



REVIEW ARTICLE

Fatty old hearts: role of cardiac lipotoxicity in age-related cardiomyopathy

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Age-related cardiomyopathy accounts for a significant part of heart failure cases. Imbalance of the energetic equilibrium of the heart along with mitochondrial dysfunction and impaired β -adrenergic receptor signaling contributes in the aggravation of cardiac function in the elderly. In this review article, studies that correlate cardiac aging with lipotoxicity are summarized. The involvement of inhibition of peroxisome proliferator-activated receptor- α , β -adrenergic receptor desensitization, and mitochondrial dysfunction as underlying mechanisms for the lipid-driven age-related cardiomyopathy are presented with the aim to indicate potential therapeutic targets for cardiac aging.

Keywords: *heart; aging; lipotoxicity; lipids*

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Age-related cardiac dysfunction is a major factor in heart failure. The elderly accounts for at least 80% of patients with ischemic heart disease, 75% of patients with congestive heart failure, and 70% of patients with atrial fibrillation (1). Heart failure with either lower or preserved ejection fraction is common for hospitalized patients with cardiac abnormalities. Cardiac aging, which is evident in both humans and mice, plays an important role for both types of heart failure (2). Mouse cardiac aging is characterized by a reduction in fractional shortening, diastolic dysfunction, left ventricular hypertrophy, increased left ventricular end-diastolic pressure, fibrosis, cardiomyocyte hypertrophy, and increased apoptosis. Several components of cardiac function, including energetic homeostasis, adrenergic signaling, and mitochondrial dysfunction, can be compromised during aging (3). Balanced cardiac lipid metabolism is critical for normal function of the heart. Any deviation toward either increased or reduced fatty acid metabolism may be detrimental for cardiac function, primarily depending on the type of pathophysiological challenge. Aging-related cardiomyopathy has been associated with downregulation of peroxisome proliferator-activated receptor (PPAR)- α (4), which is a central regulator of cardiac fatty acid metabolism (5) and cardiac lipid accumulation (6,7).

Thus, impairment of fatty acid metabolism may at least partially account for the aggravation of cardiac function that occurs with aging.

Aging-related cardiomyopathy

The cardiovascular system's functions are altered with aging, and a significant portion of the observed changes affects myocardial biology. Decreased elasticity and increased stiffness of the arterial system increases afterload on the left ventricle, as well as systolic blood pressure and leads to left ventricular hypertrophy. Although systolic function is preserved in the older humans, diastolic dysfunction and lower exercise capacity have been reported (2). Diastolic dysfunction accounts for the lower exercise capacity and increased mortality (8). Mice with cardiac aging demonstrate increased left ventricular mass with slightly lower systolic function, diastolic dysfunction, reduced exercise capacity, and worse myocardial performance (9,10). Aged mouse hearts obtained from either 129/SvJ-C57BL/6 or C57BL/6J mice have increased fibrosis, hypertrophic cardiomyocytes, increased apoptosis, and amyloid deposition (3,11). C57BL/6J mice develop contractile dysfunction approximately at 18 months of age (12). This is associated with increased cardiac fibrosis, apoptosis, and inflammation-related gene expression (12).

Cardiac fibrosis is generally associated with increased Transforming growth factor (TGF)- β levels, which is also thought to be a critical mediator of age-related cardiac fibrosis (13). In addition, other proteins such as matrix metalloproteinases (MMPs), osteopontin, and periostin have a significant contribution in cardiac fibrosis (14). Induction of inflammatory markers has also been associated with aging (15), although it seems that there is a significant mouse-strain-dependent variation on the extent of inflammation (16). Similarly, the existence of apoptotic cardiomyocytes seems to vary with studies showing either increased (17) or unchanged (12) apoptosis during cardiac aging. Also, oxidative stress has been associated with cardiac aging and particularly with aging-related cardiac hypertrophy (18). Finally, reduced responsiveness to adrenergic stimulus and increased plasma catecholamine levels have been reported with aging (3). Thus, cardiac aging is accompanied by dysregulation of various pathways that have been attributed causative role in several types of cardiomyopathy.

