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Role of Lipotoxicity in Endothelial Dysfunction

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SUMMARY

Lipotoxicity, caused in large part by overnutrition, directly leads to endothelial dysfunction. Excess lipids in both the circulation and at the tissue level contribute to endothelial dysfunction that underlies much of the pathophysiology of both metabolic disease, including obesity and diabetes and their CV complications. Direct lipotoxic effects on other organs as well as secondary insults from endothelial dysfunction synergize to cause substantial morbidity and mortality. Lifestyle interventions, including reduced calorie intake, diet, and exercise as well as a variety of pharmacologic interventions targeting various mechanisms underlying lipotoxicity in vascular endothelium significantly modify metabolic and CV risk.

Keywords

Lipotoxicity; Endothelial dysfunction; Metabolic disorders; Cardiovascular disease

INTRODUCTION

CV disease is the leading cause of death in the United States. Reciprocal relationships between endothelial dysfunction and insulin resistance tightly link metabolic diseases, including obesity and diabetes, with their CV complications.¹ Obesity and diabetes per se increase the risk of CV morbidity and mortality at least 3-fold.²

Vascular endothelium plays an important role in maintaining vascular homeostasis and actively participates in the delivery of hormones, nutrients, and oxygen to metabolic target

tissues. These functions are regulated by secretion of endothelium-dependent relaxing factors, endothelium-dependent hyperpolarizing factors, and endothelium-dependent contracting factors.³ Imbalance among these factors contributes to endothelial dysfunction that is associated with cardiac dysfunction, coronary artery disease, hypertension, diabetes, and neurologic disorders, leading to increased mortality and morbidity.^{1,4} Excess circulating lipids (hyperlipidemia) caused by both overnutrition and disordered metabolism are important independent cause of both endothelial dysfunction and insulin resistance. High levels of lipids, including triglycerides (TGs), NEFAs, and low-density lipoprotein cholesterol (LDL-C) damage vascular tissues and their functions. This is known as lipotoxicity.⁵⁻⁷ Lipotoxicity may be defined as pathologic changes at the cellular and organ levels that results from excess lipids in the circulation or in tissues.

Endothelial dysfunction caused by lipotoxicity is mediated through several diverse mechanisms that include increased oxidative stress and proinflammatory responses. The effect of lipotoxicity on endothelial dysfunction is magnified even further in patients with obesity, metabolic syndrome, and diabetes.^{1,8,9} This article discusses

1. Lipotoxic effects in vascular endothelium
2. Molecular mechanisms underlying the pathophysiology of endothelial dysfunction from lipotoxicity
3. Lifestyle intervention and therapeutic approaches that may oppose lipotoxicity-induced endothelial dysfunction and its CV and metabolic complications.

HYPERLIPIDEMIA AND ENDOTHELIAL DYSFUNCTION

Triglycerides

An elevated serum TG level is a risk factor for coronary heart disease.^{10,11} Lipoprotein-associated TGs circulate in the plasma as very low-density lipoproteins or chylomicrons. Insulin receptor substrate-1 (IRS-1) knockout mice, a nonobese animal model of insulin resistance, have impairments in endothelium-dependent vascular relaxation and hypertriglyceridemia (HTG) with low activity of lipoprotein lipase (LPL).¹² This suggests that insulin resistance may play an important role in hypertriglyceridemia and endothelial dysfunction that may accelerate the progression of atherosclerosis. Through genome-wide association studies, Johansen and colleagues¹³ identified common variants in APOA5, Glucokinase regulator, LPL, and APOB that are associated with HTG. Mutations of lipoproteins, LPL and glycosylphosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) are associated with HTG.^{14,15} LPL hydrolyzes TGs into glycerol and free fatty acids (FFAs). GPIHBP1 is necessary for TG hydrolysis.¹⁶ In addition to genetic variations, diet, lack of exercise, and various medications affect HTG.¹⁷ Lipodystrophy, Cushing syndrome, and medications, including β -blockers and tamoxifen, induce HTG.¹⁸ A meta-analysis of population-based prospective studies shows that plasma TG level is a risk factor for CV disease independent of high-density lipoprotein (HDL).¹⁹ The TG level associated with postprandial lipemia suppresses flow-mediated dilation in patients with hypothyroidism.²⁰ Flow-mediated dilation is negatively correlated with TG and thiobarbituric acid reactive substances level in the plasma. Thus, endothelial dysfunction

after an oral fat challenge in these patients is due to HTG and reactive oxygen species.²⁰ A clinical study with 109 patients who had coronary heart disease during statin therapy compared ezetimibe add-on therapy to placebo. Endothelial function was assessed after 3 months. Flow-mediated dilation in patients treated with ezetimibe is improved and blood TG level is significantly reduced.²¹ Ezetimibe improves postprandial hyperlipidemia and endothelial dysfunction.²² In a previous report, the intima-media thickness of patients who received combination therapy of ezetimibe and simvastatin is not different from that in the patients treated with simvastatin only despite significant lowering effect of low-density lipoprotein (LDL), as shown in the Ezetimibe and Simvastatin in Hypercholesterolemia Enhances Atherosclerosis Regression trial.²³ This suggests that TG level independent of LDL may lead to endothelial dysfunction that contributes to development of atherosclerosis. TG increases inflammatory responses by activation of leukocytes.^{24–26} Intramuscular TG content is also associated with insulin resistance in skeletal muscle^{27,28} and endothelial dysfunction.²⁹ Accumulation of TG in the heart is associated with heart failure in animals and humans.^{30,31} Thus, intracellular and plasma level of TG is associated with impairment of glucose tolerance and insulin resistance as well as CV dysfunction.

