Lipid signaling and lipotoxicity in metabolic inflammation: indications for metabolic disease pathogenesis and treatment

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Running title: Lipid signaling and lipotoxicity in metabolic inflammation
Abstract

Lipids encompass a wide variety of molecules such as fatty acids, sterols, phospholipids and triglycerides. These molecules represent a highly efficient energy resource and can act as structural elements of membranes, or as signaling molecules that regulate metabolic homeostasis through many mechanisms. Cells possess an integrated set of response systems to adapt to stresses such as those imposed by nutrient fluctuations during feeding-fasting cycles. While lipids are pivotal for these homeostatic processes, they can also contribute to detrimental metabolic outcomes. When metabolic stress becomes chronic and adaptive mechanisms are overwhelmed, as occurs during prolonged nutrient excess or obesity, lipid influx can exceed the adipose tissue storage capacity, and result in accumulation of harmful lipid species at ectopic sites such as liver and muscle. As lipid metabolism and immune responses are highly integrated, accumulation of harmful lipids or signaling intermediates can interfere with immune regulation in multiple tissues, causing a vicious cycle of immune-metabolic dysregulation. In this review, we summarize the role of lipotoxicity in metabolic inflammation at the molecular and tissue level, describe the significance of anti-inflammatory lipids in metabolic homeostasis, and discuss the potential of therapeutic approaches targeting pathways at the intersection of lipid metabolism and immune function.

Keywords: Lipids, Inflammation, Obesity, Diabetes, Insulin resistance, Lipotoxicity, Metabolic disease, Signaling lipids
The term *lipid* is used to identify a large set of hydrophobic and amphiphilic molecules such as free fatty acids, sterols, fatty acid esters, and phospholipids. These molecules are involved in forming fundamental structures in cells and tissues, providing energy for the metabolic needs of organisms, and regulating several homeostatic processes within and outside of cells including organelle homeostasis, immune function, inter-organ communication, energy metabolism, and cell survival. However, when the balance in their metabolism and composition is altered by environmental or metabolic stress, lifestyle, genetic or epigenetic factors, lipids can also become critical components of pathophysiological cascades that are detrimental to healthy cell and tissue function. Hence, although lipids play fundamental physiological roles, in excess or in improper composition, they can be highly damaging, leading to organelle dysfunction, cell death, chronic inflammation, and disturbances in energy and substrate metabolism and survival responses. In this review, we primarily focus on the roles of lipid classes that regulate immune responses and signaling mechanisms, which perpetuate a vicious cycle of metabolic and inflammatory disturbances leading to disease. We also describe the role of lipid-induced toxicity in relevant tissues and the significance of anti-inflammatory lipids, and discuss potential approaches to target these systems for metabolic disease treatment.

**Lipid associated metaflammation**

Lipotoxicity, generally defined as an increased concentration of harmful lipids, impairs cellular homeostasis and disrupts tissue function. This is a vast area of study that encompasses many fundamental processes in the cell and involves multiple mechanistic models. Here, we will focus mainly on lipotoxicity as it relates to the integration of metabolic and immune responses, which is critical for health and also plays a role in metabolic diseases (1). Chronic low-grade metabolic inflammation, termed “metaflammation,” is considered one of the hallmarks of metabolic diseases such as obesity and diabetes, and it occurs in several metabolic tissues including adipose tissue, liver, muscle, brain, and gut. Among other potential mechanisms, it is now well-established that immunometabolic pathways are highly
responsive to lipids and linked to lipotoxicity (1). Just as lipotoxicity gives rise to metaflammation, alterations in lipid metabolism and signaling can also converge on common immune and stress responses (2), thus creating vicious pathological cycles that contribute to many diseases.

Perturbations in fatty acid and cholesterol fluxes lead to higher representation of harmful lipid classes in cells and in the circulation, especially saturated fatty acids and oxidized cholesterol (Figure 1). These species have been studied extensively for their effects on cellular function, including inflammatory responses and organ performance. For example, the saturated fatty acid palmitate can be imported into cells via fatty acid transport protein 1 (FATP1), and overexpression of FATP1 in the heart leads to lipotoxicity-mediated cardiomyopathy (3). Forced exposure to fatty acids can also be a driver for pathologies at other sites or other metabolic diseases, and the discussion below is predominantly framed in this context.

The mechanisms underlying the harmful effects of excess lipid flux are related in part to the impact of lipids on the biophysical properties of cellular organelles. For example, the endoplasmic reticulum (ER), which is one of the major hubs for lipid biosynthesis and esterification, is a critical organelle mediating both metabolic and inflammatory adaptive responses to proteotoxic, nutritional and energy-related stresses. In the setting of chronic nutrient stress, lipid synthesis is dysregulated in the ER, leading to changes in phospholipid composition of the ER membrane. These changes cause disruption of calcium signaling, prolonged ER stress, and decreased translation of ER-associated proteins (4, 5). Similarly, saturated fatty acids and cholesterol loading increase ER stress and associated cell death (6-9). ER stress responses also intersect with inflammatory pathways via activation of numerous inflammatory kinases such as JNK, PKR, and IKK (10, 11) and activation of inflammatory mediators and the inflammasome (Figure 1). The adaptive responses of ER and the unfolded protein response (UPR) exhibit a peculiar pattern of defects in obesity and diabetes, in the context of chronic inflammation. This is evident in both type 1 and type 2 diabetes (10, 12-17). For instance, ER stress propagation via increased induced nitric
oxide synthase (iNOS) activity and subsequent inactivation of the key ER regulator inositol-requiring protein 1 (IRE1α) via nitrosylation (18) represents one example of indirect impact of lipotoxicity on chronic inflammatory processes.

