Lipotoxicity in the Heart

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Introduction
Heart failure can occur in metabolic diseases, independent of coronary artery disease or valvular dysfunction. Although long-chain fatty acids are the principal fuel of the adult vertebrate heart, mismatch between the uptake and utilization of fatty acids is associated with cardiac dysfunction. Cardiomyopathy is observed in both rare genetic metabolic abnormalities and highly prevalent diseases characterized by elevated serum triglycerides and non-esterified fatty acids, such as obesity and type 2 diabetes. In these disorders, an imbalance between fatty acid uptake and utilization leads to the inappropriate accumulation of free fatty acids and neutral lipids within cardiomyocytes. Through the process of lipotoxicity, this lipid overload causes cellular dysfunction, cell death, and eventual organ dysfunction. This review focuses on lipotoxicity in the heart, with an emphasis on the contribution of this process to the pathogenesis of cardiomyopathy associated with obesity, diabetes, and the metabolic syndrome. The magnitude of the current worldwide epidemic of obesity and type 2 diabetes suggests that understanding the pathogenesis of cardiac complications associated with these diseases will contribute substantially to improvements in health care.

Cardiomyopathy in Diabetic and Obese Humans
Epidemiologic and clinical studies are consistent with the existence of a specific cardiomyopathy that is attributable to the diabetic milieu. Diabetes is associated with an increased relative risk (2.4-fold) of developing heart failure in the absence of valvular or congenital heart disease, alcoholism, hypertension, or significant epicardial coronary atherosclerosis [5]. Moreover, among patients with idiopathic cardiomyopathy there is an increased frequency of diabetes, compared with normal control populations (22% among cardiomyopathy patients, 11% among age- and sex-matched controls) [6]. Diabetic cardiomyopathy may contribute to the increased (threefold over the non diabetic population) relative risk of death in patients with type 2 diabetes who have suffered a previous myocardial infarction [7] and to the strikingly worse long-term survival of people with diabetes after coronary artery bypass graft surgery [8].

In patients with diabetes, the diagnosis of diabetic cardiomyopathy is based on evidence for diastolic and/or systolic dysfunction in the absence of other potential causes for cardiac dysfunction, such as atherosclerosis or...
valve dysfunction. Noninvasive transthoracic echocardiography and invasive hemodynamic studies have been used to characterize diabetic cardiomyopathy. This disorder begins with prolonged ventricular relaxation, delayed mitral valve opening, and elevated left ventricular end-diastolic pressure, all signs of impaired diastolic function, which are accompanied by evidence of left ventricular remodeling [9]. This early, often asymptomatic manifestation may progress over an extended period of time to result in both diastolic and systolic dysfunction [10].

Obesity is also an independent risk factor for the development of clinical heart failure, even when controlling for traditional risk factors such as co-incident diabetes and hypertension [11]. Echocardiographic studies document abnormalities in obese individuals that range from left ventricular hypertrophy to diastolic dysfunction to systolic dysfunction [12]. Cardiac dysfunction in these patients may result from a combination of hemodynamic load, neurohormonal activation, and altered metabolism.

Although the molecular basis of cardiomyopathy associated with diabetes and obesity is not known, systemic metabolic disturbances likely play an important role in the pathogenesis. In addition to hyperglycemia, type 1 and type 2 diabetes are associated with elevated fasting serum TAGs [13] and FFAs [14,15]. Hyperlipidemia is also reported in the postprandial state [15] and under conditions of hyperinsulinemic euglycemic clamp [16]. In obese individuals, lipid metabolic disturbances include impaired TAG clearance and abnormal FFA fluxes as well as fasting hypertriglyceridemia [17,18]. In both diabetes and obesity, high serum TAGs and FFAs increase the delivery of lipid to non-adipose tissues such as the myocardium. Mismatch between FFA uptake and utilization leads to intracellular lipid accumulation, often manifest as increased TAG stores.

