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Mechanisms of Lipotoxicity in the Cardiovascular System

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Abstract Cardiovascular diseases account for approximately one third of all deaths globally. Obese and diabetic patients have a high likelihood of dying from complications associated with cardiovascular dysfunction. Obesity and diabetes increase circulating lipids that upon tissue uptake, may be stored as triglyceride, or may be metabolized in other pathways, leading to the generation of toxic intermediates. Excess lipid utilization or activation of signaling pathways by lipid metabolites may disrupt cellular homeostasis and contribute to cell death, defining the concept of lipotoxicity. Lipotoxicity occurs in multiple organs, including cardiac and vascular tissues, and a number of specific mechanisms have been proposed to explain lipotoxic tissue injury. In addition, recent data suggests that increased tissue lipids may also be protective in certain contexts. This review will highlight recent progress toward elucidating the relationship between nutrient oversupply, lipotoxicity, and cardiovascular dysfunction. The review will focus in two sections on the vasculature and cardiomyocytes respectively.

Keywords Heart · Vasculature · Nutrient oversupply · Lipotoxicity · Pathways and mechanisms · Cardiac function · Vascular dysfunction · Cardiovascular disease · Ceramide · Adiponectin · Nitric oxide · Reactive oxygen species · MicroRNA · Epigenetics · Autophagy · Insulin resistance · Obesity · Inflammation · Mitochondria · Endothelial dysfunction · Glucotoxicity · Cardiac lipoprotection · Diabetes · Hypertension

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Introduction

Cardiovascular complications are the leading causes of morbidity and mortality in individuals with diet-induced obesity (DIO), insulin resistance, and type 2 diabetes mellitus (T2DM). Complications include pathologies specific to large vessels (atherosclerosis, cardiomyopathy) and small vessels (retinopathy, nephropathy, neuropathy). There are multiple mechanisms that contribute to these clinical outcomes, including hyperglycemia, hyperinsulinemia, insulin resistance, inflammation, changes in circulating concentrations of adipokines, and alterations in intracellular signaling pathways. Increased circulating concentrations of lipids and altered tissue metabolism of lipids are consistent features of these prevalent conditions and contribute importantly to cardiovascular complications. Impairment in tissue homeostasis that is attributable to alterations in lipid utilization, or lipid-induced changes in intracellular signaling, have been broadly termed lipotoxicity. This review will focus on the mechanisms linking increased tissue lipid uptake, metabolism, and utilization with vascular dysfunction and changes in cardiac contractility. We will not focus on atherosclerosis, which has been the subject of many excellent reviews.

Part 1. Lipotoxicity and Vascular Dysfunction

Endothelial dysfunction is a hallmark of obesity and diabetes-related vascular dysfunction. A central aspect of endothelial dysfunction is reduced nitric oxide (NO) bioavailability. Multiple alterations in the metabolic milieu, such as hyperglycemia; oxidative stress; activation of the renin-angiotensin system and increased pro-inflammatory cytokines that are present in individuals with diet-induced obesity (DIO); type 2 diabetes mellitus (T2DM); and insulin resistance, independently and synergistically contribute to decreasing NO bioavailability. This section will focus on

specific mechanisms that are attributable to lipotoxicity. We will review key mechanisms by which lipotoxicity may decrease endothelial NO synthase (eNOS) gene expression and eNOS catalytic activity, leading to endothelial cell dysfunction. These include vascular insulin resistance, inflammation, oxidative stress, accumulation of toxic lipid metabolites, and mitochondrial dysfunction (Fig. 1).

Insulin Resistance in Endothelial Cells

It is generally accepted that vascular insulin resistance correlates with endothelial dysfunction [1]. This relationship is driven by a pathway-selective inhibition of insulin-mediated activation of eNOS by PI3K and Akt, whereas MAPK signaling to endothelin 1 (ET-1) is intact or even augmented [2••]. The resulting endothelial cell dysfunction renders the vascular wall more susceptible to atherosclerosis and less responsive to agonist-induced vasodilation [3•, 4•].

Excessive free fatty acids (FFAs) can promote *de novo* synthesis of diacylglycerol that activates classic and novel protein kinase C (PKC) isoforms [5, 6]. PKC activation inhibits insulin and vascular endothelial growth factor (VEGF)-mediated activation of PI3K and Akt [2••, 7], which limits cGMP formation from nitric oxide (eNOS generated) activation of guanylate cyclase [8•]. An examination of multiple targets in the insulin-signaling pathway in endothelial cells revealed that general (phorbol ester) and specific (angiotensin II) PKC activation [4•] (particularly the PKCα isoform) phosphorylates a novel site (Thr-86) on the p85α subunit of PI3K. Phosphorylation of Thr-86 reduces the binding of p85α to insulin receptor (IR) substrate 1 (IRS1), and decreases insulin and VEGF signaling via PI3K to eNOS. Whole-body IRS1 knockout (KO) mice are hypertensive and endothelial specific IRS1 KO mice display endothelial dysfunction [9], supporting the functional importance of intact IRS1-mediated PI3K signaling to eNOS.

