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## Cardiac Lipotoxicity: Molecular Pathways and Therapeutic Implications

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### Abstract

Diabetes and obesity are both associated with lipotoxic cardiomyopathy exclusive of coronary artery disease and hypertension. Lipotoxicities have become a public health concern and are responsible for a significant portion of clinical cardiac disease. These abnormalities may be the result of a toxic metabolic shift to more fatty acid and less glucose oxidation with concomitant accumulation of toxic lipids. Lipids can directly alter cellular structures and activate downstream pathways leading to toxicity. Recent data have implicated fatty acids and fatty acyl coenzyme A, diacylglycerol and ceramide in cellular lipotoxicity, which may be caused by apoptosis, defective insulin signaling, endoplasmic reticulum stress, activation of protein kinase C, MAPK activation or modulation of PPARs.

### Keywords

heart failure; lipotoxicity; fatty acid oxidation; diacylglycerol; ceramide; apoptosis; insulin signaling; endoplasmic reticulum stress; protein kinase C; MAPK; PPAR

## INTRODUCTION

The purpose of this review is to discuss how lipids, which are important for cardiac function, can also be detrimental when their amount or distribution is in excess and may lead to abnormal cardiac structure and function. The heart, under normal conditions, is primarily dependent on fatty acids (FAs). On the other hand, the preferred substrate of cardiac energy metabolism switches from FAs to glucose for ATP production in pathological situations such as ischemia, advanced hypertrophy and heart failure. Although this “flexibility” is considered as an adaptive process, several studies have demonstrated that both inhibition and profound elevation of cardiac FA oxidation, which is regulated by multiple factors (Figure 1) can be pathogenic. Defective cardiac lipid uptake<sup>1</sup> impairs optimal heart function. This finding may be explained, in part, by the limitation of the normal hearts for substituting glucose for lipids. In contrast, excessive FA oxidation is also harmful<sup>2, 3</sup>. Whether this is

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due to lipid toxicity with inappropriate regulation of lipid uptake or overwhelming FA oxidation alone will be discussed in this review.

### The delivery and uptake of lipids to the heart

FAs are the primary energetic lipids metabolically used by the heart. In fact, FAs are estimated to supply approximately 70% of the heart's energy requirements<sup>2</sup>. Moreover, studies from more than four decades ago suggest that esterified FAs are the major source of cardiac lipids in humans<sup>4</sup>. FA supplies for the heart are derived from dietary fat, as well as by hepatic FAs synthesized from carbohydrates, and FAs released following adipose tissue lipolysis. In the circulation, most FAs are present either esterified to glycerol as a component of lipoprotein triglycerides (TG) and phospholipids, or unesterified, free fatty acids (FFAs) that circulate bound to albumin. Some FAs are acquired by tissues, including the heart, as a component of either whole lipoprotein particles or smaller lipid vesicles that dissociate from larger lipoproteins during lipolysis. Studies in humans demonstrated extraction of esterified i.e. lipoprotein-associated FAs by the heart<sup>4</sup>. In isolated hearts, most triglyceride uptake requires extracellular lipolysis<sup>5</sup>. TGs are the primary source of energy-producing FAs and circulate in the bloodstream within chylomicrons and very low density lipoproteins (VLDL). Lipoprotein-derived FAs are acquired by the heart following TG hydrolysis, which is mediated by endothelial-bound lipoprotein lipase (LpL). Intracellular esterified lipids stored in the form of triglycerides are converted to FFAs via the intracellular enzymes hormone sensitive lipase (HSL) and adipose triglyceride lipase (ATGL)<sup>6, 7</sup>, which seems to be a major circuit for providing FAs in the ATP production machinery<sup>8, 9</sup>.

Lipoprotein-derived FFAs are created when plasma lipoproteins interact with endothelial cell-associated LpL<sup>10</sup>. The heart is a particularly rich source of this enzyme. Of note, mice with expression of LpL solely in cardiomyocytes have normal plasma triglyceride levels<sup>11</sup>. In the neonatal and presumable prenatal period, cardiac LpL expression is very low as the heart primarily consumes glucose. Shortly after birth and with the consumption of high-fat milk, LpL is rapidly induced in cardiac tissue<sup>12-14</sup>. Fasting reduces adipose tissue LpL activity, but increases cardiac LpL<sup>15</sup>. This is thought to be a response to the increased circulating fraction of TG and FFA under starvation. Active cardiac LpL increases in the setting of defective glucose utilization and hypoinsulinemia<sup>16, 17</sup>. In contrast, insulin is needed to maintain LpL expression in isolated cardiomyocytes<sup>18</sup>. Hypertension reduces cardiac LpL as the heart shifts to greater glucose utilization<sup>19</sup>. Cardiac-specific loss of LpL converts the heart's "metabolic preference" towards greater glucose uptake and oxidation<sup>20</sup>, while constitutive cardiomyocyte-specific expression of glycosylphosphatidylinositol (GPI)-anchored LpL leads to cardiomyopathy<sup>21</sup>.

FFA uptake occurs via cell surface receptors, the most well characterized being CD36<sup>22</sup>, and via biophysical non-receptor transport, also known as "flip-flop"<sup>23</sup>. CD36 knockout (CD36<sup>-/-</sup>) mice have reduced FFA uptake into muscle and heart<sup>22</sup>. Humans with a genetic defect in CD36 also have defective cardiac long chain FA uptake and an approximately 3-fold increase in glucose uptake<sup>24</sup>. Although CD36-deficient humans tend to have more hypertriglyceridemia and insulin resistance, they have not been reported to have cardiomyopathy<sup>25</sup>. Cardiac function in CD36<sup>-/-</sup> mice is also normal. One group reported that CD36<sup>-/-</sup> hearts respond normally to ischemia reperfusion<sup>26</sup>, an observation leading the investigators to conclude that glucose uptake compensates for defective FA uptake. However, other investigators reported that CD36 deficiency led to impaired cardiac contraction during ischemia, which was corrected by inclusion of caprylic acid, a medium chain FA, to the perfusate<sup>27</sup>. Differences in the conditions for these two studies likely contributed to the opposing results.