Animal Models of Diabetic Cardiomyopathy
As in human diabetes, animal models of type 1 and type 2 diabetes are characterized by significant elevations in serum FFAs, TAGs, and glucose. Dyslipidemia likely contributes to the observed alterations in steady-state cardiac tissue lipid content. Rodent and dog models of streptozotocin- and alloxan-induced diabetes, used to model metabolic changes in poorly controlled type 1 diabetes, demonstrate greater FFA uptake and diminished TAG hydrolysis than that in control hearts [19]. These hearts show a twofold to fourfold increase in cardiac myocyte FFA [20] and TAG [21] content compared with control hearts. Similarly elevated cardiac TAG content has been reported in mouse and rat models in which disruption of normal leptin signaling axis results in obesity and a diabetic state that resembles type 2 diabetes [22,23]. Furthermore, electrospray ionization mass spectrometry analysis of cardiac tissue lipids provides evidence for remodeling of TAG molecular species in the streptozotocin-treated rat model [24] and phospholipid molecular species in the Zucker diabetic fatty (fa/fa) rat with nonfunctional leptin receptors [25].

In addition, a number of studies have documented increases in cardiac fatty acid metabolism in rodent models of type 1 and type 2 diabetes. Under physiologic conditions, 60% to 70% of cardiac ATP is generated through fatty acid oxidation [26], although the heart is capable of utilizing glucose, ketones, and amino acids as well. The metabolic plasticity to vary the contribution of these different substrates under different workloads and under pathophysiologic circumstances may be important for optimal cardiac performance. In models of type 1 [19,21] and type 2 diabetes [27], the heart demonstrates approximately a twofold increase in fatty acid oxidation with concomitant decrease in glucose oxidation. In the former models, insufficient insulin may lead to this change in substrate utilization, whereas in the latter models, insulin resistance at the level of the cardiac myocyte may be key. In either situation, increased cycling through fatty acid oxidation pathways and diminished glucose utilization may be deleterious.

Cardiac myocyte lipid accumulation in diabetic rat, mouse, and dog models has been associated with cardiomyocyte cell death and cardiomyopathy [22,23,28–31]. The Zucker rat, streptozotocin-treated mouse or rat, and db/db mouse are characterized by cardiac hypertrophy, progressive diminution of systolic performance, and accompanying diastolic dysfunction, findings documented by a combination of echocardiography, cardiac catheterization, and isolated working heart preparations. These animal models are thought to recapitulate pathophysiology relevant to end-stage diabetic cardiomyopathy. On the other hand, isolated diastolic dysfunction in 10- to 11-week-old ob/ob mice may serve as a better model for the early changes in diabetic cardiomyopathy.

In each of these models, pleiotropic metabolic abnormalities may contribute to cardiac dysfunction. The observation of diminished cardiac TAG content and improved cardiac performance, following treatment of Zucker diabetic fatty rats with a thiazolidinedione [22] or in transgenic mice with increased cardiac capacity for lipoprotein secretion [29], suggests that lipid accumulation plays a direct causal role in the genesis of cardiomyopathy.

