REVIEW

On the role of macrophages in the control of adipocyte energy metabolism

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Abstract

The crosstalk between macrophages (MΦ) and adipocytes within white adipose tissue (WAT) influences obesity-associated insulin resistance and other associated metabolic disorders, such as atherosclerosis, hypertension and type 2 diabetes. MΦ infiltration is increased in WAT during obesity, which is linked to decreased mitochondrial content and activity. The mechanistic interplay between MΦ and mitochondrial function of adipocytes is under intense investigation, as MΦ and inflammatory pathways exhibit a pivotal role in the reprogramming of WAT metabolism in physiological responses during cold, fasting and exercise. Thus, the underlying immunometabolic pathways may offer therapeutic targets to correct obesity and metabolic disease. Here, I review the current knowledge on the quantity and the quality of human adipose tissue macrophages (ATMΦ) and their impact on the bioenergetics of human adipocytes. The effects of ATMΦ and their secreted factors on mitochondrial function of white adipocytes are discussed, including recent research on MΦ as part of an immune signaling cascade involved in the 'browning' of WAT, which is defined as the conversion from white, energy-storing adipocytes into brown, energy-dissipating adipocytes.

Key Words

- inflammation
- oxidative phosphorylation
- glycolysis
- cellular energy metabolism
- obesity
- diabetes

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Introduction

White adipose tissue (WAT) is a metabolically active tissue that modifies systemic metabolism significantly by regulating the storage and release of lipids. Free fatty acids serve as a major fuel source during times of energy scarcity and high energy demands, such as exercise, cold exposure and immune responses. The dysregulation of fatty acid release contributes to dyslipidemia, resulting in ectopic fat deposition into various organs. Ectopic fat in turn impairs organ functionality, as seen during many metabolic diseases. Importantly, WAT also releases metabolites other than fatty acids (e.g. lactate as glycolytic end-product) (1). Beyond the direct metabolic effects, WAT also mediates endocrine crosstalk by secretion of various adipokines (e.g. adiponectin and leptin) (2, 3).

The crucial role of mitochondrial activity for WAT function is well established and impacts the capacity of lipid storage (4, 5) and secretory function (6, 7, 8). Clinical studies substantiate the strong association between decreased mitochondrial content and oxygen consumption of WAT/adipocytes, which is in particular evident during metabolic complications such as insulin resistance, type 2 diabetes (T2DM) and cardiovascular diseases (9, 10, 11, 12, 13). A crucial hallmark in the development of obesity-associated metabolic disorders is the chronic, low-grade inflammation of WAT (14, 15). Although obesity-associated inflammation and macrophage (MΦ) infiltration affect many tissues (such as liver, muscle, brain and pancreas (16, 17, 18, 19, 20)), the infiltration into WAT is disproportionately increased. Notably, it has been suggested that the obesity-associated inflammation of human WAT compromises mitochondrial function (21, 22, 23, 24).

Adipose tissue macrophages (ATMΦ) also reside in the WAT of lean and healthy individuals, suggesting a

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fundamental physiological role for ATMΦ, beyond the context of pathology (Fig. 1). Some inflammatory processes appear to be important for healthy WAT expansion (25). The ATMΦ-secreted cytokines and chemokines act in an autocrine and paracrine manner, the latter by controlling the inflammatory response of other immune cells or possibly impacting the metabolism of adjacent adipocytes. Recent mouse studies suggest the secretion of ATMΦ factors that metabolically enhance adipocytes during cold, stress and exercise (26, 27, 28), which has been broadly termed the 'browning' of WAT. Some mechanistic aspects of MΦ-induced browning have been questioned (29, 30), but most studies collectively support a role for MΦ in the energy metabolism of adipocytes, in particular controlling adipocyte mitochondrial function (26, 27, 31, 32, 33, 34).

Taken together, there is accumulating evidence that ATMΦ enhances or suppresses the mitochondrial function in WAT. The understanding of how adipocyte energy metabolism and mitochondria are regulated during physiological and pathophysiological adaptation requires the mechanistic understanding of the immunometabolic interaction between ATMΦ and adipocytes. The molecular networks of this interaction may offer potential interference points to correct imbalanced metabolism during pathological situations such as obesity and T2DM.

Adipose tissue macrophages (ATMΦ)

MΦ number increases in human white adipose tissue during obesity

ATMΦ are numerically the dominant type of immune cells in human WAT, and obesity further enhances MΦ numbers in WAT, which contributes to obesity-related immune imbalances (Fig. 1A). However, the data on the

