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Review Series

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Huaizhu Wu[1,2] and Christie M. Ballantyne[1,3,4]

[1]Department of Medicine, [2]Department of Pediatrics, and [3]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas, USA. [4]Center for Cardiovascular Disease Prevention, Methodist DeBakey Heart and Vascular Center, Houston Methodist Hospital and Baylor College of Medicine, Houston, Texas, USA.

Obesity is associated with chronic inflammation, which contributes to insulin resistance and type 2 diabetes mellitus. Under normal conditions, skeletal muscle is responsible for the majority of insulin-stimulated whole-body glucose disposal; thus, dysregulation of skeletal muscle metabolism can strongly influence whole-body glucose homeostasis and insulin sensitivity. Increasing evidence suggests that inflammation occurs in skeletal muscle in obesity and is mainly manifested by increased immune cell infiltration and proinflammatory activation in intermyocellular and perimuscular adipose tissue. By secreting proinflammatory molecules, immune cells may induce myocyte inflammation, adversely regulate myocyte metabolism, and contribute to insulin resistance via paracrine effects. Increased influx of fatty acids and inflammatory molecules from other tissues, particularly visceral adipose tissue, can also induce muscle inflammation and negatively regulate myocyte metabolism, leading to insulin resistance.

Introduction

Obesity is becoming a global epidemic, increasing the health burden of associated complications of insulin resistance and diseases such as cardiovascular disease (1). Because insulin resistance leads to type 2 diabetes (T2D) (2), T2D incidence and prevalence are also increasing rapidly. The number of adults with diagnosed diabetes in the United States nearly quadrupled over 32 years, from 5.5 million in 1980 to 21.3 million in 2012; 90% to 95% of these individuals have T2D (3).

Molecular links between obesity and insulin resistance and T2D remain incompletely understood but may include chronic inflammation, particularly in adipose tissue (AT) (4–8). AT inflammation may contribute to whole-body insulin resistance and T2D via the endocrine effects of inflammatory molecules secreted by AT (known as adipokines) on insulin sensitivity in various tissues, particularly skeletal muscle (SM) and liver. Additionally, dysregulation of preadipocyte/adipocyte functions accelerates fat spillover from AT to SM and liver, resulting in ectopic fat deposition and insulin resistance in these tissues, which contribute to systemic insulin resistance and T2D (5, 7, 9–13). SM is the most important organ for whole-body glucose homeostasis (14, 15) and is responsible for approximately 80% of insulin-stimulated whole-body glucose uptake and disposal under normal conditions (15–18). Insulin resistance in SM is the major defect in T2D (16–18) and is therefore central to systemic insulin resistance and T2D.

While studies have focused on the roles of intramyocellular lipids, mitochondrial defects, and endocrine effects of adipokines on SM insulin resistance (10, 12, 15), emerging evidence indicates that inflammation also occurs in SM in the setting of obesity and may exert autocrine or paracrine effects on myocyte metabolic functions. In this Review we focus on obesity-linked SM inflammation and its roles in muscle insulin resistance.

Inflammation in SM

Although obesity-linked inflammation is less well studied and documented in SM than in AT, available evidence suggests that SM myocytes can secrete large numbers of cytokines and other molecules and may become inflamed in obesity. In addition, immune cells can infiltrate into SM and increase SM inflammation in obesity.

SM as a secretory organ and myocyte secretion of inflammatory molecules. Similar to adipocytes, SM myocytes express and secrete numerous cytokines such as IL-6, IL-8, and IL-15 and other molecules such as FGF21, irisin, myonectin, and myostatin (known as myokines; see Table 1 and refs. 19, 20). Whereas most adipokines are proinflammatory, regulated by obesity, and involved in the development of obesity-linked metabolic dysfunction (4, 5, 11), most myokines are regulated mainly by exercise and muscle extraction, counteract the detrimental effects of adipokines, and have beneficial effects on glucose and lipid metabolism and inflammation (19, 20).

Myokines may affect myocytes and immune cells locally via autocrine or paracrine actions and other cells such as adipocytes and hepatocytes via endocrine effects. IL-6 is the most well-studied myokine. Exercise and muscle extraction dramatically enhance IL-6 secretion from muscle and can increase plasma IL-6 levels up to 100-fold (19–21). Acute treatment of myocytes or intravenous infusion of healthy humans with IL-6 increases basal and insulin-stimulated glucose uptake by myocytes and improves whole-body insulin sensitivity (19, 22). IL-6 also increases lipolysis and fatty acid (FA) oxidation in myocytes and adipocytes; induces UCP1 expression in mouse white AT, which is indicative of browning (20, 22–25); and mediates antiinflammatory effects by inducing expres-

Table 1. Selected myokines

Myokines	Major autocrine, paracrine, or endocrine effects on metabolism and inflammation	Regulation in SM
Cytokines/chemokines		
IL-6	Promotes muscle hypertrophy Acutely: enhances insulin sensitivity and insulin-stimulated glucose uptake; promotes lipolysis and FA oxidation in myocytes, adipocytes, and whole body (19, 20, 22–25); inhibits inflammation (19, 26) Chronically: induces insulin resistance; promotes inflammation in skeletal muscle and liver (162, 163)	Increased with exercise (19, 20), decreased in obesity and T2D (30, 31), increased in obesity and T2D (32–34)
IL-8	Induces angiogenesis and leukocyte recruitment (19, 24)	Upregulated with exercise (19, 20), increased in obesity with T2D (34)
IL-15	Promotes muscle hypertrophy; reduces lipid deposition in white AT (19, 24)	Increased with exercise (19, 20); increased in obesity with T2D (34); no change in obese subjects with insulin resistance (164)
TNF-α	Inhibits insulin sensitivity and insulin-stimulated glucose uptake; impairs mitochondrial ultrastructure and functions; proinflammatory in myocytes, adipocytes, and immune cells (40–45)	Increased in obesity with insulin resistance or with T2D (31, 34, 35, 38)
GROα	Induces immune cell infiltration; proinflammatory (34)	Increased in obesity with T2D (34)
MCP-1	Induces immune cell infiltration; proinflammatory (34, 35, 37, 76)	Increased in obesity with insulin resistance or with T2D (34, 35)
RANTES	Induces immune cell infiltration; proinflammatory (9, 35, 77)	Increased in obesity with insulin resistance (35)
Other molecules		
FGF21	Induces white AT browning; protects from diet-induced obesity and insulin resistance (29, 165)	Increased with stress, insulin challenge, obesity, and T2D (165)
Irisin	Induces white AT browning; increases myocyte proliferation, GLUT4 expression, and mitochondrial uncoupling and biogenesis (24, 27–29)	Induced with muscle contraction (28); decreased in obesity (166)
Myonectin	Promotes FA uptake by adipocytes and hepatocytes with reduction of circulating FFAs (167)	Increased with exercise; decreased in obesity (167)
Myostatin	Inhibits muscle hypertrophy; maintains metabolic homeostasis and modulates AT function and mass (19, 24)	Decreased with exercise; increased in obesity (19, 168)

