Fat in flames:

Influence of cytokines and pattern recognition receptors on adipocyte lipolysis

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Abstract:

Adipose tissue has the largest capacity to store energy in the body and provides energy through the release of free fatty acids during times of energy need. Different types of immune cells are recruited to adipose tissue under various physiological conditions indicating that these cells contribute to the regulation of adipose tissue. One major pathway influenced by a number of immune cells is the release of free fatty acids through lipolysis during both physiological (e.g. cold stress) and pathophysiological processes (e.g. obesity, type 2 diabetes). Adipose tissue expansion during obesity leads to immune cell infiltration and adipose tissue remodeling, a homeostatic process that promotes inflammation in adipose tissue. The release of pro-inflammatory cytokines stimulates lipolysis and causes insulin resistance leading to adipose tissue dysfunction and systemic disruptions of metabolism. This review will focus on the interactions of cytokines and other inflammatory molecules that regulate adipose tissue lipolysis during physiological and pathophysiological states.

Keywords: Adipocyte, lipolysis, cytokines
Introduction:

Lipolysis is the hydrolysis of triglycerides (TGs) to free fatty acids (FFAs) and glycerol. A variety of enzymes are involved in this process that vary depending on the cell type. In adipocytes, lipolysis occurs via the actions of adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase (Figure 1). The lipolytic pathway is highly regulated by both hormonal and nutritional factors. Stimulation of lipolysis provides FFA release from adipocytes that is critical in times of negative energy balance, including exercise and fasting. However, the inability to inhibit lipolysis when FFA are not needed can have serious metabolic consequences including the development of type 2 diabetes mellitus (T2DM). A great deal of progress on dissecting the molecular regulation of adipose tissue lipolysis has occurred in the past two decades and has been recently reviewed (2, 52). The regulation of lipolysis occurs by multiple mechanisms including modulation of transcription and translation, post-translational modifications, cellular localization, protein–protein interactions, and protein stability/degradation (2, 52).

There are many stimulators of adipocyte lipolysis including catecholamines, natriuretic peptides, growth hormone, glucocorticoids, and TNFα. Whereas, the primary anti-lipolytic pathway is regulated by insulin. The mobilization of FFA from adipose tissue is recognized as playing an important role in insulin resistance and T2DM and support the notion that dysregulation of adipose tissue lipolysis is a critical factor in metabolic disease states.

The primary source of lipolytic regulators produced in adipose tissue are cytokines. Table 1 summarizes influential cytokines that regulate adipocyte lipolysis. Enhanced adipose tissue cytokine release during obesity drives low-grade inflammation resulting in systemic impairments of glucose and insulin tolerance and lipid metabolism (53). However,
the metabolic response to obesity is varied, and there is a considerable proportion of obese individuals that lack obesity associated disease and are metabolically healthy (57). Recent studies implicate lipid storage, extracellular matrix composition and inflammation as factors that distinguish metabolically healthy versus metabolically unhealthy obesity (31, 38, 47, 58). Secretion of cytokines from immune cells in adipose tissue appears to be a key component linking inflammation, lipid storage, unregulated lipolysis, ectopic fat and metabolic disease through both paracrine and endocrine mechanisms (42). Storage of lipids in adipose tissue protects against the development of metabolic disease (33), while the inability to store lipids in adipose tissue redistributes body fat leading to ectopic storage and metabolic impairment (6). This review will focus on how cytokines influence adipocyte lipolysis and how this relates to the development of insulin resistance and obesity associated comorbidities.

**TNFα stimulated lipolysis:**

Tumor necrosis factor α (TNFα) was the first cytokine shown to be produced in adipose tissue and increased in conditions of obesity and insulin resistance (28). TNFα is a potent mediator of adipose tissue and systemic insulin resistance (28). TNFα induces insulin resistance through multiple mechanisms that include inhibition of adipogenesis (81), inhibition of insulin sensitive glucose uptake in mature adipocytes (27), inhibition of glucose transporter 4 (GLUT4) (76), insulin receptor (IR), and insulin receptor substrate-1 (IRS-1) expression (75), and the ability to alter IR and IRS-1 phosphorylation (27). Accordingly, animals that lack TNFα (26) have improved systemic insulin sensitivity and glucose tolerance. TNFα signals through TNFα receptor 1 and 2, and leads to transcriptional changes mediated through activation of nuclear factor-κB (NF-κB) and
extracellular signal related kinase (ERK) signaling (Figures 1 and 2). Mice that lack TNF receptor 1 and 2 are protected against insulin resistance and glucose intolerance (85). In both human and murine adipocytes, signaling through TNFR1 appears to have the largest contribution to insulin resistance (55, 90).