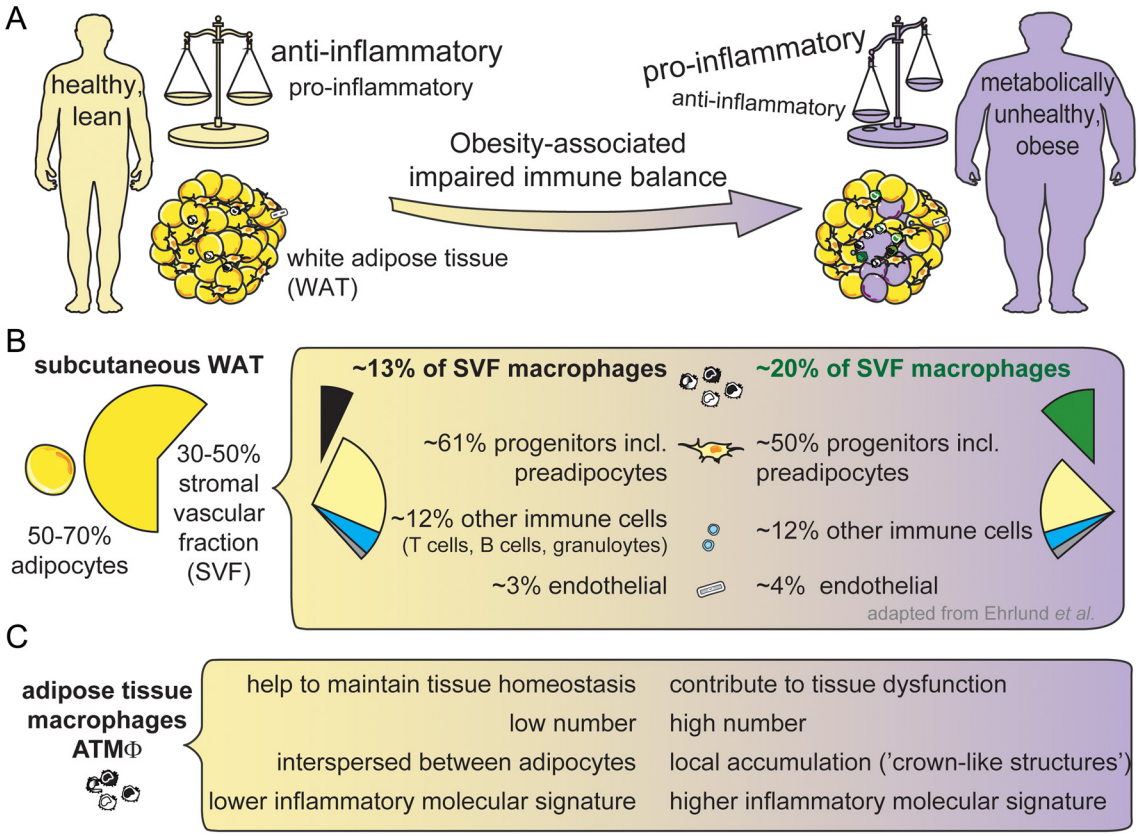

Figure 1
Obesity-associated impaired immune balance in white adipose tissue. (A) Obesity is associated with an impaired immune balance toward pro-inflammatory in WAT. All fat depots are affected, but mostly the viscWAT. (B) ATMΦ amount is low in lean scWAT (~13% of SVF). However, MΦ are numerically the dominant type of immune cells representing half of the immune cells. MΦ increase in obese WAT, for example in human scWAT from 13 to 20% of the SVF (36). (C) The roles of ATMΦ in lean (left) and obese (right) WAT. The number of MΦ is low and they are interspersed between adipocytes in WAT of lean subjects, contrasting the higher number and local accumulation of MΦ in crown-like structures during obesity, which is fostered by proliferation, high immigration and low emigration. The low inflammatory profile (surface markers, cytokine expression and secretion, e.g. IL4, IL10) in lean subjects transforms into higher inflammatory status (e.g. TNFα, IL6, IL1β) during obesity.

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cellular composition of WAT (and thus the amount of ATMΦ) vary quantitatively between studies, depending on donor, fat depot, WAT isolation/processing method and molecular readout. The relative amount of MΦ varies from as little as ≤1% (CD11c$^+$ cells, immunohistochemistry) in lean human scWAT (35), to up to 40% in obese scWAT, as seen in the first report from Weisberg *et al.* (CD68$^+$ cells, immunohistochemistry) (24). The recent publication from Ehrlund *et al.* found that the stromal vascular fraction (SVF) of scWAT from lean donors consists of ~60% progenitors including preadipocytes, ~3% endothelial cells, ~25% immune cells (and an undetermined rest) (36) (Fig. 1B). Half of this immune cell population is represented by MΦ (CD45$^+$/CD14$^+$ cells), whereas the other half is represented by T cells, B cells, mast cells, neutrophils and eosinophils. This study also reports that MΦ content significantly increases during obesity to ~20% of SVF in scWAT (36). The identified numbers of ~13% MΦ in lean scWAT and ~20% in obese scWAT (Fig. 1B) agree well with other reports (35, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46). Several publications show increased MΦ numbers in WAT during obesity that are more pronounced in viscWAT than in scWAT (47, 48, 49). The ATMΦ numbers in both viscWAT and scWAT correlate with BMI (24, 40, 50). Although ATMΦ increase significantly in viscWAT during obesity, a recent publication also notes that the relative contribution of MΦ to the SVF is much smaller in viscWAT (lean: 3%; obese: 7%) as compared to scWAT (51). Comparing immune cell populations in viscWAT from lean, middle-aged, male mice to cynomolgus macaques and healthy humans revealed that MΦ are the dominant immune cell type in murine viscWAT, whereas in humans and cynomolgus macaques, T cells dominate, followed by MΦ as the second largest immune cell population (52). Considering these cross-species comparisons, MΦ may not always be the most abundant immune cell type in adipose tissue. Nevertheless, MΦ are present in scWAT and viscWAT with increasing numbers during obesity. Furthermore, the obese condition alters their quality, comprising the mode of activation and the diversity of the secretome.

The local accumulation of ATMΦ in obese WAT

Excessive energy intake (overnutrition) is broadly accepted as an inducer of increased ATMΦ infiltration in obese WAT, causing adipocyte hypertrophy and hypoxia, eventually leading to adipocyte dysfunction, cell death and fibrosis. This scenario is accompanied by higher levels of chemoattractant cytokines such as chemokine (C-C motif) ligand 2 (CCL2/MCP-1), chemokine (C-C motif) ligand 3 (CCL3/MIP1a) and others. These cytokines provide a chemotactic gradient that attracts monocytes into WAT (39, 53, 54, 55). Inside WAT, monocytes enhance the chemotactic gradient by secreting their own chemokines, thereby attracting additional MΦ and setting up a feed-forward inflammatory process. Between lean and obese, not only the number of MΦ changes, but also their localization: In lean WAT, ATMΦ are interstitially spaced, contrasting the local accumulation of ATMΦ in so-called 'crown-like structures' around dead, apoptotic adipocytes and/or fibrotic areas in obese WAT (35, 50, 56). Mouse studies indicate that the increased MΦ content in obese WAT presumably results from several processes: higher rates of recruited/infiltrating monocytes (e.g. via CCL2, see above) (57, 58, 59), proliferation of WAT-resident monocytes (60, 61) and lower emigration rates of ATMΦ out of obese WAT (e.g. via netrin 1) (62).