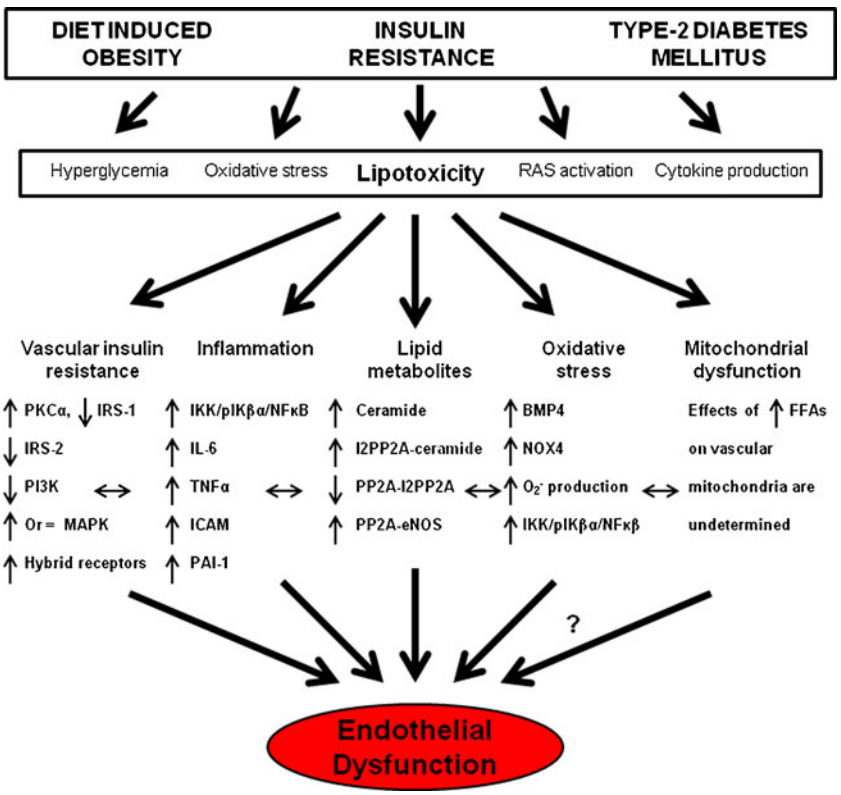

Fig. 1 Multiple mechanisms contribute to endothelial dysfunction that exists in subjects with diet-induced obesity, insulin resistance, and type 2 diabetes mellitus. Of these, lipotoxicity can decrease endothelial function by: (i) impairing agonist-induced signaling to eNOS in endothelial cells and blood vessels; (ii) increasing inflammation; (iii) stimulating accrual of toxic sphingolipids e.g., ceramide; (iv) promoting oxidative stress to an extent that overwhelms the antioxidant environment; and/or by (v) potentially precipitating mitochondrial dysfunction. Cross talk between these pathways is likely. Definition of abbreviations: FFA—Free fatty acids; PKCα—Protein kinase C alpha; IRS-1—Insulin receptor substrate-1; IRS-2—Insulin receptor substrate-2; PI3K—Phosphoinositide 3-kinase; MAPK—Mitogen activated protein kinase; IKK—Inhibitor of kappa B kinase; p—phosphorylated; NFκB—Nuclear factor kappa-beta; IL-6—Interleukin-6; TNFα—Tumor necrosis factor-alpha; ICAM—Intercellular adhesion molecule; PAI-1—Plasminogen activator inhibitor-1; I2PP2A—Inhibitor 2 of protein phosphatase 2A; PP2A—Protein phosphatase 2A; eNOS—Endothelial nitric oxide synthase; BMP4—Bone morphogenetic protein 4; NOX4—NADPH oxidase 4. For lipid metabolites, the arrows represent the interactions between ceramide, I2PP2A, PP2A, and eNOS

IR substrate 2 (IRS2) also plays an important role in relaying the insulin signal to eNOS. Endothelial cell IRS2 expression, Akt activation, and p-eNOS are decreased and capillary recruitment and insulin delivery are impaired in fat-fed vs. lean mice [10••]. These defects are neutralized when fat-fed mice are treated with a prostacyclin analog that increases eNOS expression, and all findings are recapitulated in endothelial-specific IRS2 KO mice. Taken together, these observations provide strong evidence linking obesity and its associated increase in circulating FFAs to impaired insulin-mediated signaling to eNOS in endothelial cells to an extent that could be physiologically relevant.

Other genetic models have been used to demonstrate important functional consequences of disrupted IR-mediated signaling to eNOS. Mice with germ line haploinsufficiency of the IR ($IR^{+/-}$ mice) display hypertension, mild insulin resistance, reduced basal and insulin-stimulated eNOS phosphorylation in the vasculature, and an age-dependent decrease in arterial vasorelaxation that is associated with an increase in endothelial cell-derived NADPH oxidase-mediated $O_2^{\bullet-}$ production [11•, 12]. Endothelial regeneration in response to wire-induced denudation of the femoral artery is delayed in $IR^{+/-}$ vs. wild-type (WT) mice, but this component of the $IR^{+/-}$ phenotype was rescued by transfusion of angiogenic progenitor cells obtained from insulin-sensitive, but not from insulin-resistant, animals [13••]. In a murine model wherein apolipoprotein (apo) E-deficient mice are crossed to mice with IR deletion in endothelial cells, atherosclerotic lesion size, endothelium-dependent dysfunction, and VCAM-1 expression are most severe in double-KO mice [3•]. Thus, defective IR signaling in the vascular endothelium, in the absence of changes in systemic metabolism, promotes early events in atherogenesis and accelerates the progression of atherosclerotic disease. However, not all studies in genetic models of IR disruption support the hypothesis that vascular insulin resistance is sufficient to induce vascular dysfunction. IR-null mice with transgenic re-expression of the IR in brain, liver, and pancreatic β-cells (*TTr-IR*$^{-/-}$ mice) exhibit preserved glucose homeostasis that is associated with hyperinsulinemia [14]. In these mice, no differences in blood pressure or in the gene expression levels of ET-1 or eNOS, or in eNOS phosphorylation, are observed between groups, despite a complete loss of insulin-stimulated activation of intracellular signaling kinases. Furthermore, endothelium-dependent vasorelaxation and indices of oxidant stress are unchanged in vessels from *TTr-IR*$^{-/-}$ vs. WT controls [15••]. These observations suggest complex interactions between IR haploinsufficiency, loss of IR or IRS isoforms, and the presence or absence of hyperinsulinemia in the vascular adaptation to genetic perturbation of insulin signaling.