Adipose tissue macrophages (ATM) produce the majority of TNFα in adipose tissue (93). It has been known for over a decade that adipose tissue expression of TNFα increases with macrophage number and is associated with the development of insulin resistance (25, 79). ATMs increase acutely during time of lipolysis including fasting and pharmacological activation (37). During a weight loss time course in diet-induced obese C57BL/6 mice, ATMs are reduced by day 21 and continue to decrease until day 60 (37). Interestingly, over this same time course tissue TNFα levels are not reduced as would be expected with decreased ATMs (37). Whether TNFα expression is increased at the level of gene expression, or a different cell type is contributing to the maintenance of TNFα expression is not known. However, it is well established that TNFα reduces LPL expression in adipocytes (32). The coupling of reduced LPL expression and lipolysis may be necessary for fat pad contraction during weight loss. It is unknown whether the expression of TNFα and pro-inflammatory cytokines in adipose tissue are reverted to that of lean phenotype after prolonged weight loss. Future studies will likely investigate the impact of inflammatory cytokines on subsequent weight cycling and lipid distribution.

In addition to prominent effects on insulin sensitivity and LPL in adipocytes, TNFα also alters adipocyte metabolism through the induction of lipolysis in many species including humans (16-18, 65, 71, 73, 97). Unlike the acute effects of beta-adrenergic signaling-induced lipolysis, TNFα-induced lipolysis occurs ~3 hours after treatment.
indicating a transcription dependent mechanism (96). Multiple mechanisms have been
demonstrated to play a role in TNFα-induced lipolysis including intracellular signaling
cascades, metabolites and lipid droplet associated proteins (Figure 1). Both ATGL and HSL
contribute to TNFα-induced lipolysis (96). To achieve maximal lipolysis in response to
TNFα, the ATGL regulating protein comparative gene identification 58 is required and
expression of the inhibitory protein G0/G1 switch 2 must be decreased in 3T3-L1
adipocytes (96). Treatment of adipocytes with TNFα activates ERK signaling pathways, and
blocking ERK activation results in a reduction of lipolysis in primary human adipocyte and
3T3-L1 adipocytes (72, 97). ERK inhibition is also associated with a TNFα-induced
reduction of the lipid droplet associated protein, perilipin 1 (97). In primary human
adipocytes, inhibition of NF-κB subunit p65 nuclear translocation results in a reduction of
glycerol release in response to TNFα (41). Interestingly, in addition to reducing lipolysis,
the combination of TNFα and NF-κB inhibition resulted in a significant decrease of perilipin
and HSL gene and protein expression, but did not alter ATGL gene expression (41).

Metabolites also play a role in regulating lipolysis. Adenosine reduces lipolysis
through activation of Gi proteins that inhibit adenylyl cyclase (Figure 1) (24). In primary
rat adipocytes, TNFα reduces Gi proteins in a proteasome dependent manner (3, 16) that
results in a loss of inhibitory signaling through the adenosine A1 receptors and enhanced
lipolysis. In 3T3-L1 adipocytes, the lipolytic effects of TNFα requires glucose and lipolysis
increases with greater concentrations of glucose in the cell culture media (18).
Additionally, this effect of glucose is specific to sugars that can support lactate production.
Like glucose, mannose also supports higher rates of glycerol release and release of lactate
from adipocytes, while sugars that do not support lactate formation (galactose and
fructose) limit TNFα-induced glycerol release (18). Consistent with the previous observations, inhibition of glycolysis inhibits glycerol release in response to TNFα (18). Phosphorylation of ERKs in response to TNFα occurs both in the presence and absence of glucose, and a decrease in perilipin occurs in the presence and absence of glucose (18). These data indicate that metabolites produced by glycolysis support lipolysis, however the acute signaling and mechanisms underlying these findings remain unknown. It is interesting to note that the induction of lipolysis via β-adrenergic receptors does not require glucose (18). Collectively, these studies indicate that the NF-κB and ERK signaling pathways as well as glucose metabolism contribute to TNFα-induced lipolysis. Interestingly, co-culture of the macrophages with 3T3-L1 adipocytes, causes increased basal glucose uptake in 3T3-L1 adipocytes, increase GLUT1 protein content and insulin resistance (46). This insulin resistance is significantly rescued by antibody mediated TNF depletion (46). Currently, the effects of TNF targeted therapies on lipolysis have not been studied in clinical trials. However, with the increased use of these medications for inflammatory conditions there may be potential to examine their effects. The influence of TNFα on lipolysis has been demonstrated across multiple cell lines, but the importance of each pathway is not known and likely varies depending on the type and/or source of adipocytes. Integration of these pathways is needed to understand the acute regulation of TNFα-induced lipolysis and how it is linked to substrate metabolism.

