. Ginhoux F, Greter M, Leboeuf M, Nandi S, See P, Gokhan S, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science. 2010;330(6005):841–5. https://doi.org/10.1126/science.1194637.
25. 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(10):1977–92. https://doi.org/10.1084/jem.20131199.
26. Felix I, Jokela H, Karhula J, Kotaja N, Savontaus E, Salmi M, et al. Single-Cell Proteomics Reveals the Defined Heterogeneity of Resident Macrophages in White Adipose Tissue. Front Immunol. 2021. https://doi.org/10.3389/fimmu.2021.719979.
27. 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(1):4434. https://doi.org/10.1038/s41467-021-24684-7.
28. Satoh T, Kidoya H, Naito H, Yamamoto M, Takemura N, Nakagawa K, et al. Critical role of Trib1 in differentiation of tissue-resident M2-like macrophages. Nature. 2013;495(7442):524–8. https://doi.org/10.1038/nature11930.
29. Nawaz A, Bilal M, Fujisaka S, Kado T, Aslam MR, Ahmed S, et al. Depletion of CD206(+) M2-like macrophages induces fibro-adipogenic progenitors activation and muscle regeneration. Nat Commun. 2022;13(1):7058. https://doi.org/10.1038/s41467-022-34191-y.
30. Odegaard JI, Chawla A. Pleiotropic actions of insulin resistance and inflammation in metabolic homeostasis. Science. 2013;339(6116):172–7. https://doi.org/10.1126/science.1230721.
31. 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. https://doi.org/10.1038/nature05894.
32. Odegaard JI, Ricardo-Gonzalez RR, Red Eagle A, Vats D, Morel CR, Goforth MH, et al. Alternative M2 activation of Kupffer cells by PPARdelta ameliorates obesity-induced insulin resistance. Cell Metab. 2008;7(6):496–507. https://doi.org/10.1016/j.cmet.2008.04.003.
33. Kang K, Reilly SM, Karabacak V, Gangl MR, Fitzgerald K, Hatano B, et al. Adipocyte-derived Th2 cytokines and myeloid PPARdelta regulate macrophage polarization and insulin sensitivity. Cell Metab. 2008;7(6):485–95. https://doi.org/10.1016/j.cmet.2008.04.002.
34. Kubota T, Inoue M, Kubota N, Takamoto I, Mineyama T, Iwayama K, et al. Downregulation of macrophage Irs2 by hyperinsulinemia impairs IL-4-indeuced M2a-subtype macrophage activation in obesity. Nat Commun. 2018;9(1):4863. https://doi.org/10.1038/s41467-018-07358-9.
35. 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. https://doi.org/10.1172/JCI45444.
36. 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. https://doi.org/10.1074/jbc.M601284200.

37. Kamei Y, Ohizumi H, Fujitani Y, Nemoto T, Tanaka T, Takahashi N, et al. PPARgamma coactivator 1beta/ERR ligand 1 is an ERR protein ligand, whose expression induces a high-energy expenditure and antagonizes obesity. Proc Natl Acad Sci U S A. 2003;100(21):12378–83. https://doi.org/10.1073/pnas.2135217100.
38. 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. https://doi.org/10.1126/science.abe9383.
39. 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. https://doi.org/10.1172/JCI19246.
40. Harman-Boehm I, Bluher M, Redel H, Sion-Vardy N, Ovadia S, Avinoach E, et al. Macrophage infiltration into omental versus subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity. J Clin Endocrinol Metab. 2007;92(6):2240–7. https://doi.org/10.1210/jc.2006-1811.
41. 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. https://doi.org/10.1016/j.cell.2019.05.054.
42. 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. https://doi.org/10.1016/j.cmet.2014.08.010.
43. Xu X, Grijalva A, Skowronski A, van Eijk M, Serlie MJ, Ferrante AW Jr. Obesity activates a program of lysosomal-dependent lipid metabolism in adipose tissue macrophages independently of classic activation. Cell Metab. 2013;18(6):816–30. https://doi.org/10.1016/j.cmet.2013.11.001.
44. 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. https://doi.org/10.2337/db09-0287.
45. 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. https://doi.org/10.2337/db09-1402.
46. 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. https://doi.org/10.2337/db08-1475.
47. 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(22):E5096–105. https://doi.org/10.1073/pnas.1802611115.
48. Weinstock A, Moura Silva H, Moore KJ, Schmidt AM, Fisher EA. Leukocyte heterogeneity in adipose tissue. Incl Obes Circ Res. 2020;126(11):1590–612. https://doi.org/10.1161/CIRCRESAHA.120.316203.
49. Burl RB, Ramseyer VD, Rondini EA, Pique-Regi R, Lee YH, Granneman JG. Deconstructing adipogenesis induced by beta3-adrenergic receptor activation with single-cell expression profiling. Cell Metab. 2018;28(2):300-9 e4. https://doi.org/10.1016/j.cmet.2018.05.025.
50. Weinstock A, Brown EJ, Garabedian ML, Pena S, Sharma M, Lafaille J, 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. https://doi.org/10.20900/immunometab20190008.
51. 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(5):639–53. https://doi.org/10.1038/s41590-021-00922-4.
52. 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. https://doi.org/10.1096/fj.202001970R.
53. Emont MP, Jacobs C, Essene AL, Pant D, Tenen D, Colleluori G, et al. A single-cell atlas of human and mouse white adipose tissue. Nature. 2022;603(7903):926–33. https://doi.org/10.1038/s41586-022-04518-2.
54. Kambara K, Ohashi W, Tomita K, Takashina M, Fujisaka S, Hayashi R, et al. In vivo depletion of CD206+ M2 macrophages exaggerates lung injury in endotoxemic mice. Am J Pathol. 2015;185(1):162–71. https://doi.org/10.1016/j.ajpath.2014.09.005.
55. 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):372-84 e12. https://doi.org/10.1016/j.cell.2017.08.035.
56. 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. https://doi.org/10.1016/j.bbrc.2019.05.113.
57. Thomou T, Mori MA, Dreyfuss JM, Konishi M, Sakaguchi M, Wolfrum C, et al. Adipose-derived circulating miRNAs regulate gene expression in other tissues. Nature. 2017;542(7642):450–5. https://doi.org/10.1038/nature21365.
58. Ying W, Gao H, Dos Reis FCG, Bandyopadhyay G, Ofrecio JM, Luo Z, et al. MiR-690, an exosomal-derived miRNA from M2-polarized macrophages, improves insulin sensitivity in obese mice. Cell Metab. 2021;33(4):781-90 e5. https://doi.org/10.1016/j.cmet.2020.12.019.
59. Lee YS, Olefsky J. Chronic tissue inflammation and metabolic disease. Genes Dev. 2021;35(5–6):307–28. https://doi.org/10.1101/gad.346312.120.
60. Glass CK, Olefsky JM. Inflammation and lipid signaling in the etiology of insulin resistance. Cell Metab. 2012;15(5):635–45. https://doi.org/10.1016/j.cmet.2012.04.001.