Cross Talk Between IR and Insulin-Like Growth Factor 1 Receptors (IGF-1Rs)

Insulin-like growth factor 1 receptors (IGF-1Rs) exist in the vasculature and heterodimerize with IRs to form hybrid receptors in insulin resistant conditions associated with DIO and T2DM. Because these hybrid receptors bind to IGF-1 but not insulin with high affinity, they may attenuate insulin-mediated NO production by the endothelium. Thus, arteries from mice with germ line haploinsufficiency of the IGF-1R (IGF-$1R^{+/-}$ mice) and endothelium-specific homozygous or heterozygous disruption of the IGF-1R display blunted phenylephrine-induced vasocontraction, increased basal NO production, and enhanced insulin-mediated NO production [16•], which are reversed in mice with double KO of $IR^{+/-}$ and IGF-$1R^{+/-}$ [16•]. These data suggest that modulation of IR/IGF-1R interactions could be a potential therapeutic target for ameliorating vascular dysfunction that is associated with insulin-resistance.

Insulin Resistance in Intact Arteries

Jiang et al. first reported that selective resistance via the IR-PI3K-Akt pathway exists in vessels from obese vs. lean rats [2••]. This issue has been examined more recently in aortae from lean and fat-fed mice [15••, 17•, 18–20]. When directly examined, the ability of insulin to stimulate Akt phosphorylation was variable, ranging from full activation of Akt phosphorylation to modest inhibition of Akt phosphorylation following insulin stimulation. Despite the variability in Akt phosphorylation, all of these studies revealed striking suppression both in basal and insulin-stimulated eNOS phosphorylation. Functional consequences of reduced vascular NO bioavailability in obese mice observed in those studies include arterial inflammation [17•, 18, 20], impaired arterial vasorelaxation [15••, 19], and hypertension [15••]. These data provide evidence that a dissociation may exist between insulin-mediated signaling and Akt in the vasculature, and impaired eNOS enzyme function in insulin-resistant conditions associated with DIO and T2DM [15••, 17•, 18–20].

We reasoned that additional systemic metabolic changes could account for the profound failure in insulin-mediated eNOS phosphorylation in arteries from obese vs. lean mice, despite intact signaling in the vasculature via Akt. Because fasting hyperglycemia was mild in fat-fed vs. lean mice, and $O_2^{\bullet-}$, NADPH-oxidase activity, AMP-dependent kinase (AMPK), and Protein Kinase A (PKA) are similar in vessels from lean and obese mice in our study [15••], we explored whether elevated FFAs in general, or a toxic metabolite of FFA such as the sphingolipid ceramide [21, 22], could contribute to these changes. When vascular ceramide biosynthesis was prevented in fat-fed mice using pharmacological and

genetic approaches, impaired eNOS phosphorylation, endothelial dysfunction, and systemic hypertension were prevented [23••]. To determine molecular mechanisms whereby ceramide might exert its effects, bovine aortic endothelial cells (BAECs) were incubated with palmitate. Palmitate increases de novo ceramide synthesis, which reduces agonist-stimulated (e.g., insulin and vascular endothelial cell growth factor) eNOS phosphorylation at S1177 and S617, eNOS dimer formation, and NO production in a ceramide-dependent manner [23••]. In contrast to findings of others using different cell lines, the changes we observed are not due to impaired upstream signaling to eNOS from Akt, AMPK, or ERK 1/2, or to $O_2^{\bullet-}$-mediated peroxynitrite formation. Instead, ceramide induces colocalization of the protein phosphatase 2A (PP2A) with eNOS, prevents eNOS from associating with Akt and Hsp90, decreases the phosphorylation of the pool of Akt that associates directly with eNOS, and impairs full eNOS phosphorylation. Ceramide might initiate PP2A association with eNOS by disrupting the interaction between inhibitor 2 of PP2A (I2PP2A) and PP2A. Collectively, these results identify an important role for endogenous ceramide accumulation in the pathogenesis of vascular dysfunction, and extended earlier observations, implicating impaired AMPK phosphorylation in ceramide-mediated vascular dysfunction [24].

Inflammation

Palmitic, oleic, and linoleic acids are abundant fatty acids in human serum that comprise~70 % of the total circulating FFAs [25]. Palmitate signals via the toll-like receptor 4 (TLR4), a pattern recognition receptor that is essential for initiating inflammatory responses associated with innate immunity [26, 27]. When palmitate signals via TLR4, inhibitor of κB-kinase (IKK) is activated, which phosphorylates and degrades IκBα, allowing nuclear translocation of NF-κβ, a transcriptional activator of the expression of many genes involved in inflammation [26]. The subsequent increase in the expression and synthesis of inflammatory mediators such as IL-6, TNFα, ICAM, and PAI-1 are associated with decreased endothelial NO production [17•, 28•, 29]. Increased p-IκBα, IL-6, and ICAM are observed in lysates of aorta from fat-fed vs. lean WT, but not $TLR4^{-/-}$, mice [17•, 18]. The Kim group subsequently demonstrated in endothelial cells and confirmed in vascular tissue from obese WT and $TLR4^{-/-}$ mice that an important link between palmitate-mediated TLR4 activation and IKK / p-IκBα / NF-κβ signaling involves NADPH oxidase-mediated $O_2^{\bullet-}$ production [30]. NOX4 is the most abundant isoform of NADPH oxidase in vascular tissue. Bone morphogenic protein 4 (BMP4) induces and activates NADPH oxidase in endothelial cells. Administration of BMP4 to mice induces arterial dysfunction and hypertension in an NADPH-oxidase dependent manner [31•]. Maloney et al. show that endothelial cells treated with palmitate and aortae from fat-fed mice display elevated BMP4 and NOX4 and indices of IKK / p-IκBα / NF-κβ signaling in a TLR4-dependent manner. In addition to stimulating NF-κβ-mediated inflammatory responses, NADPH oxidase-mediated $O_2^{\bullet-}$ production may impair NO bioavailability and precipitate vascular dysfunction by additional mechanisms. When endogenous cellular antioxidants are incapable of responding adequately to the imposed oxidant load, $O_2^{\bullet-}$ may combine with NO to form peroxynitrite, which disrupts the eNOS dimer, thereby reducing eNOS activity and arterial vasorelaxation [19, 32, 33]. Thus, the FFA palmitate signals via TLR4 to increase oxidant load and increase NF-κβ-mediated signaling in endothelial cells. Some studies question whether toll-like receptors directly bind saturated fatty acids [34], and others suggest that saturated fatty acids may not induce inflammation via TLR-mediated signaling [35]. For example, FFAs have been shown to amplify the inflammatory responses of monocytes / macrophages to bacterial lipopolysaccharide via a TLR4–independent mechanism that is dependent upon ceramide generation, which in turn activates PKC and MAPK [27]. Despite these controversies, the balance of the evidence favors the conclusion that FFA-mediated signaling may contribute to endothelial dysfunction by mechanisms that involve the activation of inflammatory pathways.