CD36 is required downstream of LpL for the uptake of at least a part of lipoprotein-derived FFAs, as well as for non-esterified FFAs<sup>28</sup>. It is possible that a transporter other than CD36 functions in normal or CD36<sup>-/-</sup> mice. Most likely, this transporter is a member of the fatty acid transport protein (FATP) gene family. Consistent with this hypothesis, FATP1 expression is increased in CD36<sup>-/-</sup> hearts suggesting a compensatory regulation of gene expression<sup>29</sup>. However, FATP1 knockout mice<sup>30</sup> do not have a cardiac phenotype. FATP6 has been reported to be the FATP most robustly expressed in the heart<sup>31</sup>. Further, intracellular accumulation of FFAs requires trapping and esterification to CoA which also involves fatty acid transport proteins (FATPs) and long chain acylCoA synthase (ACS).

Figure 2 depicts the major proteins that are involved in fatty acid uptake and delivery to the mitochondria for oxidation and ATP production.

### Toxic lipids and lipid intermediates

Not all lipids are toxic. Specifically, the status of TG as a toxic molecule is unresolved. Despite the fact that increased TG levels correlate with insulin resistance in the setting of obesity and the metabolic syndrome, genetic experiments suggest that TG is by itself solely a marker for general lipid overload by the cells. Saturated LCFAs, particularly palmitic acid (PA, 16:0), are considered to be a more potent cause of lipotoxicity than unsaturated LCFA, such as oleic acid (OA, 18:1). This has been correlated with increased intracellular ceramide levels due to distinct enzyme specificity; PA leads to increased ceramide formation compared to OA<sup>32-34</sup>. Cardioliptoxic animal models are characterized by increased myocardial lipid content in the absence of obesity. Transgenic mice expressing a GPI-anchored form of human LpL driven by the cardiac muscle-specific  $\alpha$ -myosin heavy chain ( $\alpha$ MHC) promoter ( $\alpha$ MHC-LpLGPI) have normal plasma lipid levels but increased lipid uptake from circulating lipoproteins and intracellular cardiomyocyte lipid accumulation. Ceramide accounts, at least partially, for the aggravating consequences of the increased cardiac lipid accumulation of the MHC-LpLGPI mice<sup>35</sup>. Treatment of these mice with myriocin, a *de novo* ceramide synthesis inhibitor, normalizes intramyocardial ceramide levels and corrects cardiac hypertrophy<sup>35</sup>. However, this treatment improved survival only slightly, indicating that additional mechanisms, which are triggered by lipids other than ceramide may also mediate cardiac lipotoxicity.

Di-Acyl Glycerol (DAG) is another lipid that mediates FA-induced toxicity and is implicated as a cause of insulin resistance in skeletal muscle and liver<sup>36-38</sup>. In TG synthesis, DAG acyl transference (DGAT) adds the final FA onto DAG and converts the toxic lipid intermediate DAG to TG. Two non-homologous DGAT genes have been cloned, DGAT1<sup>39</sup> and DGAT2<sup>40</sup>. Overexpression of DGAT1 in hearts of lipotoxic models, such as the ACS-overexpressing mice prevents from cardiac dysfunction although increased lipid accumulation still occurs<sup>41</sup>. This suggests a role for this enzyme in clearing toxic lipid intermediates into the stable and non-toxic storage for TG. On the other hand, DGAT1 deficient mice are resistant to weight gain when fed a western-style high fat diet<sup>42, 43</sup>. This condition is associated with increased total energy expenditure due to increased physical activity in these animals. Thus, based on these data, ceramide and diacylglycerol rather than TG appear to be toxic and contributing to deteriorating cardiac function.

### Models of lipid toxicity

Two human conditions are thought to be caused by excess cardiac lipid accumulation: obesity-related cardiomyopathy, a dilated cardiomyopathy associated with normal coronary arteries and sudden cardiac death<sup>44</sup>, and diabetic cardiomyopathy with decreased cardiac function disproportional to coronary artery disease<sup>45</sup>. In studies of pathological specimens<sup>46</sup>, cardiac lipid uptake and oxidation<sup>47</sup>, and MR cardiac TG analysis<sup>48, 49</sup> establish that cardiac

dysfunction is associated with defects in cardiac lipid metabolism and lack of intracellular TG-derived FAs mobilization that leads to TG accumulation.

It is not very clear whether cardiomyopathy is due to excess FA oxidation or accumulation of toxic lipids or both. Oxidation of long chain FAs such as palmitate requires the transfer of FA-CoA into the mitochondria by carnitine palmitoyltransferase (Cpt)-1. Cpt-1-mediated FA consumption is critical as shown in Cpt-1 $\beta^{+/-}$  mice that have aggravated pressure overload-induced cardiac hypertrophy caused by lipotoxicity<sup>50</sup>. Cpt-1 function is inhibited through a negative feed-back loop by malonyl-CoA, a product of acetyl-CoA carboxylase (ACC). Malonyl-CoA was reduced and cardiac function was improved in mice with cardiomyocyte-specific ACC2 ablation that underwent pressure overload via transverse aortic constriction<sup>51</sup>. ACC2 $^{-/-}$  have increased FAO and this led to reduced left ventricular mass<sup>52</sup>. Pharmacological inhibitors of Cpt-1, such as etomoxir, ethyl-2-tetradecyl glycidate and oxfenicine switch energy metabolism from FA to glucose oxidation<sup>53</sup>. It has been proposed that such metabolic change from oxidation of FA to the energetically less O<sub>2</sub>-consuming glucose or lactate oxidation is beneficial in the post-ischemic heart failure<sup>54</sup>. On the other hand prolonged inhibition of Cpt-1 by etomoxir, in combination with high-fat diet, resulted in excess triglyceride accumulation and lipotoxicity in skeletal muscle of rats<sup>55</sup>.