Beyond the alteration of organelle function, lipotoxicity can also influence metaflammation and insulin action via direct effects on intracellular signaling pathways. For example, palmitate exposure is implicated in synthesis of diacylglycerols (DAGs), and ceramide (19) which can activate novel protein kinase C (PKC) isoforms (20) (Figure 1) such as PKC-θ and PKC-ε, and have been linked to T cell activation and LPS responses (21, 22) as well as insulin action and metabolic responses (23). While the exact mechanisms underlying these signaling events and the lipid species that engage PKCs remains under debate (23, 24), there is strong evidence supporting the involvement of PKCs in both metabolic and inflammatory responses that are relevant to obesity and type 2 diabetes. Palmitate accumulation also leads to ceramide biosynthesis, which can activate inflammatory pathways and inhibit insulin action (Figure 1). Ceramides inhibit Akt-mediated insulin signaling as well as mitochondrial fatty acid oxidation by disrupting mitochondrial electron transport (25-28). Furthermore, inhibition of ceramide synthesis via myriocin treatment improves glucose and energy metabolism via recovery of insulin signaling in liver and muscle (29). Interestingly, TLR4 signaling can also lead to increased expression of ceramide biosynthetic enzymes (30), suggesting the importance of this pathway in mediating metaflammation and insulin resistance and the reciprocal and highly integrated operation of lipid and immune signaling pathways (Figure 1).

Finally, lipids can influence cell fate and function by engaging receptors on the cell surface or stress kinases within the cytoplasm. Palmitate can directly activate inflammatory pathways by increasing TLR4 signaling (31) and by stimulating signaling molecules such as protein kinase R (PKR) (10) (Figure 1). In response to harmful lipids such as palmitate and oxidized cholesterol, PKR can activate JNK, leading to engagement of downstream transcription factor activator protein 1 (AP-1) and expression of genes that
mediate inflammation and apoptosis, and promote inflammasome activity (32-34). It is unlikely that a single receptor or molecular event underlies these lipotoxic responses, however, it is possible to envision common signaling intermediates that mediate the vast array of downstream biological outcomes of lipotoxicity. One such potential mechanism involves upregulation of small nucleolar RNAs (snoRNAs) in the cytoplasm (35). Interestingly, PKR is the only kinase that also has direct double stranded RNA binding activity, and snoRNAs are enriched in PKR immunoprecipitates after palmitate treatment, suggesting palmitate sensing by PKR may involve direct binding of snoRNAs to PKR for its activation in the metabolic disease context (36). How snoRNAs are involved in signaling lipotoxicity at the molecular level is yet unclear, but cells with impaired ability to produce snoRNAs are resistant to lipotoxicity induced ER stress and death (37), indicating the potential of snoRNAs serving as mediators of broad lipotoxic responses. This is one of the most interesting emerging areas of research related to mechanisms of lipotoxicity and metabolic regulation.

Lipotoxicity Associated Inflammation in Metabolic Tissues

Continuous cycles of nutritional exposures and changing environmental factors require integrated metabolic, stress, and immune responses in many critical organs. Under chronic energy and substrate excess, metabolic stress is unresolved, yielding maladaptive outcomes, such as unresolved inflammation, impaired hormone action, lipid accumulation and loss of function. Here, we will not discuss specific conditions such as insulin resistance, fatty liver disease and cardiovascular pathologies which are covered in detail in excellent recent reviews (2, 19, 38-42) beyond specific examples relevant to metaflammation in few representative sites (Figure 2).