An important link between lipid accumulation, inflammation, and NO bioavailability might involve IKK-mediated ceramide biosynthesis. Holland et al. show that lard oil infusion increases skeletal muscle and liver ceramide synthesis in WT but not TLR4-null mice; and that palmitate incubation increases ceramide synthesis and transcript levels of enzymes involved in ceramide biosynthesis (*Sptlc1, Sptlc2, Des1*) in cells with intact IKK kinase signaling, but not in IKK kinase-dead cells [36•]. To determine if these findings could be translated to the whole animal, mice were fed standard or high-fat chow for 4 months, and then a subgroup was switched to a diet supplemented with the IKKβ inhibitor sodium salicylate. Although body weights did not change, insulin and glucose tolerance were improved, and ceramide accumulation in skeletal muscle and liver was prevented. Vascular function was not determined in this study, but based on studies in myriocin-treated animals on a high-fat diet, it would be predicted that sodium salicylate would be expected to improve vascular function. Other approaches to suppressing flux through this pathway have also proved beneficial. Diosgenin, a steroidal sapogenin, reduces palmitate-induced IKKβ and NF-κβ phosphorylation in endothelial cells to an extent that impaired the production of inflammatory markers and restored insulin-stimulated IRS-1 tyrosine phosphorylation [29]. Diosgenin restores insulin-stimulated NO production and endothelium-dependent vasorelaxation in arterial rings treated with

palmitate, indicating the potential relevance of targeting this pathway in vascular diseases associated with a lipotoxic milieu. Collectively, palmitate signaling via toll-like receptor or related signaling pathways may induce vascular dysfunction by promoting: (i) IKK / p-IκBα / NF-κβ signaling that promotes inflammation; (ii) NADPH-oxidase-mediated $O_2^{\bullet-}$ production that may uncouple eNOS function and exacerbate oxidative stress; and (iii) ceramide biosynthesis which may lead to PP2A-mediated dephosphorylation of eNOS.

Cross Talk Between Adiponectin and Ceramide

In addition to its insulin-sensitizing, antiapoptotic, and anti-inflammatory functions, adiponectin appears to regulate sphingolipid metabolism, and this link might have consequences concerning arterial function. Once formed, ceramide can be hydrolyzed by ceramidases to produce sphingosine. Sphingosine kinase can then phosphorylate sphingosine to sphingosine-1-phosphate (SIP). Ceramide and sphingosine are pro-apoptotic, whereas SIP is generally regarded as pro-survival. This led to the hypothesis that the ceramide / SIP balance might be important concerning cell fate [37]. Hepatic ceramide accumulation in lipid-infused and fat-fed rodents can be normalized by administering recombinant adiponectin; prevented in mice that transgenically overexpress adiponectin; and amplified in adiponectin null mice [38••]. The adiponectin receptors AdipoR1 and AdipoR2 belong to the progesterone and adiponectin Q receptor (PAQR) family [39]. Because some PAQR family members enhance ceramidase activity, investigators sought to determine whether AdipoR1 and AdipoR2 might mediate the ability of adiponectin to lower ceramide. Adiponectin increases ceramidase activity in mouse embryonic fibroblasts (MEFs) with intact adiponectin receptors, but fails to do so in MEFs in which both isoforms are deleted. Consistent with lower ceramidase activity in AdipoR1-deficient and AdipoR2-deficient MEFs, there was diminished accumulation of SIP, such that the ceramide / SIP ratio was 5-fold greater relative to WT cells. The disrupted ceramide-to-SIP balance increases the susceptibility of AdipoR1/2 deficient cells to palmitate-induced cell death that is prevented when these MEFs are treated with SIP. This signaling pathway might also be relevant in endothelial cells. For example, SIP signals through the endothelial differentiation gene-1 receptor to the heterotrimeric G protein G_i, to activate Akt and eNOS, and to increase NO production [40•]. Although this would be predicted to increase arterial vasorelaxation, in vivo studies focusing on vascular function, in which this pathway is manipulated, remain to be performed. Indirect support comes from studies in which adiponectin delivery to mice with T2DM can improve arterial vasorelaxation by increasing NO bioavailability [41].

Mitochondria

Mitochondria regulate cell survival and ion homeostasis. Endothelial mitochondria are responsible for generating reactive oxygen species and maintaining the cytosolic Ca^{2+} concentration. Evidence that changes in mitochondrial morphology and function correlate with endothelial cell dysfunction in the context of diabetes is emerging [42•, 43]. Increased mitochondrial fission or attenuated fusion disrupts the mitochondrial network and leads to endothelial dysfunction. Mitochondrial fission may increase reactive oxygen species generation and Ca^{2+} overload, which could potentially disrupt endothelial function and cell survival [44]. Palmitate exposure but not hyperglycemia or hyperinsulinemia, induces mitochondrial fragmentation in differentiated C_2C_{12} muscle cells and increases reactive oxygen species production [45]. Similar findings are observed in skeletal muscle of mice with genetic obesity and DIO. Increased expression of *Fis1* and mitochondrial fragmentation are observed in endothelial cells isolated from patients with T2DM [43]. Mitochondrial-reactive oxygen species generation was increased, and agonist-stimulated NO production suppressed, in endothelial cells from diabetics that correlated with reduced flow-mediated dilation. While hyperglycemia was present in the diabetic patients in this study, triglycerides were moderately, but not significantly, elevated and FFAs were not measured. Thus, hyperglycemia correlates with mitochondrial fragmentation and endothelial cell dysfunction. It remains to be established if lipid excess per se contributes to this phenomenon.