Nonesterified Fatty Acids

Increased plasma NEFAs and accelerated rates of lipolysis are characteristics of type 2 diabetes mellitus and obesity.^{32–34} Acute elevation of plasma fatty acids leads to impairment of glucose uptake,³⁵ inhibition of hepatic gluconeogenesis,^{36,37} and endothelial dysfunction.^{38,39} Inhibition of lipolysis by using acipimox, a niacin derivative, improves glucose tolerance, insulin sensitivity, insulin-stimulated capillary recruitment, and acetylcholine-stimulated vasodilation.^{40,41} Likewise, intralipid plus heparin impairs 20% of methacholine, but not single-nucleotide polymorphism-stimulated, vasodilation in lean healthy human subjects.³⁹ This suggests that fasting NEFA impairs glucose metabolism as well as endothelial function. NEFA levels independently predict all-cause and CV mortality in subjects with angiographic coronary artery disease.⁴² Some other studies, however,^{43,44} show fasting NEFA levels were not correlated with obesity when normalized to fat mass.⁴⁵ NEFA is liberated from adipose tissue under starvation conditions by hydrolysis of TG. Elevated fasting NEFA level is associated with obesity.⁴⁶ After a meal, dietary fat is hydrolyzed by LPL to NEFA and glycerol that are taken up by adipose tissue and re-esterified intracellularly. Some NEFA escapes, however, in a process called *spillover*.⁴⁷ A recent study shows that rates of delivery of NEFA were reduced in obese men compared with lean subjects when normalized to fat mass. The reduced lipid trafficking into adipose tissue leads to ectopic fat deposition.⁴⁸ Thus, postprandial fat content is determined by dietary fat, 40% to 50% of which may be from spillover.⁴⁸ Both fasting and postprandial NEFAs interact with glucose metabolism; NEFA level may be a predictor for CV disease that is associated with obesity and diabetes.

Cholesterol

Non-HDL cholesterol contributes to impairment of nitric oxide (NO) bioavailability due to increased production of reactive oxygen species and inhibition of endothelial NO synthase (eNOS) activity that leads to endothelial dysfunction and atherosclerosis.^{49–51} Patients with high cholesterol have impaired endothelium-dependent vasodilation but not endothelial

independent vasodilation.⁵⁰ This suggests that elevated cholesterol levels impair vascular function, which contributes to atherosclerosis. Plasma LDL levels in patients with type 2 diabetes mellitus are similar to those of nondiabetics, but LDL catabolism is significantly reduced.⁵² Insulin treatment in type 2 diabetes mellitus and lipid lowering by diet or pharmacologic treatments, including statins, restores catabolism of LDL^{52,53} and improves endothelial function.⁵⁴ Oxidized LDL (ox-LDL)/LDL-C ratio in type 2 diabetes mellitus is reduced by insulin therapy.⁵⁵ Ox-LDL displaces eNOS from caveolae to impair eNOS activity stimulated by acetylcholine.⁵⁶ Ox-LDL-mediated inhibition of eNOS is restored by HDL.⁵⁷ Ox-LDL is taken up by macrophages to promote foam cell formation and reduces cholesterol efflux and reverse cholesterol transport.^{58,59} These processes contribute to atherosclerosis and CV diseases. A decreased HDL level is associated with obesity and HTG, insulin resistance and diabetes that may be due to increased HDL catabolism.^{60–62} In a more recent study, HDL isolated from patients with CAD fails to activate eNOS and does not have anti-inflammatory and endothelial repair activity when compared with HDL isolated from healthy subjects.⁶³ HDL isolated from patients with type 2 diabetes mellitus is not able to reverse the ox-LDL-mediated inhibition of endothelium-dependent vasorelaxation.⁶⁴ This suggests not all HDL is beneficial. Thus, deregulated cholesterol metabolism affects endothelial function and blood flow that contributes to atherosclerosis and CV events.

LIPOTOXICITY IN TISSUES

Cardiomyopathy

Obesity, insulin resistance, and diabetes are associated with cardiomyopathy that leads to premature morbidity and mortality. Obesity-mediated endothelial dysfunction, dysregulated autonomic regulation, and altered hormonal profiles contribute to the impairment of cardiac function.^{65–67} Obese people have 2 to 3 times higher risk for developing heart failure than normal weight people.⁶⁸ Excess lipid intake or dyslipidemia causes ectopic lipid accumulation in cardiomyocytes. This results in cell death, increased ER stress, mitochondrial dysfunction, accumulation of ceramide, and increased exposure to reactive oxygen species.⁶⁹ Accumulation of TG in myocytes results in alternative substrate metabolism. Thus, cardiac energy metabolism is shifted toward more O₂ consumption and lower ATP production that leads to cardiac dysfunction. Ceramide metabolized from FFA is a major mediator of lipotoxicity that is blocked by myriocin or by stimulation of sphingomyelinase.^{69,70} Dietary fat determines lipid profiles in the plasma and tissues and causes various pathophysiologic conditions. In recent studies, a diet with high fat/low carbohydrate is more preventive in progression of heart failure than a diet with low fat/high carbohydrate.^{71–75} A diet high in polyunsaturated fatty acid reduces coronary heart disease, whereas a diet with high cholesterol increases coronary heart disease.^{76–79} Studies using various high fat diet and rodent models are well described in a recent review.⁷⁵ More studies regarding recommendations for optimal diet to patients with cardiomyopathy are necessary.