A number of spontaneous and targeted rodent mutations have reproduced dilated cardiomyopathy with excess lipid accumulation. Rodents such as the Zucker rat<sup>56, 57</sup> and the db/db mouse<sup>58</sup> that have genetic defects in leptin signaling pathways demonstrate reduced cardiac glucose oxidation, increased FA oxidation, lipid accumulation, and cardiac dysfunction. More recently, studies have shown that decreased cardiac function linked to aging is blunted in CD36 $^{-/-}$  mice<sup>59</sup>.

A number of genetically modified mice develop dilated cardiomyopathy associated with lipid accumulation. Several models have transgenic expression of proteins that increase lipid uptake by the heart. Cardiomyocyte-specific expression of long chain acyl CoA synthase 1 (ACS1)<sup>60</sup>, the enzyme that traps FA intracellularly by esterification to CoA, leads to both systolic and diastolic heart dysfunction. Fatty acid transport protein 1 (FATP1) overexpression also causes lipotoxic cardiomyopathy<sup>61</sup>; this protein is postulated to modulate FA trapping and uptake. Cardiomyocyte-anchored LpL is associated with greater uptake of plasma lipids and dilated cardiomyopathy<sup>21</sup>. All three genetically modified mice in the studies cited have increased cardiac lipid content, but also greater FA oxidation.

Additional models were created by overexpression of the FA metabolic transcription factors PPAR $\alpha$  and PPAR $\gamma$ . Cardiomyocyte-specific PPAR $\alpha$  transgenic mice have an increase in FA oxidation genes, greater FA oxidation, and less glucose oxidation and GLUT4 expression<sup>62</sup>. Cardiomyocyte specific PPAR $\alpha$  transgenic mice fed with a long-chain-fatty-acid-containing diet exerted a severe lipotoxic cardiomyopathic phenotype in diabetic background, whereas the lipotoxic effect was ameliorated when diet was switched to a medium-chain triglyceride-enriched diet<sup>63</sup>. Reduction of lipid uptake in the PPAR $\alpha$  transgenic model using either a whole body deletion of CD36<sup>29</sup> or a cardiac specific-deletion of LpL<sup>64</sup> corrected the cardiomyopathy. PPAR $\gamma$  transgenic mice have a similar increase in FA metabolic genes, but no decrease in GLUT4<sup>65</sup>. Although it seems paradoxical that these mice develop lipotoxicity rather than reduced cardiac lipid stores, lipid uptake pathways involving either CD36 or LpL are upregulated by PPARs and increased lipid uptake must exceed oxidation. Interestingly, cardiomyocyte-specific overexpression of PPAR $\gamma$  on a PPAR $\alpha$  $^{-/-}$  genetic background led to improvement in fatty acid oxidation, cardiac function and survival rates despite similar cardiac TG and toxic lipids, DAG and ceramide, accumulation, as compared to PPAR $\gamma$ <sup>66</sup>. Notably, acylcarnitine content was decreased and so apoptosis, ROS levels, and endoplasmic reticulum stress

marker were. Although these models suggest that lipid accumulation is the culprit, the well-known toxic effects of excess lipid oxidation in perfused heart models<sup>67</sup>, especially in the setting of ischemia, does not rule out toxicity due to excess lipid oxidation.

If lipid accumulation occurs in the setting of reduced FA oxidation, it confirms that excess lipids are at least one cause of toxicity. Two lines of genetically modified mice that develop cardiac dysfunction and lipid accumulation have been created. Using a line of mice in which LpL expression was driven by a fragment of its natural promoter, a line of mice expressing LpL specifically in cardiac muscle was created<sup>11</sup>. Lacking any other mutations, these mice appear normal. However, when FA oxidation was reduced by crossing onto the PPAR $\alpha$  knockout background, cardiac dysfunction ensued<sup>68</sup>. Further, lipid oxidation decreased, lipid uptake was presumably not affected, and cardiomyocyte lipid accumulation occurred. Similarly, when FA oxidation was reduced by a tissue-specific knockout of PPAR $\delta$ , lipid accumulation and cardiomyopathy occurred<sup>69</sup>. On the other hand, constitutive cardiomyocyte-specific expression of PPAR $\delta$  induced the expression of FAO-associated genes and did not lead to lipid accumulation and cardiac dysfunction<sup>70</sup>. Besides elevated FAO, the prevention of cardiac lipid accumulation and organ dysfunction in the  $\alpha$ MHC-PPAR $\delta$  mice may be attributed to increased expression of Angiotensin-like 4<sup>71</sup>, which is an inhibitor of LpL and therefore may minimize cardiac lipid uptake. Accordingly, ATGL<sup>-/-</sup> mice show reduced FAO and massive cardiac lipid accumulation that are associated with severe cardiac dysfunction. Cardiac dysfunction in these mice is corrected upon pharmacological activation of PPAR $\alpha$ , also indicating that FA-mediated PPAR $\alpha$  activation relies on intracellular TG lipolysis<sup>72</sup>. Consistently, cardiomyocyte-ATGL overexpression seems to be beneficial for mice that undergo pressure overload<sup>9</sup>. However, this benefit cannot be attributed to increased FAO, which is surprisingly reduced, possibly as a secondary event to the increased glucose catabolism. In mice with total gene deletion of the other important cardiac lipase, HSL, cardiac TG lipase activity was decreased, but cardiac TG was not dramatically changed and there was no overt cardiac phenotype<sup>73</sup>. These data implicate lipid accumulation, rather than excess oxidation, as a cause of heart dysfunction.