Interleukin 1β, Interleukin 1α, Interleukin 18:

The secretion of both of interleukin-1β (IL-1β) and interleukin-18 (IL-18) is regulated by caspase-1 (68). In the obese condition, activation of nucleotide-binding oligomerization domain-like receptor, pyrin domain containing (NLRP3) inflammasome
regulates caspase-1 activation to influence the synthesis of IL-1β and IL-18 (88). The NLRP3 inflammasome is activated in response to metabolic danger signals (e.g. ceramides, fatty acids and hyperglycemia) and thus plays an important role in linking impairments of metabolism during obesity and T2DM with cytokine secretion and insulin resistance (88, 94, 99). Knockout of the NLRP3 inflammasome reduces adipose tissue inflammation during diet-induced obesity and leads to improvements in insulin sensitivity in skeletal muscle, liver, and adipose tissue (78, 88, 94). Although the NLRP3 inflammasome is primarily expressed in macrophages and dendritic cells, its components are present in adipocytes (19). Caspase-1 -/- and NLRP3 -/- adipocytes have increased capacity to store lipids and increased expression of GLUT4, adiponectin and peroxisome proliferator-activated receptor γ (PPARγ), which are associated with improved adipose tissue function (77). The NLRP3 inflammasome and caspase-1 influence adipogenesis and the storage of lipids in adipocytes, however the in vivo function of adipocyte expressed NLRP3 and its contribution to insulin sensitivity has not been investigated.

Initial studies using recombinant IL-1 demonstrated its ability to increase lipolytic activity in adipocytes (11, 60). More recently, studies in mouse and human primary adipocytes showed that IL-1β represses adipogenesis and decreases the expression of PPARγ, adiponectin and GLUT4 (39, 77). In response to chronic treatment with IL-1β, mouse 3T3-L1 and 3T3-F442A and human adipocytes have reduced phosphorylation of IR, IRS-1, protein kinase B (AKT) and ERK1/2 in a dose dependent manner (39, 77). Along with changes in insulin signaling, IL-1β also increases the basal rate of lipolysis in 3T3-L1 but not 3T3-F442A adipocytes (39). Interleukin 1α (IL-1α) is known to have similar effects on insulin resistance (21). IL-1α and β signal through the IL-1 receptor 1, whose activation
results in NK-κB dependent transcription (Figure 2). Mice that lack the IL-1 receptor are protected against inflammation associated with diet-induced obesity and have improved glucose tolerance. The effects induced by IL-1β and IL-1α may be in part mediated by their ability to induce interleukin-6 (IL-6) expression and subsequently signal transducer and activator of transcription 3 (STAT3) activation, which are known to contribute to lipolysis (13, 84). Unlike IL-1β & IL-1α, IL-18 does not influence adipogenesis or appear to cause insulin resistance in adipocytes (77). IL-18 modestly enhances insulin-stimulated glucose uptake and modestly counteracts the reduction of insulin-stimulated glucose uptake induced by TNFα in 3T3-L1 adipocytes (89). Yet, there is also evidence that IL-18 suppresses adiponectin gene expression and secretion in cultured mouse adipocytes (4). It is likely that IL-18 does not share the lipolytic properties of IL-1β and IL-1α since mice that lack IL-18 develop obesity and insulin resistant due to hyperphagia (50). There is growing interest in IL-1 targeted therapies for the management of inflammatory disease. Interestingly, glyburide, an insulin secretagogue, is also an inhibitor of inflammasome activation (40). It appears to have anti-inflammatory effects in addition to its ability to increase insulin secretion and lower blood glucose, triglycerides, and free fatty acids. The contributions of inflammasome inhibition versus effects on insulin secretion have not been examined. Anakinra, an IL-1 receptor antagonist, has been shown to enhance insulin secretion and lower HbA1C, however it has minimal effects on triglycerides and blood cholesterol (40). Thus, IL-1β and IL-1α and IL-18 appear to have divergent metabolic effects that merit further study to determine the impact of inflammasome activation on adipocytes and adipose tissue.

