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Creating and curing fatty hearts

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Abstract

Purpose of review—Diseases associated with ectopic disposition of lipids are becoming an increasingly important medical problem as the incidence of type 2 diabetes and obesity increases. One of the organs affected by lipotoxicity is the heart and this review presents an update on human and animal studies of this problem.

Recent findings—Human studies have clearly correlated heart dysfunction with the content of triglyceride. More recently human heart samples have been used to assess gene changes associated with altered lipid accumulation. Genetically altered mice have been created that develop lipotoxic cardiomyopathies and newer investigations are attempting to delineate curative therapies.

Summary—Human studies will confirm the metabolic changes associated with lipotoxic cardiomyopathy and, hopefully, animal studies will guide treatment options.

Keywords

cardiomyopathy; ceramide; lipotoxicity; triglyceride

Introduction

A number of disorders associated with obesity – non-alcoholic fatty liver disease, type 2 diabetes, and lipotoxic cardiomyopathy – are attributed to excess lipid accumulation. The association between sudden death and dilated cardiomyopathy with obesity was described decades ago. More recently, studies in humans have related cardiac lipid content with reduced heart function. In addition, human studies have begun to uncover metabolic alterations in these tissues that might lend insights into the primary pathological process, that is the link between lipid accumulation and impaired heart function.

A second approach to understanding cardiac and other forms of lipotoxicity has been to create the disease by dietary or genetic alterations in rodents. A number of such models have been created by increasing lipid uptake into the heart even without elevated lipid levels in the circulation. More recently, investigators have used pharmacologic and genetic methods to cure cardiac lipotoxicity; the objective is to define and eventually correct the abnormal signaling pathways.

Human hearts have abnormal lipid accumulation and alterations in lipid metabolism

During the past decade a number of studies of human hearts have associated increased lipid oxidation with obesity and type 2 diabetes [1]. More recently, techniques developed to quantify triglyceride levels within the working human heart using magnetic resonance spectroscopy have confirmed that cardiac triglyceride content is a marker for impaired heart function [2].

As was found in animal models of obesity and diabetes, patients with metabolic syndrome have elevated myocardial lipid content that appears to contribute to cardiac dysfunction. A recent study by Marfella *et al.* [3^{***}] correlated the relationship between intramyocardial triglyceride content and lipogenic transcription factors in patients with metabolic syndrome undergoing aortic valve replacement. In this study, biopsies from patients with metabolic syndrome and pathologic cardiac remodeling had increased myocyte triglyceride content, poor cardiac function, and increased oxidative stress compared to patients without metabolic syndrome. Patients with metabolic syndrome also had increased expression of SREBP1c and PPAR γ . SREBP1c and PPAR γ are transcription factors that act in concert to promote lipogenesis during periods of excess energy or overnutrition. As this study suggests, the caloric excess that occurs in metabolic syndrome may stimulate these two transcription factors, which in turn promote lipogenesis and fat storage. This, in turn, increases the ectopic deposition of lipid in the heart, ultimately leading to its dysfunction. In addition, the upregulation of PPAR γ suggests that metabolic syndrome patients could be especially sensitive to cardiac effects of PPAR γ agonists, drugs that are known to increase heart failure.

Effects of defective cardiac fatty acid oxidation

There are at least three methods to inhibit oxidation of fatty acids in the heart: reduction of lipid uptake, prevention of use of stored and recycled triglyceride, and inhibition of fatty acid oxidation pathways. This latter process can be blocked by reducing PPARs, inhibiting fatty acid oxidative enzymes, and increasing intracellular metabolites that block these enzymes.

Due to the limited ability of the heart to synthesize fatty acid, cardiac fatty acids are obtained either via hydrolysis of triglyceride contained in circulating lipoproteins or from free fatty acids associated with albumin. Although some free fatty acids are likely to enter cardiomyocytes via nonregulated pathways designated as 'flip-flop', there is a consensus that receptor-mediated pathways are of major importance. Although there may be a number of cardiac free fatty acid transporters, the best characterized is FATP/CD36. Loss of this protein is associated with a marked reduction in cardiac fatty acid uptake in rodents (*Cd36*^{-/-} mice) and in humans.