Therefore, the aged myocardium is more susceptible to hemodynamic and ischemic stress compared to young myocardium (19,20). The vulnerability of aged hearts to stress has been correlated with increased reactive oxygen species (ROS) levels (21) and higher susceptibility to mitochondrial permeability transition pore (mPTP) opening (22). Nevertheless, mPTP opening may eventually cause mitochondrial swelling, ATP depletion, apoptosis, and

cell death (23,24). Thus, cardiac aging is associated with myocardial dysfunction and increased sensitivity to cardiac stress.

Cardiac fatty acid metabolism and lipotoxicity

The heart normally consumes a large amount of ATP in order to pump more than 7,000 liters of blood on a daily basis (25). For the production of ATP that is needed for this massive amount of work, the heart oxidizes fatty acids, glucose, lactate, ketone bodies, and amino acids as energy-providing substrates. Fatty acid oxidation (FAO) is a major component of the energy production process as it accounts for the generation of approximately 70% of cardiac ATP (25,26).

FA utilization in healthy hearts is a complex process that includes several steps: FA uptake, conversion of free FA to FA-CoA, storage of FAs in triglycerides (TG), TG lipolysis, transfer of fatty acids into the mitochondria, β -oxidation, and oxidative phosphorylation for ATP production (Fig. 1). The flawless transfer of fatty acids from cellular uptake to mitochondrial oxidation prevents accumulation of excess lipids. A study using positron emission tomography in humans showed that aging decreases myocardial FA utilization and FAO without any difference in myocardial glucose utilization (27). Several types of cardiac dysfunction, such as ischemia, obesity, diabetes, sepsis, and heart failure, are associated with impaired FAO,