sion of antiinflammatory cytokines such as IL-10 and inhibiting expression of proinflammatory cytokines such as TNF-α (19, 26). However, IL-6 is generally considered proinflammatory and can induce insulin resistance, particularly under chronic conditions related to obesity (see below). Irisin, another myokine enhanced by muscle contraction, may increase glucose transporter 4 (GLUT4) expression and mitochondrial uncoupling and biogenesis in myocytes, and also induce browning of white AT (24, 27–29).

Studies on the effects of obesity on myokine expression are limited and have had inconsistent results. For example, obesity in rats decreased IL-6 and IL-15 expression in SM (30). Similarly, cultured myocytes from SM of obese subjects with impaired glucose tolerance or T2D expressed lower IL-6 levels than those from healthy controls (31). However, more studies found increased IL-6 expression or release in SM of obese subjects with impaired glucose tolerance or T2D compared with healthy controls (32–34). Differentiated myocytes can express numerous proinflammatory molecules (Table 1), particularly under stimulation of inflammatory cytokines and free FAs (FFAs) (32, 34–37). Differentiated cultured myocytes isolated from obese subjects with insulin resistance or T2D secrete more cytokines such as TNF-α and chemokines such as monocyte chemoattractant protein 1 (MCP-1) than myocytes from lean controls (31, 34, 38). Higher TNF-α levels were also observed in SM of rats fed a fructose-rich diet (39), and TNF-α can induce insulin resistance and mitochondrial dysfunc-

tion in myocytes (40–45). Thus, the obesity-linked increases in TNF-α secretion by myocytes may contribute to myocyte insulin resistance via autocrine effects. Therefore, obesity is associated with increased inflammation in myocytes, which may secrete elevated levels of proinflammatory molecules and contribute to muscle inflammation. Nevertheless, changes in myocyte secretion of cytokines do not appear to constitute the major component of SM inflammation in obesity (see below), and the role of various myokines in SM inflammation remains to be further investigated.

Infiltration of immune cells into SM. Increased immune cell infiltration is the main characteristic of obesity-linked inflammation in AT (4, 5, 9, 11, 46–51). Growing evidence indicates that immune cells also accumulate in SM and may constitute the predominant inflammatory cells in SM in obesity (2, 11, 35, 52, 53). Increased macrophage and T cell levels have been reported in SM of obese humans with insulin resistance or T2D (35–37, 53–55). In fact, a short-term high-fat, high-calorie diet or overfeeding with induction of insulin resistance increased macrophage markers in SM in healthy subjects (56, 57). In mice, obesity and insulin resistance induced by a high-fat diet (HFD) was consistently associated with increased accumulation of immune cells including macrophages and T cells in SM (11, 35, 37, 47, 49, 53, 58–62). Similar to humans, mice fed a short-term HFD have increased macrophage content in SM (35, 53, 60). Mast cells and eosinophils were observed in mouse SM but showed no changes with obesity (53, 63). Changes

in other immune cells including neutrophils, B cells, NK cells, and invariant NKT (iNKT) cells, which are found in visceral AT (2, 13, 64), have not been reported in SM in the setting of obesity.

Histologically, macrophages and T lymphocytes are primarily located in muscle AT between myocytes or surrounding the muscle, so-called intermyocellular/intermuscular AT (IMAT) or perimuscular AT (PMAT) (11, 35, 47, 49, 59). Both IMAT and PMAT are adjacent to myocytes and differ from subcutaneous AT (65). Both are extramyocellular fat that expands substantially in obesity and decreases following weight loss (66), and both depots are highly correlated with insulin resistance and expression of MCP-1 and C-reactive protein (65, 67–70). Macrophages and T cells within these adipose depots are markedly increased in obesity (35, 49, 53) and can form crown-like structures surrounding dead or dying adipocytes (35). Additionally, macrophages and T lymphocytes can be found in SM between myofibers at a lower frequency (35–37, 53). Obesity-linked changes in immune cells and inflammatory markers are much greater in muscle AT than in muscle (35), which may help explain the low levels of immune cells and inflammation in SM in human subjects with small-muscle biopsies (71), as well as why alterations in BMI or lifestyle intervention–induced weight loss do not alter macrophage numbers in SM in obese subjects in some studies (72, 73).

Similar to those in visceral AT, immune cells in SM tend to polarize into proinflammatory phenotypes in obesity. Most macrophages in SM are CD11c+ and display classically activated (M1-like) phenotypes (35, 37, 47, 53, 62). Both CD4+ and CD8+ T cells are increased in SM of obese mice. While the proportion of IFN-γ–expressing Th1 cells is increased, the proportion of Tregs is decreased in SM in mice with obesity (35). Accordingly, proinflammatory markers related to immune cell activation such as TNF-α, IL-1β, and IFN-γ are increased (32, 33, 35, 37, 47, 53, 58, 60, 61), while antiinflammatory markers such as IL-10 are reduced in SM in obesity (60). Although in vitro studies show capacity of differentiated myocytes to express proinflammatory molecules (32, 34–37), studies in mouse models of obesity indicate that levels of most proinflammatory markers are much higher and show greater obesity-linked changes in PMAT than in muscle (35, 37), suggesting that in vivo obesity-linked SM proinflammatory molecules may be mainly derived from immune cells in muscle adipose depots.