Adipose Tissue

Fatty acids are esterified and compartmentalized in dynamic organelles called lipid droplets (LDs), such that their use can be coupled to cellular metabolism and signaling (43). During times of excess nutrient
availability, LDs act as a depot for excess fatty acids and cholesterol that are otherwise harmful to the cells (43). In higher organisms, this can occur in all cells but the most dramatic example is the adipose tissue, a specialized organ for lipid deposition. In the setting of increased metabolic demand, adipocytes hydrolyze neutralized lipids in LDs through lipolysis, liberating fatty acids for use in other tissues, feeding mitochondrial fatty acid oxidation pathways, and creating metabolic intermediates that serve as substrates or signaling molecules (24). The proper functioning of this rheostat is necessary to keep all other metabolic organs in check. In the presence of excess nutrients and energy, or in obesity, the capacity of adipose tissue can be overwhelmed, causing stress, injury, and abnormalities in function. For example, insulin resistance under these conditions leads to higher levels of basal lipolysis and a decreased capacity to esterify fatty acids for storage or neutralization via down-regulation of synthetic machinery (44-46). This dysfunctional state contributes to systemic lipotoxicity, as released or absorbed excess dietary fatty acids move into circulation and are deposited into organs that are not well-equipped to store lipids (Figure 2). The purpose of esterification is to prevent harmful effects of fatty acids by forming a neutral pool (47), and accordingly, increasing the storage capacity of lipid droplets in adipocytes and macrophages by driving synthesis can protect against diabetes and insulin resistance (48-50). However, above a certain threshold, the accumulation of such lipids induces stress and metaflammation, mediated by several stress kinases such as PKC, JNK, and PKR, molecular sensors or receptors such as TLRs and signaling proteins such as cyclic AMP-responsive element-binding protein 3-like protein 3 (CREBH) and SOCS proteins (10, 31, 51-56). In addition, obesity leads to a local lipotoxic environment through dysregulated release of fatty acids, leading to alteration of the secretory output and an immune phenotype of adipose tissue characterized by increased release of cytokines such as TNFα, and MCP-1, decreased secretion of anti-inflammatory adipokines such as adiponectin, and recruitment of inflammatory macrophages, T-cells, and other immune effectors (38). Reciprocally, pro-inflammatory cytokines such as TNFα regulate lipid metabolism in adipocytes via increasing lipolysis (57) which continuously exposes the organs to fatty acids. Increased lipolysis from adipose tissue is also linked to secretion of the adipokine aP2 (FABP4)
(58, 59), which is an important mediator of immunometabolic responses locally at the adipose tissue, and links the lipolytic state to glucose metabolism in the liver and elsewhere (60-62). Overall, while adipocytes are the specialized site for neutralizing fatty acids, this capacity is not infinite and these cells are not impervious to excess lipid accumulation which drives inflammatory responses locally and at distant tissues. The mechanisms and specific mediators in this context are not well understood, and are an area of great interest.

Studies of mouse models of obesity have demonstrated that the lipotoxic milieu of adipose tissue promotes drastic changes in the resident immune cell profile and function. Specifically, adipose tissue is infiltrated by macrophages, which shift from an anti-inflammatory profile (also referred to as M2-like polarized) to a pro-inflammatory (aka M1-like polarized) phenotype, and several immune cell types are implicated in adipose tissue dysfunction such as B and T lymphocytes, neutrophils, eosinophils, mast cells, and NK cells (63). As explained above, palmitate, ceramide, and DAGs are broadly-studied lipid mediators that lead to activation of immune cells (Figure 1), further suggesting a lipotoxic inflammatory connection in adipose tissue. Apart from direct activation of sensors and stress kinases, lipids can also act as antigens that are presented to immune cells by CD1d. Recently, CD1d positive adipocytes have been implicated in lipid derived antigen presentation to a subclass of natural killer T (NKT) cells called invariant NKT (iNKT) cells (64). However, the role of specific iNKT cells in regulation of adipose tissue inflammation has been a subject of debate, with some studies suggesting a beneficial effect of iNKT cells (64, 65) and others implicating iNKT cells in skewing the adipose phenotype to a more inflammatory and dysfunctional state (66). The identity of lipid molecules that are subject to antigen presentation and how they mount an inflammatory cascade is an exciting and understudied area and may provide clarity on the role of this particular mechanism on adipose tissue and systemic metabolism. Overall, these findings indicate that lipids can directly or indirectly modulate both innate and acquired immune responses due to close interactions between these systems, with implications for systemic metabolic homeostasis.
Compared to white adipose tissue, there is much less information on the lipotoxic events in the brown adipose tissue (BAT), which has a lower storage but higher turnover capacity. BAT is best known for its function in non-shivering thermogenesis and energy expenditure. At cold temperatures, BAT increases fatty acid uptake and fatty acid oxidation to keep up with thermogenic needs. Recently, a surprisingly large capacity for postprandial lipid uptake via triglyceride rich proteins was demonstrated in BAT, suggesting another layer of metabolic regulation in BAT function which can lead to intracellular stress and intermediary products that would require a strong defense mechanism (67). Indeed, it is known that in dietary and genetic models of metabolic disease such as ob/ob mice, BAT becomes a white adipose-like tissue due to increased triglyceride accumulation leading to inflammation and dysfunction (68, 69) (Figure 2). The mechanisms that are in place to preserve the functional integrity of BAT and defend against inflammation and stress, and how these relate to pathophysiology of metabolic disease are critical but incompletely answered questions and open to further research.

**Liver**

In the attempt to understand the mechanisms and outcomes of lipotoxicity, the liver is one of the most highly explored organs. Since nonalcoholic fatty liver disease (NAFLD) is highly prevalent, and is closely associated with a cluster of metabolic diseases such as obesity, insulin resistance, and diabetes, understanding hepatic lipotoxicity is of paramount importance. A subclassification of NAFLD is nonalcoholic steatohepatitis (NASH), which is characterized by presence of inflammatory cells in liver histology. NASH progression is mediated by interplay between lipid mediated toxicity and inflammatory responses leading to liver injury (70) (Figure 2). The signaling events in this process involve DAGs and ceramide (71, 72) which are discussed above for their significance in inflammatory signaling. Numerous studies have also demonstrated the importance of inflammatory responses in hepatic lipotoxicity associated with local or systemic TNFα and IL-1β exposure (73, 74) or as a result of dysbiosis (75-77). In liver, ER is a pivotal node at the crossroads of inflammation and lipid metabolism. ER dysfunction may contribute to metabolic dysregulation of liver through iNOS mediated tyrosine nitrosylation of IRE1
(18) or engaging inflammatory signaling cascades (10, 78, 79) and the inflammasome (80, 81). Similarly, JNK as well as the δ and γ isoforms of p38 are critical mediators of inflammation and metabolic deterioration in mouse models of obesity or NASH, highlighting engagement of the stress pathways in lipotoxic responses (82-84). In mouse models of fatty liver disease, the mitochondria exhibit dysfunctional β-oxidation. This might be partially due to increased ER-mitochondria connections and associated calcium overload in mitochondria in obesity (85). In these contexts, it is unequivocal that inflammatory pathways are highly critical to obesity-induced metabolic dysfunction, and blocking these can reverse the disease. A recent study in mouse models as well as humans also showed that weight loss can result in dramatic resolution of obesity-induced liver inflammation along with insulin action (86). Interestingly, this study also showed that residual adipose tissue inflammation and insulin resistance persists for extended periods of time despite weight loss at this site (86).

