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COMPREHENSIVE INVITED REVIEW

Cardiac Aging: From Molecular Mechanisms to Significance in Human Health and Disease

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Abstract

Cardiovascular diseases (CVDs) are the major causes of death in the western world. The incidence of cardiovascular disease as well as the rate of cardiovascular mortality and morbidity increase exponentially in the elderly population, suggesting that age *per se* is a major risk factor of CVDs. The physiologic changes of human cardiac aging mainly include left ventricular hypertrophy, diastolic dysfunction, valvular degeneration, increased cardiac fibrosis, increased prevalence of atrial fibrillation, and decreased maximal exercise capacity. Many of these changes are closely recapitulated in animal models commonly used in an aging study, including rodents, flies, and monkeys. The application of genetically modified aged mice has provided direct evidence of several critical molecular mechanisms involved in cardiac aging, such as mitochondrial oxidative stress, insulin/insulin-like growth factor/PI3K pathway, adrenergic and renin angiotensin II signaling, and nutrient signaling pathways. This article also reviews the central role of mitochondrial oxidative stress in CVDs and the plausible mechanisms underlying the progression toward heart failure in the susceptible aging hearts. Finally, the understanding of the molecular mechanisms of cardiac aging may support the potential clinical application of several "anti-aging" strategies that treat CVDs and improve healthy cardiac aging.

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I. Introduction

Cardiovascular diseases (CVDs) are the leading cause of death, especially in the elderly population. Old age is a major risk factor for cardiovascular disease, which prolongs exposure to hypertension, diabetes, hypercholesterolemia, smoking, and other cardiovascular risk factors. In addition, intrinsic aging of the heart also makes the heart more susceptible to stress and contributes to increased cardiovascular mortality and morbidity in the elderly. Intrinsic cardiac aging is defined as the slowly progressive structural changes and functional declines with age, in the absence of major cardiovascular risks. Intrinsic cardiac aging is evident in rodents and flies, even though the risk factors common in humans are generally absent in these species, making these model organisms valuable for the study of the pathophysiology and genetics of intrinsic cardiac aging.

II. Aging and Epidemiology of CVDs

CVDs are highly prevalent in the western hemisphere. It was estimated that 82.6 million adults in the United States have one or more types of CVD (273). An estimated 75.4 million adults are living with high blood pressure. Coronary heart disease affects 16.3 million adults today, including 7.9 million presenting with acute coronary syndrome (myocardial infarction) and 9 million manifesting as stable angina pectoris. Heart failure accounts for 5.7 million cases per year in the United States (2006), and stroke accounts for 7 million cases (273). The 2010 update of American Heart Association's Heart Disease and Stroke Statistics, based on the data from National Center for Health Statistics (NCHS) and National Heart Lung and Blood Institute, showed that the prevalence of high blood pressure, stroke, coronary heart diseases, and heart failure increase dramatically with age in both men and women (Fig. 1A–D). Furthermore, the elderly (>60 years old) account for more than 80% of patients with coronary heart disease, more than 75% of patients with congestive heart failure, and more than 70% of patients with atrial fibrillation.

The 10th revision of the International Classification of Diseases (ICD-10) mortality data shows that 33.6% (~814,000 people) of all deaths in 2007 have CVD as the underlying cause of death (273). In the same year for both older men and women (65 years of age or older), CVD are the number one leading causes of death (273). According to the original and offspring cohort data from the Framingham Heart Study (1980 to 2003), the average incidence of first cardiovascular events in men and women increases progressively with age; from 3 per 1000 men at the age ranging from 35 to 44 years old, to 74 per 1000 men at the age ranging from 85 to 94 years old, and comparable proportions are observed in women 10 years later in life. According to the data from the NCHS, the CVDs mortality rate increases exponentially with age (exponential curve $R^2 = 0.9895$, Fig. 1E), and mean life expectancy would increase by almost 7 years if all forms of cardiovascular disease were abolished (273). These exponential increases in both the incidence of cardiovascular events and CVD mortality in the elderly population suggest that aging *per se* is a major risk factor for CVDs. With these stark statistics, further research into the effect of aging on the structure and function of the cardiovascular system is imperative.