Nephropathy

Nephrotic subjects tend to develop CV disease associated with metabolic disorders and vascular complications. Diabetic nephropathy is a major risk factor for CV disease and

increases morbidity and mortality.⁸⁰ Diabetic nephropathy progresses in 5 stages: glomerular hyperfiltration, incipient nephropathy, microalbuminuria, overt proteinuria, and end-stage renal disease.⁸¹ Endothelial dysfunction precedes manifestation of microalbuminuria and is an independent risk factor for CV disease and diabetic nephropathy.^{82,83} Hypercholesterolemia decreases NO availability, which leads to activation of podocyte and renal injury.^{84,85} The common lipid abnormalities found in chronic kidney disease patients are HTG, reduced HDL, and increased lipoprotein (a).⁸⁶ Lipoprotein (a) level is a risk factor for CVD and is associated with glomerular filtration rate.⁸⁷ Decreased catabolism of very low-density lipoproteins, chylomicrons, and their remnants leads to accumulation in the plasma. Increased ApoC(III), a potent inhibitor of LPL, leads to increased serum TG.⁸⁸ Proposed mechanisms include increased reabsorption of lipids, including, fatty acids, phospholipids, and cholesterol, that stimulates tubulointerstitial inflammation and tissue injury.^{89,90} Accumulation of oxidized lipoproteins, especially ox-LDL, causes inflammation in glomerular mesangium that contributes to recruitment of macrophages.^{91,92} Diabetic nephropathy can contribute to dyslipidemia and lipid-lowering therapies that can improve endothelial function may have beneficial effects on both renal and CV functions.

Neuropathy

Patients with diabetic neuropathy have peripheral nerve disorders with symptoms of pain, numbness, weakness, and difficulties with balance.^{93,94} It is characterized as axonopathy with distal predominance, with axons failing to regenerate.⁹⁵ Both myelinated and unmyelinated fibers undergo axonal change, leading to axonopathy.⁹⁵ Impaired autonomic nerve functions inducing a reduction of blood flow contributes to alterations in the microvasculature, leading to diabetic nephropathy and retinopathy.^{96,97} Impairment of vascular function is associated with diabetic neuropathy and CV disease.^{98,99} Loss of autonomic control is associated with impairment of ventricular function independently from endothelial dysfunction.¹⁰⁰ Accumulation of sorbitol, oxidized lipid, and poly (ADP-ribose) polymerase (PARP) and activation of lipoxygenase in peripheral nerve are observed in a high-fat diet rodent model.¹⁰¹ FFA causes toxicity in cultured neuronal and Schwann cell lines.¹⁰² Elevated FFAs due to increased lipolysis in adipocytes may increase secretion of inflammatory cytokines affecting peripheral nerve inflammation. Neuronal cells express receptors for ox-LDL and glycated LDL, including ox-LDL receptor 1, scavenger receptors, receptor for advanced glycation end products, and Toll-like receptors (TLRs).^{103,104} This suggests that ox-LDL and glycated LDL can injure neuronal cells through stimulation of inflammatory response and oxidative stress.¹⁰³ Dysregulated lipids directly and indirectly impair neuronal function through mediators, including inflammatory cytokines and active oxygen/nitrogen species produced by other tissues that contribute to neuropathy.

MOLECULAR MECHANISMS

Lipid-mediated toxicities contribute to functional abnormalities in various tissues, including vascular endothelium, cardiac, and renal tissues. The proposed mechanisms are

- Oxidative stress

- Inflammation
- Mitochondrial dysfunction
- ER stress
- Cell death

These mechanisms are interlinked and contribute to complex pathophysiology.