gp130 cytokines
The interleukin-6 (IL-6) family of cytokines is a group of functionally and structurally related proteins that consist of (IL-6), interleukin-11, interleukin-27, leukemia inhibitory factor (LIF), oncostatin M (OSM), ciliary neurotrophic factor (CNTF), cardiotrophin-1 (CT-1), cardiotrophin-like cytokine (CLC), and neuropoietin (NP) (22). These cytokines regulate a variety of complex biological processes. Because all IL-6 family members utilize glycoprotein 130 (gp130) receptor and it is required for signaling, the IL-6 family is commonly referred to as gp130 cytokines (10). IL-6 family cytokines utilize gp130 and other receptor proteins including the oncostatin receptor and LIF receptor. Once activated, signaling by gp130 family members causes STAT3 translocation to the nucleus and STAT3-dependent transcription (Figure 2). It is well known that IL-6 concentrations are increased in adipose tissue and circulating levels are associated with metabolic impairments during obesity and T2DM (59, 91). However, IL-6 levels are also increased during exercise and may contribute to some of the beneficial effects of physical activity on health (54). A study in humans demonstrated that IL-6, at concentrations that do not affect catecholamines or TNFα, induces lipolysis and enhances fatty acid oxidation (87). In the older adults, IL-6 increased the rate of appearance of free fatty acids as well as fatty acid disposal further confirming an in vivo effect of IL-6 on adipose tissue lipolysis and fatty acid oxidation in humans (56). Mouse models of IL-6 deletion have generated contradictory data. It has been reported that IL-6-/- mice develop mature onset obesity (92), while others have not observed this effect (7). Treatment of 3T3-L1 adipocytes with IL-6 increases glycerol and NEFA release. However, there is variation in the degree of magnitude observed across studies (30, 56). Also, IL-6 induced lipolysis appears to be influenced by other hormonal factors. The combined treatment of dexamethasone and growth hormone
is capable of inhibiting IL-6 induced lipolysis (56). It is noteworthy that the in vivo changes in lipolysis and lipid oxidation in humans can be mimicked in cell culture models. In addition to the lipolytic actions observed in adipocytes, treatment of L6 myotubes with IL-6 increases palmitate oxidation (56). A role for IL-6 in energy expenditure was demonstrated in IL-6/- mice under cold stress. IL-6/- mice have reduced energy expenditure under basal conditions and when challenged with cold stress have a reduced ability to maintain core temperature (95). It is interesting that uncoupling protein 1 (UCP1) expression in brown adipose tissue was not different in WT and IL-6/- mice indicating a capacity for thermogenesis; however it may be that the delivery of substrates to the tissue was reduced. The contribution of IL-6 to cold-induced lipolysis in WAT for sustaining energy production during cold-induced thermogenesis has not been addressed.

Although IL-6 the effects of IL-6 on lipolysis have been studied, it is only one member of the larger gp130 cytokine family, and the effects of other cytokine members of this family on adipose tissue lipolysis have not been well studied. LIF modifies adipocyte lipid metabolism by reducing LPL activity, slightly increasing lipogenesis and modestly increasing lipolysis in cultured murine adipocytes (48). CT-1 has recently been shown to be important in the control of glucose homeostasis and metabolism as mice that lack CT-1 have reduced energy expenditure and increased body weight (49). CT-1/- mice have reduced expression of ATGL and HSL in adipose tissue at 12 months of age and increased basal lipolysis. However, when adipocytes were isolated from 2 month old WT and CT-1/- mice there were no differences in basal lipolysis, indicating that changes in adipocyte lipolysis were secondary to weight gain (49). Delivery of recombinant CT-1 (0.2 mg/kg/day) to ob/ob mice for 10 days was shown to enhance glycerol release when
compared to pair-fed mice. Recombinant CT-1 activates lipolysis in 3T3-L1 adipocytes (44).

CNTF remodels adipocyte metabolism by promoting mitochondrial biogenesis and reducing fatty acid esterification and lipogenesis, however it had no effects on lipolysis (5). In general, the effects of gp130 cytokines on lipolysis have received minimal investigation. Hopefully, future studies will address the effects of this family of cytokines on adipocyte lipolysis as one of these cytokines, oncostatin M, is produced in immune cells in adipose tissue and highly regulated in mouse and human obesity and T2DM (66).