The physiological importance of dynamic ATMΦ for WAT biology

ATMΦ exert distinct physiological roles and beneficial effects on WAT homeostasis, for example, healthy lipid storage (25, 26, 63, 64, 65) (Fig. 1C). ATMΦ are dynamic cells and they quickly adapt their phenotype and metabolism to changing environments, for example during fasting-induced WAT lipolysis (65, 66) and overnutrition (67). ATMΦ stimulate healthy lipid storage and therefore prevent adverse ectopic lipid storage in other organs (e.g. hepatic steatosis). Anti- and pro-inflammatory signals seem to be involved in maintaining WAT homeostasis: Healthy WAT expansion is impaired by ablating tissue-resident ATMΦ (anti-inflammatory M2) (68) or reducing pro-inflammatory signals in murine WAT (25). Recently, ATMΦ function has been implicated in cold adaptation and exercise of mice (27, 28). IL4-activated MΦ appear to be part of an anti-inflammatory signaling cascade contributing to cold-induced browning and recruitment of beige adipocytes in scWAT (26, 27, 28, 63, 69, 70). The underlying molecular mechanisms, however, and some of the reported effects have been controversially discussed (29, 30). The potential role of ATMΦ in browning will be detailed in later sections.

ATMΦ display a mixed phenotype in obese WAT

One of the first studies investigating ATMΦ proposed a phenotypic switch during obesity: while resident M2-like ATMΦ dominate in lean WAT, pro-inflammatory (M1)

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ATMΦ dominate in obese WAT (71). The stressed, obese WAT is marked by elevated levels of fatty acids and LPS, which can activate TLR4 signaling to polarize MΦ toward the pro-inflammatory M1 phenotype (72). Thus, ATMΦ can resemble the phenotype of LPS and IFNγ-activated MΦ during diet-induced obesity (15, 24, 37). This simplified classification of anti- (M2) vs pro- (M1) inflammatory activated MΦ, however, does not reflect the actual situation *in vivo*, where a spectrum of mixed markers is found (73). Notably, there are also species differences on the molecular level between human and murine ATMΦ. For example, the markers commonly used for murine MΦ polarization, such as inducible nitric oxide synthase (*iNos*) and arginase 1 (*Arg1*), are barely expressed in human ATMΦ (74, 75, 76, 77). Recently, several different ATMΦ subtypes have been identified in obese human WAT expressing macrophage activation markers of both the M1 spectrum (e.g. CD11c) and the M2 spectrum (e.g. CD163, CD206) (51, 78, 79, 80, 81). Additionally, human ATMΦ displaying M2 surface markers are capable of secreting both, pro- and anti-inflammatory cytokines (82). $CD11c^+$-ATMΦ show a reduced pro-inflammatory profile after weight loss (79). Thus, in particular during obesity, ATMΦ cannot be classified using the simple dual M1/M2 model. A new category of MΦs, termed 'metabolically' activated MΦ, was recently proposed, which can be activated by the WAT-specific environment (hormones and nutrients) (83). Indeed, the WAT-specific microenvironment and/or the long retention time of MΦ in WAT during obesity may be the cause for the unique phenotype of ATMΦ. Data on monocytes/MΦ during obesity reveal higher immigration rates into obese WAT (59) and lower emigration rates (62), indicating longer exposure times for ATMΦ in the WAT microenvironment during obesity.

Dissecting the different spatiotemporal phenotypes of human ATMΦ, including their secreted cytokines, chemokines and other factors, either during acute or chronic metabolic challenges (e.g. feeding/fasting, different diets, exercise, cold), is a challenging task. However, further insights on the role of ATMΦ in WAT metabolism and dysfunction would be gained from those studies, including the potential to distinguish and classify subgroups of obese patients with high risk for certain obesity-associated metabolic complications (e.g. NAFLD, cardiovascular complications).

In summary, ATMΦ assist the maintenance of normal tissue function, such as adipokine secretion, healthy lipid storage and adaptation toward metabolic challenges (e.g. cold, exercise, fasting) (Fig. 1C). In obesity, the amount of ATMΦ increases through the combination of proliferation, immigration and retention. ATMΦ accumulate around dead adipocytes in crown-like structures and change their phenotype. Indeed, ATMΦ of the obese display altered secretion profiles, surface marker expression and metabolic function, thereby contributing to the overall (dys)function of WAT, which will eventually impact whole body metabolic homeostasis.

The bioenergetics of human white fat cells

Mitochondrial activity is important for lipid storage and secretory function of human white adipocytes

Synthesis of ATP through oxidative phosphorylation (OXPHOS) is a major function of mitochondria to provide sufficient cellular energy. Therefore, energy-demanding adipose-specific functions, such as endocrine signaling and lipid storage, highly depend on adequate mitochondrial activity. Indirectly, mitochondria also control free fatty acid (FA) levels as the consequence of lipid storage control. Beyond ATP production, mitochondria also generate metabolic intermediates that are required for *de novo* lipogenesis. For example, the mitochondrial pyruvate dehydrogenase complex (PDH) decarboxylates pyruvate to acetyl-CoA, and thereby regulates glyceroneogenesis and the metabolic switch from glucose to lipid metabolism (4). A similar regulating role of mitochondria is found for the reverse process of lipolysis, the breakdown of lipids. Lipolysis and mitochondrial activity are closely linked as mitochondria facilitate lipolysis through FA oxidation. Furthermore, free FA can uncouple mitochondrial chain activity from ATP synthesis and enhance respiratory activity, while inhibitors of mitochondrial ATP production can abolish catecholamine-stimulated lipolysis (84, 85, 86).