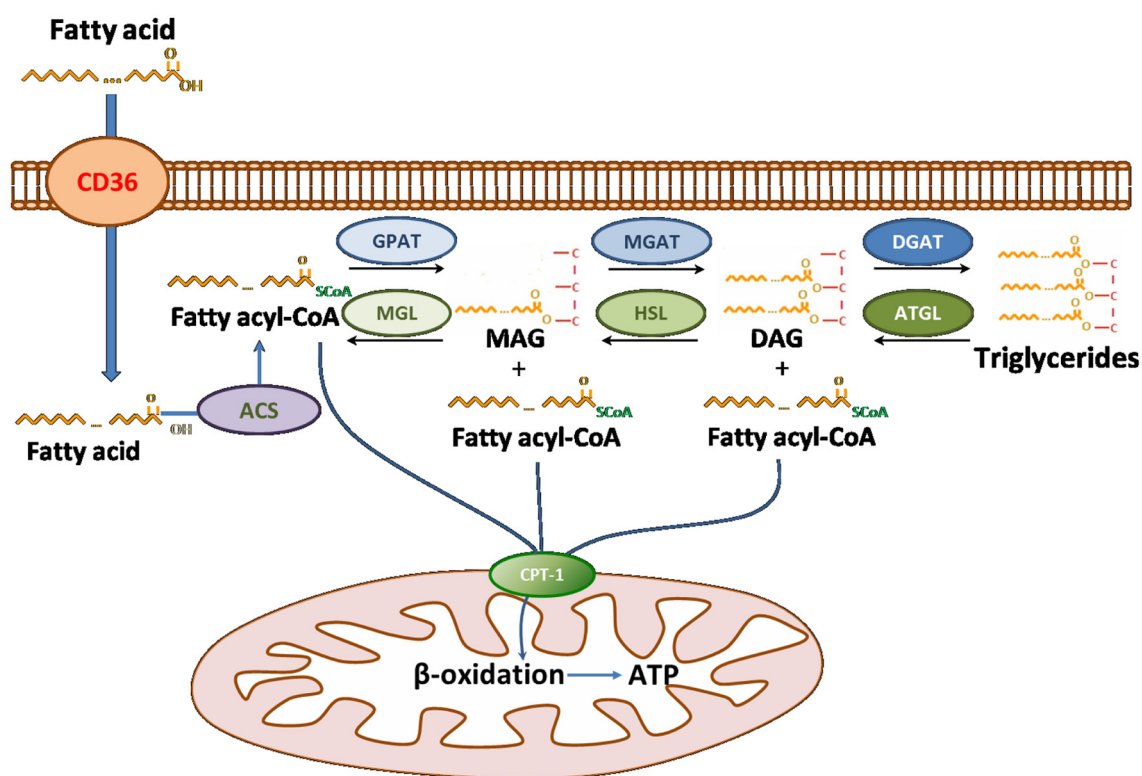


Fig. 1. Summary of cardiomyocyte fatty acid metabolism in healthy hearts.

which frequently leads to lipid accumulation characterized as cardiac lipotoxicity (28,29). Although cardiac lipotoxicity is accompanied by increased accumulation of neutral lipids, several studies have dissociated myocardial TG accumulation from lipid-driven cardiac dysfunction (30–33). In fact, cardiac lipotoxicity has been attributed to other lipids, such as saturated free fatty acids, ceramides, diacylglycerols (DAGs), and acylcarnitines (29,32,34,35), which change in parallel with cardiac TG.

Although cardiac toxic lipids have been associated with cardiac dysfunction (29), it has not been studied thoroughly whether they mediate aging-related cardiomyopathy, as well as what lipid-driven signaling mechanisms may be involved. Various studies have established a correlation between cardiac lipid accumulation and aging in humans and animal models (6,7). Aged hearts have increased expression of the cardiac fatty acid transporter, cluster of differentiation 36 (CD36) (36). Genetic ablation of CD36 prevented age-related cardiomyopathy, which indicates the involvement of increased cardiomyocyte lipid uptake in this process (36). Several lipids have been related to lipotoxic cardiomyopathy, such as palmitic acid, acylcarnitine, unesterified cholesterol, lysolecithin, ceramide, and DAGs. These lipids can trigger apoptosis, inflammation, and mitochondrial dysfunction (29). Interestingly, ob/ob mice, which are models of hyperphagia and type 2 diabetes, develop symptoms of cardiac aging much earlier, compared to wild-type mice fed with regular chow diet (37). This phenotype is associated with increased cardiac TG and DAG accumulation. Cardiac ceramide levels have been shown to be increased in a senescence-accelerated mouse model that develops cardiac hypertrophy (38). Thus, toxic lipids, such as DAGs and ceramides, are increased with cardiac aging.

Either ceramides or DAGs can activate Protein Kinase C (PKC) signaling (39) that has been associated with myocardial aging (40). PKCs constitute a lipid-sensitive Ser/Thr kinase family, which is involved in a broad range of G protein-coupled receptor (GPCR)-mediated responses and have been linked with several types of cardiomyopathy (24, 41–46). All PKC isoforms share a common structural motif consisting of a conserved kinase domain linked at the N-terminal domain, via a hinge region, with a more variable regulatory domain that in most cases contains lipid/phorbol ester binding and calcium-dependent phospholipid binding subdomains. PKCs are classified into three subcategories: conventional PKCs (cPKCs – PKC α , PKC β I, PKC β II, and PKC γ), novel PKCs (nPKCs – PKC δ , PKC ϵ , PKC θ , and PKC η), and atypical PKCs (aPKCs – PKC ζ and PKC ι/λ) (39). All PKC isoforms, except PKC θ and PKC ι/λ , have been detected in cardiomyocytes from humans or other animal models (41,44, 46–48). A number of studies have demonstrated that PKC activation relies on binding of DAG or ceramide and translocation to the membrane. Briefly, PKC movement to the membrane involves activation by Ca²⁺

binding to the C2 domain and DAG or ceramide binding to the C1 domain for membrane penetration. DAG binding results in formation of a hydrophobic cap facilitating membrane insertion and stabilizing the protein-membrane interface without causing a conformational change.