### Signaling effects of lipids linked to cardiac dysfunction

Several lipids have been implicated in pathological processes that might contribute to lipotoxic cardiomyopathy: FA/fatty acyl CoA, acylcarnitine, unesterified cholesterol, lysolecithin, ceramide, and DAG. These lipids are thought to cause apoptosis, inflammation, mitochondrial dysfunction, and/or defective intracellular signaling (Figure 3).

**Apoptosis pathways**—Myocardial dysfunction has been attributed, among other factors, to apoptosis induced by abnormal conditions such as obesity, diabetes, and aging<sup>74</sup>. In most of these abnormalities, cardiac lipid overload is associated with and thought to contribute to the initiation of the apoptotic cascade. Studies, primarily in isolated cardiomyocytes, have implicated saturated FAs as a primary cause of apoptosis. Treatment of isolated neonatal rat ventricular myocytes with palmitic acid alters mitochondrial physiology and leads to apoptosis associated with cardiolipin loss, cytochrome *c* release, mitochondrial swelling, and DNA laddering<sup>75, 76</sup>. These pathways are summarized in Figure 3.

Saturated FA-induced apoptosis has been attributed to changes in cellular lipid content and/or excess lipid oxidation. Ceramide, one lipid class proposed to be toxic, is synthesized via two major pathways. In the first pathway, increased cellular palmitate drives *de novo* ceramide synthesis. Palmitate is initially converted to palmitoyl-CoA. Then, serine palmitoyltransferase (SPT), the rate limiting enzyme of ceramide *de novo* synthesis, catalyzes the condensation of palmitoyl-CoA and serine, producing 3-keto-sphinganine<sup>77</sup>. Subsequent reactions lead to the sequential synthesis of sphinganine, dihydroceramide, and

ceramide<sup>78, 79</sup>. In the second pathway, ceramide is released from sphingomyelin after it is hydrolyzed by sphingomyelinase<sup>80</sup>.

Support for a non-toxic effect of greater FA oxidation in the non-ischemic heart is provided by studies associated with modulation of 5'-AMP-activated protein kinase (AMPK). Activation of AMPK with 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR) attenuated the apoptotic effect of palmitate on cardiomyocytes<sup>75</sup>. This specific effect of AMPK was attributed to reduced malonyl-CoA levels, increased FA transport and oxidation in mitochondria, and, presumably, decreased intracellular levels of toxic lipids<sup>75</sup>. Nevertheless, AMPK is a "master regulator" of mechanisms relevant to cardiac energy production through FA or glucose catabolism. AMPK is activated by an increase in intracellular AMP:ATP ratio<sup>81</sup> and has been correlated with both cardiac FA uptake and oxidation. Activation of AMPK with AICAR increases protein levels of CD36 in cardiac myocytes and, subsequently, long chain FA transport in the heart<sup>82</sup>. AMPK-mediated increase of long chain FA uptake by mouse cardiomyocytes correlates with CD36 expression<sup>83</sup>. AMPK-mediated increase of FA uptake by cardiac cells also takes place by recruiting LpL to the coronary lumen, an event that increases LpL activity<sup>84</sup>.

ROS generation has also been implicated in palmitate-induced programmed cell death<sup>85,86</sup>. In CHO cells, reduction of palmitate-generated ROS by two ROS scavengers prevents apoptosis. In contrast, in neonatal rat cardiomyocytes, palmitate-induced apoptosis was neither associated with increased ROS nor rescued by antioxidants. In cultured aortic endothelial cells, FFAs increased ROS production<sup>87</sup>, especially in the setting of hyperglycemia.

**Lipid-induced defective insulin signaling**—There may be several mechanisms by which insulin signaling is cardioprotective and anti-apoptotic: 1) augmenting glucose oxidation, especially during ischemia, 2) direct activation of survival pathways downstream of Akt, 3) changes in cardiac perfusion due to eNOS activation. Insulin is an important regulator of myocardial substrate metabolism, but it also exerts regulatory effects on intracellular Ca<sup>2+</sup> handling and cell survival. Via either of these pathways, defective insulin signaling might exacerbate lipotoxic cardiomyopathy. Insulin resistance is one of the earliest observed cardiac defects found in mice given a high-fat diet<sup>88</sup>.

Lipid accumulation in the heart can lead to the development of cardiomyocyte insulin resistance characterized by predominant utilization of FA for cardiac energy, decreased glucose uptake, defective contractile response to insulin, and decreased cardiac efficiency caused by oxygen waste for noncontractile purposes<sup>89–93</sup>. In contrast, mice with a cardiac-specific deletion of insulin receptors demonstrate increased glucose uptake and oxidation, and develop smaller hearts<sup>94</sup>.

The FA-induced impairment of the insulin/IRS1/PI3K/Akt pathway has been suggested to be a causative factor in diabetic cardiomyopathy. The mechanism that underlies the effect of the accumulated myocardial FA on insulin signaling has not been elucidated; however, several mechanisms have been proposed based on findings in cells besides cardiomyocytes. ROS play a major role in the development of insulin resistance, as was shown by the amelioration of insulin resistance following ROS attenuation<sup>95</sup>. Both ceramide and DAG have also been implicated in defective insulin signaling and reduced glucose uptake in muscle.

Ceramide-mediated insulin resistance occurs in saturated fat feeding<sup>96</sup>. Although the mechanism is not completely clear, ceramide prevents insulin-mediated activation of Akt/PKB<sup>97–99</sup>. Ceramide may do this by blocking phosphorylation of Akt/PKB and/or by

stimulating protein phosphatase 2A, thereby leading to the dephosphorylation of Akt/PKB<sup>100</sup>. In C2C12 myotubes, overexpression of acid ceramidase, which catalyzes the conversion of ceramide to sphingosine i.e. the reduction of intracellular ceramide levels, attenuated the inhibitory effects of saturated FFAs on insulin signaling<sup>101</sup>.