One emerging aspect of hepatic lipotoxicity is free cholesterol accumulation due to disturbances in cholesterol homeostasis and transport (87-89), which has been underappreciated until recently in liver metabolic dysfunction. This phenomenon is supported by association studies in humans demonstrating dysregulation of cholesterol metabolism genes and free cholesterol levels with NAFLD (90). Excess free cholesterol causes damage in liver through JNK-1 and TLR4 dependent mechanisms (91). Cholesterol loading of mitochondria in liver is also argued to contribute to TNFα-mediated steatohepatitis (92). Additionally, Kupffer cells, the liver resident macrophages, have been shown to form crown like structures around dying hepatocytes and accumulate cholesterol in NASH (93). However, whether lipogenesis or inflammation constitute the initiating events in NASH and are causal to pathology has been subject to much debate. While the value of such debate itself could be debated, time course analysis of inflammatory and lipogenic changes in mouse models of hepatosteatosis (94), and inhibition of steatosis by liver macrophage depletion (74) suggest that inflammatory events occur very early in the course of disease. In agreement with this, recent studies examining Arginase 2-deficient (Arg2−/−) mice suggest that
inflammation can lead to *de novo* hepatic lipogenesis (95). Arg2−/− mice develop spontaneous steatosis with inflammation and increased lipogenic gene expression. Depletion of liver macrophages leads to decreased lipid droplet accumulation in liver, suggesting that inflammation may precede lipid accumulation (96). Other studies have suggested that this may not be the case and even concluded that inflammation may not be a major player in this context due to lack of inflammatory connections to liver insulin resistance in the respective mouse models studied (97-99). The likely scenario is that lipogenesis and inflammatory responses cannot be separated in real life — they regulate each other, both contribute to abnormal liver metabolism and disease, and both are part of a regulatory as well as maladaptive cycle (2).

For example, high fat diet feeding exacerbates inflammation and liver injury in Arg2−/− mice suggesting a further contribution of lipotoxicity in forming a futile cycle. Also, adipose-derived acetyl CoA has been shown to accumulate in the liver in the setting of insulin resistance, and this is linked to aberrant hepatic glucose production (100). In this case, the effect of acetyl-CoA was dependent on IL-6 action, as neutralization of this cytokine reversed the disease phenotype demonstrating yet another example of inflammatory pathways interacting with lipids. Many other examples exist in literature and are reviewed elsewhere (2, 101).

In discussing tissue lipid accumulation, it is important to note that lipid droplet formation in hepatocytes may also be an adaptive response in the liver as it is in adipose tissue, and to a certain tolerable threshold and composition, may represent a way to preserve the function of the hepatocytes. Consistent with this, hepatic lipid droplet formation should not be expected to be, and in fact is not, uniformly associated with adverse metabolic outcomes (102-104).