Pattern recognition receptors:

The systemic response to pattern associated molecular patterns (PAMPs) found on pathogens including bacteria, viruses and fungi is characterized by the mobilization of energy resources including glucose and fatty acids. Toll-like receptors (TLR) are one class of pattern recognition receptors and human adipose tissue as a whole expresses most TLRs (36). However, 3T3-L1 adipocytes are only responsive to TLR1, TLR3, TLR4, and TLR2/6 agonists, but not TLR5 agonists (36). Stimulation of adipocytes with lipopolysaccharide (LPS) causes nuclear translocation of NF-κB and associated changes in NF-κB responsive genes (36). Specifically, TLR ligand stimulation results in enhanced gene expression and secretion of cytokines and chemokines from adipocytes and both c-Jun N-terminal kinase and mitogen activated protein kinase signaling pathways regulate this process (35). Interestingly, the stage of adipocyte development influences the response to stimulation with TLR ligands (36). In adipose tissue, preadipocytes exhibit the highest secretion of IL-6, while mature adipocytes exhibit the highest monocyte chemoattractant protein-1 (MCP-1) secretion. Adipocytes generated from human bone marrow mesenchymal stem cells express TLR 1-10 (14). Similar to 3T3-L1 adipocytes, adipocytes derived from
mesenchymal stem cells are responsive to TLR 1-4 and TLR 6 agonists (14). However, these cells are also responsive to TLR5 agonists and do not respond to TLR7-9 agonists (14). Lipolysis measured by glycerol release is induced in these cells by the danger associated molecular patterns poly (I:C) that signals through TLR3, and LPS. Interestingly, both poly (I:C) and LPS had a comparable induction of lipolysis (14). Adipocytes appear very responsive to TLR3 and TLR4 stimulation, yet is not known whether other TLR activators would produce a comparative lipolytic response. Because there is variation in the gene expression response to these stimuli, there also are likely variations in the physiological responses.

In addition to the TLRs, nucleotide-binding oligomerization domain-containing protein (NOD) 1 and 2 are also expressed in adipocytes. NOD1 and NOD2 expression is increased during adipogenesis in 3T3-L1 and primary human cells (29). Activation of NOD1 suppresses adipocyte differentiation and expression of PPARγ, CCAAT/enhancer binding protein α, fatty acid binding protein 4 and leptin in a dose-dependent manner (29). NOD2 does not suppresses adipocyte differentiation in 3T3-L1 adipocytes, but does suppress differentiation of adipose-derived adult stem cells, indicating some possible species-specific effects (29). NOD1 activation results in increased NF-κB activity, and the expression and secretion of MCP-1 and regulated on activation, normal T cell expressed and secreted (RANTES) from 3T3-L1 adipocytes (98). Similarly, glycerol and free fatty acid release is increased in response to NOD1 agonists (61). The lipolytic actions of NOD1 agonist are dependent on NF-κB, protein kinase A, and hormone sensitive lipase (61). In human primary adipocytes, the same trend of increased MCP-1 and IL-6 expression is observed (98). Interestingly, NOD1/2−/− mice have reduced gonadal adipose tissue mass,
improved insulin sensitivity, and reduced adipocyte size and liver lipid accumulation during high-fat feeding (67). Systemic administration of NOD1 ligand (FK156) during hyperinsulinemic-euglycemic clamp studies in mice results in a reduced rate of glucose infusion and reduced glucose disposal, while the NOD 2 ligand muramyl dipeptide had only modest effects (67).