DAG blocks upstream signaling events by promoting the serine phosphorylation of IRS-1, resulting in its deactivation<sup>102–104</sup>. This process, at least in skeletal muscle, may be mediated by activation of PKC $\theta$ <sup>105</sup> or other PKCs<sup>106</sup>. Systemic insulin resistance in patients with heart failure was accompanied by increased toxic lipid intermediates, DAG and ceramide. Mechanical unloading after left ventricular assist device implantation was shown to decrease myocardial levels of DAG and ceramide and improved insulin/phosphatidylinositol-3 kinase/Akt pathway activation<sup>107</sup>.

It may be that the insulin-resistance associated with lipotoxicity is an adaptive process. Insulin signaling may exacerbate lipid accumulation in the heart by increasing FA uptake. Besides its well-known function in glucose uptake, regulation, and catabolism, insulin redistributes CD36 from intracellular stores to cell membranes of rat cardiac myocytes<sup>108</sup>, a process that might increase intracellular FA stores. This process occurs via the actions of the forkhead transcription factor FoxO1 in muscle cells<sup>109</sup>. Moreover, following insulin stimulation, long chain FAs that are taken up by cardiomyocytes are less likely to be oxidized, thereby leading to the increased storage of toxic lipids.

**PKC activation**—Several lipids are associated with activation of protein kinase C (PKC). PKCs are a family of 12 serine/threonine protein kinases that are capable of modifying the activity of many cellular proteins. Several PKCs are highly expressed in adult myocardium and have been implicated in the regulation of contractility, gene expression, and growth<sup>110</sup>. A number of studies have demonstrated that PKC activation relies on binding of DAG<sup>36–38</sup> or ceramide<sup>111–121</sup> and translocation to the membrane. Overexpression of PKC $\beta$ , specifically in the myocardium of transgenic mice, leads to a cardiomyopathy associated with myocardial necrosis and thickened left and right ventricular walls resulting in an increase in the number of cardiomyocytes and the size of the interstitial extracellular matrix<sup>122</sup>. A later study showed that male Sprague-Dawley rats that were assigned to a high-fat diet (saturated fat from coconut oil) had increased activation of PKC $\beta$ 2, as evidenced by greater membrane translocation and cardiac hypertrophy<sup>123</sup>. Thus, lipid-mediated activation of PKC could exacerbate lipotoxic cardiomyopathy.

Several PKC isoforms are activated in failing hearts<sup>124</sup>. PKC $\alpha$  and PKC $\epsilon$  confer negative inotropic effects in cardiomyocytes<sup>125, 126</sup>. PKC $\beta$  impairs Ca<sup>2+</sup> handling, increases cardiomyocyte necrosis and ventricular wall thickening<sup>122, 123, 127, 128</sup>. Genetic<sup>128–130</sup> and pharmacologic<sup>128, 130, 131</sup> inhibition of PKCs improves cardiac responsiveness to catecholamines and heart function in cardiomyopathic mice. Of note, myocardial tissue from heart failure patients<sup>107</sup>, cardiolipotoxic mice<sup>32</sup> and a palmitate treated-human cardiomyocyte cell line<sup>32</sup> have increased PKC $\alpha$  and PKC $\delta$  activation. In addition cardiac lipotoxicity animal models and palmitate-treated cells show compromised  $\beta$ -adrenergic receptor responsiveness, which is corrected by inhibition of the PKC pathway<sup>32</sup>. Thus, PKC signaling is activated by toxic lipids and is associated with heart failure.

**MAP Kinases**—Mitogen Activated Protein Kinases (MAPKs) constitute a major group of kinases participating in critical intracellular signal transduction and regulation pathways. Members of the MAPK family, including Erk1, Erk2, JNK1, JNK2, and p38, have been associated with the control of survival signaling in cardiomyocytes following oxidative stress and are known to modulate insulin signaling<sup>132, 133</sup>. Erk and JNK activation also occurs in a genetic heart failure animal model (mutated lamin A/C gene) and treatment of

the animals with Erk and JNK inhibitors prevented left ventricular end-systolic dilatation, increased ejection fraction, and decreased myocardial fibrosis<sup>134</sup>. Although MAPKs have been implicated in cardiac development and disease, as well as in cardiomyocyte apoptosis<sup>135, 136</sup>, they may also play a role in FA-induced toxicity. Palmitate treatment of primary neonatal rat ventricular myocytes was found to activate Erk1/2, p38, and JNK<sup>137</sup>. However, a MEK1/2 inhibitor or a p38 kinase inhibitor had no effect on baseline or palmitate-induced apoptosis<sup>137</sup>. Activated JNK1 has been implicated in the induction of apoptosis in rat cardiomyocytes that undergo ischemia/reoxygenation stress<sup>138</sup>. The same study showed that the apoptotic effect of ceramide in rat cardiomyocytes can be mediated by activation of JNK and can be attenuated by administration of antisense JNK1 or JNK2. JNK interacts with proapoptotic Bax on the mitochondrial membrane<sup>136</sup>. Treatment of the same cells with a low concentration of oleate along with palmitate inhibited both palmitate-induced JNK activation and apoptotic events<sup>137</sup>. Inhibition of JNK has also been associated with improved FAO in the heart<sup>139</sup> and adipose tissue<sup>140</sup>. These data suggest that MAPKs may be involved in lipid-mediated apoptosis or suppression of FAO and thus may, at least partially, account for compromised cardiac function.