Mitochondria are also important players in the regulation of Ca^{2+} homeostasis (87), tying into the well-documented calcium-dependent processes in adipocytes during insulin-stimulated glucose uptake, leptin secretion and adipogenesis (88, 89, 90, 91, 92). Furthermore, adequate mitochondrial activity is required to execute the endocrine function of WAT (e.g. adiponectin secretion (6)). Finally, the basic processes of adipocyte differentiation and maturation are closely linked to the initiation of *de novo* mitochondrial biogenesis and reactive oxygen species (ROS) production (93, 94). Collectively, mitochondrial activity of adipocytes has an impact on all the essential and specialized functions of WAT, even those that control distantly the processes that maintain systemic homeostasis.

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The bioenergetic profile of (pre-)adipocytes and the regulation by nutrients and hormones

As most proliferating progenitor cells, human subcutaneous preadipocytes depend mainly on glycolytic ATP production (~85% from glycolysis vs 15% from OXPHOS, Fig. 2) (95). During adipogenic differentiation, the mitochondrial content increases several fold (96) and the relative contribution of OXPHOS to total ATP production increases to 45–73% in human adipocytes (95, 97). Comparing mitochondrial oxygen consumption rates (OCRs) revealed four- to five-fold higher OCR in adipocytes as compared with preadipocytes (SGBS and primary cells) (95, 98). Of note, at least under *in vitro* conditions, glycolysis seems to be the preferred energy-producing pathway in both, preadipocytes and mature adipocytes. Adipocytes partially switch from OXPHOS to glycolysis in the presence of glucose. In the absence of glucose, however, only adipocytes, but not preadipocytes, are able to maintain their ATP demand by increasing mitochondrial activity. Therefore, mitochondria in human adipocytes allow for the high flexibility in substrate choice to maintain their energy metabolism (95). Visceral adipocytes show lower mitochondrial activity than subcutaneous, when calculated per cell and normalized for mitochondrial content (99, 100). When comparing isolated mitochondria from subcutaneous and visceral adipocytes, no significant difference in mitochondrial function was observed (9). This indicates that differences in energy metabolism between visceral and subcutaneous adipocytes, and WAT depots, do not depend on intrinsic mitochondrial capacity. Instead, cellular capacity of OXPHOS may depend on mitochondrial mass per cell (e.g. higher mitochondrial density in visceral than subcutaneous adipocytes (99)), the control of mitochondrial function at the cellular level (e.g. higher beta-3 adrenergic receptor mRNA levels in viscWAT than in scWAT (101)) and the depot-specific surrounding (e.g. higher vascularization in viscWAT than scWAT (102)), including the inflammatory environment created by MΦ (higher concentration of cytokines such as IL6 in viscWAT than scWAT (103)).

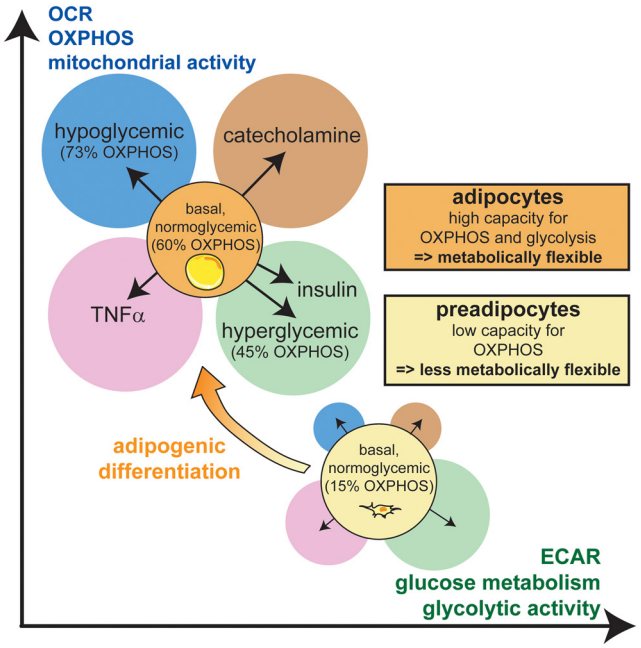

Figure 2
The dynamics of adipocyte energy metabolism. Oxygen consumption rates (OCRs), representing mitochondrial function, are plotted against extracellular acidification rates (ECARs), representing an estimate for glycolytic activity. Both pathways fuel cellular ATP demands, are complementary and display metabolic flexibility, in particular in healthy, lean adipocytes (orange). Preadipocytes (yellow) display lower OCR, higher ECAR and less metabolic flexibility. During adipogenic differentiation, glycolytic ATP production is replaced by oxidative phosphorylation (OXPHOS). OXPHOS increases about five-fold, and its contribution to cellular ATP increases from 15% in preadipocytes (yellow circle) to ~60% in adipocytes (orange circle) under basal, normoglycemic conditions. Adipocytes in lean WAT display high flexibility of OCR and ECAR, depending on nutrient availability (e.g. glucose: 45% OXPHOS in hyperglycemic (green circle) and 73% OXPHOS in hypoglycemic (blue circle) conditions), depending on hormonal input (e.g. catecholamine (brown circle) induces simultaneous glycolysis and OXPHOS = increased metabolic activity; insulin (green circle) suppresses OXPHOS and increases glycolysis = metabolic shift), and depending on inflammatory mediators (e.g. TNFα (pink circle) is suspected to reduce OCR and ECAR = metabolic depression).

Upon adrenergic activation, subcutaneous adipocytes of lean humans display increased OCR that associates with increased lipolysis (13). In parallel, extracellular acidification rates (ECARs), which estimate glycolytic activity, are increased (13). Notably, the extracellular acidification may also derive from increased carbon dioxide production of the TCA cycle (dissolved as carbonic acid), and therefore, partially unrelated to glycolysis. Insulin stimulation of subcutaneous adipocytes from obese donors leads to increased glycolytic activity and simultaneously, to decreased ATP-linked respiration (104). Whether this response is different in adipocytes from lean donors, or different in visceral adipocytes, needs to be determined. Overall, the high capacity of mitochondrial OXPHOS, that is linked to trigacylglycerol/FA cycling activity and induced by hormones and nutrients, is essential for metabolic flexibility of WAT (105, 106, 107), representing a marker of healthy adipocytes (Fig. 2).