III. Physiology of Cardiac Aging

A. Ventricular changes

According to Framingham Heart Study and Baltimore Longitudinal Study on Aging, based on the data from apparently healthy adults, there was an age-dependent increase in left ventricular wall thickness measured by echocardiography in both men and women (Fig. 2A), indicating increased prevalence of left ventricular hypertrophy (LVH) with age, even in the absence of clinical hypertension, which is the most common causative risk factor of LVH. Left ventricular (LV) filling in early diastole, measured as peak E wave by Doppler echocardiography, is gradually compromised with increasing age in both genders (Fig. 2B), with possible causes due to fibrosis and

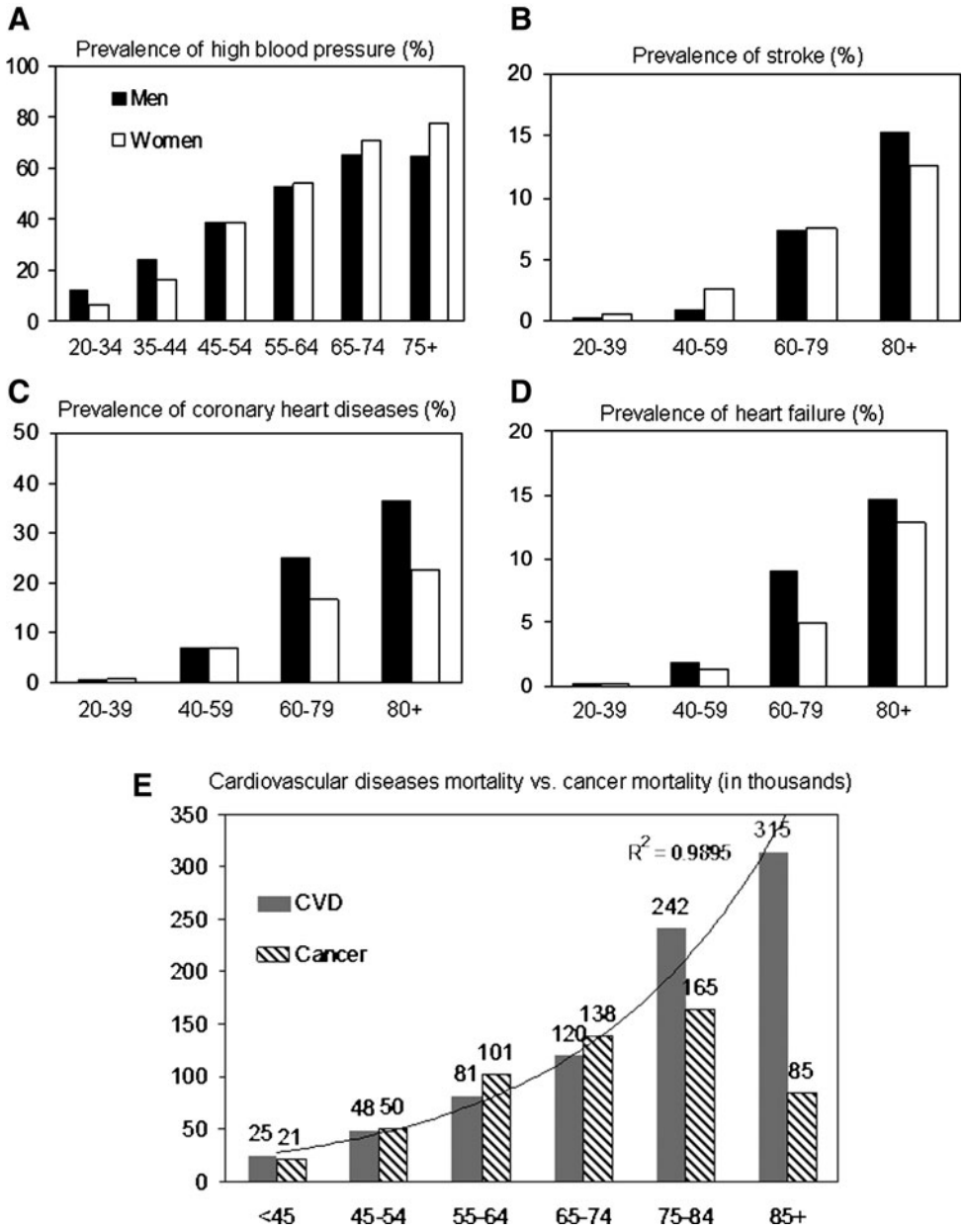

FIG. 1. The prevalence of various CVDS with age. The prevalence of high blood pressure **(A)**, stroke **(B)**, coronary heart diseases **(C)**, and heart failure **(D)** significantly increase with age in both men and women. There is an exponential increase in CVD mortality in the elderly population **(E)**. Data source: NCHS and NHLBI (273). CVD, cardiovascular disease; NCHS, National Center for Health Statistics; NHLBI, National Heart Lung and Blood Institute.