*Skeletal muscle and heart*

High levels of circulating fatty acids and triglycerides are associated with muscle insulin resistance in mice and humans (105-107). In both experimental models and in obese and diabetic individuals, there is increased TNFα expression and accumulation of inflammatory cells in the muscle tissue (Figure 2) which
can, at least in part, be rescued by exercise (108, 109). In addition, adipocytes can accumulate in the muscle tissue, providing an opportunity for paracrine communication (110). Chronic exposure to a high concentration of saturated fatty acids leads to inhibition of insulin receptor signaling through inhibition of IRS-1 via decreased tyrosine phosphorylation in muscle cells (107, 111, 112). Saturated fatty acids also engage several stress and inflammatory signaling molecules such as PKC, JNK, ERK, STAT3, and iNOS, and increase IL-6, TNFα, and IL1β, expression which in turn impact metabolism (113, 114). DAG and long-chain-acyl CoAs have been implicated as the culprits in muscle lipotoxicity since they activate stress kinases such as PKC that contribute to insulin resistance (111, 115, 116). Chronic metabolic stress leads to mitochondrial dysfunction, and lipid accumulation is suggested to occur due to defective mitochondrial β-oxidation (117). However, this notion has been challenged by studies in which lipid trafficking into mitochondria is perturbed by knocking out malonyl-CoA decarboxylase (MCD) (118). Mice lacking MCD are resistant to developing diet induced glucose intolerance, although they have increased intramuscular lipid accumulation. These data led to the proposal that the problem of mitochondrial dysfunction in this context may in fact be increased yet maladaptive β-oxidation, such that a blockade in the TCA cycle and consecutive accumulation of acyl-CoAs and acylcarnitines in the mitochondria would result in insulin resistance (118). Regardless, impaired mitochondrial function can also promote chronic inflammatory responses through many mechanisms including the activation of the inflammasome and generation of excess reactive oxygen species (ROS) (81, 119) (Figure 1). Overall, lipid mediated changes during insulin resistance in muscle converge with immune pathways and directly or indirectly regulate inflammatory signaling. An intriguing exception where excess lipid accumulation in muscle does not correlate with metabolic deterioration occurs in athletes, suggesting active adaptive mechanisms including robust mitochondrial beta-oxidation (120). In depth understanding of such adaptive mechanisms could help identify targets for alleviating insulin resistance.
The heart has a robust capacity to utilize fatty acids for metabolic functioning (39). However, a prolonged increase in circulating fatty acids and triglycerides and accumulation of pericardial adipose tissue can trigger inflammatory signaling in heart and cause cardiac dysfunction in metabolic disease that features mitochondrial dysfunction, increased reactive oxygen species and NFκB activity (Figure 2) (121). Epicardial fat can also secrete proinflammatory cytokines to drive inflammatory cell infiltration and exacerbate heart disease (122-124). TLR4 has been implicated as the mediator of fatty acid induced lipid accumulation and inflammatory responses in the heart (125). Similarly, excessive triglyceride accumulation in the cardiac muscle was demonstrated to cause exhaustion of mitochondrial capacity via deregulation of PPARα signaling involved in oxidative pathways (126, 127) and accumulation of harmful lipids such as DAG and ceramides which can activate PKCs and inhibit insulin signaling (128). Interestingly, driving triglyceride synthesis via DGAT1 expression in cardiac tissue renders mice resistant to developing lipotoxic cardiomyopathy and decreases accumulation of DAG and ceramide (129), suggesting an adaptive role for increasing lipogenic capacity or altering the profile of lipids in cardiomyocytes.

Lipotoxicity mediated inflammation also drives cardiovascular disease pathogenesis in the context of atherosclerosis. Exposure to saturated fatty acids such as palmitate or modified lipoproteins leads to ER stress in macrophages, which drives apoptosis and further promotion of inflammation in atherosclerotic plaques (130). Dysregulation of cholesterol fluxes and accumulation of oxidized low-density lipoprotein (LDL) particles in arterial walls leads to inflammatory cell recruitment and atherogenic plaques (131). In an attempt to limit plaque formation, macrophages take up oxidized LDL particles, which causes formation of foam cells that secrete inflammatory cytokines and leads to a futile cycle of inflammation and formation of more foam cells. Cholesterol crystals are also indicators of progressed atherosclerotic plaques, and can activate the inflammasome in macrophages in the context of atherosclerosis (132) (Figure 1). The role of cholesterol and inflammation in cardiovascular disease is extensively reviewed elsewhere and will not be covered further here (131, 133, 134). It is however important to note that
diabetes is a central risk factor for cardiovascular disease, and our current understanding of how diabetes drives myocardial perturbations is insufficient. Further study on diabetes-associated changes in heart and the significance of cardiovascular lipotoxicity and inflammation may help determine alternative and novel clinical approaches to cardiovascular disease.

Other organs

As has been reviewed elsewhere (135-137), lipotoxicity plays an important role in islet dysfunction in obesity. Although fatty acids regulate insulin secretion from islets at different levels such as vesicle trafficking and calcium influx via their metabolites (138), chronic elevation of such lipids leads to β-cell failure together with inflammatory etiology (139). Human data suggests that non-insulin dependent diabetic patients have increased IL-1β expression and macrophage recruitment in their islets (139-141). It has also been proposed that β-cell failure in type 2 diabetes has an inflammatory component that is promoted by lipotoxicity (Figure 2) (142). Moreover, in a study in which mice were infused with ethyl palmitate in order to elevate circulating palmitate levels, glucose stimulated insulin secretion was defective in a TLR4/MyD88 dependent manner (143). Palmitate elevation also led to macrophage infiltration of the islets. These observations laid the groundwork for a new perspective for lipotoxicity induced inflammation in islets in type 2 diabetes. Recent findings also suggest a role for abnormal cholesterol metabolism in β-cell failure in type 2 diabetes patients (144, 145). A role for cholesterol accumulation in islet inflammation and β-cell dysfunction was shown in mice that are deficient in cholesterol transporters ABCA1 and ABCG1. In this model, excessive accumulation of cholesterol in islets lead to macrophage recruitment and increased IL-1β expression as well as defective glucose stimulated insulin secretion (146). This intriguing hypothesis that dysregulated cholesterol fluxes drive metabolic inflammation in islets may have profound translational implications for the utility of cholesterol management strategies in beta cell preservation and diabetes.
Similarly, many of the pathways defined earlier apply to the central nervous system (CNS), in that obesity and exposure to excess lipids can create chronic inflammation and cause ER stress in the brain (147-149) (Figure 2). In both humans and preclinical models, obesity-induced inflammatory changes are evident in the brain (150, 151) and hypothalamic ER stress contributes to defective insulin and leptin action (147, 149, 152). Interestingly, amelioration of ER stress via chemical chaperones can reverse some of these effects (152). One lipotoxic inducer of hypothalamic ER stress was proposed to be ceramide. CNS levels of ceramide are increased in obese mouse models, and central administration of these lipids leads to weight gain via inhibition of BAT function (153). Saturated fatty acids have been shown to increase hypothalamic inflammation via TLR4 (148). In contrast, increasing fatty acid oxidation in hypothalamic neurons can decrease palmitate-induced inflammation and toxicity (154). These observations highlight the need for more research to understand the mechanism of lipotoxic inflammation in the CNS leading to metabolic dysregulation.