The role of the adipocyte as a participant in the response to innate immune activators is ambiguous. However, it is obvious that there is a lipolytic response to both pro-inflammatory cytokines and pathogen associated molecular patterns in adipocytes. It is intriguing that in addition to a significant lipolytic response in adipocytes there is also a global remodeling of lipid metabolism during infection. In the liver, these proinflammatory signals can shift metabolism toward fatty acid synthesis and away from fatty acid oxidation and ketogenesis (34). Lipoproteins including very-low density lipoprotein, low-density lipoprotein, and high-density lipoprotein can bind LPS, and this occurs in both the lipid and protein fractions of lipoproteins (34). The extent to which adipose tissue lipolysis contributes to the formation of VLDL and subsequent neutralization of pathogens is not known. Adipose tissue derived lipid substrate may also influence the metabolism of immune cells. Cells that regulate inflammation, particularly regulatory T cells and M2-polarized macrophages rely on fatty acid oxidation (69, 89). Thus, the lipids released from adipose tissue could support the metabolism of these cells and the resolution of inflammation. In addition to the metabolites secreted by adipose tissue, there are also a variety of adipokines that have endocrine functions. Mice that lack adiponectin have an exaggerated inflammatory response to systemic LPS administration (80), and mice that lack leptin have increased mortality in response to sepsis (9). These studies demonstrate
Adipokines have the potential to influence the systemic inflammatory response (9). Adipose tissue constitutes a large portion of body mass and is an immunologically active compartment. Thiazolidinediones (TZDs) are a unique class of insulin sensitizing drugs that increase adipogenesis and lipid storage in adipose tissue and reduce lipolysis. They have also been shown to have anti-inflammatory effects during sepsis. Yet, it is not known whether adipocytes contribute to the beneficial effects of TZDs through adiponectin secretion. Also, many of the anti-inflammatory actions attributed to TZDs may be due to their effects on leukocytes (12, 20, 63). Future research will be needed to assess the contribution of adipose tissue to the systemic inflammatory response and distinguish the metabolic influence of adipose tissue on inflammation from its hormonal influence upon systemic inflammatory responses.

**Alternatively activated macrophages:**

Although pro-inflammatory cytokines and pattern recognition receptors have the ability to induce insulin resistance and adipose tissue lipolysis, anti-inflammatory cytokines also play a role in regulating lipolysis as well. Lipolysis is necessary to fuel thermogenesis in order to maintain body temperature during cold exposure. Most research on thermogenesis has focused on modulation by the central nervous system, however recent evidence indicates that alternatively activated macrophages play a role in this process. It is well established that interleukin-4 (IL-4) and interleukin-13 (IL-13) are linked to the alternative activation of macrophages and signal via STAT6 (86). IL-4 signals through the IL-4 receptor resulting in translocation of signal transducer and activator of transcription 6 (STAT6) to the nucleus and STAT6 dependent gene transcription (Figure 2). STAT6−/− mice are resistant to the development of obesity, despite developing insulin resistance and...
glucose intolerance (64). Recent observations indicate that mice lacking IL-4 receptors on
macrophages have a reduced ability to maintain core body temperature in response to a
cold stimulus (51). These experiments also suggest that these cytokines do not act directly
on adipocytes, but that catecholamine production by adipose tissue macrophages supports
the lipolysis that is required for thermogenesis (51). However, others have reported direct
effects of IL-4 on 3T3-L1 adipocytes to inhibit adipogenesis and increase lipolysis (82).
Future studies will be needed to identify and understand the primary targets of IL-4 in
adipose tissue. It is interesting that both pro-inflammatory signals and anti-inflammatory
signals have the capacity to influence lipolysis in adipocyte and adipose tissue. An
important discriminator of these signals is the stimuli that activate them. The IL-4
macrophage catecholamine pathway is responsive to cold stimuli and necessary for the
maintenance of core body temperature. The proinflammatory pathway is responsive to
adipose tissue and systemic inflammation, which may proceed in a chronic fashion and
may not be coupled to the increased oxidative capacity associated with thermogenesis.

Although interleukin-10 (IL-10) is frequently used as a measure of adipose tissue
inflammatory balance, this cytokine has received a very limited amount of attention for its
potential role in adipocyte regulation. IL-10 is known to inhibit the actions of TNFα on
insulin resistance and improve insulin signaling in cultured 3T3-L1 adipocytes (45).
Similarly, IL-10 inhibits NF-κB activation in response to LPS (43). Its role in lipolysis may
be as a negative regulator of lipolytic signaling by inflammatory stimuli, rather than having
direct effects. IL-10 signaling occurs through the IL-10 receptor, and like gp130 cytokines
activates STAT3 (Figure 2). In some circumstances, STAT3 activation appears to have pro-
inflammatory effects on adipocytes. However, STAT3 regulation is complex and it will be
critical to determine how different signaling pathways determine the specificity of STAT3 activation. In primary human adipocytes, there is no STAT3 activation in response to IL-10 indicating that there may be species-specific differences in IL-10 signaling (83).