decreased elasticity of the ventricle as well as a delay in active ventricular relaxation. Reduced rates of calcium reuptake by myocardial sarcoplasmic reticulum calcium ATPase (SERCA2a) will delay ventricular relaxation, thus further exacerbating compromised LV filling in early diastole. To maintain LV filling, atrial contraction (A wave) is gradually increased in aging, which also increases atrial pressure and adversely contributes to atrial hypertrophy and an increased risk of atrial fibrillation. The prevalence of atrial fibrillation increases as well with aging (172–174). Doppler echocardiographic measurement of the E/A ratio, the ratio between early (E) and late (A) diastolic LV filling, declines dramatically with age (Fig. 2C) (70,71). This decline in the E/A ratio suggests that a greater portion of blood filling in the LV results from late diastolic filling as opposed to early diastolic filling, which is clinically interpreted as an indication of diastolic dysfunction. Diastolic dysfunction is highly prevalent in older adults (43), and it adversely affects exercise capacity in the geriatric population. It also predisposes to the development of diastolic heart failure, which is defined as heart failure with preserved ejection fraction (systolic function). Diastolic heart failure accounts for more than half of the heart failure cases in patients older than 75 years.

Aging also contributes to the decline of the maximal heart rate during exhaustive exercise, but does not appear to affect the resting heart rate when in the supine position (87). While systolic function determined from ejection fraction is relatively preserved at rest, exercise capacity significantly declines with age, as does cardiovascular reserve after prolonged exercise (63). This is attributed to a modest decrease in ejection fraction after maximal exercise (Fig. 2D) and a prominent decline in maximal heart rate at peak exercise (Fig. 2E). The cardiac index is another measure of systolic function calculated as the cardiac output normalized to the body surface area. Healthy adults exhibit a reduction in maximum