Oxidative Stress

Increased lipids in the plasma, ectopic lipid accumulation, and intracellular lipid droplets cause oxidative stress.^{27,105} Reactive oxygen and nitrogen species react with proteins, lipid, and carbohydrates to modify structure and function of cellular components.^{106,107} Lipids affect cellular redox status through metabolic pathways. Vascular endothelium is an important redox-sensitive tissue because it transports various metabolites between tissues. Nicotinamide adenine dinucleotide phosphate-oxidase (NAD(P)H oxidase) creates oxidative stress by producing superoxide that can be converted to hydrogen peroxide and reacts with NO.^{108–110} This reaction determines bioavailability of NO, a major determinant of endothelial dysfunction.¹⁰⁹ In the Zucker fatty rat, plasma FFA and TG levels are higher than lean controls causing impairment of acetylcholine-stimulated vasodilation.¹¹¹ Treatment of primary vascular endothelial cells with palmitate stimulates production of superoxide and increased expression of NAD(P)H oxidase subunits.^{109,111} Inhibition of NAD(P)H oxidase activity with apocynin or diphenyleneiodium or by knockdown of NAD(P)H oxidase (NOX) 4 (a NOX subunit) suppresses production of superoxide or expression of NAD(P)H oxidase subunits. These data suggest that saturated fatty acids stimulate production of reactive oxygen species that leads to reduction of NO, bioavailability, and subsequent endothelial dysfunction. In addition to increased expression of NAD(P)H oxidase subunits, diacylglycerol stimulates activation of protein kinase C, a kinase that phosphorylates p47phox. Phosphorylation of p47phox is important for the activation of NAD(P)H oxidase and assembly of multiple NAD(P)H oxidase subunits to form an NAD(P)H oxidase holoenzyme.^{112,113} Another mechanism to increase oxidative stress requires mitochondrial dysfunction. The mitochondrial respiratory chain (mainly complex I and III) produces superoxide when fatty acids are oxidized.^{114,115} Fatty acids are metabolized by β -oxidation to produce NADH (Nicotinamide adenine dinucleotide) and FADH₂ (Flavin adenine dinucleotide) that enter the electron transport chain. This generates ATP by oxidative phosphorylation in mitochondria, where oxygen serves as an electron acceptor. During this process, a proton gradient is formed across the mitochondrial membrane where addition of a single electron to oxygen generates superoxide.^{114,116,117} Accumulated protons leak from the intermembrane space to the matrix, resulting in reduced proton motive force that generates heat instead of ATP. This is called uncoupling. Uncoupling protein 2 knockout mice have increased diet-induced atherosclerosis, endothelial dysfunction, and decreased antioxidative capacity.¹¹⁸ Additionally, uncoupling of eNOS and stimulation of xanthine oxidase produces superoxide.^{119–121} Increased reactive oxygen species increases lipid peroxidation and oxidized cholesterol and reduces NO availability to promote vascular insulin resistance and endothelial dysfunction.

Inflammation

Obesity and type 2 diabetes mellitus are characterized by a chronic proinflammatory state.^{122,123} Increased plasma or intracellular lipid contents cause proinflammatory responses. Inflammation is closely linked to atherosclerosis, a major sequelae of dyslipidemia-mediated endothelial dysfunction.¹²⁴ NEFA stimulates TLRs 2/4 that activate I κ B kinase (IKK β)/nuclear factor (NF)- κ B and c-Jun N-terminal kinase (JNK)/activator protein-1, pathways leading to increased expression of other cytokines and cell adhesion molecules, including tumor necrosis factor (TNF)- α , interleukin (IL)-1 β , intercellular adhesion molecule, vascular cell adhesion molecule, and E-selectin in endothelial cells.¹²⁵ NEFA-mediated proinflammatory signaling inhibits insulin signaling that leads to impairment of glucose tolerance, vascular insulin resistance, and lipogenesis.^{125–127} Activation of TLRs by NEFA occurs in macrophages to facilitate atherogenic processes that exacerbate pathophysiology in metabolic and CV tissues.¹²⁸ NEFA activates bone morphogenic proteins 2 and 4 that contribute to vascular calcification and differentiation of smooth muscle cells associated with vascular stiffness.^{109,129} Ox-LDL also acts as a ligand for TLRs to stimulate proinflammatory responses.¹³⁰ The mechanisms for inflammatory signaling to inhibit insulin signaling are increased serine phosphorylation of IRS-1 and IRS-2, facilitated degradation of IRS-1 and IRS-2, and ceramide production.¹³¹ Increased serine phosphorylation of IRS-1 and IRS-2 leads to inhibition of interaction with insulin receptor, plasma membrane, and phosphatidylinositol 3-kinases that are linked to reduced activation of Akt and impairment of eNOS activation.^{132–135} This mechanism contributes to impairment of glucose uptake and blood flow in skeletal muscle.^{38,119,136} Activation of TLRs by NEFA or LPS increases ceramide production that contributes to inhibition of insulin-stimulated phosphorylation of Akt as well as facilitation of apoptosis.^{131,137}

Mitochondrial Dysfunction

Mitochondria are intracellular organelles that oxidize fatty acid. Electron transfer from NAD(P)H to NAD(P)⁺ couples with production of ATP, an energy source for movement and cellular enzymatic process.¹¹⁷ When metabolic stress conditions occur, cells adapt by increasing mitochondrial biogenesis.^{138,139} More mitochondria may compensate for increased cellular energy needs. eNOS and NO play important roles in mitochondrial biogenesis through AMP-activated protein kinase (AMPK)/nuclear respiratory factor 1/ peroxisome proliferator-activated receptor (PPAR) γ coactivator 1 α (PGC1 α)–mediated mechanisms.^{140,141} Maladaptation of this process leads to mitochondrial dysfunction and decreased ATP production. Thus, mitochondrial dysfunction is associated with decreased fat oxidation and accumulation of intramuscular lipids.¹⁴² Mitochondria also play important roles in apoptosis (discussed later).

