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Concise Original Report

Bioactive lipids and metabolic syndrome—a symposium report

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Recent research has shed light on the cellular and molecular functions of bioactive lipids that go far beyond what was known about their role as dietary lipids. Bioactive lipids regulate inflammation and its resolution as signaling molecules. Genetic studies have identified key factors that can increase the risk of cardiovascular diseases and metabolic syndrome through their effects on lipogenesis. Lipid scientists have explored how these signaling pathways affect lipid metabolism in the liver, adipose tissue, and macrophages by utilizing a variety of techniques in both humans and animal models, including novel lipidomics approaches and molecular dynamics models. Dissecting out these lipid pathways can help identify mechanisms that can be targeted to prevent or treat cardiometabolic conditions. Continued investigation of the multitude of functions mediated by bioactive lipids may reveal additional components of these pathways that can provide a greater understanding of metabolic homeostasis.

Keywords: inflammation; bioactive lipids; cardiometabolic disease; lipid mediators; lipid metabolism

Introduction

With the global incidence of cardiometabolic diseases continuing to rise, there is an urgent need to develop novel therapeutics that can mitigate or better manage these conditions. Obesity, type 2 diabetes (T2DM), atherosclerosis, and nonalcoholic fatty liver disease (NAFLD)/nonalcoholic steatohepatitis (NASH) result from ectopic lipid accumulation, glucose intolerance, and hypertriglyceridemia,[1–3] with inflammation often serving as the core of these pathologies.[4]

Bioactive lipids play a key role in maintaining immune function and tissue homeostasis. They act as physiological mediators of intra and intercellular functions that give rise to inflammation.[5,6] Bioactive lipids thus represent essential regulators of a multitude of cellular functions that exert unique effects on lipogenesis and glucose metabolism.[7]

Genetic association studies in people with cardiometabolic disease have revealed a number of genes involved in lipid metabolism that can confer a higher risk of developing these conditions.[8] These findings have propelled exploration of inflammatory pathways, such as the regulation and balance of proinflammatory and proresolving mediators, and their contribution to metabolic homeostasis.[9] Additional markers and pathways under investigation include lipid-modifying enzymes, branched fatty acid esters of hydroxy fatty acids (FAHFAs), sn-1,2-diacylglycerols, phosphocholine (PC)-containing oxidized phospholipids (OxPLs), prostaglandin E_2, monoacylglycerol acyltransferase 2 (MGAT2), and lysophosphatidic acid (LPA).

Recent findings have revealed a variety of promising therapeutic targets for several cardiometabolic diseases and have enhanced our understanding of the complex processes involved in lipid metabolism.
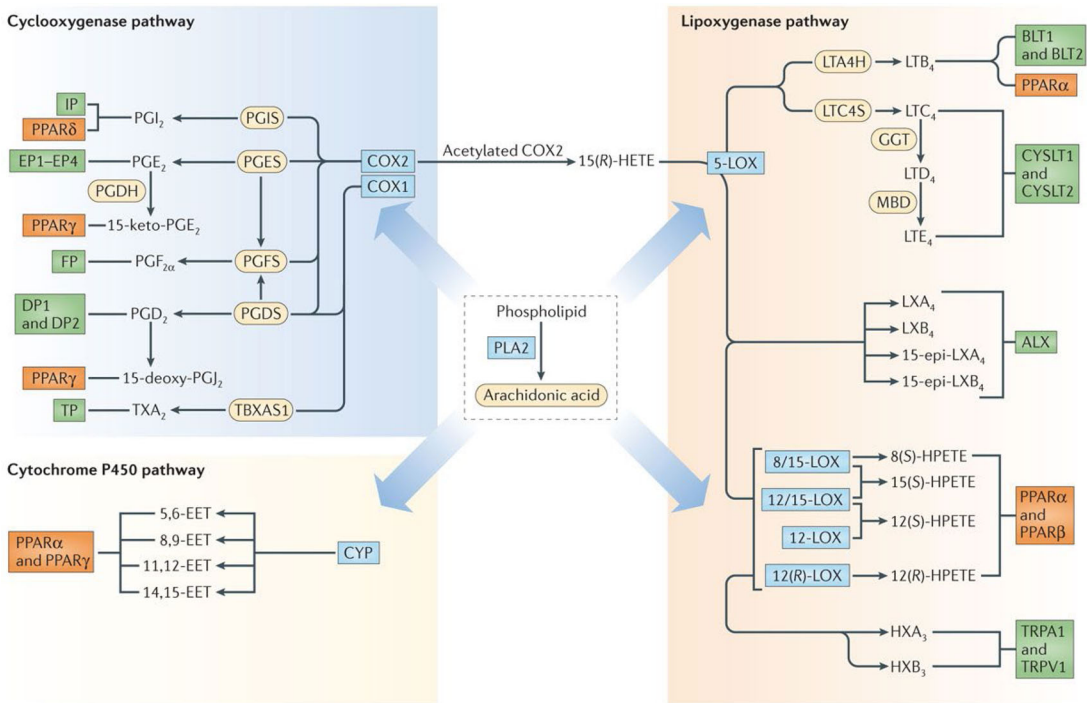
Figure 1. Eicosanoid biosynthesis and receptor signaling. Adapted from Ref. 6.

Additional investigation can provide great insights into how the body normally regulates inflammation in critical metabolic centers.[10]

On June 8, 2021, experts in lipid science presented virtually at a New York Academy of Sciences symposium "Bioactive Lipids and Metabolic Syndrome," organized by **Gregory Tesz, Michelle Clasquin,** and **Min Wan** to discuss the latest data on the mechanisms by which bioactive lipids regulate different signaling pathways and how these pathways could be targeted in developing novel therapeutics for cardiometabolic disease. This report summarizes the speakers' presentations at the one-day symposium.

Allosteric regulation and specificity of phospholipase A_2 in initiating inflammation

Edward A. Dennis of the University of California, San Diego opened the day's discussion with a keynote presentation addressing the role of bioactive lipids in metabolic disease. Research in Dennis' laboratory focuses on the phospholipase A_2

enzyme superfamily, which initiates the lipid aspect of inflammation.[11]

Macrophages are key components of both signaling and inflammation. Liposaccharide treatment activates TLR4 and, in turn, $cPLA_2$, which induces COX-2, leading to eicosanoid production.[12] ATP activation of $P2X_7$ results in sustained calcium influx and rapid eicosanoid production.

During eicosanoid biosynthesis, release of free arachidonic acid via PLA_2 leads to activation of primary and secondary prostaglandins; these bind to G-protein coupled receptors (GPCRs) or peroxisome proliferator-activated receptors (PPARs) within the cyclooxygenase or lipoxygenase pathways (Fig. 1).[6] Therapeutic mimetics of resolvins and protectins of eicosanoid pathways are being developed to treat metabolic disease with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) representing abundant fish oil omega-3 fatty acids (FAs) that may serve as precursors of anti-inflammatory agents.