The effect of loss of CD36 on heart function is unclear. *Cd36*^{-/-} mice appear normal and a recent study suggested that these mice have less hypertrophy with aging [4]. The importance of continued fatty acid uptake and oxidation is unclear. In the setting of ischemia it has long

been known that whereas fatty acid oxidation produces more ATP, this is a more oxygen-requiring process than glycolysis. Two groups that used isolated perfused hearts to study ischemia in *Cd36*^{-/-} mice came to opposite conclusions. Irie *et al.* [5] found that hearts from *Cd36*^{-/-} mice with depressed fatty acid uptake and oxidation rates had a worse outcome in the setting of ischemia/reperfusion, whereas a similar study by Kuang *et al.* [6] found the opposite. This discrepancy between these two studies may be related to the fact that Irie *et al.* [5] did not use insulin in the perfusate, thereby limiting the amount of glucose entering the heart, whereas Kuang *et al.* [6] used supra-physiologic levels of insulin and as a result stimulated excess uptake and adequate compensation.

A second method to alter lipid uptake relies on the elimination of lipoprotein lipase (LpL). Using heart-specific LpL knockout mice, Yamashita *et al.* [7] studied how and if the heart compensated for hypertension induced by either angiotensin infusion or deoxycorticosterone/salt. Normal hearts markedly increase glucose uptake with increased afterload, but the LpL knockout hearts were unable to do this; they appeared to have already reached maximal uptake. The LpL knockout mice also developed heart failure, an outcome thought to be due to a deficiency in energetic substrates.

Fatty acid oxidation, at least theoretically, should be reduced if cellular malonyl CoA, the inhibitor of CPT1, is increased. Malonyl CoA decarboxylase (MCD) is responsible for decarboxylation and degradation of malonyl CoA; MCD deficiency should increase malonyl CoA and inhibit fatty acid oxidation. Using an isolated working heart perfusion model, Dyck *et al.* [8] studied whether hearts from MCD knockout (*Mcd*^{-/-}) mice had altered cardiac metabolism and impaired cardiac ability to handle ischemic injury. Whereas acute pharmacologic inhibition of MCD had been previously shown by the same group to inhibit fatty acid oxidation and increase glucose oxidation in the heart, long-term inhibition in the *Mcd*^{-/-} mouse did not reveal any differences in fatty acid or glucose oxidation despite achieving low levels of MCD activity and elevated levels of malonyl CoA. The comparable fatty acid and glucose oxidation rates between *Mcd*^{-/-} and wild-type mice were explained by a compensatory increase in some PPAR α -regulated genes (CD36, UCP-3, CPT1) that restored fatty acid oxidation. Although substrate oxidation rates and cardiac function were similar under aerobic conditions, ischemia/reperfusion increased glucose oxidation to a greater extent in *Mcd*^{-/-} mice and attenuated ischemic injury. In spite of the fact that fatty acid oxidation rates between *Mcd*^{-/-} and wild-type mice were unchanged during the ischemia/reperfusion protocol, the authors concluded that the inability of *Mcd*^{-/-} hearts to increase fatty acid oxidation led to higher levels of glucose oxidation with greater reliance of the ATP pool on glucose oxidation. In some ways the results are reminiscent of studies from Goodwin *et al.* [9] who noted that the primary response of isolated hearts to overload was increased glucose uptake and oxidation with no change in fatty acid oxidation.

Additional models of cardiac lipotoxicity

Cardiac lipotoxicity has been created by increasing the trapping or uptake of lipid via overexpression of acyl CoA synthetase, fatty acid transport protein 1, and LpL. In addition, both PPAR α and PPAR γ [10] expression in cardiomyocytes leads to accumulation of lipid. Thus, uptake is out of proportion to oxidation. Recently, a new model was created by Yan *et*

al. [11**] using GLUT-1 transgenic mice. This group had previously reported that these mice had improved response to in-vitro ischemia/reperfusion, presumably due to greater glucose uptake. When GLUT-1 transgenic mice were fed a high fat diet they developed more advanced heart dysfunction than wild-type mice. GLUT-1 transgenic mice have greater glucose oxidation and diminished fatty acid oxidation associated with downregulation of PPAR α and upregulation of acetyl-coA carboxylase (ACC). When exposed to diet-induced obesity GLUT-1 transgenic hearts developed oxidative stress and contractile dysfunction. This was associated with failure to increase fatty acid oxidation and accumulation of more lipid within the heart, that is an example of glucose-induced gluco-lipotoxicity.

The study underscored two important observations. First, high glucose oxidation by itself did not prove to be toxic to the heart and second, contractile dysfunction occurred only when myocardial plasticity in fuel substrate selection was compromised in the setting of high circulating fatty acids.