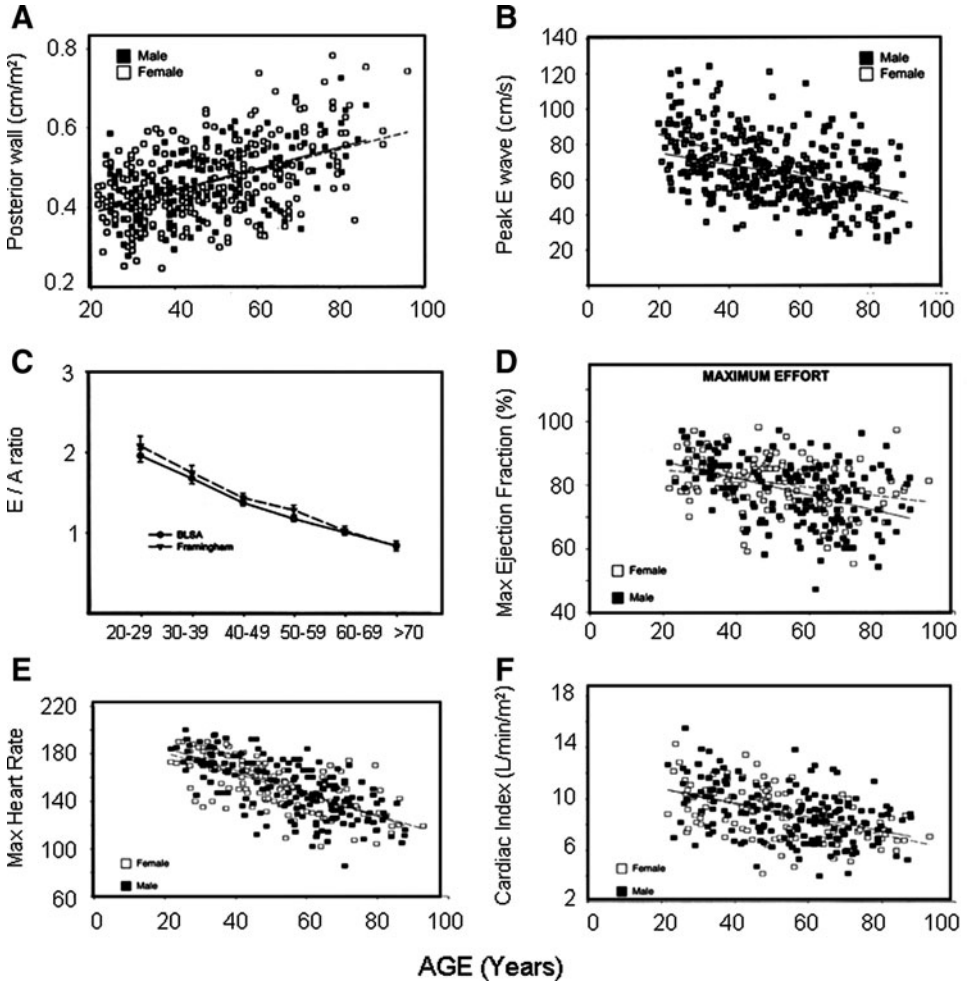

FIG. 2. Age-dependent changes in cardiac structure and function. (A). Posterior wall thickness (cm/m^2) by M-mode echocardiography significantly increases with age in both men and women, **(B)**. Peak E wave (early diastolic filling) declines with age. **(C)**. Doppler E/A ratio of mitral inflow, an indicator of diastolic function, decreased with age in apparently healthy participants in both the Baltimore Longitudinal Study on Aging and the Framingham Heart Study. Ejection fraction **(D)** and heart rate **(E)** after maximal exercise decrease with age in both genders. **(F)**. Cardiac index (Cardiac output normalized to body surface area) decreases with age. [Reproduced with permission from Lakatta and Levy (2003)].

cardiac index with age, which is mostly due to a decline in maximal heart rate (Fig. 2F).

B. Valvular changes

Age-dependent valvular changes mainly include myxomatous degeneration and collagen deposition called valvular sclerosis. Aortic valve sclerosis is present in 30%–80% of elderly individuals (145,227,306). It is most accurately detected by echocardiographic examination. Some of the echocardiographic features include calcification of aortic valve leaflets and aortic annulus that increase with age (94,246). While a systolic ejection murmur can sometimes be detected during physical examination, many patients with aortic valve sclerosis do not exhibit this murmur. Consequently, echocardiography is a more effective diagnostic tool for detection of this condition. Individuals with hypertension, LVH, hyperlipidemia, smoking, end-stage renal disease, and congenital bicuspid aortic valves are at an increased risk of having aortic valve sclerosis progression (242). In addition, elderly patients with aortic valve sclerosis have an increased occurrence of cardiovascular events and mortality (17,246), as aortic sclerosis often develops in parallel with progression of atherosclerosis in other vessels (245). Unlike stenosis, aortic valve sclerosis does not obstruct blood flow. However, it can advance into aortic stenosis when severe thickening, stiffening, and calcification of the leaflet result in obstruction of the aortic valve. The presence of this progression is an indicator of increased risks of CVDs. Increased leaflet calcification and decreased leaflet mobility may serve as early signs of this progression.

In the elderly, fibrosis and valvular calcification are the most common factors contributing to the development of aortic stenosis, which occurs when the aortic valve opening narrows due to the stiffening and calcification of the aortic valve leaflets (242). This narrowing prevents blood from being pumped effectively, creating a pressure gradient between the aorta and the left ventricle. To compensate for this hindrance, the walls of the left ventricle thicken with myocardial hypertrophy to maintain sufficient systolic function. Later in progression, the increased wall stress causes the left ventricle to dilate, leading to deterioration of systolic function. The prevalence of aortic valve stenosis increases in an age-dependent manner, with the estimated prevalence of 2% for severe stenosis, 5% for moderate stenosis, and 9% for severe stenosis in elderly patients. In addition, aortic regurgitation, also related to the calcification of the aortic cusps and annulus, also increases with age, and is present in 13%–16% of the elderly population (227). The presence of aortic regurgitation indicates that the aortic valve does not close properly; the flow of blood back to the left ventricle from the aorta during diastole results in the left ventricle working harder and increasing in size.

Mitral annular calcification (MAC), commonly found with aortic valve sclerosis given their overlapping pathological

appearance (85,139), is a degenerative process involving the fibrous annulus of the mitral valve, and is associated with aging. Frequently found during an echocardiographic examination, MAC results when calcium deposits accumulate along and beneath the mitral valve annulus (97). Individuals with hypertension, end-stage renal disease, aortic stenosis, and mitral valve prolapse are commonly found to have MAC. Individuals with MAC have increased risks of mitral stenosis and regurgitation, heart failure, atrial fibrillation, conduction system diseases, stroke, coronary and vascular diseases, mortality, and adverse cardiovascular events (145).