Genetic Mouse Models of Metabolic Cardiomyopathy
More direct evidence for the role of excess lipids in the pathogenesis of cardiomyopathy comes from several transgenic mouse models in which lipid overload in the heart causes cardiomyopathy, in the absence of systemic metabolic disturbances and in the absence of impairment in fatty acid oxidation. First, cardiac-restricted overexpression of long-chain acyl-CoA synthetase 1 (MHC-ACS) [32], a protein that participates in vectorial trafficking of FFA across membranes, leads to increased FFA uptake that exceeds the ability of the heart to use this substrate and results in cardiac myocyte lipid accumulation. Cardiomyocyte apoptosis associated with early lipid accumulation in
the MHC-ACS model likely contributes to the progression of systolic dysfunction and may be mediated, in part, through increases in cardiac ceramide content. In a second model, cardiac-restricted overexpression of peroxisome proliferator activated receptor-α (MHC-PPAR-α) [33•] leads to transcriptional activation of genes that function in FFA transport and metabolism, increasing in both substrate uptake and utilization. However, the net effect in this model is intramyocellular neutral lipid accumulation, accompanied by evidence for oxidative stress and systolic heart failure. A third model, in which a glycosylphosphatidylinositol-linked lipoprotein lipase is overexpressed in cardiac myocytes (hLPLGPI) [34•], is characterized by increased uptake of very low-density lipoprotein (VLDL)-derived lipid into the heart and consequent cardiomyocyte cell death and cardiomyopathy with systolic dysfunction. Recently, our laboratory described a fourth model with cardiac myocyte overexpression of the fatty acid transport protein 1 (FATP1), a protein that facilitates movement of long-chain fatty acids across the cell membrane [35•]. In this model, increased FFA uptake leads predominantly to increased cardiac fatty acid oxidation, and FFA, but not TAG, accumulation in the heart. This model differs from other models of metabolic cardiomyopathy in that the perturbations of cardiac lipid homeostasis are associated with isolated diastolic cardiac dysfunction that may recapitulate lipid-induced changes in early diabetic cardiomyopathy.

In addition to their use as discovery platforms in which to evaluate mechanisms of cardiac lipotoxicity, these models have been used to assess potential therapeutic approaches to lipotoxic cardiomyopathy. Cardiomyopathy in MHC-PPAR-α mice is exacerbated by diets enriched in long-chain TAGs, an effect that was not observed with medium-chain TAGs [36]. The reversibility of cardiomyopathy upon lowering dietary lipid or switching to substrates of shorter chain length suggests that dietary strategies may contribute to improvement in metabolic cardiomyopathy. Furthermore, the observation that a PPAR-γ agonist simultaneously reduced cardiac lipid uptake and improved cardiac function in hLPLGPI mice suggests further that diversion of lipid to other tissues, such as adipose depots may also be beneficial [37]. Even more dramatic is the beneficial effect of pharmacologic doses of leptin in the MHC-ACS model [38•], which completely reverses lipid accumulation and cardiomyopathy in this model. This effect likely results from a combination of leptin’s actions to lower serum TAGs, to divert lipid away from the heart toward adipose depots, and to increase fatty acid oxidation in the heart. Together these studies provide intriguing insights that may be extended to diabetic cardiomyopathy.

Cardiomyocyte Response to Lipid Overload
Studies using cultured cells to model the lipotoxic response have helped elucidate mechanisms involved in the response to fatty acid overload. This reductionist approach using a variety of cell culture systems, including immortalized cell lines and isolated primary cells, has facilitated both the identification of specific fatty acids that induce lipotoxic cell death and the identification of metabolic and signaling pathways involved in the cellular response to lipid overload.

Long-chain saturated fatty acids (ie, palmitate), but not unsaturated fatty acids (ie, oleate), induce cell death in a variety of cell types, including endothelial cells [39], Chinese hamster ovary (CHO) cells [40], pancreatic β-cells [41,42], hepatocytes [43], and cardiomyocytes [44–48]. In addition, co-treatment of CHO cells or pancreatic β-cells with palmitate and an unsaturated fatty acid (either oleate or linoleate) diminishes palmitate-induced cell death [49–51]. In breast cancer and pancreatic β-cells, this protective effect is due to activation of a phosphoinositide 3-kinase-dependent cellular survival pathway [51,52]. In CHO cells, oleate facilitates the channeling of palmitate to cytosolic lipid droplets, presumably sequestering it away from pathways leading to cell death [49]. Thus, unsaturated fatty acids may help prevent lipotoxic cell death through both the activation of a cellular survival pathway and the channeling of palmitate to lipid droplets.