Taken together, compelling evidence supports the association of obesity with increased inflammation in SM in both humans and rodents. Myocytes have the capacity to express cytokines and may secrete more proinflammatory cytokines in obesity. However, increased SM inflammation in obesity may mainly result from increased infiltration of immune cells, particularly macrophages and T lymphocytes that are primarily localized in muscle adipose depots (IMAT/PMAT) and tend to polarize into proinflammatory phenotypes (Figure 1).

Regulation of inflammation in SM in obesity

Despite the evidence for increased SM inflammation in obesity, the underlying mechanisms remain largely unexamined. Below, we detail potential roles for various mediators in SM inflammation.

Chemokines, adhesion molecules, and immune cell infiltration. Similar to what is observed in visceral AT (61, 74, 75), inflammation, including immune cell infiltration, starts early in SM during

obesity development (35, 53, 56, 57, 60). Macrophage infiltration precedes T cell infiltration (35). Infiltration of leukocytes from the circulation into tissues requires attractant signals such as chemokines, and chemokines such as MCP-1 increase early in SM and visceral AT of mice fed a HFD. In visceral AT, the increase in MCP-1 appears to precede the increases in macrophages and the activation marker TNF-α (74, 75), suggesting that the initial increase in chemokines may derive from tissue-resident cells. Adipocytes and myocytes, the main resident cells in AT and SM, respectively, can express chemokines including MCP-1 (9, 32, 35–37, 61). Under stimulation with inflammatory molecules or FFAs or in obesity, adipocytes and myocytes secrete more chemokines (9, 32, 34–37, 61), which induce immune cell migration (9, 37, 61). Therefore, chemokines secreted by myocytes or adipocytes may play crucial roles in immune cell infiltration and inflammation in SM and visceral AT. MCP-1 overexpression in myocytes or adipocytes increases inflammation with enhanced immune cell infiltration in SM or visceral AT in mice (37, 76), while MCP-1 knockout prevents HFD-induced increases in muscle or AT macrophages (53). The RANTES/CCR5 pathway is also upregulated in SM and visceral AT in obesity (9, 35, 77) and may play a role in obesity-linked inflammation in visceral AT (77). The initiating signals that trigger SM or AT inflammation are not well known and may include FAs, particularly HFD-derived saturated FAs, which can induce expression of inflammatory molecules including chemokines in myocytes and adipocytes (34, 37). In addition to myocyte or adipocyte secretion of chemokines, as obesity progresses, recruited immune cells may also secrete chemokines, which may further increase inflammation in SM and AT.

The arachidonic acid–derived leukotriene LTB4, which is increased in SM, visceral AT, and liver of obese mice, also contributes to macrophage infiltration of visceral AT in obesity (78). Interactions of adhesion molecules on immune cells and their ligands on endothelial cells are crucial for immune cell migration. Lymphocyte function–associated antigen-1 (LFA-1), a β2 integrin mainly expressed on immune cells, plays an essential role in T cell accumulation and inflammation in SM and visceral AT of obese mice, likely by interacting with ICAM-1 on endothelial cells or antigen-presenting cells (35, 79).

While infiltration of circulating Ly-6Chi monocytes is important in obesity-linked inflammation and accumulation of proinflammatory CD11c+ macrophages in AT in mice (48, 80), the role of Ly-6Clo monocytes remains to be determined. In the circulation, Ly-6Clo, but not Ly-6Chi, monocytes express CD11c (81, 82). Circulating CD11c+/Ly-6Clo monocytes are increased with obesity and hyperlipidemia, infiltrate into atherosclerotic aortas, become CD11c+ macrophages/dendritic cells, and contribute to atherogenesis in mice (46, 81–83). Infiltration of CD11c+/Ly-6Clo monocytes likely also plays a role in CD11c+ macrophage accumulation and inflammation in visceral AT and SM in obesity. In addition, macrophages and T cells proliferate in visceral AT (79, 84, 85), and potential proliferation in SM warrants investigation.