ER Stress

Obesity and type 2 diabetes mellitus cause increased ER stress in animal models and humans.^{143–146} ER stress is involved in both impairment of energy metabolism and inflammation.¹⁴⁷ The lipid peroxidation product, 4-hydroxy-trans-2 nonenal (HNE), increases ER stress. Treatment of endothelial cells with phenylbutric acid or taurine-

conjugated ursodeoxycholic reduces HNE-induced leukocyte rolling and adhesion.¹⁴⁸ ER stress leads to unfolded protein responses (UPRs) by (1) increasing expression of molecular chaperones, (2) facilitating degradation of proteins, and (3) inhibiting protein synthesis.¹⁴⁷ Molecules in the ER, including ATF6, IRE-1 α /XBP-1, and p-PERK/p-eIF2 α , are involved in UPRs to protect cells from stress conditions. When UPR is insufficient to protect cells from ER stress, cells are subjected to pathologic conditions, including inflammation, apoptosis, and lipid accumulation, that promote atherogenesis.^{144,149–152} Activation of JNK is a well-known mechanism underlying obesity-induced insulin resistance and ER stress.^{143,144,153} JNK directly phosphorylates IRS-1 at serine 307, leading to inhibition of insulin signaling pathways that contribute to inflammatory responses, impairment of glucose tolerance, and endothelial dysfunction.^{125,154–156}

Apoptosis

Excess lipids increase ceramide, reactive oxygen species, lipid peroxidation, and membrane destabilization that lead to cell death through apoptosis. Inhibition of ceramide synthesis by myriocin reverses high fat/high cholesterol-mediated endothelial dysfunction and atherosclerosis.¹⁵⁷ Antiapoptotic molecules, including Bcl2 and Bax, are phosphorylated by Akt. This action is inhibited by ceramide to cause apoptosis.^{158,159} Ceramide increases iNOS expression through NF- κ B, leading to production of peroxynitrite that is toxic to cells.^{160,161} Ox-LDL promotes endothelial cell death and atherosclerosis. This is opposed by HDL and ApoA.^{162,163} Endothelial dysfunction and subsequent reduction of NO inhibits caspase activity by nitrosylation.^{164,165} Thus, tissue damage by lipotoxicity is a major mechanism underlying the pathophysiology caused by dyslipidemia and diabetes.

ROLE OF EXERCISE IN OPPOSING LIPOTOXICITY AND ENDOTHELIAL DYSFUNCTION

Exercise plays a pivotal role in improving lipotoxicity and endothelial dysfunction.^{166,167} Increased lipid accumulation with intramyocellular TGs in obese individuals with limited physical activity leads to reduced β -oxidation. Activation of peroxidation pathways predisposes to insulin resistance and its complications.¹⁶⁸ Intermediates of peroxidation pathways (thiobarbituric acid derivative, 4-hydroxynonenol, and malondialdehyde) may lead to increased levels of TNF- α and other proinflammatory factors that mediate insulin resistance and endothelial dysfunction.¹⁶⁸ Exercise and endurance training help increase turnover of stored excess lipids by increasing β -oxidation and reducing peroxidation pathways. In one study, even low-intensity exercise of 2 h/wk for 3 months had a positive impact on opposing lipotoxicity and increasing total fat oxidation.¹⁶⁹ Exercise has beneficial effects not only on skeletal muscle lipotoxicity but also on cardiac muscle. Two weeks of intensive swimming by mice led to an increase in diacylglycerol acyltransferase expression with greater TG uptake and oxidation in cardiac muscle. This was accompanied by decreased plasma cholesterol, TG, FFA, ceramide, and diacylglycerol. Lipid uptake genes, CD36 and LPL; PDK4 (Pyruvate dehydrogenase kinase, isozyme4), a regulator of glucose oxidation and acyl coenzyme A oxidase; PPAR α mRNA; and mRNA for PGC1 α (a regulator of mitochondrial biogenesis) increased with 2 weeks of physical activity. Adipose TG lipase, the enzyme that initiates intracellular TG lipolysis, was induced with exercise.¹⁷⁰

The other benefits of exercise to oppose lipotoxicity include increased expression of superoxide dismutase.^{171,172} Oxidative enzyme activity in skeletal muscle improves with moderate intensity physical activity in previously sedentary men and women with 4 months of physical activity and moderate weight loss. Exercise improves enzymatic activity of the mitochondrial electron transport chain and increased surface area of the inner mitochondrial membrane with increased cardiolipin levels leading to mitochondrial biogenesis.¹⁷³

AMPK is a fuel-sensing enzyme that is activated in response to exercise. It is also expressed in adipose tissue, liver, and other organs in response to exercise in intensity-dependent matter. Even low-intensity exercise, when prolonged, can also increase expression of AMPK. AMPK activity causes increased glucose uptake in muscle, promotes fatty acid oxidation, exerts anti-inflammatory and anti-fibrinolytic actions, raises HDL cholesterol, and decreases protein synthesis in skeletal muscle. AMPK also has central actions to decrease food intake in response to leptin action in the hypothalamus.¹⁷⁴ Hypothalamic insulin and leptin sensitivity is also improved by anti-inflammatory effect of cytokines, IL-6 and IL-10, that are positively influenced by exercise.¹⁷⁵ Other beneficial effects of exercise include increase in GLUT4 expression¹⁷⁶ and effects on muscle glycogen synthase activity. These actions of exercise oppose lipotoxicity and translate into improved CV outcomes.