Summary, Part 1

Endothelial dysfunction is a well-established characteristic of insulin resistance and obesity. Multiple mechanisms conspire to impair vascular function in this prevalent condition. Recent findings provide strong evidence that increased circulating lipids may impair vascular function in vivo by impairing insulin signaling, promoting ceramide accumulation, increasing inflammation and disrupting mitochondrial dynamics. It is likely that these changes are not parallel pathways, but are interrelated and synergistically interacting to promote vascular dysfunction (Fig. 1).

Part 2. Lipotoxicity and Cardiac Function

Maintenance of cardiac contractile function requires a constant energy supply through mitochondrial generation of adenosine-5'-triphosphate (ATP). In the healthy heart, the primary substrate for ATP synthesis is fatty acids. However, throughout development and in response to various stimuli, the heart exhibits metabolic flexibility to alter substrate

preference and mitochondrial efficiency. A consequence of modern civilization is the relationship between nutrient excess, obesity, diabetes, and increased cardiovascular disease, which threatens to reduce advances in life expectancy. These trends were evident at least 200 years ago, when the descriptions of failing human hearts in obese individuals were recorded: "It is the state in which the heart with very fat people, and even with persons moderately fat, is found oppressed, and suffocated by an enormous mass of fat [46]."

Much progress has been made toward elucidating mechanisms by which obesity increases the risk of heart failure, and specifically, the contribution of free fatty acids (FFAs) [47•, 48•]. A number of recent reviews describing the relationship between circulating fatty acid concentrations, their saturation content, and duration of exposure leading either to maladaptive responses in normal hearts [49•] or cardioprotection in failing hearts [50•] have been recently published. These divergent responses underscore the importance of further studies to fully understand the mechanisms linking nutrient excess, and specifically lipid excess, to cellular regulation. We recently summarized proposed mechanisms involved in cardiac lipid utilization and the development of lipotoxicity in the heart that lead to contractile dysfunction [48•]. These mechanisms include altered AMPK signaling, ceramide accumulation, endoplasmic reticulum (ER)-stress, reactive oxygen species (ROS), and mitochondrial dysfunction. This review will provide an update on recent progress in the elucidation of these mechanisms and present additional novel pathways such as autophagy, microRNA, and epigenetics.

Human Myocardial Steatosis and Cardiac Function

Myocardial steatosis has been used as a biomarker for cardiac lipotoxicity in humans. Advances in magnetic resonance spectroscopy and positron emission tomography [51, 52], have increased our understanding of the pathophysiology of myocardial lipid accumulation and metabolic fate of circulating fatty acids. Analysis of cardiac tissue obtained at the time of surgery has also provided molecular insights into lipid-mediated cellular mechanisms that impair cardiac function in subjects with obesity and T2DM, with or without heart failure [53•, 54, 55]. Of note, changes in cardiac lipid content can also be induced in otherwise healthy individuals subjected to short-term hyperinsulinemia and hyperglycemia [56], or by temporally increasing circulating FFAs in healthy individuals exercising in the fasted state [57]. Conversely, exercise can result in reduced lipid accumulation in obese individuals, leading to improved cardiac function [58•].

Heart failure is associated with increased lipid accumulation in cardiac tissue, which correlates with increased expression of genes for fatty-acid transport proteins such as CD36 [59]. Lipid accumulation in the failing heart may be reversed by mechanical unloading, which partially alleviates mitochondrial dysfunction and insulin resistance [60]. However, reducing circulating FFAs during heart failure is not sufficient to restore cardiac function [61•]. Thus, the accumulation of lipid in normal or diseased hearts reflects complex interactions between the metabolic and neurohumoral milieu and changes in mechanisms governing cardiac lipid uptake and metabolism. Moreover, as will be discussed below, the functional implications of myocardial steatosis are context-dependent, where it may be maladaptive, or could represent adaptations that might be cardioprotective.

Recent Advances in Previously Described Molecular Pathways That Are Implicated in Cardiac Lipotoxicity

A number of mechanisms that contribute to the development of lipid induced cellular toxicity have been identified. These are summarized in Table 1. Recent advances in our understanding of the contribution of these pathways to the pathophysiology of lipotoxicity in the heart are now summarized.

AMP-Dependent Kinase (AMPK) – A Key Metabolic Sensor

Accumulating evidence suggests that AMPK may play a protective role that mitigates cardiac contractile dysfunction in models of diet-induced obesity. AMPK deficiency exacerbates contractile dysfunction and impaired glucose metabolism in mice fed a high-fat diet (HFD) for 20 weeks [62]. Obesity is associated with hypoadiponectinemia. Adiponectin coordinates fatty acid utilization via AMPK-dependent mechanisms that regulate mitochondrial fatty acyl-CoA import (generation of malonyl-CoA by acetyl-CoA carboxylase), and through induction of CD36 translocation that increases fatty acid uptake [63]. This effect of adiponectin likely increases complete oxidation of fatty acids and limits the likelihood that toxic lipid intermediates will accumulate. Trimetazidine, an inhibitor of long-chain 3-ketoacyl coenzyme A thiolase activates AMPK, reduces lipid accumulation, and restores contractile function in *db/db* diabetic mice [64]. The mechanism of action of trimetazidine also involves amelioration of oxidative stress via increased antioxidant defenses, such as MnSOD. Thus, modulation of AMPK could represent a novel therapeutic approach to mitigate the adverse consequences of myocardial lipotoxicity.