The four main types of phospholipase A_2 (PLA_2) include $cPLA_2$, which is located in the cytoplasm

that releases arachidonic acid, iPLA$_2$, also located in the cytoplasm that releases unsaturated FAs, sPLA$_2$, which acts on the outside of the cell and releases both saturated and unsaturated FAs, and Lp-PLA$_2$, found in the bloodstream and associated with low-density lipoprotein (LDL), releasing oxidized FAs.[13–15] cPLA$_2$ is well known for producing prostaglandins and leukotrienes, which have proinflammatory effects.[11]

Water-soluble enzymes like PLA$_2$ need to associate with membranes or micelles and thereby exhibit surface dilution kinetics, which is dependent on both surfactant and phospholipid levels.[16,17] Dennis and his team used deuterium exchange mass spectroscopy to better understand how phospholipid substrates and membranes interact with lipid enzymes to affect metabolic pathways. Using molecular dynamics simulations based on the crystal structure of Lp-PLA$_2$, they were able to visualize enzyme docking and how the helix interacts and shifts in response to specific membrane vesicles; these results were consistent across several enzymes, including cPLA$_2$, iPLA$_2$, and sPLA-A$_2$[14,15] (and unpublished data). Membranes cause conformational changes in water-soluble enzymes, and phospholipids move quickly by lateral diffusion in the membrane until one molecule binds firmly in the active site.[15] These data demonstrate that membranes interact allosterically with these enzymes.[18]

A novel lipidomics assay revealed that enzyme activity is associated with high specificity for the *sn*-2 FA chains within membrane substrates.[19] Additionally, the binding site for the *sn*-2 acyl chain of each PLA$_2$ enzyme determines its binding specificity, rather than its catalytic residues or polar head group binding sites. Dennis and his team also showed that different PLA$_2$ enzymes exhibit specificity toward different FAs, including arachidonic acid, EPA, and DHA, as well as linolenic acid, antibacterial saturated FAs, or oxidized FAs in LDL (unpublished data). Therefore, each PLA$_2$ enzyme associates allosterically with membranes to pull in phospholipid substrates into their catalytic site and, therefore, hydrophobic subsites determine enzyme specificity.

Dennis summarized his presentation by highlighting the importance of these findings to the inflammatory processes associated with metabolic disease. The novel lipidomics platform that his team has developed provides a paradigm to evaluate protein–membrane lipid interactions.

Diabetes, cardiovascular, and NASH triad

De novo *lipogenesis in adipose tissue and the production of signaling lipids with beneficial metabolic and anti-inflammatory effects*

In the first session of the day, **Barbara B. Kahn** of Harvard Medical School discussed her investigation of the link between obesity and T2DM by studying *de novo* lipogenesis in adipose tissue, which is reduced in people with insulin resistance and increased in those who are insulin sensitive. Kahn and her team are specifically interested in the GLUT4 glucose transporter, as it is downregulated in adipocytes but not muscle tissue in humans and rodents with obesity and T2DM.

In collaboration with Ulf Smith, Kahn's team found a tight correlation between GLUT4 protein levels in adipose tissue of humans and glucose infusion rate, a measurement of whole-body insulin sensitivity, suggesting that GLUT4 levels in adipose tissue may be an early predictor of T2DM in people. Kahn's team knocked out *Glut4* specifically in adipocytes in mice and found insulin resistance and diabetes,[20] whereas when they overexpressed *Glut4* selectively in adipocytes, the mice showed enhanced glucose tolerance and protection against T2DM.[21]

A gene set enrichment analysis revealed that the pathway most highly regulated was FA synthesis, which is driven by carbohydrate response element binding protein (ChREBP).[22] Adipose-specific knockdown of ChREBP demonstrated that this transcription factor is a critical regulator of FA synthesis in adipose tissue; in addition, these results indicate that driving glucose into FAs in adipocytes is necessary for insulin sensitivity.[22,23]

Kahn's lab, in a project led by Mark Herman, discovered that overexpression of GLUT4 in adipocytes in mice induced ChREBP expression. With Alan Saghatelian and Edwin Homan they found that this resulted increased production of a novel class of lipids that they called branched FAHFAs. Kahn's team evaluated one subfamily in the "FAHFA-ome", specifically palmitic acid esters of hydroxy stearic acids (PAHSAs), to explore the characteristics and biological effects of these beneficial FAHFAs (Fig. 2).[24] The team found that FAHFAs are most highly expressed in white and brown fat. Additional work with Smith revealed that
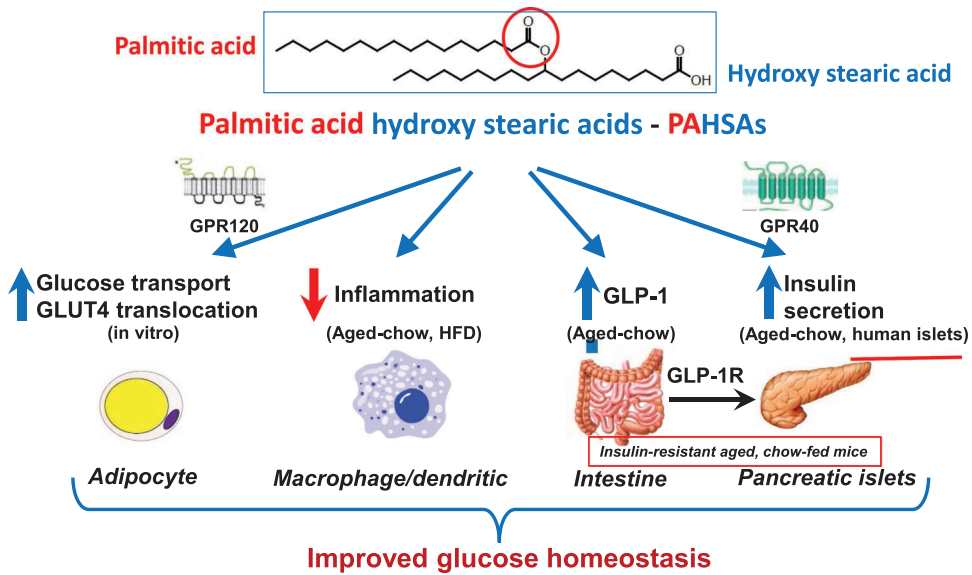
Figure 2. Palmitic acid hydroxy stearic acids (PAHSAs) are signaling lipids that promote glucose transport and GLUT4 translocation to the plasma membrane in adipocytes, have anti-inflammatory effects in macrophages and dendritic cells, promote GLP1 secretion from intestinal enteroendocrine cells, and augment glucose-stimulated insulin secretion from pancreatic islets. These secretory effects occur in mice that are insulin resistant due to aging and in human islets but not in mice on a high-fat diet (HFD). In HFD-fed mice, PAHSAs increase hepatic and systemic insulin sensitivity. Adapted from Refs. 24 and 25.

serum PAHSA levels are reduced in people who are insulin resistant and, in fact, serum PAHSA levels are tightly correlated with insulin sensitivity in people; PAHSA levels in adipose tissue revealed similar patterns also exhibiting correlations with systemic insulin sensitivity.[24]

Further evidence has revealed that PAHSAs exert antidiabetic and anti-inflammatory effects by increasing glucose transport and GLUT4 translocation from intracellular storage pools to the plasma membrane in adipocytes, reducing inflammation, inhibiting hepatic glucose production, increasing GLP-1 secretion, and improving insulin secretion, thus improving overall glucose homeostasis. While improvements in GLP1 and insulin secretion are not seen in mice on a high-fat diet, PAHSAs exert beneficial effects via insulin sensitization.[25,26] They also showed that PAHSAs potentiate insulin secretion following glucose stimulation in pancreatic islets from humans.[24] PAHSAs also improve hepatic and systemic insulin sensitivity, without altering weight gain or adiposity in mice on a high-fat diet, suggesting that they act as insulin sensitizers.[26]

A key factor driving insulin resistance is increased hepatic glucose production, which is caused by increased glycogenolysis and gluconeo-genesis and decreased glycogen synthesis. Usually, insulin exerts direct effects on the liver to regulate these processes through insulin signaling and indirect effects by inhibiting lipolysis in adipose tissue, which limits gluconeogenic substrate delivery to the liver.[27,28] In mice fed a high-fat diet, PAHSAs reduced adipose tissue inflammation by blocking antigen presentation and cytokine production.[24] PAHSA treatment also increased insulin action to suppress serum FAs in both chow-fed mice and those fed a high-fat diet.[26] Intralipid infusion prevented the lowering of FAs by PAHSAs, and this blocked their effects on insulin action to suppress hepatic glucose production in mice fed a high-fat diet. These results indicate that PAHSAs exert their effects by inhibiting lipolysis, thereby reducing hepatic glucose production.[26] PAHSAs also activate glucose transport and mediate insulin secretion via lipid-sensing GPCRs GPR120 and GPR40, respectively.[24,25]