Immune cell activation. Macrophages and T lymphocytes not only are increased in number but also display proinflammatory phenotypes in SM and visceral AT in obesity. The tissue inflammatory milieu, including increased cytokines, macrophage/T cell interactions, and increased FFAs and metabolites, may play key roles in immune cell proinflammatory activation in obesity (Figure 1).
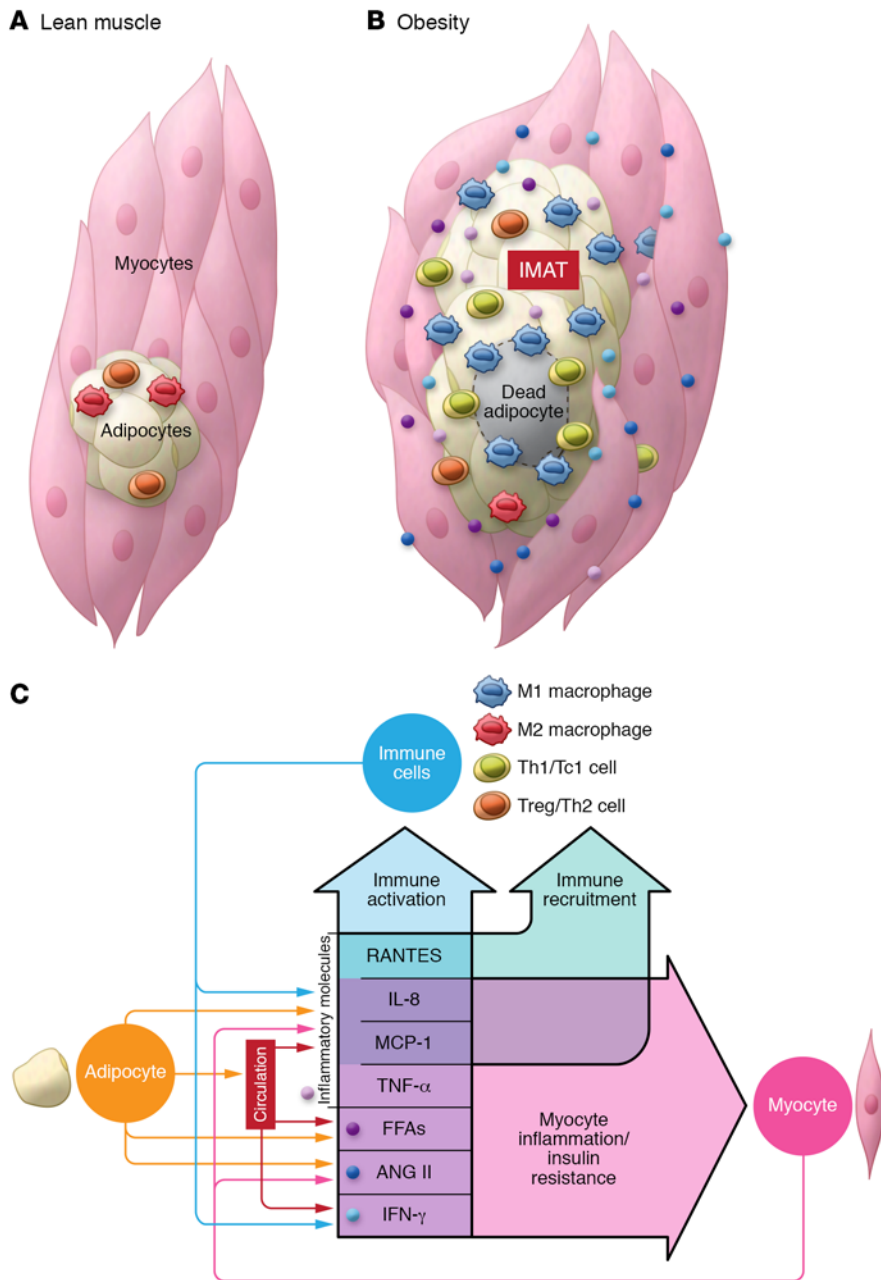
Figure 1. Inflammation in skeletal muscle in obesity. (**A**) In lean conditions, few immune cells with resting or antiinflammatory phenotypes reside in skeletal muscle. (**B**) As obesity develops and progresses along with expansion of visceral and subcutaneous AT, adipose depots expand between muscle fibers or surrounding muscle, so-called IMAT/PMAT. In obesity, immune cells including macrophages and T cells infiltrate into IMAT/PMAT and polarize into proinflammatory phenotypes, leading to increased inflammation in skeletal muscle. At the same time, myocytes may become inflamed and express proinflammatory cytokines and chemokines. (**C**) Chemokines and cytokines secreted by myocytes, adipocytes, and immune cells, along with FFAs that are transferred into skeletal muscle and ANG II produced within skeletal muscle, may themselves further accelerate immune cell recruitment and activation and myocyte inflammation, forming a feed-forward loop of inflammation in skeletal muscle.

Cytokines and signaling pathways in immune cell activation. Cytokines play central roles in immune cell activation. IFN-γ and TNF-α are crucial for macrophage polarization into M1 proinflammatory phenotypes, while IL-4, IL-13, and IL-10 are crucial for macrophage polarization into alternatively activated (M2) phenotypes (86). IL-12 is critical for T cell polarization to Th1, whereas IL-4 is critical for T cell polarization to Th2 phenotypes. TNF-α, the signature cytokine of M1-polarized macrophages, and IFN-γ, the signature cytokine of Th1, are both increased in SM and visceral AT in obesity and are involved in obesity-linked AT inflammation, including macrophage activation (35, 58, 87). These cytokines may also induce immune cell activation and play crucial roles in muscle inflammation. IL-10 is reduced in SM in obesity, and overexpression of IL-10 in SM attenuates obesity-induced macrophage activation in muscle (60).

TNF-α exerts proinflammatory effects mainly by activating IκB kinase/NF-κB (IKK/NF-κB) and JNK pathways. The IKK complex, which consists of the catalytic subunits IKKα and IKKβ and the regulatory subunit IKKγ, activates NF-κB transcription activity by phosphorylating and degrading the inhibitory protein IκB. Ablation of IKKβ in myeloid cells protects mice from obesity-induced inflammation (88). Activation of NF-κB in obesity also leads to increases in IKKε, a non-canonical IKK, in macrophages, adipocytes, and liver. Knockout or inhibition of IKKε in mice attenuates obesity-linked inflammation including reductions in accumulation and M1 polarization of macrophages in visceral AT and liver (89, 90).

Obesity increases JNK activity in muscle and AT (89, 91) and increases phosphorylated JNK levels in circulating monocytes (47). Ablation of JNK1 alone or both JNK1 and JNK2 in hematopoietic cells or myeloid cells dramatically decreases obesity-induced

inflammation in mice (92, 93). Tissue culture studies support a crucial role of JNK in macrophage polarization to M1, but not M2, phenotypes (47, 92, 93).

IFN-γ exerts proinflammatory effects primarily through activating the JAK/STAT1 pathway. Upon binding its receptor, IFN-γ mainly activates JAK1 and JAK2, which phosphorylate and activate STAT1. STAT1 plays a pivotal role in M1 polarization and Th1 polarization (86). Short-term treatment of obese mice with a JAK1/JAK2 inhibitor decreases inflammation in SM (35), supporting an important role of the JAK/STAT pathway in obesity-linked muscle inflammation.

Cytokines may be the main mediators by which macrophages and T lymphocytes influence each other's inflammatory status. For example, knockout of LFA-1 in mice reduces obesity-induced T cell infiltration and Th1 polarization, along with decreased IFN-γ levels, but does not change total macrophage content, in SM and visceral AT. However, macrophage expression of proinflammatory markers such as MCP-1 and TNF-α is decreased (35, 79), possibly because of reduced induction of macrophage activation by decreased Th1 cytokine in muscle.

T cells, particularly CD8^{+} memory T cells including those in AT, may become activated and proliferate under the stimulation of cytokines IL-12 and IL-18, which are mainly expressed by macrophages and dendritic cells and are increased in obesity (79). In addition, macrophages and dendritic cells can activate T cells through the MHC/antigen/TCR pathway. MHC-II and CD11c, which are mainly expressed on M1-like macrophages/dendritic cells, play important roles in macrophage/dendritic cell–induced T cell activation in obese AT (46, 84). Moreover, MHC-II is upregulated on obese adipocytes, which also contribute to T cell activation in obese AT (94). The potential role of these pathways in obesity-linked SM inflammation remains to be examined.