The effect of exercise to oppose endothelial dysfunction is mediated, in part, by increased bioavailability of NO in the vasculature.¹⁷⁷ Regular physical activity improves blood glucose control and can prevent or delay type 2 diabetes mellitus, along with beneficial effects on blood pressure, lipids, CV morbidity, mortality, and quality of life. The American College of Sports Medicine and the American Diabetes Association recommend combined aerobic and resistance training exercise and aerobic activity of moderate intensity, 40% to 60% of V O₂max, 3 days a week, with no more than 2 consecutive days between bouts of physical activity.¹⁷⁸

PHARMACOLOGIC TREATMENTS

Endothelial function is an important predictor of clinical outcomes, especially in populations with high CV risk. Therefore, targeting established and modifiable risk factors for lipotoxicity and endothelial dysfunction is a rational primary strategy for preventing CV complications associated with dyslipidemias and insulin-resistance. Combined therapy with statins or PPAR agonists and renin-angiotensin-aldosterone system (RAAS) blockade has additive beneficial effects on endothelial dysfunction, dyslipidemia, and insulin resistance compared with monotherapy in patients with CV risk factors that are mediated through both distinct and interrelated mechanisms.¹⁷⁹ In addition to primary prevention based on caloric restriction, weight loss, physical exercise, and smoking cessation, pharmacotherapies, including insulin sensitizers, hypolipidemic agents, and/or inhibitors of the renin-angiotensin system, effectively modify risk factors to result in beneficial effects on both metabolic and vascular homeostasis. A combinatorial therapeutic strategy aimed at improving circulating lipid profile, lowering oxidative stress, and attenuating inflammation by distinct cellular mechanisms may be helpful in improving the risk/benefit profile of pharmacotherapy for endothelial dysfunction.

Statins

Statins comprise either natural or synthetic compounds (Box 1) acting as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors. HMG-CoA reductase converts HMG-CoA to mevalonic acid in the cholesterol biosynthetic pathway, the rate-limiting step in cholesterol synthesis. By inhibiting HMG-CoA reductase activity, statins decrease hepatic sterol synthesis. This decreases hepatocellular cholesterol. Hepatocytes respond to decreased intracellular cholesterol concentration by increasing synthesis of LDL receptors to enhance hepatic LDL reuptake from the circulation. The net result is an increased fractional catabolism of LDL that reduces serum LDL-C concentration and total cholesterol.¹⁸⁰ Several large randomized controlled trials have unequivocally demonstrated that inhibition of HMG-CoA reductase with subsequent lowering of LDL-C has large beneficial impacts on CV risk.^{181–183} In addition to LDL lowering, statins have several pleiotropic effects associated with improvements in markers of vascular disease risk, including

- Inflammation
- Plaque stability
- Endothelial function^{184–188}

Box 1

Statins

Natural compounds

- Lovastatin
- Simvastatin
- Pravastatin

Synthetic compounds

- Atorvastatin
- Fluvastatin
- Rosuvastatin

Direct actions of statins to affect Rho/Rho kinase pathways in human leukocytes have been reported.^{189,190} Several other in vitro and in vivo studies have suggested that statins exert physiologic effects independent from LDL cholesterol lowering. For example, beneficial vascular effects of statins may depend on their ability to modulate levels of C-reactive protein (CRP). CRP induces synthesis of cytokines, cell adhesion molecules, and tissue factor in monocytes and endothelial cells^{188,191} and upregulates angiotensin II type 1 (AT1) receptors in vascular smooth muscle cells.¹⁹² Therapy with statins decreases IL-1 β -induced plasma CRP levels independently of cholesterol lowering in transgenic mice expressing human CRP.¹⁹³ These direct anti-inflammatory effects in vivo occur at the transcriptional level and have been confirmed in cultured human liver slices and in human hepatoma cells.¹⁹³ Improved endothelial function in response to statins may also result from

prevention of NO oxidation and upregulation of eNOS expression,^{194,195} partly by eNOS mRNA stabilization.¹⁹⁶ Additional mechanisms by which statins exert their beneficial effects on endothelial function may involve eNOS coupling and vascular bioavailability of BH4 (tetrahydrobiopterin), a critical cofactor that is required to maintain eNOS enzymatic coupling activity.^{197,198} This effect has been attributed to statin-mediated upregulation of GTPCH I (guanosine triphosphate cyclohydrolase), the rate-limiting enzyme in BH4 biosynthesis, initially demonstrated in cultured human endothelial cells.¹⁹⁹ Improved endothelial function in response to statin therapy has also been linked to increased NO and reduced peroxynitrite and O₂⁻ generation in cell culture^{198,200,201} as well as in animal models.²⁰² Clinical evidence supports the concept that improved endothelial function with treatment with statins is associated with early reduction of systemic oxidative stress,²⁰³ partly as a result of reduction in NOX activity in the arterial wall.²⁰⁴ In addition, statins are known to downregulate AT1 receptor density,²⁰⁵ and this may result in a direct NOX inhibitory action through blockade of Rac isoprenylation.