Corrections of cardirolipotoxicity

An obvious method to correct lipotoxicity should be to reduce lipid uptake by the heart or increase cardiac fatty acid oxidation; unless the real culprit is lipid oxidation and not accumulation of toxic lipids. Using cardiac-specific PPAR α transgenic mice – denoted MHC-PPAR α because the transgene is driven by the α -myosin heavy chain promoter – Kelly and colleagues cured the mice by elimination of lipid uptake. When MHC-PPAR α was crossed onto the *Cd36*^{-/-} background, lipid accumulation and heart dysfunction ceased [12], however, several PPAR downstream targets associated with fatty acid oxidation were still upregulated. More recently, elimination of lipoprotein-derived lipid uptake by crossing MHC-PPAR α onto the heart-specific LpL knockout background corrected both the toxicity and induction of genes that are associated with cardiac abnormal function [13]. Thus, loss of LpL likely affects uptake of specific lipids required as PPAR agonists in the hearts.

Release of stored triglyceride from tissues, maybe especially the heart, requires initial hydrolysis by adipose triglyceride lipase (ATGL) followed by hydrolysis of diacylglycerol by hormone-sensitive lipase (HSL). This accounts for the marked cardiac triglyceride engorgement in ATGL knockout mice and the lack of triglyceride accumulation in tissues of HSL knockout mice. However, HSL still could modulate triglyceride content of the heart for the following reasons: HSL has some activity against triglyceride and greater HSL activity could prevent recycling of diacylglycerol back into triglyceride. These actions of HSL might especially be evident with increased HSL expression. Ueno *et al.* [14*] investigated the role of HSL in cardiac lipotoxicity in diabetic mice. They used heart-specific HSL overexpressing (MHC-HSL) mice and induced diabetes by administering streptozotocin (STZ). Diabetic hearts accumulated more lipid droplets and also developed increased collagen. However, the hearts were not enlarged, so this model differs from the enlarged dilated hearts found in lipotoxicity models. Neither fatty acids nor diacylglycerols were increased in hearts from STZ-treated mice. Diabetic MHC-HSL mice had no lipid droplet or collagen accumulation. Unlike in hearts from diabetic wild-type mice, PPAR α mRNA was not increased in diabetic MHC-HSL mice. Diabetic MHC-HSL mice also had lower expression of genes involved in lipoapoptosis (NF- κ B, iNOS), and greater expression of

ROS scavengers (MT1, MT2). So removal of lipids by overexpression of HSL was beneficial. Similarly, overexpression of HSL in the liver, which was associated with free fatty acid loss from hepatocytes, corrected fatty liver [15].

Ceramide is a toxic lipid that is increased in most models of lipotoxicity. Ceramide is proapoptotic in several cell systems. Park *et al.* [16] treated cardiolipotoxic mice that express a membrane anchored form of LpL, denoted LpL^{GPI}, with the ceramide biosynthesis inhibitor myriocin and also crossed the LpL^{GPI} mice with animals that had a heterozygous deletion of one subunit of serine palmitoyltransferase. This improved cardiac function and reduced, but did not eliminate, excess mortality. So ceramide appears to be at least one cardiotoxic lipid. However, when ceramide levels were assessed in the right atrial appendage of patients undergoing cardiac surgery, cardiac ceramide levels were not increased with diabetes and obesity [17]. Curiously several enzymes in the ceramide, sphingomyelin pathway including serine palmitoyltransferase, sphingosine kinase 1, neutral sphingomyelinase and ceramidases were upregulated.

A novel approach to finding pathways responsible for cellular lipotoxicity was employed by Brookheart *et al.* [18^{**}]. Chinese hamster ovary (CHO) cells were infected with a retroviral [reverse orientation splice acceptor (R-OSA) β -geo] promoter trap vector. This vector contains a gene (β -geo) that was generated by in-frame ligation of the β -galactosidase gene with the neomycin phospho-transferase gene and is flanked by a splice acceptor at its 5' end and a polyadenylation signal at its 3' end. Successful insertions were assessed by positive X-Gal staining (blue color) of cells that were screened following G-418 selection. This allowed them to inhibit randomly an array of specific genes. Transduced cells were screened for palmitate-resistant mutants that survived for more than 2 days; palmitate-treated control cells died within 2 days. Specific resistance to palmitate was confirmed by treating mutant cells with other apoptotic inducers. RACE PCR and directed PCR analysis of the mutant cDNA identified one allele of the gadd7 gene that was trapped. Gadd7 is transcribed to a noncoding (nc) RNA. To further evaluate the importance of gadd7 in the induction of oxidative and endoplasmic reticulum stress, gadd7 expression was knocked down by stable transfection of cells with shRNA and cell death rate was reduced. The investigators then showed that ROS induces gadd7 expression in palmitate-treated cells. However, gadd7 is also required for palmitate-induced endoplasmic reticulum stress. Thus there is a bidirectional communication between ROS and gadd7. Aside from the relationship to lipotoxicity, this study shows an important function for a noncoding RNA, and implicates gadd7 in palmitate-induced ROS generation and endoplasmic reticulum stress.