FFAs and signaling pathways in immune cell activation. In addition to increased cytokines, increased influx of FFAs (derived from lipolysis in AT or from a HFD; see below) usually occurs in SM in obesity. FAs, particularly long-chain saturated FAs, have been consistently shown to induce inflammation, thereby also likely contributing to immune cell activation in SM in obesity. Palmitic acid or a mixture of long-chain FAs increases macrophage expression of proinflammatory molecules and induces M1 polarization, possibly via engagement of TLR2 and TLR4 and subsequent activation of NF-κB and JNK pathways (47, 92, 93, 95). In addition, palmitic acid and its metabolite ceramide activate the NLRP3 inflammasome, a cytosolic multiprotein complex that activates caspase-1, leading to maturation and secretion of the proinflammatory cytokines IL-1β and IL-18 (96). Consistently, in addition to NF-κB and JNK, TLR2/4 and the inflammasome play crucial roles in obesity-linked macrophage proinflammatory activation and inflammation (47, 95, 96).

Influx of FAs into SM and triglyceride-rich lipoproteins. In obesity, elevated levels of circulating FFAs, mainly derived from lipolysis in adipocytes, lead to increased FA influx into SM, which not only induces inflammation in immune cells (see above) and myocytes in muscle, but also causes insulin resistance in myocytes (see below). In addition, obesity is usually associated with hypertriglyceridemia, with elevated levels of triglyceride-rich lipoproteins (TGRLs), including enterocyte-derived chylomicrons and hepatocyte-derived VLDLs, which may also release more FAs into SM and contribute to muscle inflammation and insulin resis-

tance. Indeed, hypertriglyceridemia correlates with and may be a causal factor for insulin resistance and T2D (97). Diets enriched with saturated fat or carbohydrates tend to cause increased levels of TGRLs (98). Besides a diet high in saturated fat, a diet high in carbohydrates, particularly fructose, also induces inflammation in muscle (39, 99). In addition to the potential direct effect of high carbohydrates, elevated levels of TGRLs may contribute to muscle inflammation induced by a high-carbohydrate diet.

Under physiologic conditions, triglyceride in TGRLs is hydrolyzed by lipoprotein lipase (LPL) and releases FFAs, which are transferred into SM mainly as an energy source and into adipocytes, where they are re-esterified into triglyceride for storage (100). Increased blood TGRL levels (with no or modest changes in LPL activity; ref. 100) in obesity or increased LPL activity is expected to enhance TGRL-derived FA transfer into SM, leading to increased muscle lipid deposition and eliciting muscle inflammation. Indeed, obesity or muscle-specific overexpression of LPL increases muscle triglyceride content, with increased FA metabolites, including diacylglycerol (DAG) and ceramide, while muscle deletion of LPL decreases lipid content in SM (101–103). LPL-mediated lipid transfer appears to involve apolipoprotein E (apoE), as apoE deficiency impairs FA delivery, leading to less lipid content and decreased inflammation in muscle (58).

The renin-angiotensin system in immune cell activation. In addition to cytokines and FAs, the renin-angiotensin system (RAS), which is activated locally in SM and AT and systemically in obesity (104, 105), has been involved in regulation of inflammation including immune cell inflammation (106–108). The classical RAS involves cleavage of angiotensinogen by renin in the circulation and formation of angiotensin I (ANG I). ANG I is converted to active ANG II by angiotensin-converting enzyme (ACE), which is mainly expressed on endothelial cells in pulmonary circulation. The nonclassical RAS involves generation of ANG 1–7 from ANG I or II by ACE2 (109, 110).

By interacting with ANG II receptors (ATRs), ANG II plays important roles in regulating blood pressure and fluid and electrolyte balance (109, 110). In addition, ANG II plays pathologic roles in fibrosis, oxidative stress, and inflammation, which all occur in obesity, via hemodynamic (blood flow reduction) or non-hemodynamic effects (109, 110). ANG II can induce activation of NF-κB, expression of MCP-1, TNF-α, and VCAM-1, and production of ROS (which activates p38 MAPK) in monocytes, endothelial cells, and cultured myocytes (106–108, 111–113). ACE inhibitors and ATR blockers (ARBs) reduce inflammation, including SM and AT inflammation induced by obesity or fructose feeding (39, 114), indicating a crucial role of ANG II in SM and AT inflammation in obesity. In contrast, ANG 1–7 exerts cellular effects mainly through the Mas receptor (109, 110) and has antiinflammatory effects including inhibition of macrophage infiltration and proinflammatory activation in AT induced by HFD or high-fructose diet (115, 116).