PAHSA treatment reduced the incidence and delayed the onset of type 1 diabetes through immune cell modulation and direct protective effects on pancreatic islets. PAHSA treatment, therefore, has beneficial effects on autoimmune T1DM and potentially other autoimmune

diseases.[29] Work with Diane Mathis and James Mohan showed that PAHSA treatment reduced the number of CD45^{+} B and T cells in the pancreas of NOD mice.[29] Histological analysis showed that PAHSA treatment improved beta-cell viability and proliferation in the context of cytokine stress, as well as reducing beta-cell apoptosis and necrosis. This occurred at least in part through PAHSA effects to reduce endoplasmic reticulum stress. PAHSA treatment affects at least ∼3000 genes associated with endoplasmic reticulum stress, glucose metabolism, and a number of other important biological processes in pancreatic islet cells. In collaboration with Matt Kolar and Alan Saghatelian, Kahn and her team identified that a gain-of-function mutation in carboxyl ester lipase, which causes a monogenic form of diabetes,[30–32] increases PAHSA hydrolysis rate.[33]

This body of work has demonstrated that modulating PAHSAs in a tissue-specific manner by targeting hydrolases may allow for more specific immunosuppressive effects. Further study has demonstrated broader effects of PAHSAs on inflammation, specifically in ulcerative colitis, in which PAHSA treatment reduced gut inflammation and colitis severity and delayed disease onset via their effects on CD4^{+} cells and cytokine production.[34] Kahn summarized the discussion by highlighting the beneficial effects of PAHSAs on improving insulin sensitivity and insulin secretion and reducing inflammation.[35]

Plasma membrane sn-*1,2-diacylglycerols mediate lipid-induced liver, muscle, and white adipocyte insulin resistance*

Gerald I. Shulman of Yale School of Medicine discussed the work that his laboratory is pursuing on the role of plasma membrane (PM) associated *sn*-1,2-diacylglycerols in mediating lipid-induced insulin resistance in liver, muscle, and white adipose tissue (WAT) through activation of protein kinase Cε (PKCε), which, in turn, phosphorylates the insulin receptor on threonine[1160] (threonine[1150] in rodents), leading to inhibition of insulin receptor kinase activity.[3]

In a series of studies using novel nuclear magnetic resonance methods, which his group developed to noninvasively assess intracellular metabolism in humans, Shulman identified glucose transport as the rate-controlling step responsible for decreased insulin-stimulated muscle glycogen synthesis in individuals with T2DM.[36–38] Further study revealed that intramyocellular lipid content was an excellent predictor of muscle insulin resistance across the lifespan of humans.[39]

Contrary to the mechanism put forward by Randle and colleagues[40] to explain FA-induced insulin resistance in muscle, Shulman's group found that increasing plasma FAs resulted in reductions in both intramyocellular glucose-6-phosphate and glucose concentrations, implicating glucose transport as the step by which FAs cause insulin resistance in humans.[41,42] They went on to show that FA-induced muscle insulin resistance could be attributed to reductions in insulin-stimulated phosphatidylinositol-3 kinase (PI3-kinase) stimulation.[42]

Shulman's group found that they could also induce muscle insulin resistance in awake rats following an acute 5-h intralipid infusion; this lipid-induced insulin resistance tracked closely with increases in intramuscular diacylglycerol (DAG) content and PKCε and PKCθ activation but was independent of changes in intramuscular ceramide or triglyceride content, thus disassociating these metabolites for lipid-induced insulin resistance.[43] Further work identified the underlying molecular mechanisms by which increase in hepatic DAG content mediates hepatic insulin resistance by showing that knockdown of PKCε in the liver protected high-fat diet–fed rats from hepatic insulin resistance and that the insulin receptor kinase catalytic loop is phosphorylated on threonine[1160] (threonine[1150] is the murine homologue) by PKCε.[43–51]

The Shulman group created a *Insr[T1150A]* knockin mouse and demonstrated that this single amino acid substitution in the insulin receptor protected these mice from developing high-fat diet-feeding-induced liver insulin resistance despite the presence of hepatic steatosis, demonstrating a critical role for PKCε-induced phosphorylation of IRK[T1150] in mediating lipid-induced hepatic insulin resistance.[52,53] In addition, they also found that DAG activation of PKCθ, which is also present in skeletal muscle in addition to PKCε, leads to inhibition of insulin signaling at the level of IRS-1-associated PI3-kinase activity.[43]

DAGs exist as three different stereoisomers (*sn*-1,2, *sn*-1,3, and *sn*-2,3), and it has been shown that only the *sn*-1,2-DAG stereoisomers lead to
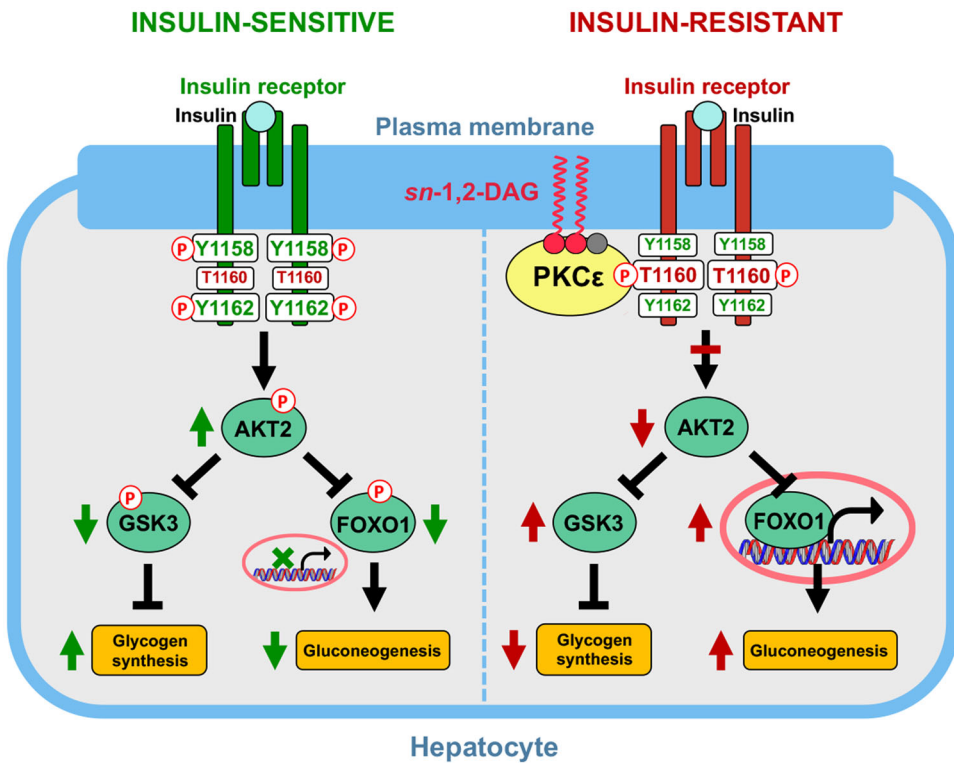
Figure 3. Downstream effects of *sn*-1,2-DAG on glycogen synthesis and gluconeogenesis. Figure from Ref. 53.