Autophagy

"Self-eating", or autophagy, is a highly conserved process that when disrupted leads to cellular dysfunction. Saturated fatty acids can induce autophagy in endothelial cells via

Table 1 Proposed mechanisms of lipid-induced regulation of cardiovascular cellular function

Mechanism	Observation
AMPK	Activation protects against FA-induced cell death [62, 64]
Autophagy	Enhanced by FA-induced activation of PKC [65]
	Enhanced by WD via *O*-GlcNAcylation of autophagy proteins [102]
	Inhibition protects against FA-induced cell death in endothelium [66•]
Ceramide	Enhances PP2A-eNOS mediated endothelial dysfunction [23••]
	Inhibition protects against FA-induced cell death [67•, 69]
Endoplasmic reticulum (ER) stress	Enhanced by FA-induced activation of Ca^{2+}-mediated activation of BCL-2, BAX, and BAK [48•]
Leptin	Treatment protects against cardiac dysfunction by reducing lipid accumulation and restoring PPAR signaling [73]
Lipid (TAG) storage	Feeding unsaturated FA, protects against saturated FA-induced cell death by sequestering lipids in intracellular droplets [106, 107•]
	Perlipins regulate FA-storage to regulate and maintain normal oxidative balance [108, 109]
MicroRNA	FA-induced changes in miR regulation results in insulin resistance [91••]
Mitochondrial function	FA-induced mitochondrial fragmentation and increased reactive oxygen species (ROS) [45]

FA: = fatty acid; TAG: = triacylglycerol; WD: = western diet

PKC signaling independent of mammalian target of rapamycin (mTOR) [65]. Inhibition of autophagy in endothelial cells ameliorates palmitate-induced cell death [66•]. Studies are required to determine whether similar mechanisms are present in cardiomyocytes.

Ceramide

When fatty acid uptake exceeds oxidative or storage capacity of a tissue, lipids may be shunted into other pathways, such as ceramide, that may alter intracellular signaling pathways and promote apoptotic cell death. Pharmacologic or genetic inhibition of ceramide synthesis improves cardiac structure, function and metabolism in a transgenic mouse model of lipotoxic cardiomyopathy [67•]. Treatment of high-fat fed animals with fenretinide, which inhibits Des1, the rate-limiting enzyme of ceramide biosynthesis, reduced tissue ceramide levels and increased insulin action [68]. The impact of fenretinide on obesity-associated cardiac dysfunction remains to be elucidated. Decreasing ceramide levels using the serine palmitoyl transferase I (SPT I) inhibitor, myriocin, protects against cardiac dysfunction and is associated with increased cardiac glucose utilization [69]. Thus, inhibiting ceramide biosynthesis remains a promising approach for limiting the adverse consequences of myocardial lipotoxicity.

Leptin

Classic studies revealed that leptin may dramatically reverse the adverse effects of lipotoxicity in transgenic models [70•]. Recent studies from our group and others have shown that leptin signaling to the brain is required to reverse steatosis, altered substrate metabolism, mitochondrial dysfunction, and cardiac dysfunction in obese mice with altered leptin signaling [71•, 72]. In addition, leptin replacement, but not caloric restriction, reduced cardiac lipid accumulation in *ob/ob* mice in part, due to a failure of caloric restriction to rescue defects in PPAR signaling in cardiomyocytes [73].

PPAR Signaling

Lipids are ligands for transcriptional regulators of the PPAR family; thus, regulating FFA delivery significantly impacts PPAR function. In a genetic mouse model of cardiac triglyceride accumulation (adipose triglyceride lipase, $ATGL^{-/-}$), activation of PPARα, but not PPARγ, reversed mitochondrial dysfunction and prevented heart failure [74]. Conversely, ATGL-overexpressing mice that exhibit increased triglyceride lipolysis are protected from pressure-overload induced cardiac dysfunction [75] in the absence of any change in diacylglycerol or ceramide levels. Loss of diacylglycerol acyl transferase 1, an important regulator of triglyceride synthesis, reduces PPAR signaling in mice, leading to decreased fatty acid oxidation and ceramide production. These hearts are protected from HFD-induced myocardial dysfunction [76]. Whereas transgenic overexpression of PPARγ increased lipotoxic cardiac injury, deletion of PPARα in these mice resulted in improved cardiac function that was associated with increased FA oxidation and increased triglyceride synthesis [77•]. Thus, PPAR signaling has pleiotropic and context-dependent effects on cardiac substrate utilization and mitochondrial function, which might

complicate the utility of modulation of this pathway as a general approach to ameliorating lipotoxic cardiomyopathy. The related transcriptional co-activators PGC-1α and PGC-1β modulate changes in mitochondria volume, oxidative capacity, and the coupling of ATP synthesis that occur in response to HFD and diabetes in the *ob/ob* model of leptin deficiency [78]. Additional studies are required to determine if modulation of PGC1 signaling represents a feasible therapeutic approach for ameliorating the adverse consequences of myocardial lipotoxicity.

p53

In the streptozotocin model of type 1 diabetes (T1D), the progressive decrease in cardiac contractile function was prevented by genetic deletion of p53, or by reducing OXPHOS complex IV levels via direct suppression of the p53 transcriptional target, cytochrome c oxidase 2 (SCO2). This protection was associated with a decrease in myocardial lipid accumulation and complex IV induction [79]. Interestingly, pressure overload itself can lead to p53-mediated activation of the inflammatory response in adipose tissue, suggesting an interesting cross talk between the heart and peripheral adipose tissue metabolism [80••, 81••]. A role for this pathway in the pathogenesis of lipotoxic cardiomyopathy remains to be elucidated.