The impact of inflammation on insulin resistance in SM

Local muscle inflammation may alter myocyte insulin sensitivity via paracrine or autocrine effects. TNF-α or conditioned medium from Th1 cells or activated macrophages decreases myocyte insulin sensitivity (35, 40, 47). In mice, local inflammation induced by
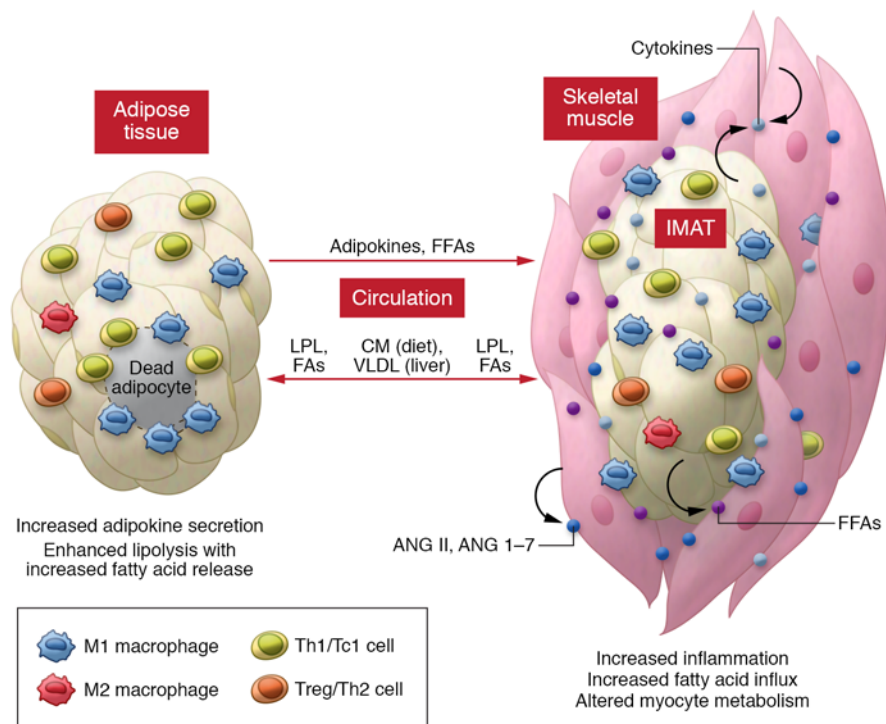
Figure 2. Inflammatory effects on myocytes in obesity. In obesity, increased infiltration and activation of immune cells in skeletal muscle (mainly in IMAT/PMAT) and myocyte inflammation lead to increased secretion of proinflammatory cytokines, which negatively regulate myocyte metabolic functions through paracrine or autocrine effects. Inflammation in visceral AT, with increased secretion of inflammatory adipokines, may also adversely affect myocyte metabolic function through endocrine effects. In addition, inflammatory effects on adipocytes in visceral AT and IMAT/PMAT may accelerate FFA release and transfer into myocytes, resulting in myocyte inflammation and metabolic dysfunction. Furthermore, elevated levels of TGRLs, including diet/enterocyte-derived chylomicrons (CM) and liver-derived VLDL may undergo enhanced LPL-mediated triglyceride hydrolysis, increasing FA release and transfer into skeletal muscle (and AT) and contributing to myocyte inflammation and metabolic dysfunctions. Activation of the RAS in skeletal muscle with local production of ANG II and ANG 1–7 may also regulate myocyte inflammation and metabolic functions.

muscle MCP-1 overexpression impairs muscle insulin signaling in some (37) but not all (76) studies, while local inflammation reduced by muscle-specific IL-10 overexpression improves obesity-induced muscle insulin resistance (60). In addition, inflammatory molecules from other tissues, particularly visceral AT, may influence SM metabolic functions through endocrine effects and adverse effects on adipocytes with increased release of FFAs into circulation. FFAs are then transferred from the circulation into muscle, where they induce myocyte inflammation and metabolic dysfunction. In obesity, elevated TGRLs release large amounts of FAs into SM and can also adversely affect myocyte metabolism. Moreover, activated RAS with local production of ANG II and ANG 1–7 may regulate insulin sensitivity in SM myocytes (Figure 2).

SM myocytes express TLR2 and TLR4 (52), which play essential roles in FA-induced effects. Individuals with obesity and T2D have increased TLR4 expression and signaling in SM (32). Inhibition or deletion of TLR4 protects against lipid-induced insulin resistance in cultured myocytes or mouse SM (117). ANG II may contribute to SM insulin resistance by reducing blood flow to SM and by directly impairing myocyte insulin signaling via increasing mitochondrial ROS production and activating inflammatory pathways (109, 110, 118, 119). ACE inhibitor or ARB treatment of human subjects with hypertension and insulin resistance is usually associated with improvement of insulin resistance (109, 118). Elevation of ANG II in animals increases oxidative stress and inflammation and decreases insulin sensitivity in SM while blockade of RAS reverses these effects (113). In contrast to ANG II, ANG 1–7 has beneficial effects on insulin sensitivity including direct insulin-sensitizing effects on SM (109, 110, 115, 120).

Below we summarize potential roles of various inflammatory pathways, which are depicted in Figure 3, in SM myocyte insulin resistance.

The IKK/NF-κB pathway. The main activators for the IKK/NF-κB pathway in obesity may include TNF-α, IL-1β, FAs, and ANG II. SM myocytes from obese subjects with T2D show enhanced activation of the IKK/NF-κB pathway (31). In cultured cells, overexpression or activation of IKKβ impairs insulin signaling (121), whereas inhibition of the IKK/NF-κB pathway prevents palmitic acid– or TNF-α–induced insulin resistance (117, 121, 122). In mice, inhibition or reduction of IKKβ prevents obesity- or lipid-induced insulin resistance in SM (121, 123), indicating a role of the IKK/NF-κB pathway in insulin resistance. The IKK/NF-κB pathway may cause insulin resistance via increased IKK-mediated serine phosphorylation of insulin receptor substrate 1 (IRS-1) or insulin receptor (IR), leading to impairment in insulin-induced tyrosine phosphorylation and subsequent inhibition of downstream insulin signaling, suppressed expression of molecules such as GLUT4 in the insulin signaling cascade, and induced expression of molecules such as inducible NOS, which may promote IRS-1 serine phosphorylation and induce nitration of IRS-1 tyrosine residues, leading to impaired insulin signaling (2, 122, 124).

Though the studies above establish signaling mechanisms by which the IKK/NF-κB pathway may influence obesity, the in vivo role of the muscle IKK/NF-κB pathway in obesity-linked insulin resistance remains unclear. Muscle-specific deletion or overexpression of IKKβ in mice does not impact muscle insulin sensitivity and systemic glucose tolerance (125, 126). Therefore, the beneficial effects of anti-IKK therapy on muscle insulin sensitivity are likely derived from its antiinflammatory effects on immune cells (121, 123), consistent with the observation that ablation of IKKβ in myeloid cells protects mice from obesity-induced insulin resistance (88).