**ER stress**—Accumulation of FAs has been linked to the induction of endoplasmic reticulum (ER) stress. Specifically, palmitate-induced ER stress has been considered to be a secondary event that follows oxidative stress and generation of ROS, and results in an induction of eukaryotic elongation factor (eEF) 1A-1, which interferes with the integrity of the cytoskeleton and causes cellular death<sup>141</sup>. A more direct mechanism of palmitate-induced ER stress has also been proposed by the same study. This mechanism involves the incorporation of palmitate in phospholipid and TG species in microsomal membranes such that ER membrane integrity is compromised and protein-folding chaperones are redistributed to the cytosol<sup>142</sup>. Another study has reported that the esterification of palmitate can directly cause ER fission<sup>143</sup>. Myocardial ER-stress markers were elevated in a rat heart failure model (left anterior descending coronary arteries ligation) and their expression was alleviated by treatment of the rats with atorvastatin, which improved left ventricular function and reduced cardiac fibrosis<sup>144</sup>. Thus, ER-stress driven by cardiac lipid accumulation may contribute in the development of heart failure.

**PPARs**—Nuclear receptors, particularly PPARs, have a major role in the control of fatty acid oxidation. The PPAR family consists of three members, PPAR $\alpha$ , PPAR $\beta/\delta$ , usually reported as PPAR $\delta$ , and PPAR $\gamma$ . PPAR $\alpha$  has been tightly associated with increased fatty acid oxidation in the heart<sup>62</sup> and skeletal muscle<sup>145</sup>. Similarly, PPAR $\delta$  has been shown to activate fatty acid oxidation in the heart<sup>146</sup>. PPAR $\gamma$ , besides its well-known role as a major regulator of lipogenesis<sup>65, 147</sup>, contributes also in fatty acid oxidation in cardiac<sup>66</sup> and skeletal muscle<sup>148</sup>. PPAR $\alpha$ -mediated fatty acid oxidation in the heart and other tissues relies on the activation of peroxisomal and mitochondrial enzymes such as, acyl-CoA oxidase (AOX) and carnitine palmitoyl-transferase I (CptI). A major  $\gamma$ -activator of PPAR $\alpha$ -mediated fatty acid oxidation, at the transcriptional level, is the PPAR $\gamma$ -coactivator-1 (PGC-1)<sup>149</sup>. Heart failure<sup>150</sup>, as well as myocardial infarction<sup>151</sup>, hypoxia<sup>152, 153</sup>, inflammatory markers such as IL-1 $\beta$ <sup>154</sup>, IL-6<sup>154</sup>, NF- $\kappa$ B<sup>155</sup>, and reactive oxygen species<sup>155</sup> downregulate PPAR $\alpha$  expression. PPAR $\alpha$  gene expression levels and subsequent fatty acid oxidation are upregulated by estrogen related receptor (ERR) $\alpha$ , which acts in conjunction with PGC-1 $\alpha$  and binds directly to the PPAR $\alpha$  promoter<sup>156</sup>. In addition, ERR $\alpha$  gene expression is induced by PGC-1 $\alpha$ <sup>156, 157</sup> at the transcriptional level indicating a positive feedback loop in the coordination of PGC-1 $\alpha$  and ERR $\alpha$  towards an increase of PPAR $\alpha$  gene expression.

Fatty acid oxidation can also be triggered by nuclear receptors other than PPAR $\alpha$ , such as PPAR $\gamma$  and PPAR $\delta$ . Mice with constitutive PPAR $\gamma$  expression specifically in the hearts of



PPAR $\alpha$ <sup>-/-</sup> mice ( $\alpha$ MHC-PPAR $\gamma$ /PPAR $\alpha$ <sup>-/-</sup>) have elevated fatty acid oxidation levels, as well as improved cardiac function and survival as compared to the cardiolipotoxic  $\alpha$ MHC-PPAR $\gamma$  mice although lipids still accumulate in the heart<sup>66</sup>. These observations indicated that overexpression of PPAR $\gamma$  can substitute for PPAR $\alpha$  suppression or even deletion. PPAR $\gamma$  agonists have also increased fatty acid oxidation in type 2 diabetic human muscle cells<sup>148</sup>. Co-activation of PPAR $\delta$  and PGC-1 $\alpha$  can induce hepatic fatty acid oxidation. Thus, one method to overcome a marked reduction in PPAR $\alpha$ , which occurs in heart failure, might be via overexpression of other members of the PPAR gene family.

Both PPAR $\alpha$  and PGC-1 $\alpha$  gene expression levels are increased by AMPK<sup>158–160</sup>, which has itself been correlated with elevated cardiac transporter-mediated fatty acid uptake<sup>82</sup> and oxidation<sup>161</sup> levels. Mice that express a dominant negative form of AMPK cannot increase mitochondrial biogenesis in response to energy starvation<sup>162</sup>. Similarly, mice that express an inactive AMPK show impaired fasting-induced expression of lipid oxidative genes<sup>163</sup>. On the other hand mice carrying a constitutively active AMPK have elevated expression of fatty acid oxidation genes<sup>163–165</sup>.

## CONCLUSIONS

Despite its preference for lipids, the heart is also vulnerable to the pathological effects of lipid overload. Surprisingly, pathways required for acquisition of cardiac lipids are relatively poorly understood. FA uptake requires CD36 and LpL acting either in series or in parallel. The heart, like other tissues, can obtain more lipid than can be oxidized or stored in a non-toxic manner. It is unknown which specific lipid or array of different lipids activate processes that cause cardiac dysfunction. Pharmacologic and dietary studies that include genetic manipulation have implicated ceramide, DAG, and FA/fatty acyl CoAs in apoptosis, defective insulin signaling, and mitochondrial dysfunction. *In vivo*, one process or a combination of dysfunctional processes might be needed for clinical pathology.