Role of PPAR α in aging-related cardiomyopathy

Several proteins of the energy production machinery that mediates processing of FAs and ATP production are regulated at the transcriptional level by PPAR α (49). PPAR α is activated by FAs that are released via lipolysis from the intracellular TG (50,51). Inhibition of PPAR α , as shown in PPAR α ^{-/-} mice, decreases myocardial fatty acid metabolism (52–54) and reduces cardiac ATP levels (53,55). Although some studies have reported normal cardiac function at baseline for the PPAR α ^{-/-} mice (54–56), others showed that these mice have reduced cardiac function at baseline associated with fibrosis (53,57) and oxidative stress (58,59). Antioxidant therapy alleviated left ventricular dysfunction, indicating that oxidative damage accounts for the cardiac dysfunction seen in mice with PPAR α ablation (59).

Cardiac abnormalities that occur in PPAR α ^{-/-} mice progress further during aging (53) and decrease longevity (60). Nevertheless, aging is associated with decreased cardiac PPAR α levels (4,61). Metabolomic analysis indicated an age-dependent decrease in cardiac glucose content, signs of decreased ketone supply, and altered FA synthesis (62). The importance of PPAR α inhibition in accelerating cardiac aging was demonstrated in 20-month-old rats that were treated with the lipid lowering drug atorvastatin, which increases PPAR α expression (63). The treatment with atorvastatin reduced cardiac hypertrophy, collagen deposition, oxidative stress, expression of inflammatory cytokines, and the aging marker β -galactosidase. The conclusion about the association of PPAR α inhibition and cardiac aging was further emphasized when pretreatment with PPAR inhibitors attenuated the effect of atorvastatin on the inhibition of inflammatory cytokines (63). This indicated that the beneficial effects of atorvastatin on cardiac aging may be attributed to either lipid lowering effect or the anti-inflammatory role of PPAR signaling (64,65).

Furthermore, cardiac aging that is observed in mice with cardiomyocyte-specific overexpression of human aldose reductase (*α MHC-hAR*) is associated with reduced cardiac expression of PPAR α gene targets, such as pyruvate dehydrogenase kinase 4 and acetyl-CoA oxidase (66), and increased PPAR γ signaling that promotes cardiac lipid accumulation characterized by increased TG, ceramides, and acyl-carnitine levels (67). Moreover, genetic ablation of *Ppar α* in *α MHC-hAR* mice resulted in earlier onset of cardiac dysfunction (66). Increased cardiac toxic lipid levels, such as ceramides, have also been reported in

senescence-accelerated mice that have lower expression of PPAR α (38). Another study also showed that PPAR α activation reduces inflammation in aged mice (61). Thus, although reduced cardiac PPAR α expression has been associated with aging-related cardiomyopathy, the underlying mechanisms that mediate the beneficial effect of PPAR α have not been fully elucidated.

β -Adrenergic signaling and cardiac aging

A component of cardiac aging pathophysiology is the impairment of β -AR signaling (3). Normally, stress increases the release of adrenal norepinephrine and epinephrine that target cardiac β -ARs, which belong to the GPCR family. Increased release of catecholamines by the sympathetic nervous system stimulates β -ARs and increases contractile force and heart rate. Activated β -ARs induce adenylyl cyclase activation and cAMP formation. β -ARs are subsequently phosphorylated and deactivated by kinases designated G protein-coupled receptor kinases (GRKs) (68). GRKs can be activated by PKCs (69). However, GRKs do not seem to be involved in cardiac aging in humans (70). PKCs can also deactivate β -ARs directly via a ligand-independent cascade (heterologous desensitization) (71). Desensitization of β -AR is followed by internalization of the receptor. This is a key step required either for restoration (72) or for its proteasomal degradation (73). Failing hearts demonstrate reduced cardiac β -AR-mediated responsiveness to catecholamines and abnormal myocardial β -AR signaling (74), which coincide with increased catecholamine production.