Other Signal Transduction Pathways

HFD may induce myocardial insulin resistance by a mechanism involving diacylglycerol activation of PKC signaling and p70S6K activation, which might feedback to inhibit insulin signaling [82]. The resulting defect in insulin-stimulated glucose oxidation was not associated with changes in contractile function, ex vivo or in vivo. Lipids also activate PKC, resulting in decreased β-adrenergic receptor abundance and reduced cardiac contractile responsiveness to catecholamines [83]. Pharmacologic inhibition of β-adrenergic receptors with metoprolol in streptozotocin-treated mice inhibited CPT1, but did not prevent cardiac lipid accumulation or oxidative stress. Of note, metoprolol treatment activated PKB mediated signaling to reduce caspase activation and promote cell survival [84]. Finally, in a recent study in a mouse model of longstanding diet-induced obesity (6 months of 60 % high-fat feeding), cardiac dysfunction was prevented in mice with cardiac-specific deletion of FOXO1, suggesting an important role for nuclear FOXO signaling in mediating obesity and diabetes-related cardiac dysfunction [85•]. Whether or not this pathway plays a role in the early metabolic changes that accompany obesity-induced cardiac dysfunction [86•], or is applicable to lower degrees of dietary fat overload, remains to be established.

Novel Molecular Pathways Implicated in Lipotoxicity

Two novel areas that have recently illuminated our understanding of the interactions between obesity, diabetes, and lipotoxicity are microRNAs (miRs) and epigenetics, which describes the regulation of gene expression through modifications DNA or chromatin.

A number of recent studies have provided interesting insights into the role of microRNAs in the regulation of cardiac and systemic lipid metabolism [81••, 87, 88, 89•], reviewed in [90]. Recent work examining livers of obese mice, found upregulation of miR-143 in the liver as well as in the heart [91••], and genetic reduction of this miR rendered animals resistant to diet-induced obesity and insulin resistance. The role of this microRNA in the pathogenesis of obesity-related cardiac dysfunction remains to be elucidated. Studies examining the relationship between other miRs, insulin resistance, and obesity-related cardiovascular dysfunction remain to be conducted. MicroRNAs are important regulators of lipoprotein metabolism, which play an important role in the delivery of fatty acid substrates to the heart [92]. Interestingly, miR delivery appears to be dependent upon lipoproteins [93•]; thus, it will be of great interest to determine if the altered lipid environment that accompanies obesity and diabetes may regulate the uptake and delivery of miRs to tissues.

Strong evidence supports a role for transgenerational epigenetic mechanisms that may contribute to the current and rapid rise in obesity, diabetes, and its related complications. Analysis of the role of epigenetics in the interaction between obesity on cardiac dysfunction is relatively understudied. High-fat diets induce metabolic defects in offspring fed a healthy diet. This is true for both maternal [94] and paternal [95] overnutrition, and may persist for at least three generations in rodents [96]. The adverse effects in the cardiovascular system resulting from parental HFD feeding are seen in otherwise normal offspring fed a healthy diet [97•] and in the absence of obesity [98]. Together, these findings suggest that high-fat and Western diets may not only have direct cellular lipotoxic effects, but may predispose future generations via epigenetic mechanisms that lead to delayed lipotoxicity and mitochondrial dysfunction that may increase the susceptibility to developing obesity, diabetes and heart disease.

Glucotoxicity Versus Lipotoxicity

Diets that are enriched for fat and carbohydrate (Western diets) appear to be those that most consistently induce left ventricular dysfunction in rodent models [99•], raising the concept that the total nutrient burden to the heart could accelerate the maladaptation that is observed. For example,

an obesogenic western diet impairs Krebs cycle flux and alters gene expression, favoring oxidative stress and ultimately programmed cell death [100]. These studies in animals on a high-fat/high-sucrose "Western diet" underscore the need to account for the contribution of increased generation or flux of glucose-derived metabolites. For example, increased flux via the hexosamine biosynthetic pathway, which increases post-translational modifications of proteins via *O*-GlcNAcylation has classically been studied in models with increased glucose delivery. This pathway is also increased in conditions associated with increased exposure to fatty acids [101]. Indeed, chronic feeding of Western diets increases *O*-GlcNAc modifications of a number of proteins, including those involved in autophagic flux [102]. In transgenic models, increasing carbohydrate delivery alone might not be deleterious to cardiac function. But these mice with enhanced glucose uptake, and which are protected against cardiac dysfunction in response to hemodynamic challenges, rapidly develop contractile dysfunction when challenged with a HFD [103•]. Similarly, models with a balanced increased myocardial fatty acid utilization and oxidation do not develop left ventricular dysfunction under non-stressed conditions or in response to pressure overload [104•]. Taken together, it is likely that cardiac maladaptation that develops in the context of diet-induced obesity reflects the synergistic interactions of substrate overload and interrelated signaling pathways that are activated by lipid-derived and glucose-derived intermediates.

Cardiac Lipoprotection

Although there is strong evidence linking diet-induced obesity and lipotoxicity with initiating heart failure, studies in models of heart failure have suggested that increased dietary fat might be cardioprotective. For example, feeding rabbits a diet rich in unsaturated fatty acids prevented pressure or volume overload-induced heart failure [105]. The concept that dietary fatty acid composition or myocardial fat storage may be cardioprotective was recently reviewed [50•]. It has long been appreciated that co-feeding cells with saturated and unsaturated fatty acids can promote lipid droplet accumulation and protection from cell toxicity [106, 107•]. Lipid droplet-binding proteins (perilipins, Plin) may play an important role in the mechanism by which lipid partitioning towards triacylglycerol (TG) storage will reduce lipotoxicity. Specifically, Plin5 antagonizes lipases, thereby preventing ROS damage while maintaining lipid droplet stability. In addition, Plin5, but not Plin1 or Plin2, directly associates with mitochondria and lipid droplets, channeling saturated fatty acids away from oxidation and toward storage [108]. In Plin5$^{-/-}$ mice fed normal chow, there is an increase in cardiac fatty acid oxidation, loss of lipid droplet formation and decline in fractional shortening with age [109], supporting a protective role for TG storage to limit the accumulation of toxic lipid intermediates.

There is also strong evidence that maintaining substrate flexibility and balance is essential in maintaining myocardial function in times of hemodynamic stress, as is the case with ischemic injury. For example, HFD feeding prevented contractile dysfunction following ischemic injury by a mechanism involving maintenance of myofilament expression [110]. In an independent study, similar cardioprotection correlated with altered cardiac mitochondrial substrate utilization through a mechanism associated with inhibition of glucose oxidation via induction of pyruvate dehydrogenase kinase 4 (PDK4) [111]. Likewise, when PDK4 was overexpressed in murine hearts, they were protected from HFD-induced lipid accumulation, likely from enhanced oxidation of fatty acids [112].