As studies proceed in this area, coincident investigations in other organs need to be observed in order to determine whether the process that leads to lipotoxic cardiomyopathy is common or heart-specific.

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## References

Papers of particular interest, published recently, have been highlighted as:

• Of importance

•• Of major importance

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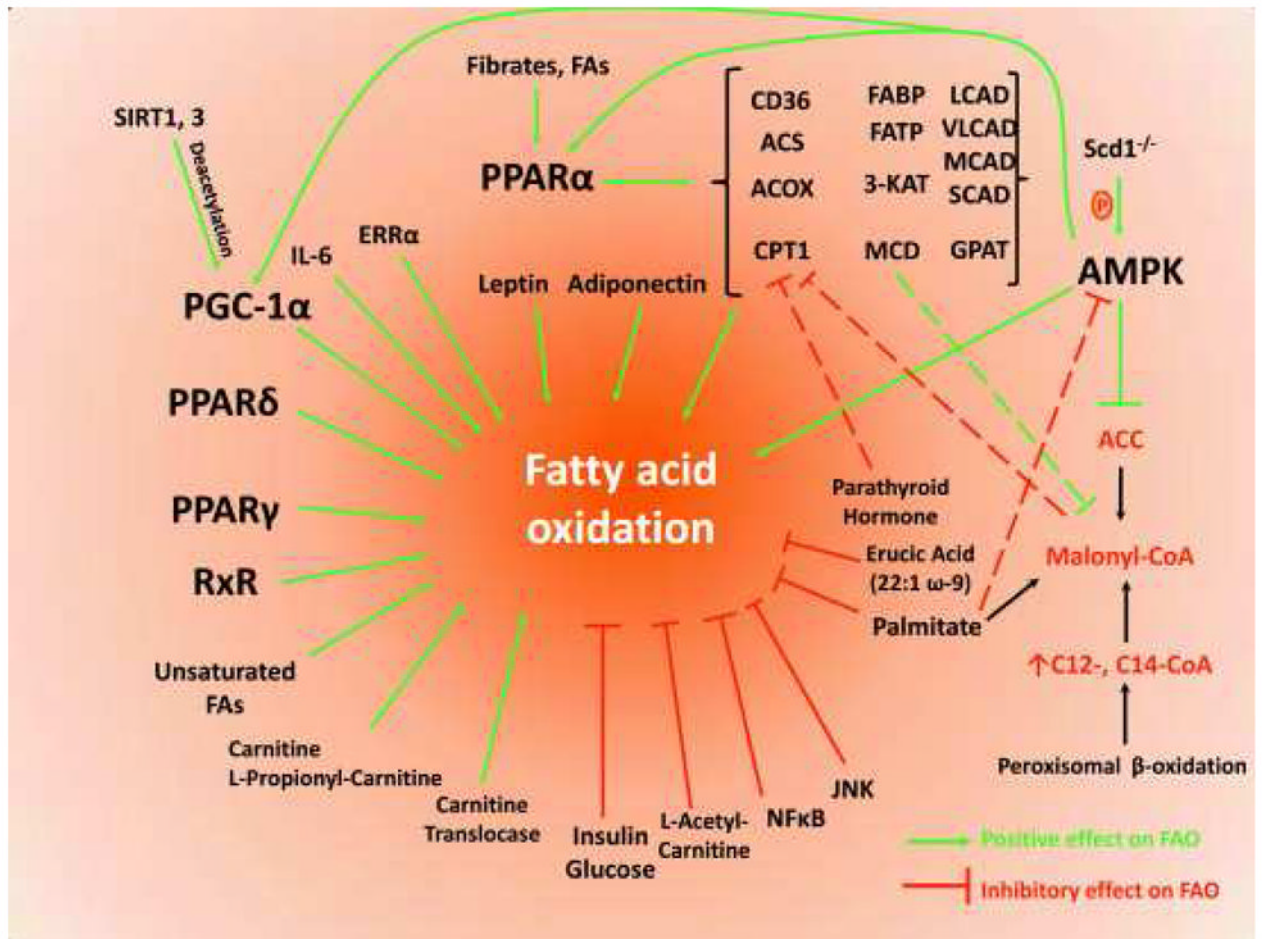
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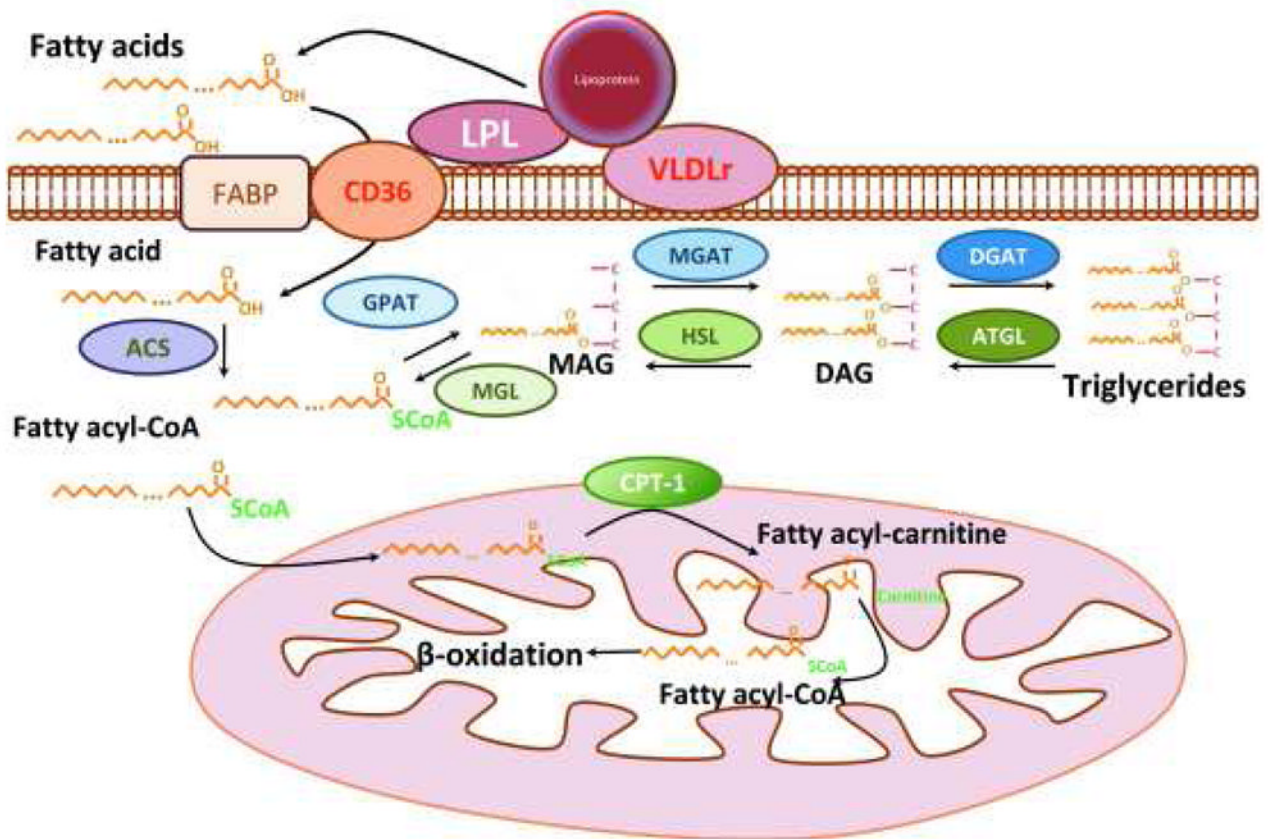
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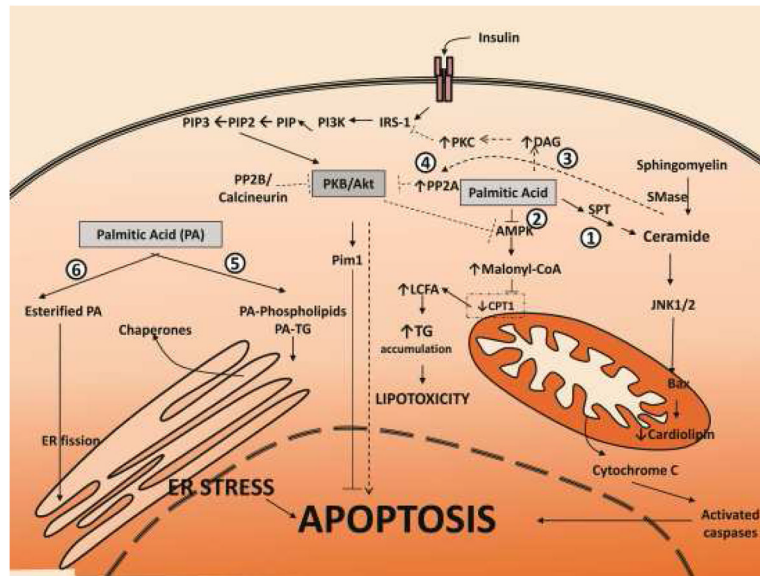