Finally, nutritional input, for example exposure to a high fat diet, leads to drastic changes in the gut microbiome (Figure 2), and these alterations are believed to contribute to the development of metabolic disease. The most compelling evidence to support this postulate is that in mice, fecal transplantation from obese to lean experimental groups is sufficient to induce weight gain (155). In obese mice, circulating levels of microbial factors such as LPS were found to be increased, potentially due to increased gut permeability (Figure 2) (156). Such factors can engage the TLR signaling pathways, which are established mediators of metaflammation. A recent study suggested that feeding mice a diet that is rich in saturated fatty acids increases LPS and other microbial factors in the circulation, and this leads to white adipose tissue inflammation through TLR4 signaling (157). These observations suggest the presence of an extra layer of regulation of metaflammation involving gut-adipose tissue communication in dietary lipid recognition. Gut-derived lipid signals such as N-acylphosphatidylethanolamines (NAPEs), which are produced upon fat ingestion, might also impact immunometabolic outcomes. For example, NAPEs act in the gut-brain axis to decrease food intake (158) and they can also suppress inflammation (159, 160).
Hence, both through its endocrine impact on systemic metabolism and by hosting the microbiota, gut is a critical determinant of metabolic and organismal health. Future research in this area is also exciting and promising in understanding systemic impact of lipids and their interactions with the immune and other response systems.

**Bioactive lipids and regulation of inflammation**

In recent years, emerging studies have offered a new understanding of lipid function as soluble signals (lipokines) regulating biological processes outside cells (Figure 3). Interestingly, several classes of these have been shown to be important for resolution of inflammation. One such lipokine with a significant role in metabolic regulation is the fatty acid C16:1n7-palmitoleate (161). When synthesis of palmitoleate is increased as a result of adipose tissue-specific upregulation of de novo lipogenesis, its levels in circulation rise, resulting in suppression of inflammation and liver lipogenesis, and stimulation of muscle glucose uptake (161-163). Furthermore, palmitoleate generation in macrophages alleviates lipotoxicity-induced ER stress and cell death and, consequently, progression of atherosclerosis (130). Another endogenously produced lipid family with potentially vast metabolic actions include the fatty acid hydroxy fatty acids (FAHFAs), which were identified in adipose tissue and the circulation of mice in which adipose tissue de novo lipogenesis was also experimentally increased (164). A subclass of these lipids called palmitic acid hydroxy stearic acids (PAHSAs) have been implicated in mitigation of adipose tissue inflammation and exert anti-diabetic and insulin sensitizing activities in mice, proving another bioactive lipid signal that controls immunometabolic aspects of diabetes.

The ω-3 fatty acids, which can be acquired via the diet, have also been shown to inhibit metabolic inflammation and alleviate insulin resistance (165). Specifically, ω-3 fatty acids stimulate a lipid sensor, G protein-coupled receptor 120 (GPR120) and inhibit TNF-α and toll-like receptor 4 (TLR4) mediated
inflammation. Treatment of mice with ω-3 decreases adipose tissue inflammation and improves insulin sensitivity (165). Discovery of a dysfunctional variant of GPR120 in human that is associated with obesity and insulin resistance also strengthened the observation that GPR120 signaling is a viable target for metabolic disease treatment and that this pathway may involve signaling by endogenous monounsaturated fatty acids such as palmitoleate (166). Additional beneficial roles are attributed to ω-3 fatty acids through their metabolism into resolvins and protectins (167, 168). Defects in the production and action of these molecules can impair resolution of inflammation and lead to impaired cellular and organismal function (168). Endo-cannabinoids are also a class of monoacylglycerols that are well known for their effect in increasing appetite via activating their receptors in central nervous system and directly impacting adipose tissue and liver in the periphery to regulate metabolism and inflammatory responses. While activation of Cannabinoid receptor type 1 (CB1) has undesirable effects in the context of metabolic disease (169-171), CB2 signaling can promote anti-inflammatory outcomes (172, 173) (Figure 3).