JNKs and MAPKs. JNKs are members of the MAPK family and can be activated by TNF-α, IL-1β, ER stress, saturated FAs, LTB4, and ANG II (78, 109, 127). JNK activity is increased in SM of obese
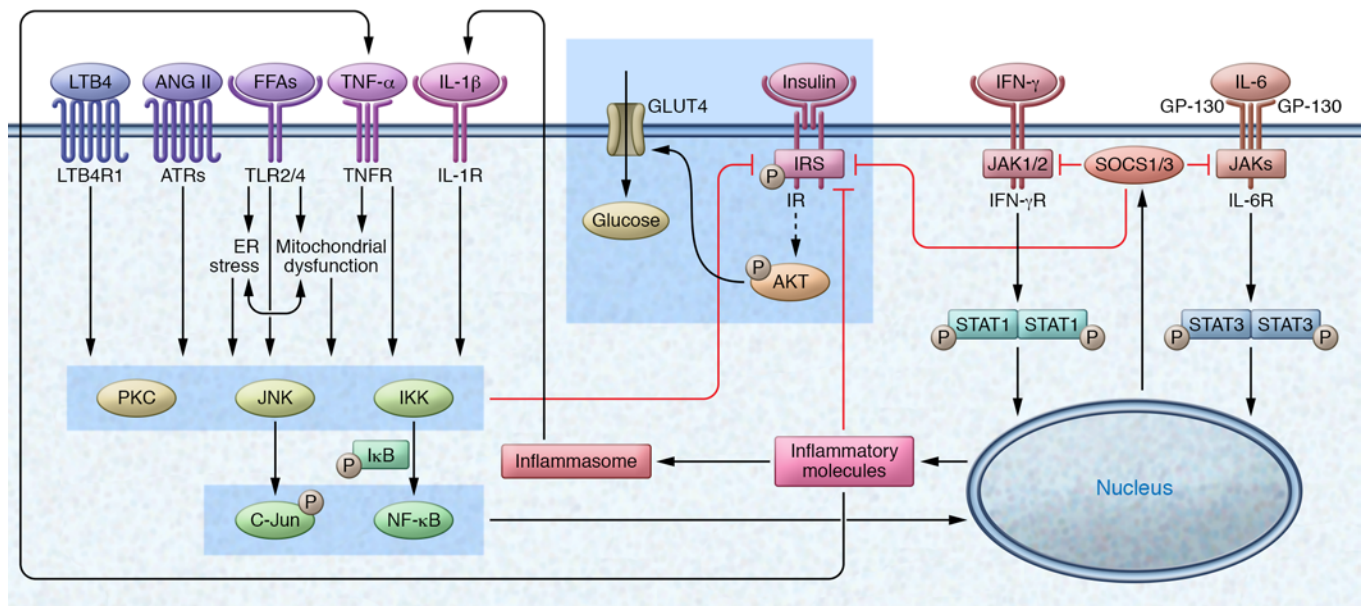
Figure 3. Inflammatory signaling mediates insulin resistance in myocytes. Increased levels of cytokines such as TNF-α and IL-1β from M1-like macrophages, saturated FFAs derived from TGRL–triglyceride hydrolysis and adipocyte lipolysis, LTB4 derived from arachidonic acid metabolism, and ANG II derived from RAS activate the PKC, JNK, and IKK/NF-κB pathways in myocytes via interactions with their receptors on the cells. These inflammatory pathways can all impair insulin signaling by increasing serine or threonine phosphorylation and disrupting insulin-stimulated tyrosine phosphorylation of IR or IRS, or by downregulating molecules involved in insulin signaling. IFN-γ from Th1 cells and IL-6 activate JAK/STAT1/3 pathways, which may also impair insulin signaling in myocytes (possibly through SOCS proteins, particularly SOCS1 and SOCS3, which interrupt the interaction of IR with IRS-1 and IRS-2, inhibit IR tyrosine kinase activity, and interact with IRS-1 and IRS-2, leading to their ubiquitin-mediated degradation). PKCs, JNK, IKK/NF-κB, and STAT1/3 may also impair insulin signaling through other, undefined inflammatory pathways. SOCSs are involved in a negative feedback loop that leads to the termination of inflammatory effects of STAT through downregulation of JAK activity, which blocks further STAT phosphorylation.

mice and humans (89, 91, 128). In cultured myocytes, palmitate-induced insulin resistance is accompanied by increased JNK activity (129), whereas JNK knockdown attenuates palmitate-induced insulin resistance (130). JNKs also contribute to oxidative stress–induced insulin resistance in isolated SM (131). One study showed that muscle-specific deletion of JNK1 in mice selectively protected muscle against obesity-linked insulin resistance (132). However, another study showed no effects of muscle-specific overexpression or deletion of JNK1 on insulin sensitivity and glucose metabolism in mice (133). JNKs may cause insulin resistance directly by inducing serine and threonine phosphorylation of IRSs, thereby disrupting the interaction of IRSs with IR to impair downstream insulin signaling (93, 127).

Other MAPKs, particularly p38 MAPK, may also be involved in obesity-linked muscle insulin resistance. TNF-α, oxidative stress, or conditioned medium from palmitate-treated macrophages impairs insulin signaling and activates p38 MAPK in myocytes. Moreover, p38 MAPK inhibition or knockdown attenuates insulin resistance (45, 134, 135).

PKCs. The roles of PKCs in metabolic functions have been reviewed in detail elsewhere (136). Studies of PKC effects on muscle insulin sensitivity have focused mainly on conventional PKCs (cPKCα, -βI, -βII, and -γ) and novel PKCs (nPKCδ, -ε, -η, and -θ), which both rely on DAG for full activation. In humans, raising plasma FA levels by lipid infusion with acute induction of muscle insulin resistance results in increased DAG content that is temporally associated with PKCθ, PKCδ, and PKCβII activation (137, 138). Obese subjects with T2D have increased PKCθ activity in

SM (139). Rats fed a HFD show increased muscle expression and translocation of PKCθ and PKCε and decreased glucose disposal (140). Palmitate treatment of cultured myocytes, with induction of insulin resistance, increases PKCθ activation (122).