activation of novel PKCs. Furthermore, DAGs can exist in multiple intracellular compartments, which can potentially lead to different metabolic effects. To identify the intracellular compartment for *sn*-1,2-DAG-mediated PKCϵ activation/insulin resistance, Shulman and his team combined a cellular fractionation method with an LC-MS/MS method to assess *sn*-1,2 DAGs, *sn*-1,3 DAGs, and *sn*-2,3 DAGs in the PM, endoplasmic reticulum, mitochondria, cytosol, and lipid droplet fractions. Using this approach, they were able to demonstrate that it was accumulation of *sn*-1,2 DAGs specifically in the PM compartment that mediated lipid-induced hepatic insulin resistance in both humans with NAFLD and rodent models of NAFLD (Fig. 3).[53]

Using antisense oligonucleotides to acutely knock-down diacylglycerol *O*-acyltransferase 2 (DGAT2) and increase PM *sn*-1,2 DAGs, in combination with liver-specific knockdown of PKCϵ expression and liver-specific expression of a constitutively activated PKCϵ awake rat studies, they where able to demonstrate that PKCϵ is both necessary and sufficient for lipid-induced hepatic

insulin resistance through insulin receptor[T1150] phosphorylation.[53] In more recent studies, the Shulman group has demonstrated that increased PM-associated *sn*-1,2-DAGs leading to activation of PKCϵ and increased IRK[T1160] phosphorylation also play an important role in causing lipid-induced insulin resistance in skeletal muscle and WAT in both humans and high-fat diet fed rodent models of insulin resistance.[47,52–55]

This concept regarding the importance of PM *sn*-1,2 DAGs in mediating lipid-induced insulin resistance also explains why under certain conditions (e.g., MTTP deficiency, CGI-58 knockdown, HDAC3 knockdown, and athlete's paradox), ectopic lipid accumulation in liver and skeletal muscle, which can mostly be attributed to increases in TAGs/DAGs in lipid droplets without any increases in PM *sn*-1,2-DAGs, is not associated with insulin resistance in these tissues since increases in *sn*-1,2-DAGs in lipid droplets do not lead to activation of PKCϵ.[56–58] Furthermore, Shulman's colleagues found that chronic activation of PKCϵ can lead to phosphorylation of many other proteins, which, in

turn, will result in additional downstream effects on insulin signaling (e.g., p70S6K phosphorylation) and protein expression.[54]

Taken together, these studies demonstrate that: (1) PM-bound *sn*-1,2-diacylglytcerols cause hepatic insulin resistance; (2) PKCε is necessary and sufficient for mediating lipid-induced hepatic insulin resistance; (3) activation of PKCε promotes hepatic insulin resistance via phosphorylating insulin receptor[T1160], which, in turn, leads to inhibition of insulin receptor kinase activity; and (4) the PM-bound *sn*-1,2-diacylglycerol-PKCε-insulin receptor[T1160] phosphorylation pathway also mediates lipid-induced skeletal muscle and WAT insulin resistance.

Shulman proposed that, since the DIYETDYYRK motif in the catalytic domain of the insulin receptor is conserved all the way down the evolutionary ladder from humans to fruit flies, there is likely an evolutionary basis for lipid-induced insulin resistance. In support of this hypothesis, his group has demonstrated that increased PM *sn*-1,2-DAG in the liver during starvation, due to increased WAT lipolysis, leads to PKCε activation and hepatic insulin resistance and decreased hepatic glycogen synthesis. The same process would be predicted to occur in skeletal muscle and WAT. Starvation-induced PM *sn*-1,2-DAG-PKCε-IRK[T1160] phosphorylation insulin resistance is thus critical for survival by preserving blood glucose for utilization by the brain and other glucose obligatory organs (e.g., red blood cells, renal medulla, etc.) when food energy is scarce.[59]

Central role of oxidized phospholipid in inflammatory diseases

Joseph L. Witztum of the University of California, San Diego presented work from his laboratory testing his hypothesis that PC-containing OxPL is a key mechanism linking hypercholesterolemia to atherosclerosis and NASH.[60,61] Previous studies in humans using vitamin E, which were designed to inhibit lipid peroxidation to treat other diseases, failed to inhibit cardiovascular disease. These negative observations decreased enthusiasm for this line of research at least at the clinical level. However, Witztum proposed that these findings resulted from a lack of understanding of the mechanisms underlying how enhanced lipid peroxidation could promote atherosclerosis.[62]

Witztum and his team observed the generation of autoantibodies to epitopes of OxLDL in APOE-deficient mice and identified the innate IgM natural antibody EO6 from a cloned panel of IgM antibodies; they also demonstrated that it bound to OxLDL lipid and protein but not native LDL.[63] Cloning the variable heavy and light genes of EO6 revealed that it had been previously cloned and found to encode the T15 idiotype, which binds to the PC moiety on the *Streptococcus pneumonia* cell wall.[64,65] They also demonstrated that EO6 recognizes the PC of OxPL when the polyunsaturated side chain is oxidatively modified[66] but did not recognize the PC of non-OxPLs. Collaboration with Edward A. Dennis revealed that E06 shows specificity to several OxPL analogs.[67]

OxPL, such as POVPC, are a common set of epitopes on OxLDL,[68] and not native LDL. On OxLDL, they are important ligands recognized by pattern recognition receptors, not only by EO6, but also by scavenger receptors on macrophages, such as CD36, and by soluble innate proteins, such as CRP. They are also present on dead and dying cells and on the apoptotic bodies shed from apoptotic cells. As they are ubiquitous in inflammatory settings, pattern recognition receptors play an important role in innate immune defenses against PC epitopes to maintain homeostasis.[69] A large body of work has shown that oxidation-specific epitopes (OSEs), such as OxPL, are present in the tissues of inflammatory diseases but not in healthy tissues.[61,70–76]

Utilizing a mouse model that overexpresses a single-chain variable fragment of the EO6 antibody (EO6-scFv), Witztum and his team found that EO6-scFv protects macrophages from OxPAPC-induced inflammation. For example, macrophages from hypercholesterolemic LDL receptor gene knockout mice exhibited a nearly 20-fold increase in IL-1β expression, which was blocked in mice that overexpressed EO6.[77] Furthermore, atherosclerosis was substantially decreased even after a full year of exposure to a high cholesterol diet.

Histological analysis of an AMLN-NASH mouse model revealed signs of NASH at 48 weeks, including an increase in serum OxPL levels.[61] People with NASH or cirrhosis also have increased plasma OxPL levels, which are correlated with disease progression; in addition, OxPL accumulation is also present in the liver and plasma.
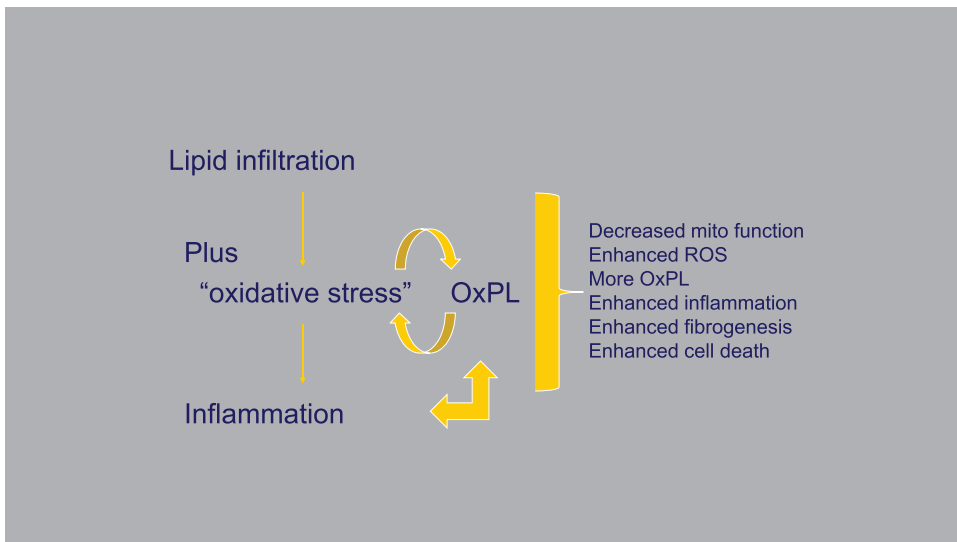
Figure 4. OxPL promotes atherogenesis and NASH via feed-forward cycles.