PPAR Agonists

The nuclear receptor transcription factors, PPARs, including PPAR α , PPAR γ , and PPAR β/δ isotypes, are each encoded by separate genes. PPARs are established drug targets for treating dyslipidemia and diabetes.²⁰⁶ PPAR α agonists (fenofibrate, gemfibrozil, ciprofibrate, and clofibrate), PPAR γ agonists (pioglitazone and rosiglitazone),²⁰⁷ and dual or pan agonists (bezafibrate, muraglitazar, ragaglitazar, tesaglitazar, and aleglitazar)²⁰⁸ have both metabolic and CV actions.

Fibrates

Although peroxisome proliferation does not occur in humans, fibrate drugs, which lower TGs and raise HDL, were found to do so via PPAR α activation.²⁰⁹ Subsequent studies established that PPAR α activation induces expression of LPL that releases fatty acids from TGs and represses apolipoprotein ApoC(III), an endogenous LPL inhibitor. PPAR α increases expression of CD36, which participates in fatty acid uptake, and fatty acid binding proteins involved in fatty acid delivery. PPAR α also regulates multiple enzymes involved in β -oxidation of fatty acids. PPAR α activation also induces expression of APOA1, a major constituent of HDL. Fibrate therapy may help limit inflammatory responses induced by FFA in vascular and inflammatory cells. In endothelial cells, PPAR α activated by synthetic agonists or LPL-mediated release of natural ligands inhibits adhesion molecule expression.^{210,211} Additional effects include suppression of monocyte-macrophage migration²¹² and monocyte chemoattractant protein-1 and reduced inflammatory markers, including CRP, lipoprotein-associated phospholipase A₂, IL-6, fibrinogen, and TNF- α .²¹³ PPAR α activation also limits inflammation in vascular smooth muscle cells that may indirectly influence atherosclerosis. PPAR α activation represses inflammation by inhibiting key proximal inflammatory mediators, including NF- κ B and activator protein-1. In vascular smooth muscle cells, PPAR α also limits cellular proliferation by targeting the cyclin-dependent kinase inhibitor and tumor suppressor, p16^{Ink4a}, resulting in inhibition of retinoblastoma protein phosphorylation, decreased G₁ phase to S phase transition, and less intimal hyperplasia in vivo. In macrophages, activation of PPAR α decreases coagulation proteins and promotes cholesterol efflux,²¹⁴ conferring additional protection from

atherosclerosis.²¹⁵ In addition, fibrates may decrease angiotensin II-mediated oxidative stress and inflammation in the vascular wall.²¹⁶ Furthermore, fibrates may improve endothelial function indirectly via increased adiponectin levels and improved insulin sensitivity and flow-mediated vasodilation.²¹⁷

Despite increasing in vitro evidence of vascular protective properties, fibrates have shown controversial results in the clinical setting: in the Veterans Affairs High-Density Lipoprotein Intervention Trial, gemfibrozil was shown to exert a statistically significant 22% relative risk reduction in CV events in a subgroup of patients with established heart disease and concomitant glucose intolerance or high fasting plasma insulin level.^{218,219} A subsequent study, the Fenofibrate Intervention and Event Lowering in Diabetes trial, did not find any significant difference in the primary endpoint between groups, although treatment with fenofibrate was associated with reduced secondary CV endpoints.²²⁰ The Action to Control Cardiovascular Risk in Diabetes, lipid arm, study reported that combining a statin and fenofibrate was no better in decreasing CV events than a statin alone.²²¹ As suggested in other fibrate trials, however, a benefit was seen in the subgroup with more significantly elevated TG levels and lower HDL levels.²²² The absence of trials focusing on patients with more significantly elevated TGs and lower HDL levels where fibrates may be most helpful, may help to resolve this issue.

Thiazolidinediones

Synthetic PPAR γ receptor agonists ameliorate lipotoxicity by multiple mechanisms. These include a decrease of circulating NEFAs by inhibition of lipolysis,^{223,224} reduction of muscle long-chain fatty acylcoenzyme A levels,^{225,226} and are distribution of fat and changes in body composition.⁵ Thiazolidinediones (TZDs) also ameliorate lipotoxicity and endothelial dysfunction via effects mediated through increased plasma adiponectin levels²²⁷ and subsequent adiponectin-mediated antiatherogenic effects.^{228,229} More recently, beneficial effects of TZDs have been related to their ability to ameliorate mitochondrial lipotoxicity, a condition characterized by reduced expression of multiple nuclear genes that encode enzymes involved in oxidative metabolism, such as PGC1,³⁴ a master regulator of mitochondrial biogenesis.²³⁰ Treatment with TZDs upregulates PGC1 α and PGC1 β mRNA expression in association with increased expression of pyruvate dehydrogenase alpha 1 and other mitochondrial oxidative phosphorylation genes.²³¹ In addition to effects on lipid metabolism, TZDs may directly regulate multiple target genes relevant to inflammation and atherosclerosis.²³² In vascular tissues, PPAR γ regulates production of endothelial mediators, including NO,²³³ and modulates expression of genes involved in cell adhesion,²³⁴ inflammation,²³⁵ oxidative stress,²³⁶ and vasoconstriction.^{237,238} In macrophages, PPAR γ anti-inflammatory actions play a protective role in atherosclerosis.²¹⁴ In the vascular compartment, PPAR γ ligands reduce AT1 receptor mRNA and protein expression,²³⁹ with decreases in reactive oxygen species and NADP oxidase activity as well as responsiveness to angiotensin II. These beneficial cellular and molecular properties of TZDs are not always supported by results from clinical trials. For example, effects of pioglitazone on CV events were investigated in PROactive, a large prospective study designed to test possible glucose-independent CV benefits in patients undergoing TZDs therapy.²⁴⁰ Although only a nonstatistically significant trend toward benefit was seen with pioglitazone for multiple