In addition to serving as signaling molecules, an alternative mechanism by which lipids regulate metabolism and alleviate inflammation is through direct engagement of transcription factors (Figure 3). For example, fatty acyl derivatives and eicosanoids are well-known ligands for nuclear receptors such as peroxisome proliferator-activated receptors (PPARs) (174). PPARs in turn regulate transcription associated with immunological and metabolic outcomes, such as fatty acid oxidation, lipid biosynthesis, and attenuation of inflammation (175-177). Oxysterols are endogenous ligands for Liver X receptors (LXRs), which are critical in liver and macrophage function in the context of metabolic and cardiovascular diseases and regulate whole body cholesterol metabolism and have anti-inflammatory roles that are partially mediated by modulation of ER membrane composition (178, 179). In addition to acting through TGR5 to inhibit NFκB mediated inflammation (180), bile acids bind to and activate farnesoid X receptor (FXR), which is a major regulator of transcription related to bile acid and cholesterol metabolism (181), and FXR activation inhibits inflammation and fibrosis in a mouse model of NASH (182).
It is clear that while the field is in its early stages, there is tremendous potential in exploring the vast diversity of lipids for their specific biology and how signaling and structural functions are intertwined. Overall, the discovery of lipid species with beneficial or detrimental effects and associated pathways involved in immunometabolic functions has already expanded the perspective on lipids that were conventionally associated with lipotoxicity, and uncoupled lipid availability from metabolic dysfunction. Identification of these lipids also offers unique opportunities to exploit them for preventive and therapeutic strategies, especially in the context of chronic immunometabolic diseases.

**Therapeutic potential of targeting inflammatory lipids in metaflammation**

Lipid metabolism and immune responses are closely integrated in multiple tissue systems through conserved pathways, perhaps due to once advantageous evolutionary adaptations at times of frequent periods of famine and high occurrence of infectious disease. In the current era, when there is an excess of available nutrients and decreased prevalence of infectious disease, excess nutrients can drive inflammatory pathways leading to chronic, low level sterile inflammation, making these adaptations disadvantageous remnants of evolution. The accelerated increase in the prevalence of obesity and associated diseases calls for deeper understanding of the complex makeup of lipid and immune responses and their interaction in order to develop therapies targeting each.

The contribution of lipotoxicity, inflammation, and associated stress responses to metabolic disease involves interplay of several dysregulated pathways that are highly integrated and co-regulated. Because of this complexity, it is challenging to distinguish which phenomena are initiating and which lie downstream, and how they can be best targeted for intervention. For example, insulin resistance leads to uncontrolled lipolysis, which is one of the earlier events in systemic lipotoxicity; thus insulin-sensitizing therapies such as TZDs can help increase lipid storage in adipose tissue to overcome lipotoxic effects (183, 184). The finding that adipose triglyceride lipase (ATGL) deficient mice are insulin sensitive
despite triglyceride accumulation (185) suggests that lipases can also be targeted for metabolic disease treatment. Fatty acids released by ATGL activity, however, are also implicated in PPARα activation and contribute to cardiac muscle homeostasis (126), and hence the prolonged general inhibition of lipolysis might have undesirable effects. Further understanding of the lipolytic pathway and the details and molecular components of its contribution to signaling and metabolism will help determine if and how lipolysis can be pharmacologically targeted in more specific and restricted manner towards treatment of diabetes. For example, lipolysis has been linked to increased FABP4 secretion from adipose tissue via a non-classical mechanism (58, 59, 186, 187). FABP4 secretion is regulated by ATGL and hormone sensitive lipase (HSL) activity and subsequent increase in fatty acid availability (59). Interestingly there is strong correlation between circulating FABP4 levels and metabolic disease in preclinical models and in humans (58, 188-198). Mechanistically, secreted FABP4 has been demonstrated to increase liver glucose production, insulin secretion, and decrease cardiomyocyte contractility (58, 199, 200), and hence, may explain some of the detrimental effects of uncontrolled lipolysis. These effects of circulating FABP4 support the possibility of using neutralizing antibodies to treat metabolic disease, and this approach has proven successful in preclinical models (58, 201, 202).

As metaflammation is the hallmark of chronic metabolic disease, immunoregulatory, anti-inflammatory or antioxidant therapies can help reduce lipid induced inflammation, cellular dysfunction and death. The most prominent and common example of this is the use of cholesterol lowering drugs such as statins that can help decrease the harmful effects of free cholesterol accumulation in liver and macrophages (203, 204). This topic is covered in detail elsewhere (133, 205). Anti-inflammatory medications such as colchicine and methotrexate have been associated with decreased cardiovascular disease (206, 207). Salicylates can inhibit the NFκB pathway and have been shown to improve glucose metabolism and diabetes (208, 209). TNFα was one of the first inflammatory molecules shown to play a role in metabolic disease (210). Targeting this pathway in pre-clinical models proved successful (211-227), although comprehensive human studies are lacking, and the existing limited studies have reported both failures and
successes in humans (228). The limitations of the human studies with existing anti-cytokine reagents were recently reviewed in an excellent manuscript (229). There are also exciting but not yet fully exploited possibilities by antagonizing lipid sensing pathways, i.e., using TLR4 antagonists (230) or PKR inhibitors (231) to alleviate inflammation along with other beneficial effects associated with the target functions. In these areas studies in humans are also highly limited at the moment. Since lipotoxicity impairs ER function and leads to a prolonged unfolded protein response which can engage stress and inflammatory pathways, it may also be considered for potential intervention strategies. The use of agents that alleviate ER stress such as tauroursodeoxycholic acid (TUDCA) and 4-phenylbutyric acid (PBA) has been tested in metabolic contexts with beneficial results on liver and CNS function (152, 232-234). TUDCA treatment in experimental models of acute pancreatitis and ischemia reperfusion in liver also demonstrated decreased JNK activity along with attenuated inflammatory responses (235, 236). In obese mice treated with TUDCA or PBA, liver JNK activity was decreased along with improved insulin sensitivity and glucose metabolism (232). Interestingly, TUDCA treatment in humans also improved liver and muscle insulin resistance (237) warranting further clinical studies (238) some of which are currently underway (e.g. NCT01829698, clinicaltrials.gov).