Most previous studies support important roles of PKCs in obesity- or lipid-induced muscle insulin resistance and inflammation. For example, inhibiting PKCθ activation prevents palmitate-induced insulin resistance and TNF-α expression in cultured myocytes (122). Dual inhibition or co-silencing of PKCθ and PKCε attenuates insulin resistance and inflammatory responses of myocytes to conditioned medium from palmitate-activated macrophages (141). Ablation of PKCθ in mice protects against lipid infusion–induced SM insulin resistance (142). Muscle PKCδ levels increase with age in mice, and muscle-specific deletion of PKCδ improves muscle insulin resistance and whole-body insulin sensitivity in aged mice (143). PKCs may induce insulin resistance by increasing serine or threonine phosphorylation of IR or IRS-1, with resultant impairment in downstream insulin signaling (136, 138, 142, 144).

JAK/STAT pathways. Major activators of JAK/STAT pathways include IFNs, other cytokines, and growth factors. JAKs are the main upstream molecules that phosphorylate and activate STATs. IFN-γ mainly induces tyrosine phosphorylation and activation of STAT1 via JAK1 and JAK2. In addition, engagement of TLR2 and TLR4 can activate JNK and MAPK (47, 91, 145–147), which can also induce STAT1 phosphorylation via JAK1 (145, 148). STAT1 phosphorylation is increased in SM of obese mice; ablation of Th1 cells in αβT cell–deficient mice blunts this effect (35).

Treating myocytes with IFN-γ or Th1-conditioned medium increases STAT1 phosphorylation and decreases insulin sensitivity (35, 149), whereas pretreating myocytes with a JAK inhibitor attenuates Th1-conditioned medium–induced STAT1 phosphorylation and improves insulin sensitivity (35). Treatment of obese mice with a JAK1/JAK2 inhibitor improves systemic insulin resistance in conjunction with reduced SM inflammation (35). However, a potential in vivo role of the muscle JAK/STAT1 pathway in obesity-linked insulin resistance remains unconfirmed.

STAT3 phosphorylation is increased in SM of humans with T2D and positively correlated with FFA levels and measures of insulin sensitivity (150). IL-6 induces STAT3 phosphorylation and activation. Palmitate, which induces insulin resistance, also induces STAT3 phosphorylation in cultured myocytes; silencing STAT3 attenuates palmitate-induced insulin resistance, indicating a role for STAT3 in myocyte insulin resistance (150). However, muscle-specific deletion of STAT3 in mice does not appear to alter obesity-linked insulin resistance (151).

The mechanisms by which the JAK/STAT pathways contribute to insulin resistance remain unclear. One possibility is their regulation of suppressor of cytokine signaling (SOCS) proteins, particularly SOCS1 and SOCS3, which are downstream of STATs and are involved in a negative feedback loop that leads to termination of inflammatory effects by downregulating JAK activity, thereby blocking further STAT phosphorylation (152). Obesity with T2D increases SOCS1 and SOCS3 expression in muscle (150, 153, 154). Overexpression of SOCS1 or SOCS3 in cultured myocytes decreases insulin-stimulated glycogen synthesis (153). SOCS1 and SOCS3 may directly inhibit insulin signaling by interrupting the interaction of IR with IRSs, inhibiting IR tyrosine kinase activity, and interacting with IRSs to induce their ubiquitin-mediated degradation (152, 153, 155). Muscle-specific deletion of SOCS3 in mice protects against obesity-induced insulin resistance (156); overexpression of SOCS3 in muscle exacerbates obesity and insulin resistance in mice (157).

Because of the negative feedback roles of SOCSs in inflammation (152), direct inhibition or knockdown of SOCS1 and/or SOCS3 is expected to improve insulin resistance, but also to enhance inflammation, which may counteract beneficial effects on metabolic functions. Indeed, macrophage-specific ablation of SOCS1 or liver-specific deletion of SOCS3 in mice increases systemic inflammation and insulin resistance (158, 159). Therefore, targeting upstream molecules such as JAK or STAT may provide more therapeutic benefits on insulin sensitivity and inflammation.

The NLRP3 inflammasome may also play a role in the regulation of myocyte insulin sensitivity, likely by mediating IL-1β production. Overexpression of perilipin 2, a lipid droplet–associated protein, resulted in increased expression of NLRP3 and impaired insulin-stimulated glucose uptake in cultured myocytes. Knocking down NLRP3 reversed this effect (160). Further studies need to be carried out to confirm the potential role and examine the exact mechanisms by which the inflammasome participates in myocyte insulin resistance in obesity.

Conclusions and perspectives

Accumulating evidence indicates that obesity is associated with increased inflammation in SM, which is mainly manifested by enhanced immune cell infiltration in IMAT/PMAT and also includes increased myocyte inflammation. Increases in IMAT/PMAT are associated with obesity, aging, inflammation, and diabetes. The immune cells in IMAT/PMAT in obesity tend to polarize into proinflammatory phenotypes with increased expression of proinflammatory molecules, which, in conjunction with inflammatory molecules from other tissues, particularly from AT, may negatively regulate myocyte metabolic functions, contributing to insulin resistance locally in SM and systemically in the whole body. Targeting IMAT/PMAT may be a promising approach to prevent T2D. Exercise and weight loss have been shown to reduce both IMAT and visceral AT accompanied with improved physical performance; these effects were independent of the change in total fat (66). We postulate that exercise combined with modest weight loss leads to major effects on IMAT/PMAT — both the absolute amount and phenotype — with important benefits on muscle insulin/glucose metabolism that may help to explain the large reduction in new-onset diabetes observed with the Diabetes Prevention Program (161).

Based on the roles of chronic inflammation in obesity-linked metabolic dysfunction, antiinflammatory therapy has also been viewed as a promising strategy for obesity-linked metabolic disease. However, most effects demonstrated in tissue culture or animal models remain to be confirmed in humans. Moreover, current knowledge about the particular pathways that mediate obesity-linked inflammation is limited and remains an obstacle to the development of novel, specific "obesity-targeted" antiinflammatory approaches. Future study is needed to identify specific inflammatory pathways closely related to obesity, which could be therapeutically targeted to treat obesity-linked metabolic disease.

Address correspondence to: Huaizhu Wu or Christie M. Ballantyne, Baylor College of Medicine, 6565 Fannin, M.S. A-601, Houston, Texas 77030, USA. Phone: 713.798.4141; E-mail: hwu@bcm.edu. (H. Wu). Phone: 713.798.5034; E-mail: cmb@bcm.edu (C.M. Ballantyne).

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