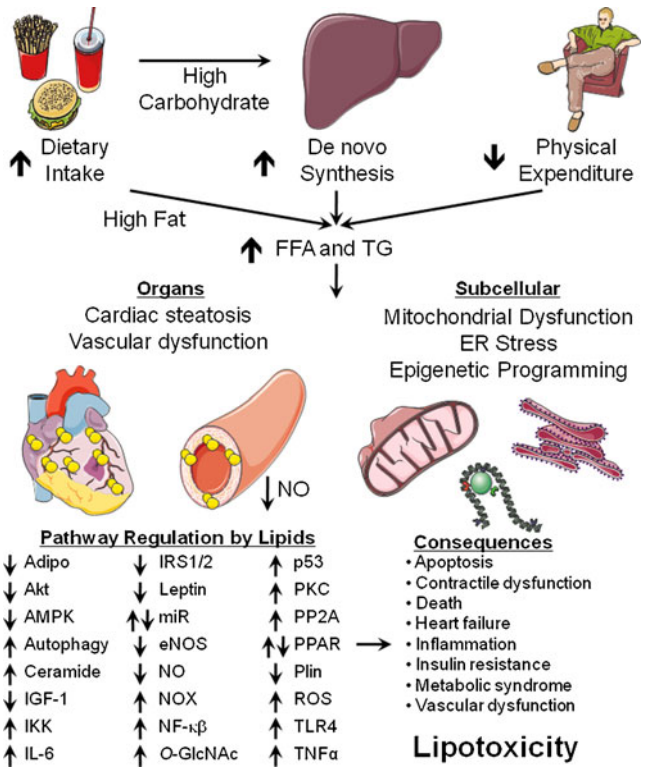

Fig. 2 Schematic representation of pathways and mechanisms of lipotoxicity. Nutrient excess, from diet, increased de novo synthesis of lipids, and reduced energy expenditure, eventually overwhelms storage and oxidation. This leads to lipid accumulation that will impair cardiac contractile function, nitric oxide signaling in the vasculature, and the function of cellular organelles. Changes in intracellular signaling result in ER stress, mitochondrial dysfunction and reactive oxygen species generation, as well as persistent modifications of DNA and histones. The overall result is dysregulation of various pathways (discussed in the text) that have far reaching consequences. Figure was produced using Servier Medical Art (www.servier.com)

Additional mechanisms linking high-fat feeding and cardioprotection in the context of left ventricular remodeling include the potential role of myocardial insulin resistance that develops in the heart when rats are fed a HFD following coronary artery ligation [113•]. Indeed, genetic reduction of insulin signaling limited left ventricular remodeling in the context of pressure overload hypertrophy via a mechanism involving the attenuation of Akt signaling [114••]. High sucrose diet-induced insulin resistance also appears to protect the heart from subsequent bouts of metabolic or hemodynamic challenge [115•]. It should be noted that although acquired insulin resistance is protective in these contexts, complete loss of insulin signaling through genetic ablation of the insulin receptor exacerbates mitochondrial dysfunction and cardiac efficiency in a model of insulin deficient diabetes [116] or pressure overload or post-myocardial infarction remodeling [117, 118].

Induction of the rate-limiting enzyme in the fatty acid desaturation pathway, stearoyl-coenzyme A desaturase-1 (SCD1), was observed in rat hearts following three months of a high sucrose diet that was sufficient to induce obesity [119]. Overexpression of SCD1 in primary cardiomyocytes protected cells from numerous lipotoxic effects, including increased ceramide synthesis, reactive oxygen species generation, and apoptosis [119], suggesting that SCD induction is an adaptive mechanism. Finally, increased expression of genes involved in fatty acid synthesis was observed in murine models of lipotoxicity and obesity-related cardiac dysfunction. It remains to be determined if these changes represent an adaptation that attempts to limit lipotoxic injury [120].

Summary, Part 2

The accumulation of lipids in the heart is associated with cardiac dysfunction in humans and rodents with diet-induced obesity. However, triglycerides per se are not likely to be toxic, but may be a biomarker for the accumulation of more toxic and reactive lipid metabolites. Multiple signaling pathways synergistically interact to impair cardiac structure and function, and emerging mechanisms such as miRNAs and epigenetic regulation will likely have important roles in the pathophysiology of lipotoxicity. The combination of these complex pathways that lead to cardiovascular dysfunction is summarized in Fig. 2. The adverse consequences of lipotoxicity are exacerbated to a greater extent by obesogenic diets that promote insulin resistance, and an overall state of nutrient overload. Seemingly paradoxically, increased dietary fat, particularly isocaloric or hypocaloric diets, may lead to cardioprotection in hearts that are already undergoing left ventricular remodeling.

Conclusion

Although there is clear overlap in the mechanisms by which increased circulating lipids or increased tissue lipid uptake may induce lipotoxicity in vascular and cardiac cells, there is no single unifying mechanism that could be easily manipulated therapeutically to treat or prevent cardiac or vascular disease. This underscores the importance of preventive strategies targeted at delaying the onset of obesity and insulin resistance. The challenge of managing the large numbers of individuals with existing vascular disease remains. Therefore, the challenge for the future will be to identify points of convergence in the pathophysiology of cardiovascular disease in obesity that are amenable to therapeutic intervention and that would have the broadest ability to modify clinical outcome. There is no doubt that further characterization of established signaling pathways, such as insulin signaling, inflammation and nitric oxide, may provide a more complete understanding towards these goals. In addition, we anticipate that new advances in emerging fields, such as microRNAs, epigenetics and autophagy, will provide a more complete understanding of how nutrient excess in obesity and diabetes precipitates cellular dysfunction in the heart and vasculature.

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- •• Of outstanding importance

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