**Figure 1.** Proteins and transcriptional factors that modulate fatty acid oxidation



**Figure 2. Lipid delivery to the cardiomyocyte**

Fatty acids derived from triglyceride-rich lipoproteins, chylomicrons, and VLDL are hydrolyzed by lipoprotein lipase. Lipoprotein-derived fatty acids or albumin-bound free fatty acids are internalized by the cells via membrane receptors such as CD36 or other transporters, or via non-receptor diffusion through the membrane, known as “flip-flop”. Internalization of whole lipoproteins or lipoprotein remnants via lipoprotein receptors is also possible. Upon release into cardiomyocytes fatty acids are converted to fatty acyl-CoAs and can then gradually be incorporated to a glycerol backbone forming mono-, di- and tri-acylglycerols (triglycerides). Fatty acyl-CoAs can be released via triglyceride lipolysis that is mediated by ATGL, HSL and MGL and with the contribution of Cpt-1 they enter mitochondria for  $\beta$ -oxidation and production of ATP.



**Figure 3. Metabolic pathways triggered by palmitic acid leading to apoptosis and cardiomyopathy**

Several mechanisms may account for the toxic effects of saturated fatty acids: **1.** Palmitic Acid is used by Serine Palmitoyl Transferase to generate cytoplasmic ceramide, which activates JNK1/2 that interacts with Bax in the mitochondrial membrane and results in the release of cytochrome C due to reduction of cardiolipin (ceramide-dependent mechanism). **2.** Palmitic acid can inhibit AMPK, which increases malonyl-CoA levels, inhibits CPT-1, and causes accumulation of fatty acids and lipotoxicity. **3.** Palmitic acid contributes to the increase of DAG that induces the upregulation of PKC. PKC inhibits the insulin-signaling pathway by blocking IRS-1. **4.** The insulin-signaling pathway can be inhibited by ceramide-mediated increased PP2A, which dephosphorylates (inactivates) the anti-apoptotic PKB/Akt; inhibition of PKB/Akt may induce apoptosis. Dephosphorylated Akt reduces AMPK activity that can lead to lipotoxicity (see above). **5.** Palmitic acid may be incorporated into phospholipid and TG species in microsomal membranes, resulting in compromised ER membrane integrity and redistribution of protein-folding chaperones to the cytosol (ER stress). **6.** Esterification of palmitate can also cause ER fission directly. Intensive ER stress may result in apoptosis.