**Metabolic flexibility and adipose tissue lipolysis:**

Obesity is a multifactorial disease associated with predisposition of many related comorbidities. It has been noted that healthy individuals display metabolic flexibility, with a respiratory quotient that increases in response to meals (indicating carbohydrate utilization as a fuel source) and decreases in response to fasting (indicating fatty acid oxidation as a fuel source). With the development of insulin resistance and T2DM, there is a loss of metabolic flexibility that is characterized by a respiratory quotient that has a reduced magnitude of change in response to fasting or meals (15). Loss of metabolic flexibility is accompanied by alterations in adipose tissue lipolysis (74). Increased lipolysis also occurs with increased expression of immune cell markers and pro-inflammatory cytokines in adipose tissue (74). These conditions are also associated with the deposition of ectopic fat in skeletal muscle and liver. NEFA themselves cause insulin resistance in skeletal muscle and can do so acutely after 2-3 days of fasting (23). Thus, strategies to control lipolysis including pharmaceuticals directly targeted to pro-inflammatory cytokines (IL-1β, TNF, etc.) that enhance fat storage and adipogenesis or that couple fatty acid oxidation to lipolysis would be of benefit for the management of obesity and metabolic syndrome. In addition to adipose tissue inflammation, bacterial PAMPs from the gut microbiota may contribute to this process as well because type 2 diabetics are known to have increased circulating levels of LPS (62). Modification of the gut microbiota may be an additional way to manage lipolysis and metabolic impairments during obesity.
Conclusions:

With an increased understanding of the connections between immune cell function and metabolic homeostasis, it is important to understand how both cytokines and pathogen associated molecular patterns affect adipocyte metabolism in order to understand the contribution of adipose tissue to acute and chronic inflammation. A growing body of literature in this area has demonstrated both pro and anti-lipolytic effects of immune related cytokines. The complex microenvironment of adipose tissue means that multiple cellular targets are present for each cytokines. Hence, cytokines are not acting singularly, and there is potential for adipocyte-secreted proteins to impact the production of pro-inflammatory cytokines. Thus, there remains much to be investigated in terms of production of cytokines, combinatorial effects on adipocytes, and cross-talk between adipocytes, leukocytes and other adipose tissue cells on the influence of adipose tissue lipolysis and inflammation.

As a metabolically active tissue, adipose tissue is able to modify systemic metabolism through the provisioning of lipids and via the endocrine actions of adipokines. Free fatty acids released from adipose tissue are a fuel source during times of energy scarcity and high energy demands including exercise and cold exposure. Dysregulation of the control of lipolysis during metabolic disease contributes to dyslipidemia and the deposition of ectopic fat that impairs tissue function. The secretion of cytokines by leukocytes in adipose tissue regulates lipolysis in both physiologic and pathophysiologic conditions. These immunometabolic interactions have profound effects on lipolysis and present an opportunity to understand underlying biology controlling lipolysis and pathological lipid metabolism during obesity and T2DM.
Acknowledgements: The authors thank Ann Liu for assistance with figure preparation and design.


17. **Green A, Dobias SB, Walters DJ, and Brasier AR.** Tumor necrosis factor increases the rate of lipolysis in primary cultures of adipocytes without altering levels of hormone-sensitive lipase. *Endocrinology* 134: 2581-2588, 1994.


Table 1: Influence of cytokines on lipolysis

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Cytokine Name</th>
<th>Model</th>
<th>Effect on Lipolysis</th>
<th>References</th>
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<td>TNFα</td>
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Figure Legends:

Figure 1. **TNFα signaling shapes lipid metabolism:** Signaling of TNFα through TNFR1 leads to activation of multiple signaling pathways including NF-κB and ERKs. Activation of these pathways leads to transcriptional changes resulting in reduced expression of lipoprotein lipase. TNFα signaling induces a loss of the lipid droplet protein perilipin, and activation of adipose triglyceride lipase and hormone sensitive lipase. These enzymes act on triacylglycerol and diacylglycerol resulting in the release of free fatty acids. To further promote lipolysis TNFα activates proteasome dependent degradation of Gi proteins that have the ability to reduce anti-lipolytic adenosine signaling.

Figure 2. **Lipolysis cytokines and signaling proteins:** Adipose tissue produces a variety of cytokines that can activate lipolysis in adipocytes. In addition to cytokines pathogen associate molecular patterns can activate adipocyte lipolysis. These cytokines bind to specific receptors and activate signaling pathways that contribute to lipolysis. Presented are the cytokines, receptors and conical signaling pathways that contribute to lipolysis.