Obesity-induced changes in the bioenergetics of white adipocytes

Decreased mitochondrial function in white adipocytes leads to dysfunction in lipid storage and endocrine

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function of WAT (2, 6) that associate with obesity-induced metabolic complications such as insulin resistance (108) (Fig. 3). Several studies demonstrate reduced mitochondrial content and activity of subcutaneous and visceral adipocytes from obese donors (9, 10, 12, 13, 109) independent of fat cell size (9, 12). Furthermore, subcutaneous adipocytes from obese donors show lower OCR responses after β-adrenergic stimulation as compared to lean individuals (13). The depression of ATP metabolism in adipocytes from obese donors is supported by data on lower mitochondrial activity, reduced lipid accumulation and insulin-stimulated glucose uptake, as compared to SGBS adipocytes, which represent a model for lean, insulin-sensitive human white subcutaneous adipocytes (110). In line with these observations, previous studies on basal heat production of primary ('floating') adipocytes from lean vs obese humans revealed an obesity-related reduction in heat output by ~50% (111). Interestingly, not only impaired mitochondrial function but also altered glycolytic activity in adipocytes is associated with obesity. Higher lactate secretion of WAT from obese patients has been reported previously, indicating higher glycolytic fluxes, impaired conversion of lactate to pyruvate and/or impaired pyruvate import into the mitochondria (1, 112, 113). This is in line with suggestions on the increased requirement of glycolytic energy production during insulin resistance (114). Under hypoxic condition, adipocytes show increased glucose uptake, leading to glycogen accumulation that has been linked to impaired adipokine secretion (115). Additionally, mitochondrial uncoupling in adipocytes, either induced by overexpressing uncoupling protein 1 (UCP1) or by administration of chemical uncouplers such as FCCP, results in less ATP yield from OXPHOS. This is usually compensated by the increase of glycolytic energy production. If the compensation fails to maintain ATP homeostasis, adipocytes show reduced lipid accumulation, possibly by diverting glucose-derived carbon flux away from fatty acid synthesis into lactate production (116, 117, 118, 119). This reduction in lipid accumulation capacity of adipocytes may lead to the adverse lipid accumulation in other organs (e.g. NAFLD), a commonly seen feature in metabolically unhealthy obese patients (120). Thus, appropriate functionality, balance and regulation of the main energy-producing pathways, oxidative phosphorylation and glycolysis, is important for metabolic flexibility to retain healthy adipocytes. Any perturbation of these metabolic processes leads to metabolic imbalances and adverse outcomes for the whole metabolic system of the body.

In summary (Fig. 3), healthy adipocytes possess the adequate mitochondrial mass and activity, allowing a wide scope of metabolic responses to hormones such as insulin and adrenaline. Mitochondrial function is required for insulin-stimulated glucose metabolism and adrenergic-stimulated OXPHOS capacity, allowing for rapid adjustments of energy metabolism. Obesity is characterized by lower mitochondria number and activity, altered basal/insulin-stimulated glucose metabolism and

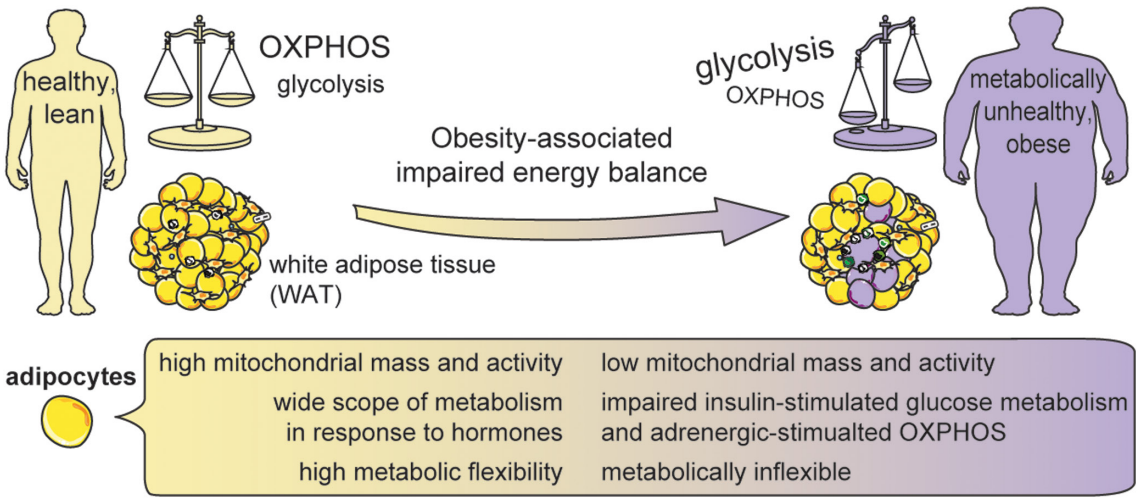

Figure 3
Obesity-associated impaired energy metabolism in white adipocytes. In lean WAT, high mitochondrial mass and activity in adipocytes allow for high metabolic flexibility. OXPHOS and glycolysis are adjusted in response to hormonal regulation (insulin and adrenergic activation). Visceral adipocytes display lower mitochondrial activity than subcutaneous adipocytes (normalize per cell and mitochondrial content). Contrasting lean WAT, obese WAT is characterized by lower mitochondrial mass and activity, impaired glucose metabolism and dampened hormonal responses. Obesity overall renders adipocytes metabolically inflexible.

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lower adrenergic-stimulated OXPHOS. Therefore, it is not surprising that unhealthy adipocytes are less metabolically flexible.