Diets and heart function

High fat feeding causes obesity and several metabolic changes in the heart. Both Park *et al.* [19] and Wright *et al.* [20] have shown that high fat diets are associated with increased cardiac lipid, insulin resistance and some degree of cardiac dysfunction. Wright *et al.* [20] studied hearts from mice that were fed a high fat diet starting at 10 weeks old and lasting for 2 and 5 weeks. Both 2 and 5-week feeding reduced glycolysis, glucose oxidation, and insulin-stimulated glucose uptake, although heart function parameters (heart rate, developed pressure and cardiac output) were not affected by this short-term trial. Although pAkt levels

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were not affected, insulin-stimulated GLUT4 translocation to the cell membrane was significantly reduced and was thought to account for the observed reduction in glucose uptake and catabolism. Palmitate oxidation and oxygen consumption were increased in isolated working hearts that were obtained from high fat-fed mice. However, mitochondrial function was not impaired as shown by normal state 3 respirations and ATP production. The expression profile of PPAR α targets was changed only in hearts from mice that were fed a high fat diet for 5 weeks. This study confirms that a switch from cardiac glucose catabolism to fatty acid utilization occurs at a very early stage and precedes the development of high fat-induced cardiac abnormalities.

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Lipotoxic models have demonstrated that elevated cardiac lipid content is associated with compromised cardiac contractility, whereas the effects of loss of lipid uptake on heart function vary. Rennison *et al.* [21^{**}], using a model of coronary artery ligation induced heart failure, found that a saturated fat-rich diet did not further aggravate cardiac dysfunction, despite elevated ceramide levels. In fact, rats fed the high saturated fat diet demonstrated improved mitochondrial function. Whereas surprising, this improvement was attributed to an increase in fatty acid ligands that stimulate PPAR α and PGC-1 α . These transcription factors were then thought to activate downstream fatty acid metabolic enzymes, which in turn increased state 3 respiration as well as the capacity of mitochondria to oxidize fatty acid. In contrast, other studies that have used PPAR α transgenic mice and PPAR α agonists to activate PPAR α have reported a decline in cardiac contractility. Conflicting results such as these highlight the contentious issue of inhibiting fatty acid oxidation as a means of protecting the failing heart.

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The lack of ceramide-induced lipotoxicity seen here had also been reported by Relling *et al.* [22] who demonstrated that acute exposure to ceramide led to an increase in peak cardiomyocyte shortening in isolated ventricular myocytes. This effect, however, was reversed with longer durations of exposure to ceramide. Therefore, the absence of lipotoxicity seen by Rennison *et al.* [21^{**}] may in fact be a consequence of the high fat diet duration and it is possible, that with continued feeding, a cardiotoxic effect might have been observed.

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Whereas diets rich in fat predispose to fatty hearts and cardiac dysfunction, diets rich in omega-3 polyunsaturated fatty acids appear to have the opposite effect. D'Alessandro *et al.* [23] examined the effects of n-3 PUFA in an insulin resistant and dyslipemic lipotoxic model. In this study, rats were fed a sucrose-rich diet for 8 months to induce cardiac lipotoxicity, and then treated in the last 2 months by replacing the corn oil in the diet with cod liver oil. The addition of n-3 PUFA corrected the altered glucose metabolism and normalized elevated levels of plasma triglyceride and free fatty acids present in these lipotoxic hearts. In addition to reducing lipid flux to the heart, fish oil also reduced levels of myocardial diacylglycerol and membrane fraction (activated) PKC (epsilon). PKC and its various isoforms have been implicated in the pathophysiology of many cardiovascular disorders such as myocardial hypertrophy, hypertension and atherosclerosis. Targeting the compartmentalization and levels of these protein kinase isoforms may provide yet another mechanism for rescuing hearts from lipid overload.

Conclusion

The epidemic of obesity and insulin resistance in the developed and developing world is likely a harbinger of secondary organ dysfunction due to lipotoxicities. Human investigations are beginning to define this disease and are likely to provide the fingerprints to diagnose this specific form of dilated cardiomyopathy. The animal models using diets or the more profound genetic alterations appear to reproduce many of the aspects of the human condition. In addition, they will hopefully direct clinical interventions leading to new nutritional and pharmacologic therapies.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 219).

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