Age-related inhibition of β -AR responsiveness occurs in both animals and humans and is characterized by reduced β -AR density and internalization (75). Isolated left ventricular cardiomyocytes from hearts of animals at different age showed that the age-related contractility impairment during β -adrenergic stimulation was associated with reduced cAMP levels.

It has been shown (34,76) that excessive cardiac lipid accumulation is associated with dilated cardiomyopathy in several animal models of cardiac lipotoxicity, which is consistent with observations in humans. Cardiac lipotoxicity is accounted for by accumulation of toxic lipids, such as DAGs and ceramides, which activate PKC α and PKC δ and impair catecholamine-stimulated cardiac contractility and relaxation (34,35). Various studies have identified palmitic acid as the FA species that primarily induces formation of DAGs and ceramides, activates PKC signaling, and promotes β -AR desensitization and cardiac dysfunction (34,51,76,77). Interestingly, a metabolomics study showed that aged rat hearts have increased utilization of palmitic acid (78) indicating a potential role for this toxic FA in aging-related cardiac dysfunction. Thus, lipid-driven mechanisms that may involve PKC signaling may account for the impairment of β -AR signaling that occurs in aged hearts.

Mitochondria and cardiac aging

Impaired mitochondrial oxidative capacity is another component of cardiac lipotoxicity that seems to have a causative role in aging (6). Aging-related cardiac mitochondrial defects have been primarily attributed to interfibrillar rather than subsarcolemmal mitochondria (79,80), which have lower abundance in aged hearts (80). Increased mitochondrial ROS generation has been proposed to be a central event in cellular aging as it was described as a major determinant of lifespan several decades ago (81). Formation of ROS accompanies dysregulation of oxidative phosphorylation and mitochondrial dysfunction. Excess electrons from complex I and III can be transferred directly to O₂ to generate superoxide anion (O⁻), which is then converted to H₂O₂ that diffuses into cytosol and nucleus and activates redox signaling. H₂O₂ can be converted to a hydroxyl radical, which is the most reactive ROS species that targets mitochondrial DNA, lipids, and proteins and contributes in mitochondrial dysfunction and aging (82).

Changes in mitochondrial biology have a significant influence in cardiomyocyte function. Nevertheless, cardiomyocytes are rich in mitochondria that serve as ATP generators. Mitochondrial function deteriorates with aging due to electron transport chain defects (83). Particularly, reduced activity of complexes I and IV has been reported and leads to mitochondrial superoxide formation, mitochondrial ROS production, and protein damage (84,85). Aged hearts have increased frequency of mitochondrial DNA mutations, oxidative damage of mitochondrial proteins, and mitochondrial structural defects (86), which have been associated with aging (87). Accordingly, mice that overexpress catalase in the mitochondria, which attenuates ROS formation and protects mitochondria from protein damage and structural defects, have increased lifespan (21); and they are protected from cardiac dysfunction (86).

Cardiolipin is a critical lipid component of the inner mitochondrial protein, where the electron transport chain exists; thus, it has a central role in oxidative phosphorylation and cellular energy production (88). It remains controversial whether the content or composition of cardiolipin is altered in mitochondria of aged hearts as there are studies that show reduced content (89–91), as well as other studies that do not (92). While it is not clear whether aging itself alters cardiolipin it seems that when stress, such as ischemia, is applied to aged hearts, cardiolipin oxidation is augmented compared to non-aged ischemic hearts (93,94). As cardiolipin oxidation, which occurs during oxidative stress, may account for disruption of the electron transport chain (95) and mitochondrial damage (96), this mechanism may underlie cardiac lipotoxicity-driven mitochondrial dysfunction during aging.