Linking ATMΦ to human adipocyte bioenergetics

Metabolically healthy (vs unhealthy) obesity is characterized by dampened inflammatory molecular signatures in WAT and lower levels of circulating inflammation markers (TNFα and hCRP) (121, 122, 123, 124). Together, this indicates the link between inflammation and dysregulated metabolism. Early observations by Weisberg *et al.* that showed obesity-associated increases in ATMΦ content, also reported on the decreased expression of several mitochondria-related genes (24). Cytokines are potential candidates mediating the crosstalk between ATMΦ and the energy metabolism of adipocytes (Fig. 4). Typical cytokines involved in WAT inflammation are TNFα, IL6 and IL1β. These cytokines promote insulin resistance and/or induce lipolysis (125, 126, 127, 128). Notably, some of these and other cytokines suppress mitochondrial function (22) (Fig. 4B). The crosstalk between ATMΦ and adipocytes is certainly bidirectional, and dysfunctional adipose mitochondria possibly promote WAT inflammation as well (129). This review, however, will focus on the effects of ATMΦ in controlling adipocyte mitochondria.

In vivo, the paracrine interactions between ATMΦ and adipose cells are complex, as MΦ are very dynamic cells with a changing cytokine profile that is influenced by adipokines (130, 131), sympathetic nerve activation (132), as well as insulin and nutrients (83). ATMΦ could represent a distinct subpopulation in WAT with a unique, not yet fully characterized phenotype that is altered during obesity (as discussed in the sections above). Thus, studying *ex vivo* the effects of MΦ-conditioned media, which represent the global secretome of MΦ, provides only a rudimentary picture of the effects that MΦ-derived products impose on fat cell bioenergetics. This *ex vivo* system, however, enables us to identify the factors, signaling pathways and mechanisms that can be further investigated and targeted *in vivo* to modulate mitochondrial function of adipocytes.

ATMΦ and secreted factors affect glucose metabolism/glycolysis of adipocytes

Using conditioned media from LPS-activated MΦ (MΦ-CM), Lumeng *et al.* observed higher basal glucose uptake in adipocytes in a murine cell culture system (3T3-L1 adipocytes and RAW264.7 or J774 macrophages) (133). In line with this, we demonstrated higher glycolytic activity in adipocytes after incubation with either LPS/INFγ-activated MΦ-CM or IL10/TGFβ-activated MΦ-CM (34), using a human model system composed of SGBS cells, a human subcutaneous adipocyte model

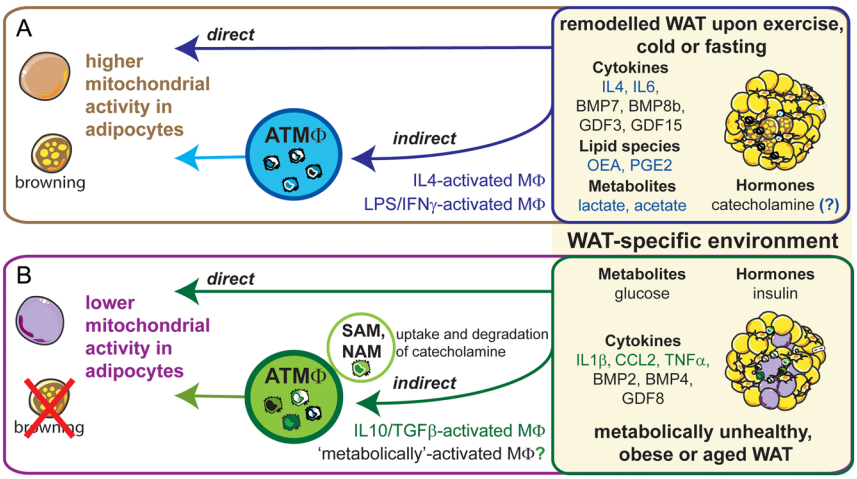

Figure 4
Control of adipocyte energy metabolism. In the WAT-specific environment (yellow background), multiple cytokines/chemokines, metabolites, lipid species and hormones from diverse cell types within WAT and/or circulation can exert either positive (upper box, A) or negative (lower box, B) effects on WAT metabolism. These factors control mitochondrial function of (pre-)adipocytes either directly and/or indirectly by first affecting the ATMΦ secretion profile. Notably, the composition of released factors depends on MΦ activation (known for factors written in blue/green). Depending on the TGFβ superfamily (BMPs and GDFs), WAT metabolism is either promoted or suppressed. A recently identified but controversially discussed mechanism of MΦ invoked browning and enhanced WAT metabolism is the secretion of catecholamine by IL4-activated MΦ during cold and exercise (upper box, A). On the contrary, NAMs/SAMs (lower box, B), which represent MΦ in close proximity of neurons/axons, may reduce local catecholamine levels and thus suppress mitochondrial function of adipocytes with age and obesity.

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and THP1 cells, a human monocytic cell line that can be differentiated into MΦ and subsequently activated with different stimuli (134). Overall, ATMΦ possess the potential to increase basal glucose uptake in adipocytes. The potential responsible factors comprise the classical inflammatory cytokines which are associated with obese WAT inflammation, such as TNFα and IL1β. Controversial reports exist for IL6, which is a cytokine that has often been associated with WAT inflammation (22, 57, 135). Furthermore, a few studies report on reduced insulin-stimulated glucose uptake after exposing adipocytes to either different MΦ-CM, or to cytokines, such as TNFα, CCL2 and IL1β, which are mostly linked to the decreased activation of insulin signaling cascades (35, 128, 133, 136, 137). Of note, many studies show the percentage or fold-changes of glucose uptake vs vehicle (0 nM insulin), not fully excluding the possibility that the reduced response in these studies may be due to increased basal glucose uptake, at least partially.