Witztum and his team utilized the EO6-scFv mice in the AMLN-fed LDL receptor gene knockout model to determine if OxPL is directly involved in the pathogenesis of NASH and, indeed, found that targeting OxPL reduced multiple manifestations of NASH.[61] These mice had reduced OxPL levels, a 50% reduction in liver fat, and a 70% reduction in collagen in the liver; ALT and AST levels were also reduced. Further analysis revealed reduced atherosclerosis in the aorta and heart despite high levels of cholesterol, indicating OxPL as a common mechanism mediating both atherosclerosis and NASH in these mice.[61,77]

Witztum summarized by discussing the underlying mechanisms that can improve NASH manifestations, including the beneficial effects of neutralizing OxPLs,[61] which appear to promote atherosclerosis and NASH via feed-forward cycles (Fig. 4). Oxidative stress generates ROS in cells, which, in turn, generates OxPL, which then generate yet more ROS and OxPL, resulting in feed-forward OxPL-mediated pathways. Among other effects, this leads to decreased mitochondrial function and enhanced inflammation, creating a vicious cycle in promoting oxidative cellular stress.[61,72] Collaboration with Sotirios Tsimikas revealed that OxPL-ApoB is a robust predictor in human populations of cardiovascular disease and stroke risk across 15 years, further demonstrating that OxPLs and other relevant OSEs may be a favorable target in thera-peutic development for metabolic and many other diseases.[78,79]

Dysregulation of resolution pathways in atherosclerosis

Sudeshna Sadhu, a graduate student at the Albany Medical College, discussed the work that she has been pursuing in the laboratory of Gabrielle Fredman on the contribution of specialized pro-resolving mediators (SPMs), such as resolvin D1 (RvD1), and efficient efferocytosis in atherosclerosis. Specifically, she discussed the balance between counter-regulators, such as lipoxins/resolvins, and proinflammatory agents, like leukotrienes (Fig. 5). In atherosclerosis, inflammation-resolution impairment is driven by defective synthesis of RvD1 and failed efferocytosis leading to larger necrotic core in advanced plaques. Exogenous administration of RvD1 to advanced atherosclerotic mice reverses the deleterious features of advanced atherosclerotic plaque.[9,80–82]

Senescent cells that undergo a phenotypic change, the senescence-associated secretory phenotype, are highly proinflammatory and proteolytic.[83–85] Inducing senescence in an animal model through sublethal ionizing gamma-irradiation demonstrated that resolution interval was delayed by 12 h and PMN infiltration in the peritoneum was increased compared to nonirradiated mice, indicating impaired
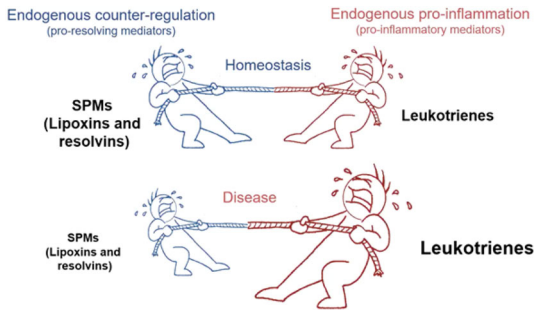
Figure 5. Proinflammatory and proresolving mediators.

inflammation resolution.[86–89] Treatment with RvD1 significantly decreased PMN infiltration in the peritoneum at 24 h in the senescence model, suggesting that enhanced efferocytosis may be a mechanism by which RvD1 is limiting PMN in the peritoneum. However, resolution was not affected by lethal radiation and bone marrow transplant.[89]

Sadhu developed and characterized an *in vitro* macrophage senescent model, and found that senescent macrophages have failed efferocytosis that can be partially rescued by RvD1; in addition, adoptive transfer of senescent macrophages prolonged inflammation.[89] These results together indicate that senescence impairs temporal resolution, senescent macrophages prolong inflammation, and RvD1 can improve resolution and restore efferocytosis.

Senescent cells accumulate and promote necrosis in advanced plaques.[90,91] Immunofluorescence analysis revealed increased p16[INK4A+] senescent cells in symptomatic plaques of people with atherosclerosis, suggesting that these cells could in fact drive plaque necrosis. Using an irradiated LDLR-deficient mouse model fed a Western diet, Sadhu found that 3 weeks of RvD1 treatment limited necrosis and p16[INK4A+] senescent cells.[89]

Evaluation of another model of senescence, in which p16[INK4A+] cells were selectively removed from LDLR-deficient mice via ganciclovir treatment, demonstrated a reduction in plaque necrosis.[89,92] In collaboration with Matthew Spite, Sadhu also found that ganciclovir treatment, and thus removal of hematopoietic p16[INK4A+] cells (like senescent macrophages), increases proresolving mediators and decreases proinflammatory mediators.[89]

Sadhu summarized her presentation by discussing how failed inflammation-resolution program and accumulation of senescent cells contribute to the progression of atherosclerosis and how these results reveal a novel mechanism that could help limit senescence in plaques.

Humanized monoacylglycerol acyltransferase 2 mice develop nonalcoholic steatohepatitis and fibrosis that responds to treatment with elafibranor

Joe Nickels of the Genesis Biotechnology Group discussed his work on the role of the monoacylglycerol acyltransferase, MGAT2, in NASH and the development of potential therapeutics targeting this enzyme. MGAT2 catalyzes the reaction of monoacylglycerol and FA CoA into DAG, which is then converted to triacylglyceride. MGAT2 is part of the monoacylglycerol pathway in the intestines for the production of triglycerides and feeds substrates into the glycerol phosphate pathway in the liver (Fig. 6).[93]

Previous work using *mMgat2* knockdown models revealed that the absence of MGAT2 increased glucose tolerance and insulin sensitivity, reduced triglyceride excursion in the small intestine, and reduced hepatic steatosis.[94] An *mMgat2* intestinal-specific knockout mouse showed similar phenotypes,[95] which were reversed after expressing human MGAT2.[96] Taken together, these results suggest that MGAT2 could represent a favorable target for developing NAFLD therapeutics.[97,98]

Nickels and his team developed a humanized huMGAT2 mouse and validated this model for its efficacy as a tool for drug discovery using elafibranor, a PPARα/δ agonist. HuMGAT2 mice fed an amylin diet for 16 weeks showed increased hepatic triglyceride accumulation and high degrees of inflammatory cytokines and hydroxyproline levels in the liver, a biomarker for collagen deposition during fibrosis. Elafibranor reversed these effects, as well as lowered levels of steatosis and immune cell infiltration. Nickels and his team also found that huMGAT2 mice were glucose intolerant and insulin resistant when fed a Western diet.