primary endpoints (10% risk reduction), a 16% reduction was found when more standard, objective secondary endpoints, such as myocardial infarction, stroke, and CV mortality, were evaluated.²⁴⁰ In addition, pioglitazone decreased plaque volume in human coronaries²⁴¹ and carotid intima-media thickness.²⁴² It is possible that these improvements may be related to pioglitazone-mediated increases in HDL.^{243,244} The effects of rosiglitazone on CV disease have been more controversial, partly because no large prospective study on CV outcomes was undertaken with this agent. Several meta-analyses of rosiglitazone data, including one by its manufacturer, suggest a possible increased risk of CV events, although the magnitude of this risk has been debated.^{245–247} Potential insight into the different clinical responses of PPAR γ agonists may come from their structure/activity relationship: as a consequence of their specific binding to the large PPAR γ /retinoid X receptor nuclear complex, TZDs may have differing transcriptional effects resulting in differences in accessory molecule recruitment and/or release.²⁴⁷ For instance, pioglitazone lowers TGs, whereas rosiglitazone does not.²⁴⁸

Dual PPAR Agonists

There is significant interest in designing molecules that target 2 or more PPAR isoforms to combine insulin-sensitizing properties with TG-lowering/ HDL-raising effects. Dual or pan PPAR ligands include compounds such as tesaglitazar, muraglitazar, and aleglitazar.²⁴⁹ Muraglitazar prevents the onset of diabetes and its complications in db/db mice²⁵⁰ and reduces hemoglobin A_{1C} and improves lipid profiles in diabetic patients.²⁵¹ Another dual PPAR modulator, tesaglitazar, reduces atherosclerosis in mice with LDL receptor deficiency,²⁵² improves metabolic abnormalities and renal function, decreases blood pressure, and prevents glomerular and interstitial lesions in obese Zucker rats²⁵³ and db/db mice.²⁵⁴ Tesaglitazar suppresses both hyperglycemia and dyslipidemia in diabetic patients.²⁵⁵ Chiglitazar improves impaired insulin and glucose tolerance and decreases plasma TG, total cholesterol, NEFAs, and LDL-C levels in animal models of obesity.²⁵⁶ Despite promising animal studies and positive clinical intervention trials, use and testing of several dual/pan PPAR modulators in humans have been discontinued because of severe side effects, including renal failure, fibrosarcomas, urinary cancer, and anemia. Aleglitazar is the only dual-PPAR α/γ agonist currently in late-stage development.^{206,257} A large prospective CV trial among patients with diabetes and acute coronary syndromes is under way with aleglitazar (clinical-trials.gov NCT01042769).

RAAS Inhibitors

In addition to well-known actions in the vasculature, the RAAS plays an important role in skeletal muscle, liver, and adipose tissue that may interfere with insulin action and lipid control. High FFA levels and increased LDL and low HDL cholesterol levels may cause further dysregulation of the RAAS and contribute to endothelial dysfunction and atherosclerosis due to dyslipidemia and lipotoxicity. Consequently, pharmacologic treatments to block RAAS activity may benefit both vascular and metabolic physiology. Angiotensin receptor blockers and angiotensin-converting enzyme inhibitors are agents that directly affect the RAAS, either by blocking the binding of angiotensin II to the AT1 receptor or decreasing the production of angiotensin II, respectively.²⁵⁸ Several large-scale clinical trials have demonstrated that the use of angiotensin receptor blockers or angiotensin-

converting enzyme inhibitors can significantly reduce the incidence of glucose intolerance, dyslipidemia, and atherosclerotic lesions in hypertensive patients and/or patients with other symptoms of the metabolic syndrome.^{259–265}

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KEY POINTS

- Lipotoxicity is caused by abnormally high levels of triacylglycerol, nonesterified fatty acid (NEFA), and cholesterol that lead to pathophysiologic conditions in metabolic and cardiovascular (CV) tissues.
- Lipotoxicity-mediated endothelial dysfunction affects tissues, including cardiac muscle, neurons, kidney, skeletal muscle, pancreatic β -cells, and liver.
- The mechanisms underlying lipotoxicity include oxidative stress, inflammation, mitochondrial dysfunction, and endoplasmic reticulum (ER) stress as well as cell death.
- Treatment with exercise, diet, or pharmacologic agents directly or indirectly reduces lipids in the plasma to ameliorate endothelial dysfunction as well as pathophysiologic conditions in metabolic and CV tissues.