The molecular identity of ATMΦ released factors reducing adipose energy metabolism

Maintenance of cellular homeostasis requires a constant production of ATP unless specific, energy-demanding tasks are performed. Therefore, increased basal glucose uptake may report increased energy demand. However, increased basal glucose uptake may equally report a compensatory mechanism to counter fit decreased OXPHOS activity (ATP-linked respiration). The latter scenario describes a switch in the energy producing pathways, rather than the increase in metabolic activity. IL10/TGFβ-activated MΦ and/or IL1β promote such a metabolic switch in adipocytes by increasing glucose uptake/glycolysis while simultaneously decreasing mitochondrial activity (22, 34) (Fig. 4B). IL1β also inhibits cAMP- and isoproterenol-induced *PGC1a* and *UCP1* mRNA levels (138, 139), further supporting the IL1β signaling pathway in suppressing oxidative metabolism of adipocytes. TNFα represents a cytokine that appears to reduce major energy-producing pathways, glycolysis and OXPHOS. The lowering in production of cellular energy subsequently results in adipocyte death, finally seen as the loss of mitochondrial membrane potential and cleaved caspase-3 (22). Notably, TNFα levels and mitochondrial mass correlate negatively in human WAT (129, 140). Whether the secretome of the 'metabolically' activated MΦ in obese WAT (83) is significantly involved in decreased adipose mitochondrial function is not known as yet (Fig. 4B).

ATMΦ and their secreted factors may, however, not only directly affect adipocyte energy metabolism, but also indirectly by altering neuronal signals into the tissue. One of those signals is catecholamine, which enhances energy dissipation. Two mechanisms have been described how MΦ may limit bioactive catecholamine in WAT and brown adipose tissue (BAT): One mechanism proposes the inhibition of neuronal innervation. BAT-specific MΦ inhibit sympathetic neuronal innervation and thereby impair catecholamine signaling in BAT, while WAT innervation is not affected (141). The other mechanism proposes neurotransmitter clearance. A distinct MΦ-type that is attached, or at least in close proximity, to axons of the SNS takes up and degrades norepinephrine (NE). These MΦ have been termed either sympathetic neuron-associated MΦ (SAMs) (30) or nerve-associated MΦ (NAMs) (142). So far, SAMs/NAMs have been identified in murine viscWAT (142) and scWAT (30), but not unequivocally in murine BAT (30). SAMs/NAMs may regulate local catecholamine concentrations and prevent catecholamine spill over into the circulation (30, 142, 143). The MΦ-mediated NE uptake and degradation system is apparently enhanced during obesity (increased number of SAMs (30)) and aging (GDF3-dependent increased expression of genes controlling NE degrading in NAMs (142)), and potentially contribute to decreased energy metabolism with age and obesity (Fig. 4B).

Mediators between ATMΦ and increased adipose energy metabolism

The interaction between ATMΦ and increased WAT energy metabolism is supported by mouse models that claim the involvement of MΦ in the 'browning' of WAT upon cold exposure, exercise and caloric restriction (26, 27, 28, 144, 145) (Fig. 4A). Browning of WAT has been classically defined as the upregulation of uncoupling protein 1 (UCP1) and the appearance of multilocular adipocytes in WAT, termed beige adipocytes. Beige adipocytes associate with mitochondrial biogenesis and higher energy turnover. UCP1 resides in the mitochondrial inner membrane and uncouples the proton motive force from ATP synthesis, thereby directly releasing energy as heat and accelerating catabolic processes. With this energy-burning machinery, the browning of WAT can restore dysregulated glucose and lipid metabolism in diverse obese and diabetic mouse models (26, 27, 28, 146). With these observations in mouse models, browning-inducing pathways have gained remarkable attention in biomedicine to treat metabolic diseases. In the context of browning, ATMΦ may release

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cytokines, which could induce UCP1 expression, higher energy turnover and energy wasting in adipocytes (Fig. 4A). Several publications implicate IL6 signaling in beige adipocyte formation and WAT browning (147, 148, 149), but some aspects of IL6-stimulated glucose uptake are controversial (147, 148, 149, 150).

Another cytokine that affects WAT energy metabolism, either directly or via MΦ, is IL4 (Fig. 4A). IL4 is secreted by MΦ and to a higher extent by eosinophils in WAT (26). It may directly control WAT metabolism, by acting either on preadipocytes to promote differentiation into beige adipocytes, or on adipocytes to induce higher ATP turnover (151, 152). Several publications place IL4-activated MΦ into immune signaling cascades that are able to induce UCP1 expression and mitochondrial activity in adipocytes. The IL4-MΦ axis can be modulated by additional factors of endocrine (e.g. released distantly from muscle (27)) and/or paracrine nature (e.g. released adjacently from other WAT cell types, including eosinophils, type 2 innate lymphoid cells, regulatory and natural killer T cells (26, 153, 154)). The underlying mechanisms how the IL4-MΦ system induces browning is not fully understood. In particular, the involvement of catecholamine-producing ATMΦ is controversially discussed (28, 29). Although some reports on catecholamine synthesis in MΦ exist (143, 155, 156), the physiological contribution during cold-induced thermogenesis seems to be of minor importance (29). Whether MΦ-mediated uptake (143) or degradation of catecholamines (as shown for SAMs/NAMs (30, 142)) is inhibited and substantially contributes to cold-induced WAT browning requires further investigations.

Additional candidates that impact mitochondrial function in adipocytes belong to the TGFβ superfamily. TGFβ3 inhibits the 'browning' of WAT and stimulates proliferation of white adipocytes (150, 157, 158, 159). Our functional work demonstrated that IL10/TGFβ-activated MΦ secreted factors decrease ATP-linked respiration in human subcutaneous adipocytes, thus providing evidence for indirect suppression of mitochondrial respiration by TGFβ (34). Several other members of the TGFβ superfamily have been proposed in the regulation of oxidative metabolism in adipocytes and the browning of WAT, affecting whole body energy metabolism. Many of these factors are indeed secreted by MΦ. Whether these factors promote or suppress energy metabolism depends on the distinct factor or receptor, as well as on the adipose depot (BAT or WAT). Examples for specific effects include bone morphogenetic proteins (BMP 2, 4, 7 and 8b) (160, 161, 162, 163, 164, 165, 166) and growth differentiation factors (GDF 1, 3, 5, 15) (142, 167, 168, 169, 170, 171, 172) (Fig. 4A and B). The effects also depend on the developmental stage of the adipocytes, whether the cytokine acts directly on the mesenchymal stem cell, on the early committed preadipocytes (brown, beige or white) or on the adipocytes, or whether the cytokine acts indirectly by changing MΦ infiltration and their phenotype (170, 173). Additionally, there are reports that these cytokines act on the central nervous system to control metabolism (164, 174).