They next evaluated one of their small molecule MGAT2 inhibitors for *in vivo* efficacy (compound 3). They found that compound 3 reduced triglycerides from entering the bloodstream at all doses evaluated using an oral lipid tolerance test. Treatment with elafibranor or compound 3 also reduced
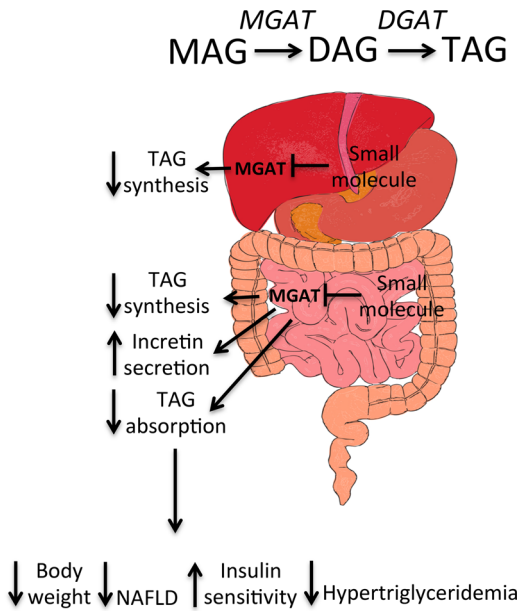
Figure 6. MGAT2 enzymatic reaction. From Ref. 97.

liver triglycerides levels, AST and ALT levels, and hydroxyproline levels.

Nickels summarized his presentation by highlighting that the huMGAT2 mRNA knockdown model may represent a "humanized model" to study various compounds aimed at improving triglyceride metabolism and preventing NAFLD progression.

Bioactive lipid mediators of inflammation

Proresolving lipid mediators

Matthew Spite of Harvard Medical School and Brigham and Women's Hospital presented his work on the role of lipid mediators in inflammation and resolution and how imbalances in these processes are associated with cardiometabolic diseases and tissue injury.

Tissue damage in the skin drives initial inflammation, which transitions over time to the reparative phase to re-establish the epithelial barrier, eventually leading to tissue maturation, where macrophages assist in repair.[99,100] People with T2DM experience delayed wound healing and also have a higher risk of cardiovascular disease, likely due to altered inflammatory responses that occur as a response to injury.[101,102]

Lipid mediators play key roles in the initiation of inflammation as well as its ultimate resolution. Over time, a lipid mediator class switch occurs, where proinflammatory lipid mediators (e.g., leukotrienes) are gradually replaced by SPMs to facilitate the resolution of inflammation and promote tissue repair.[103] Omega-3 FAs EPA and DHA, as well as aspirin, initiate the production of SPM, suggesting that SPMs contribute in part to the anti-inflammatory effects of omega-3 FAs and aspirin.[104]

Spite and his team showed that altered resolution of inflammation in obesity and diabetes could be rescued by treatment with RvD1, a resolution agonist, in part by increasing macrophage-dependent apoptotic cell clearance (i.e., efferocytosis).[105,106] Topical RvD1 also enhanced wound closure in part by reducing apoptotic cells in the skin and by promoting epithelial cell migration; longer-term treatment improved systemic metabolism by increasing glucose tolerance and improving HOMA IR.

A large body of evidence has shown that SPM improves metabolism in obesity by resolving inflammation.[107–114] In contrast, leukotriene B_4 (LTB_4), a proinflammatory lipid mediator, increases inflammation and insulin resistance in obesity. Spite and his team found that LTB_4 receptor-deficient mice are protected from insulin insensitivity induced by a high-fat diet and exhibit lower levels of liver triglycerides and inflammation.[115] A collaboration with Mark Brown's group uncovered that reduced levels of FADS1, an enzyme involved in the production of polyunsaturated FAs that are precursors to lipid mediators, worsened cardiometabolic disease in part by altering the balance of proinflammatory and -resolving lipid mediators in a diet-dependent manner. Omega-3 FAs treatment reduced atherosclerosis, and these effects were FADS1 dependent.[116]

Lipid mediator metabolomics revealed an imbalance in proinflammatory versus proresolving lipid mediators in advanced atherosclerotic lesions in humans and animal models, resulting in part from higher levels of proinflammatory leukotrienes in vulnerable compared to stable regions of the lesions.[9] Treatment with RvD1 decreased atherosclerotic plaque necrosis, increased lesional efferocytosis, and increased the ratio of proresolving mediators to proinflammatory lipid mediators to that observed in early lesions; this was associated with increased thickness of the protective fibrous cap, indicative of increased lesion stability. Several additional studies demonstrated the central role of macrophage effector functions in resolution of
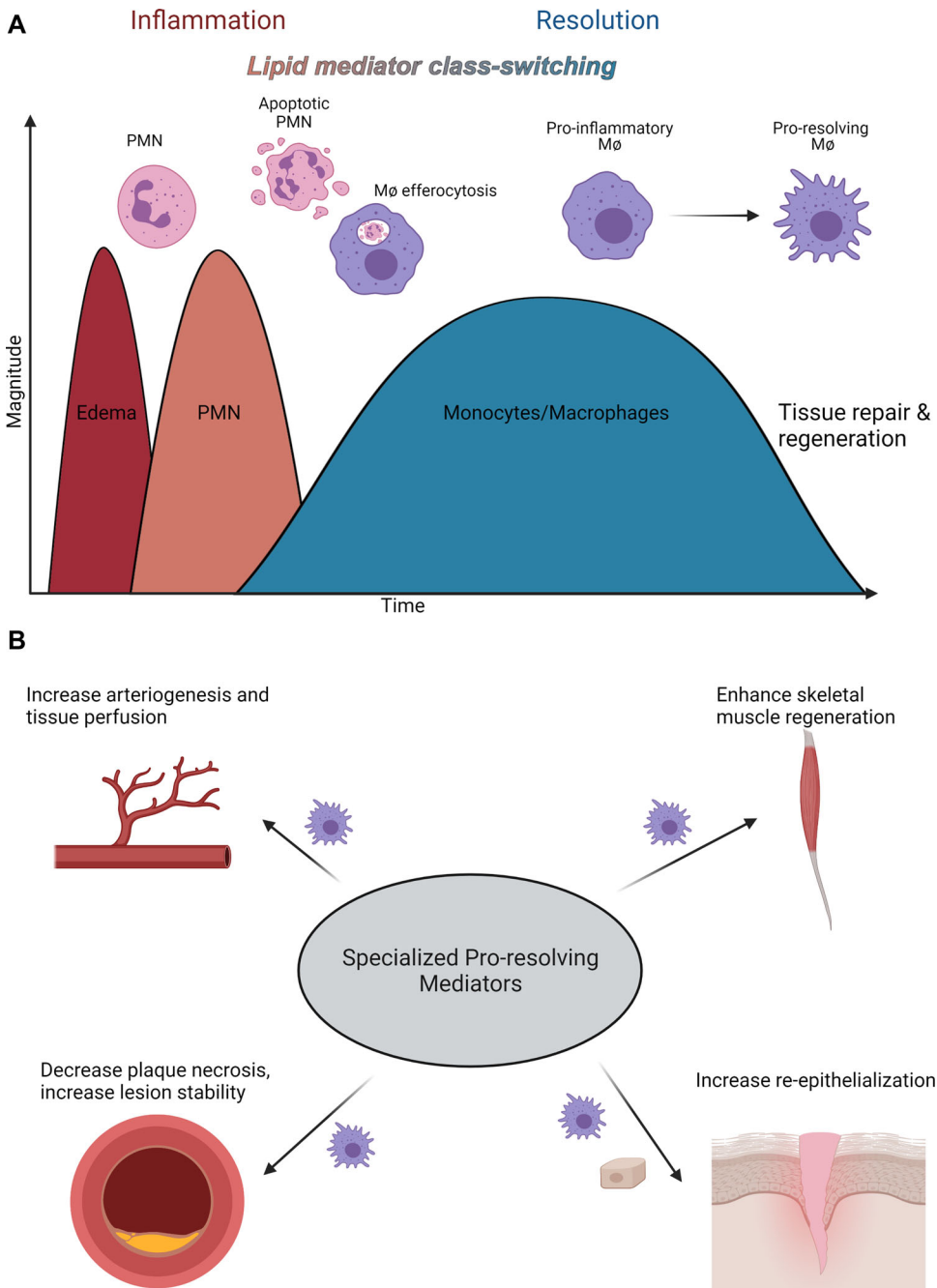
Figure 7. Resolution of inflammation is associated with lipid mediator class switching in which proinflammatory lipid mediators are replaced by specialized proresolving lipid mediators that facilitate tissue repair and regeneration in part through regulating macrophage function.