Increased frequency of mitochondrial DNA defects, particularly deletions (97), has also been associated with

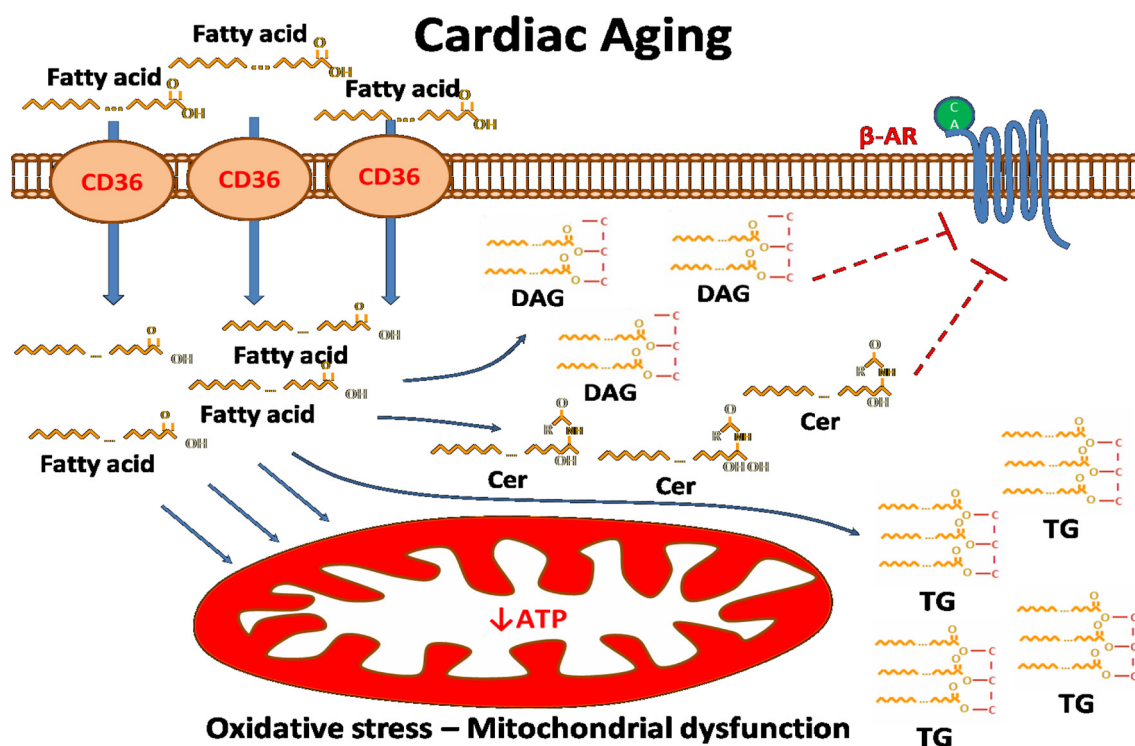


Fig. 2. Cardiac lipotoxicity contributes to cardiac aging by promoting formation of toxic lipids, ceramides (Cer), and diacylglycerols (DAGs) that compromise β -AR function and by causing mitochondrial dysfunction.

cardiac aging. This was demonstrated in mice with homozygous mutation of mitochondrial polymerase γ . These mice have increased mtDNA mutations and deletions, and they develop age-dependent cardiomyopathy and have shorter lifespan (98). The importance of mitochondrial DNA mutations for cardiac aging has also been demonstrated in humans as shown by age-associated accumulation of mtDNA deletions that have been reported in various tissues, including the heart (99,100). Thus, mitochondrial dysfunction that can be triggered by either oxidative stress or mutations of mitochondrial DNA is a contributing factor in cardiac aging.

Epilogue

In summary, cardiac FAO is important for lipid metabolism homeostasis and normal cardiac function. Inhibition of FAO leads to increased cardiac lipid content, which is often accompanied by increased levels of toxic lipids, such as Cer and DAGs. These lipids compromise cardiac function via β -AR desensitization, which is driven by activation of the PKC signaling pathway. Aging-related cardiomyopathy is associated with reduced cardiac levels of PPAR α , a master regulator of cardiac FAO, as well as with inhibition of β -AR signaling and mitochondrial dysfunction. These components of cardiac lipotoxicity that are also involved in cardiac aging indicate therapeutic targets that may alleviate age-related cardiomyopathy (Fig. 2).

Conflict of interest and funding

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