Other mediators between ATMΦ and adipocyte metabolism are metabolites. Upon activation, MΦ change their metabolomic profile (175, 176), for example upon LPS activation, more lactate and pyruvate are released (176). Lactate and acetate have been suggested as inducers of WAT browning (177, 178, 179). Lipid mediators (e.g. oleoylethanolamine (OEA), prostaglandin E2 (PGE2)) are differentially released by MΦ, depending on the mode of activation (180, 181, 182). Circulating metabolites and lipid mediators are involved in the browning of rodent WAT (177, 183, 184), indicating that these factors represent additional candidates by which MΦ modulate mitochondrial activity in white adipocytes (Fig. 4A). Although MΦ may not be the main source for some cytokines or factors that have been linked to increased or decreased energy expenditure in WAT (e.g. IFNγ, retinoic acid, catecholamine, IL17, lactate), the indirect involvement of these factors cannot be formally excluded (29, 34, 185, 186, 187, 188). For instance, we have recently found increased ATP-linked respiration in white adipocytes after exposure to the secreted factors of LPS/IFNγ-activated MΦ (34) (Fig. 4A).

In summary (Fig. 4), ATMΦ can be activated by the WAT-specific microenvironment which is impacted by circulating endocrine and auto-/paracrine factors (cytokines, nutrients and hormones). Thus, the WAT-specific environment is characterized by distinctly activated ATMΦ and MΦ-secreted factors which contribute to the microenvironment but furthermore and the regulation of WAT energy metabolism. During cold, exercise and fasting, the induction of adipose energy metabolism by enhancing beige adipocyte differentiation, inducing UCP1 expression, increasing ATP turnover and/or increasing energy dissipating pathways such as catecholamine can be mediated by activated MΦ (e.g. LPS/IFNγ- or IL4-activated MΦ) and MΦ released factors such as cytokines (IL4, IL6), metabolites (lactate, acetate) and/or lipid mediators (PGE2, OEA) (Fig. 4A). In the obese state, other activated MΦ (e.g. IL10/TGFβ- or 'metabolically' activated MΦ) and MΦ released factors such as cytokines (e.g. IL1β and TNFα) may decrease energy

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metabolism of adipocytes or limit local energy dissipating pathways by uptake and degradation of catecholamine (by SAM, NAM) (Fig. 4B). Several scenarios how these factors operate are conceivable, and they most likely overlap and work in concert, by direct action on mesenchymal stem cells, preadipocytes and adipocytes and by indirect signals via MΦ. Indirect action may also occur via additional cell types in WAT, including other immune cells (e.g. T cells), epithelial cells and neurons (not depicted in Fig. 4).

Conclusion and outlook

In the upcoming field of immunometabolism, which investigates the crosstalk between immune cell function and metabolic homeostasis, the understanding on paracrine regulation of human white adipocyte metabolism by ATMΦ, is utterly important. By identifying the ATMΦ-secreted factors that control mitochondrial function and energy metabolism in adipocytes, we may be able to find novel therapeutic targets to treat diseased WAT during obesity. This new understanding of the metabolic network in WAT needs to be resolved on the molecular level, investigating how controlling pathways are regulated under physiological and pathophysiological conditions. A detailed investigation is required on the ATMΦ phenotypes/subpopulations and how fat depot-, gender- and age-specific ATMΦ infiltration and activation are related to adipocytes, WAT and whole body metabolism in health and disease. Although this review focuses on the paracrine action of MΦ within the white adipose tissue, it should be considered that ATMΦ contribute to the overall secretion profile of WAT with factors that are released into the circulation for endocrine action causing systemic effects such as insulin resistance. It is feasible to speculate that these factors will not only impact the energy metabolism of adipocytes, but also as endocrine factors potentially impact the bioenergetics of other more distantly located target cells (such as hepatocytes and myocytes).

Furthermore, not only MΦ composition changes with obesity, but other immune cells, such as T cell, B cell, eosinophil, iNKT and neutrophils change in number and activation state, contributing to the impaired immune balance in obese WAT. Thus, the complex microenvironment of adipose tissue that controls the bioenergetics of adipocytes is composed of multiple cytokines and cell types with multiple cellular targets. Additionally, there is potentially a feed-back mechanism in place where adipocyte-secreted proteins and signals impact the immune cell secretome that in turn controls adipocyte metabolism. How the endocrine and nervous system that regulates metabolism (e.g. catecholamine, acetylcholine, insulin and glucagon) affects the crosstalk of ATMΦ and fat cells represents another promising research topic. Many other aspects require further investigation, concerning cytokine production and combinatorial effects on adipocytes, the interaction with energy storing and dissipating pathways, as well as the crosstalk between adipocytes and cell types other than ATMΦ to control adipocyte glycolysis and mitochondrial function.

Owing to the profound differences in the immune system between mice and humans, it is of major importance to consolidate murine pathways and their impact on metabolism in humans. That said, however, it is promising that certain activated MΦ not only induce energy-producing pathways (glycolysis and OXPHOS) in white adipocytes, but possibly in an UCP1-independent manner, suggesting new options to increase energy expenditure by targeting inflammatory pathways in WAT. Novel strategies in obesity therapy are required as obese and older subjects are usually characterized by the absence or low content of BAT ($UCP1^+$-cells) (189). Whether the browning capacity of human subcutaneous WAT can be enhanced to that extent that it eventually contributes significantly to systemic energy expenditure, is still an open question (190, 191). Beyond energy wasting in adipocytes, mitochondria are crucial for all cellular pathways (e.g. differentiation, apoptosis, energy dissipation, adipokine secretion), thus representing ubiquitous targets to treat obesity and its associated disorders. Collectively, targeting inflammatory pathways in fat depots could be a feasible strategy for the treatment of metabolic diseases.

Declaration of interest
The author declares that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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