An exciting and emerging translational area relates to bioactive lipids, such as palmitoleate, FAHFAs, and ω-3 fatty acids that have all been shown to have anti-diabetic and anti-inflammatory effects in mouse models of metabolic disease (discussed above). Hence supplementation with such molecules or finding agonists that target pathways associated with these lipids such as GPR120 signaling (239, 240) could help reduce lipid induced metaflammation in a variety of immunometabolic diseases including obesity and diabetes. ω-3 fatty acids are further metabolized into resolvins, which are involved in resolution of inflammation (168), a persistent process in diabetes. Hence resolvin supplementation or targeting pathways of resolvin synthesis could help alleviate systemic and local adipose tissue inflammation (241) and represent an effective approach against diabetes. Similar opportunities may also arise from exploring the products of gut microbiota (242, 243). Considering the potential transformational impact of these
approaches to manage chronic disease, further research and preclinical as well as clinical testing of these concepts represent a highly promising area of further translational possibilities.

Summary

Extensive research on immunometabolic disorders has established a strong connection between inflammatory pathways and lipids. While the current challenge remains to be the effective development and/or testing of translational tools for prevention and treatment, the future looks more promising than ever with the diversity of new possibilities against chronic metabolic diseases.
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Figure 1: Coupling of toxic and proinflammatory lipids and innate immune response. Accumulation of toxic lipid classes causes deterioration of metabolic regulation, and the effects of such lipids converge on inflammatory and stress pathways. Saturated fatty acids such as palmitate have been extensively studied for their effects in increasing inflammation and inhibiting insulin action. Palmitate is taken up by the cells via FATPs, and is involved in upregulation of cytosolic snoRNAs which are implicated in ER stress. PKR is a potential kinase that links palmitate-mediated snoRNA upregulation to ER stress induction. PKR also activates inflammasomes and JNK, and promotes AP-1 mediated inflammatory gene expression. Lipotoxicity can also lead to ROS production from mitochondria, which is linked to inflammasome activation. Palmitate directly contributes to synthesis of DAGs and ceramides. DAGs activate stress kinases, PKCs and the NFκB pathway; ceramides activate JNK signaling; and both DAGs and ceramides cause insulin resistance via inhibition of IRS1 and AKT respectively downstream of Insulin receptor (IR). Palmitate can also activate TLR4 signaling which leads to activation of...
inflammasomes and induction of inflammatory gene transcription factors Interferon regulatory factor (IRF), NFκB, and AP-1. Cholesterol is taken up by the cells via scavenger receptors (e.g. CD36). Accumulation of oxidized cholesterol or cholesterol crystals also leads to induction of TLR4, PKR, and stress kinase (JNK and p38) signaling or inflammasome activation and proinflammatory gene expression, which are central players in atherosclerosis progression.
Figure 2: Integrated organ pathology resulting from lipotoxicity and metabolic inflammation.

White adipose tissue is the designated lipid storage depot of higher organisms. In the presence of prolonged nutrient excess or metabolic disturbances, the storage capacity of white adipose tissue is exhausted, leading to ectopic deposition of lipids and lipotoxicity in several organs such as muscle, liver, pancreas and heart, as depicted and explained in detail in the text. Since the lipid homeostatic pathways converge with stress and immune responses, such responses in affected tissue systems are activated by harmful lipid species. The outcomes of lipotoxicity differ in the various target tissues, for example NASH in liver, cardiac failure in the heart, altered feeding behavior and appetite due to lipotoxicity in brain, degenerative changes in muscle and BAT, etc. Furthermore, lipotoxicity leads to sustained and unresolved inflammation, organelle dysfunction and stress, which can lead to a vicious cycle of metabolic deterioration.
Figure 3: Immunometabolic signaling capacity of lipids. The discovery of beneficial roles for specific lipids has changed the perspective on metabolic disease from being excessive fat storage disease to dysregulation of fat composition, and suggested the presence of more complex regulation of lipid subclasses in maintaining homeostatic signaling. The figure depicts several lipids that have anti-inflammatory signaling properties through various distinct mechanisms. For example, lipid ligands bind a variety of nuclear receptors: PPAR ligands are fatty acyl derivatives, LXR ligands are oxysterols, and FXR ligands are bile acids, all with ability to activate an anti-inflammatory program. Lipids are also involved in cell-to-cell and interorgan communication. Palmitoleate, FAHFAs, and ω-3 fatty acids can activate GPR120 signaling leading to inhibition of JNK and IKK mediated inflammation. FAHFAs and palmitoleate are endogenous lipids identified in mouse models of increased adipose tissue de novo lipogenesis, while ω-3 fatty acids are acquired from food intake. ω-3 fatty acids can further be metabolized into resolvins and protectins which are involved in resolution of inflammation. Bile acids and...
endo-cannabinoids bind to their respective receptors TGR5 and CB2 to inhibit inflammation and can impact metabolic homeostasis.