Mitral valve regurgitation is another abnormality common in the aging population. This condition occurs when the mitral valve fails to seal firmly, allowing the backward flow of blood into the heart and subsequently resulting in an insufficient delivery of blood to the rest of the body. Physical symptoms include shortness of breath and fatigue. Two of the leading causes of mitral valve regurgitation are myxomatous degeneration and ischemic heart disease (138). Chronic mitral valve regurgitation is one of the most common indications for valve surgery in the older population (7).

These ventricular and valvular changes in cardiac aging result in a severe compromise in the cardiac functional reserve capacity as well as lower the threshold for symptoms and signs of heart failure (63). This makes the aged heart more susceptible to stress and disease-related challenges, leading to increased prevalence of heart failure and cardiovascular mortality in the geriatric population.

IV. Animal Models of Cardiac Aging

A. Rodents

Cardiac aging in mouse models recapitulates the age-related changes found in human hearts (38,71). Using echocardiography to examine the age-related changes in cardiac structure and function in a mouse longevity cohort, we found a significant age-dependent increase in left ventricular mass index (LVMI, Fig. 3A) and left atrial dimension (71). Systolic function measured by fractional shortening showed only a modest decline with age (Fig. 3B). Diastolic function, measured by tissue Doppler imaging Ea/Aa, significantly declined with age (Fig. 3C), with a substantial increase in the proportion of diastolic dysfunction (defined by $Ea/Aa < 1$), up to 55% in mice older than 24 months.(71). Myocardial performance index (MPI), an indicator of global systolic and diastolic function, was significantly impaired with age (Fig. 3D). The oldest group of mice had a $\sim$75% increase in LVMI compared with that in the young adult group, indicating LVH in aged mice. The decline in systolic and diastolic function in the aged heart, including the increased LV end-diastolic pressure, resulted in the enlargement of the left atrium. Likewise, a greater fraction of the systole is spent on coping with the pressure changes during isovolemic phases, as reflected in the worsening of the MPI with age (71). All of the phenotypes just mentioned are also found in middle-aged mitochondrial mutator mice with "premature aging" ($Polg^{m/m}$, Fig. 3E–H, see section VI.A). Histopathologic changes in mouse hearts with age were increased interstitial and subendocardial fibrosis, along with hyaline cytoplasmic change, vacuolization of cytoplasm, variable and hypertrophic myocyte fiber size, collapse of sarcomeres, mineralization, and arteriolosclerosis (71). Morphometric analysis indicated an increased myocardial fiber size, increased fibrosis, and amyloid deposition with age, especially in the subendocardial areas (71).

Contributions to diastolic dysfunction with age include fibrosis, as just noted, and a reduced abundance of Sarco(endo)plasmic reticulum calcium ATP-ase (SERCA2), which affected the rate of diastolic calcium reuptake (71). Previous authors, who similarly noted increased LV end-diastolic dimensions and increased wall thickness in murine aging, also demonstrated changes in the extracellualr matrix, including increased matrix metalloproteinase (MMP) and decreased tissue inhibitor of metalloproteinase abundance (196). Thus, diastolic dysfunction in aging appears to be multifactorial in etiology. This is discussed in greater detail in Section VII.B, given next.

Several earlier studies of Fischer rat heart aging using pressure-volume catheter and more recent studies applying echocardiography consistently revealed age-dependent LVH, impairment of systolic and diastolic function, and increased prevalence of mitral regurgitation in rat hearts (13,36,91). Histopathology of aged rat hearts demonstrated cardiomyocytes hypertrophy and increased LV fibrosis (91), both of which reduce LV elastance and might cause diastolic dysfunction. Aging rat hearts also demonstrated decreased responsiveness to sympathetic and dobutamine stimulation (5). Interestingly, coronary blood flow and coronary reserve measured by radioactive microsphere also significantly declined with age, compatible with the findings that aging hearts are more susceptible to injury induced by pressure overload or ischemia (114).

B. Drosophila

In recent years, the adult Drosophila heart has been used to study cardiac function and aging, surmounting the limitations posed by the long lifespan of mammals. Using new techniques for analysis, including high-speed video imaging, many parameters related to cardiac functions in Drosophila were found to change with increasing age. Intact fruit flies under anesthesia in the first week after eclosion have an average heart rate of 3 Hz (345). Semi-intact and isolated hearts showed similar results (236,314). Aging is accompanied by declines in this heart rate in both genders when measured in anesthetized flies or isolated hearts. Aging in Drosophila also affects the rhythmicity of their heartbeat. Experts in fruit fly research have derived an arrhythmia index, and have found the index to increase approximately linearly with age (237). The level of disorganization of the myofibrillar arrays within the cardiomyocytes, as detected using antibody staining, also increases with aging (65,314,348). Evidence of misalignment appears by 5 weeks of age, which contributes to the impairment of age-dependent cardiac functionality (314). The power of the genetic study of Drosophila allowed Bodmer and colleagues to identify the mammalian target of rapamycin (mTOR) pathway as a critical step in the aging of the fly heart, described in greater detail in section VI C given next. The emergence of Drosophila as a genetic model for studying cardiac aging should bring about significant advancements, increasing the pace of discovery of genes regulating cardiac aging.