In general, palmitate-induced cell death is characterized by markers of apoptosis, including cytochrome C release, caspase activation, and DNA fragmentation. However, we and others have also observed concomitant necrotic cell death in response to palmitate, as evidenced by relatively modest caspase activation and increased cell membrane permeability [40,49,51]. Although relatively few studies have focused on mechanisms of palmitate-induced cell death in cardiomyocytes, recent evidence obtained using primary cardiomyocyte cultures from embryonic chicks and neonatal rats suggests that mitochondria are central to palmitate-induced apoptosis. The role of mitochondria in apoptosis is well established [53], and incubation of cardiomyocytes with palmitate is associated with loss of mitochondrial membrane potential, mitochondrial swelling, and cytochrome C release [45,46,54,55], all events involved in mitochondrial regulation of cell death. Palmitate may initiate these events via several mechanisms, including decreased synthesis of the signature mitochondrial membrane phospholipid, cardiolipin [45], increased ceramide synthesis, and increased generation of reactive oxygen species (ROS).

The de novo synthesis of the membrane sphingolipid ceramide was initially considered the most likely mechanism for palmitate-induced cell death in multiple cell types, including cardiomyocytes. Ceramide synthesis, initiated by the condensation of serine and palmitoyl CoA, has been linked in numerous studies to the induction of apoptosis by diverse stimuli [56]. Although incubation of cardiomyocytes with palmitate increases de novo production of ceramide [46,47], studies using pharmacologic and genetic blocks in this pathway revealed that ceramide synthesis is not required for palmitate-induced cell death.
Oxidative stress can lead to the loss of mitochondrial calcium (Ca²⁺) from the endoplasmic reticulum (ER) to mitochondria.

Impaired synthesis of the mitochondrial membrane phospholipid, droplets is overwhelmed. Excess intracellular palmitate results in the use of fatty acids for energy or to store fatty acids as triglycerides in lipid droplets.

Cardiomyocyte response to palmitate overload. Under conditions of palmitate overload in cardiomyocytes, cellular capacity to use fatty acids for energy or to store fatty acids as triglycerides in lipid droplets is overwhelmed. Excess intracellular palmitate results in the use of fatty acids for energy or to store fatty acids as triglycerides in lipid droplets.

Recent studies in pancreatic islets [57] and in CHO cells [40,49] suggest that palmitate-induced apoptosis is downstream of the generation of ROS. Although the involvement of ROS in palmitate-induced apoptosis in cardiomyocyte cultures is controversial [58], oxidative stress has been observed in MHC-PPAR-α mice, a model of lipotoxic cardiomyopathy [36]. Oxidative stress can lead to the loss of mitochondrial membrane potential and apoptosis [53]. In fact, the induction of apoptosis by both oxidative stress and ceramide absolutely requires calcium flux from the endoplasmic reticulum to mitochondria [59], further suggesting that palmitate overload initiates several cellular responses that eventually converge on the mitochondria (Fig. 1).

Conclusions
As reviewed here, evidence from animal models strongly implicates lipids and metabolic derangements in the pathogenesis of cardiomyopathy. However, the relationship between lipid metabolic abnormalities and cardiomyopathy in obese and diabetic humans remains to be firmly established. Furthermore, although studies in cell culture have revealed important clues as to the mechanisms involved in fatty acid-induced cell death, further research is required to fully elucidate the lipotoxic response in cardiomyocytes. Given the pandemic of obesity and diabetes, understanding the pathogenesis of cardiac complications of these diseases at both physiologic and cellular levels will be an important goal.

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References and Recommended Reading
Papers of particular interest, published recently, have been highlighted as:
• Of importance
•• Of major importance


Figure 1. Cardiomyocyte response to palmitate overload. Under conditions of palmitate overload in cardiomyocytes, cellular capacity to use fatty acids for energy or to store fatty acids as triglycerides in lipid droplets is overwhelmed. Excess intracellular palmitate results in the use of fatty acids for energy or to store fatty acids as triglycerides in lipid droplets.