inflammation in atherosclerosis, which is driven in part by lipid mediator balance (Fig. 7).[80,117–119]

Spite and his team collaborated with colleagues at Johns Hopkins University to determine the role of lipid mediators in muscle injury in which macrophages are necessary for tissue regeneration.[120,121,122] Targeted lipidomics analysis in a model of cytotoxic muscle injury revealed a

lipid mediator class switch across days 2–4, where proinflammatory lipid mediators peak early after injury and SPM production coincided with the appearance of tissue reparative macrophages. Further analysis indicated that lipid mediators have distinct time signatures across specific immune cell subsets.[122]

RvD2, an effector of macrophage subtype specificity, promotes functional recovery in skeletal muscle when given after injury.[122] Along these lines, another SPM, RvD1, promoted revascularization of injured skeletal muscle and decreased fibrosis by activating the ALX/FPR2 receptor; these effects were lost in ALX/FPR2-deficient mice.[123] RNA-seq analysis revealed that RvD1 induces a prorevascularization transcriptomic signature in macrophages. A myeloid-specific *Alx/Fpr2* knockout impaired perfusion recovery to a similar extent as that in the global knockout; there was also significant downregulation of provascular genes in macrophages in the muscle.[123] These results extend prior studies by the Spite group demonstrating that RvD2 promotes arteriogenesis during ischemia to enhance tissue perfusion.[124]

Spite summarized his discussion by highlighting the multilevel actions of proresolving lipid mediators in tissue repair and their contribution to unresolved inflammation in cardiometabolic disease. Proresolving mediators that can facilitate macrophage reparative functions could potentially be effective in treating altered tissue repair in cardiometabolic diseases.

Lysophospholipids in cardiovascular disease

Susan Smyth of UAMS discussed work that her laboratory is pursuing on the role of LPA in vascular inflammation and atherosclerosis, as genome-wide association studies revealed a link between *PLPP3* variants and myocardial infarction risk across many populations.[125]

PLPP3 encodes for an enzyme that regulates LPA. The enzyme autotaxin (ATX) regulates extracellular levels of LPA, which exerts effects on cellular functions, including migration, proliferation, and survival. LPP enzymes along the cell surface hydrolyze and inactivate LPA. Genetic polymorphisms in *PLPP3* (encoding LPP3) predict coronary artery disease.

The ATX/LPA/LPP3 signaling system regulates adipogenesis, endothelial barrier function, inflam-

mation (especially IL-6 levels), and smooth muscle cell migration.[126–130] Using an LDLR mRNA knockdown model, Smyth and her team found that LPA accumulates in very low-density lipoprotein (VLDL)/LDL fractions in diet-induced hyperlipidemia. Further analysis of an ATX mRNA knockdown model revealed lower levels of plasma LPA and VLDL/LDL fractions, indicating that changes in lipoprotein LPA content occur in an ATX-dependent manner.[131]

Additional analysis of the LDLR mRNA knockdown model revealed accumulation of LPA in vessel walls of the aorta and within plaques. As LPAR4 knockdown causes abnormal endothelial function,[132] Smyth and her team used a PCSK9 viral vector to reduce LDL receptors in wild-type controls and LPAR4-deficient mice, and fed a Western diet to elevate cholesterol levels. Despite similar increases in LDL levels, the LPAR4-deficient mice had lower atherosclerotic plaque burden in comparison to littermate control animals, suggesting a role for LPA receptor signaling in experimental atherosclerosis.[133]

Three PLPP3 SNPs in humans have been identified that enhance promoter activity.[8,134,135] Work with Chris O'Callaghan using FAIRE seq to evaluate lipid-induced epigenomic changes in foam cells identified a region that overlapped with a risk polymorphism in *PLPP3*; the protective allele interacts with C/EBP-beta and predicts higher *PLPP3* expression in foam cells.[136] Smyth proposed a model in which LPP3 expression results in more degradation of LPA and/or another bioactive lipid mediator that in turn lowers inflammation and potentially reduces atherosclerosis (Fig. 8).

Further work revealed the role of Rel response elements in the human *PLPP3* promoter,[137] and thus prompted the hypothesis of the creation of an insulator loop that brings together regulatory regions of the gene, including the final intron containing the risk polymorphisms. To test the overarching model, Smyth and her team examined the effect of reducing LPP3 expression on experimental atherosclerosis. Their work found that global LPP3 reduction in LDLR-deficient mice increases plaque LPA and plaque inflammation and promotes atherosclerosis.[138] Similarly, reduction in smooth muscle cell LPP3 expression accelerates atherosclerosis. Additional work using double ATX-LPP3
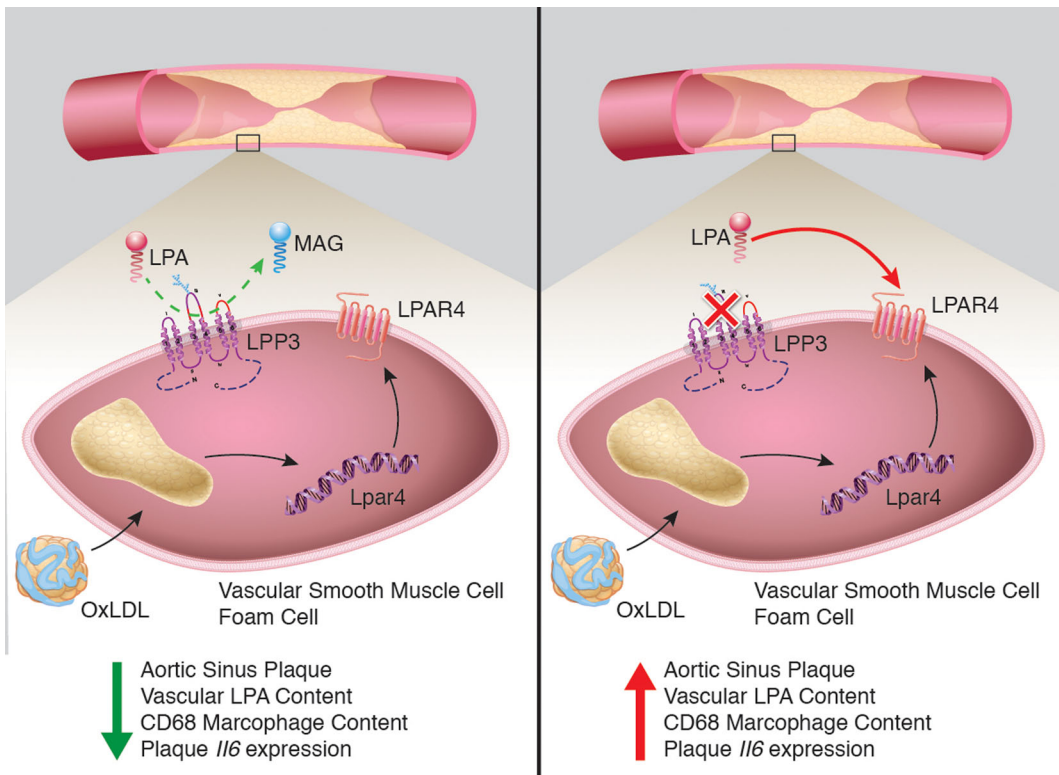
Figure 8. Proposed model of LPP3 regulation in experimental atherosclerosis.

deficient mice revealed a potential for ATX in the process (unpublished data).

Smyth summarized by highlighting the role of LPP3 regulation in vascular smooth muscle cells and foam cells. She emphasized how genetic variants of LPP3 in humans greatly affect lipid signaling and the risk of developing atherosclerosis. Her work also identified LPA, ATX, and LPP3 as mediators of cardiovascular disease.

Sphingolipids in metabolic disease

Sarah Spiegel of the Virginia Commonwealth University closed out the day's session by presenting her work and that of others on the contribution of bioactive sphingolipid metabolites, such as ceramide and sphingosine-1-phosphate (S1P), to obesity and metabolic disorders as molecular mediators (Fig. 9).[139]

Work in human and animal models has revealed that increased ceramide levels are associated with metabolic disease, and specifically that the C16:0/C24:0 ceramide ratio is predictive of heart failure. Therefore, ceramide could represent a potential biomarker of cardiovascular disease; this is now being investigated at the Mayo Clinic.[140–145] Some studies, however, have reported that plasma S1P is also increased in obese rodents and humans; thus, correlation with disease progression requires further study.

Increased ceramide is implicated in the development and progression of metabolic diseases, and the precise molecular mechanisms of ceramide elevation and its downstream effects are focuses of current research. One area of fairly extensive work has focused on AKT, a kinase that mediates many of the effects of insulin, including increasing liver gluconeogenesis and uptake of glucose in muscle and adipose tissue. Work from Summers, Holland, Scherer, and others strongly indicates that, in many tissue types, ceramide blunts insulin-induced activation of AKT by activating the protein phosphatase PP2A or alternatively by activating atypical protein kinase C, PKCζ.[146,147] PP2A also reduces adipocyte lipolysis by inhibiting
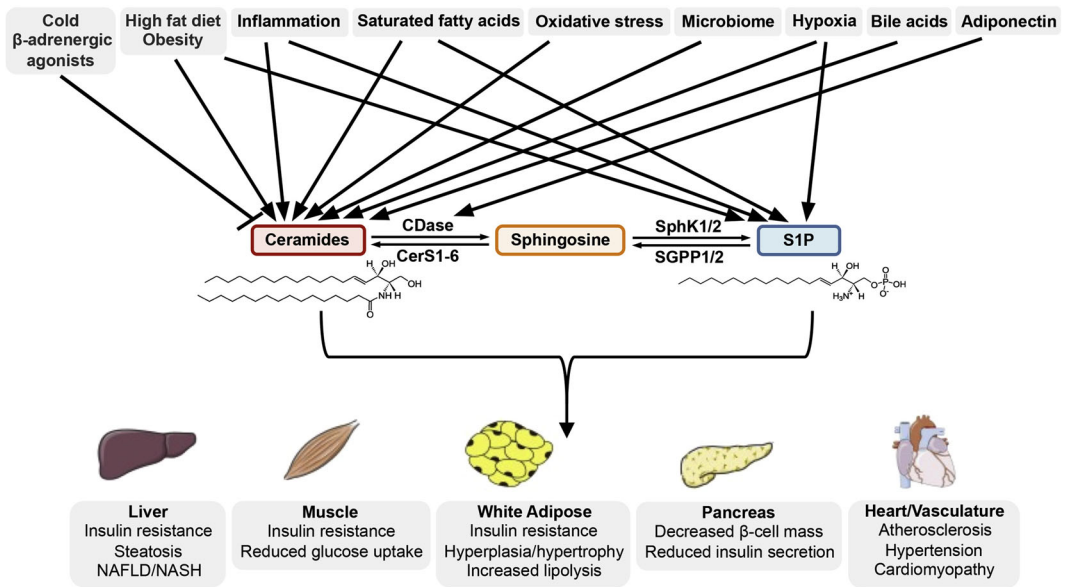
Figure 9. Involvement of ceramide and S1P in the development of metabolic diseases. From Ref. 139.

hormone-sensitive lipase.[148] PKCζ enhances lipogenesis in hepatoctyes by sterol regulatory element binding transcription factor 1c (Srebp1c)[149] and increased expression of CD36, an FA translocase that promotes their esterification.[148,150]

As mentioned, recent data suggest that not all ceramides are the same and that there are different acyl chain ceramides associated with different metabolic diseases, either as biomarkers or potential direct mediators. One prominent example of the latter is hepatic C16:0 ceramide, which binds to mitochondrial fission factor to promote mitochondrial fission, causing impairment of mitochondrial function and insulin resistance in obesity.[151]

While there is abundant evidence that ceramide functions in numerous ways to aggravate metabolic diseases, the roles for its further metabolite S1P and the sphingosine kinases (SphKs) that produce it are less well studied. Many agonists, including insulin, IGF1, and cytokines, stimulate sphingosine kinases, particularly SphK1; S1P is transported out of cells and signals in an autocrine or paracrine manner by binding to S1P receptors (S1PRs) on the cell surface.[152] This concept, termed "inside-out signaling by S1P," is now well accepted and has important implications for metabolic dysregulation, immune responses, and inflammation, and may be involved in adiponectin protection from obesity-associated

pathology. It has been suggested that the binding of adiponectin to its receptors decreases ceramide levels by enhancing their intrinsic ceramidase activity that leads to the conversion of sphingosine to S1P.[153,154] It is possible that binding of S1P to its receptor can then prevent apoptosis of pancreatic β-cells and cardiomyocytes and may also contribute to the antidiabetic action of adiponectin.

In contrast to ceramide, the binding of S1P to S1PR2/3 in hepatocytes can activate AKT to enhance insulin signaling and can also regulate the expression of PPARγ and promote lipid storage.[155] In addition to actions via the S1PRs, S1P also has intriguing intracellular actions.[156] We have shown that S1P formed in the nucleus by SphK2 inhibited histone deacetylases HDAC1/2,[157] causing an increase in acetylation of histones and upregulation of hepatic genes encoding nuclear receptors and enzymes involved in lipid metabolism.[158] We also found that the prodrug FTY720/fingolimod, which is phosphorylated *in vivo* by SphK2 to an S1P, mimetic protects from insulin resistance in part by inhibiting HDAC and decreasing FA synthase expression.[159]

Despite these beneficial roles, SphKs and S1P have deleterious functions likely due to their known effects on immune cell trafficking and proinflammatory signaling.[152] An overload of saturated FA

increases liver SphK1 in mice and in people with NASH. It has been suggested that activation of S1PR1 by S1P in hepatocytes leads to NF-κB activation and elevated cytokine/chemokine production that potentially contributes to proinflammatory signaling in NASH.[160]

The role of S1P in adipose tissue is also complicated. Increased SphK activity can initially remove harmful ceramide; however, in adipocytes, S1P and S1P/S1PR signaling modulates inflammation, differentiation, and pathways affecting lipolysis, glucose uptake, and mitochondrial biogenesis.[161,162] S1PR signaling in endothelial cells is also involved in metabolic disorders and T2D. Impaired vasodilation in hypertension is a serious complication of metabolic diseases, and was shown that ApoM-S1P acts as a biased agonist that antagonizes the cytokine-induced NF-κB pathway and also prolongs S1PR1 signaling resulting in sustained AKT and eNOS activation leading to enhanced barrier function and vasodilation counteracting ceramide effects. Indeed, a biased agonist of S1PR1, SAR247799, that mimicked ApoM-S1P effects in endothelial cells, is now in clinical trials for T2D.[163,164]

This work illustrates the Janus face of S1P and the kinases that produce it in metabolic diseases based on cell type, signaling effectors, and S1P synthesis/degradation. Spiegel summarized by discussing the link between ceramide and S1P in the development of metabolic disease, with ceramide at the nexus of obesity and S1P as a key regulator of inflammation. She also discussed gaps that should be addressed with further research to better understand the biological functions of ceramide and S1P in metabolic diseases.

Acknowledgments

Competing interests

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