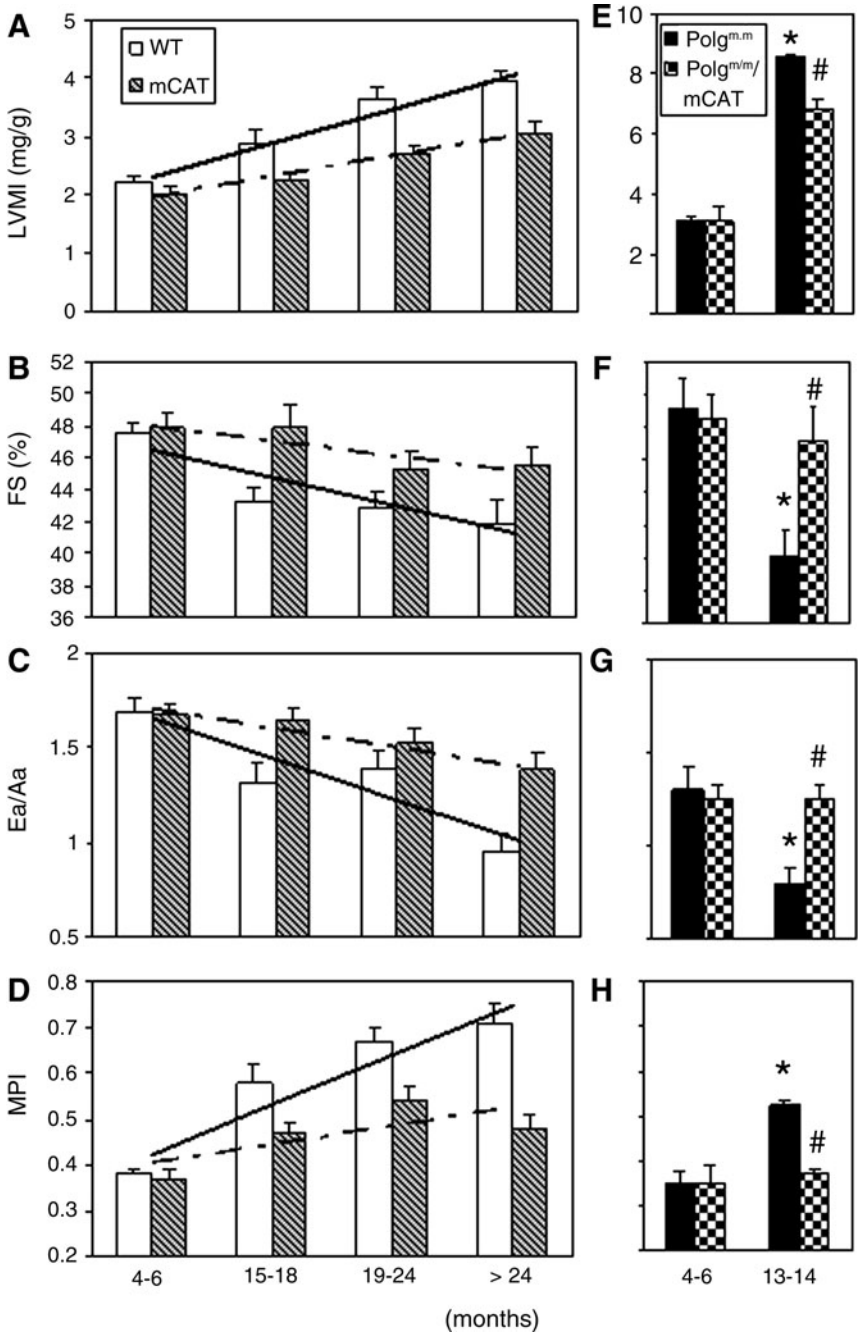

FIG. 3. Echocardiographic changes in aging and mitochondrial mutator mice. Echocardiographic analysis of WT and mCAT C57Bl/6 mice in a longevity cohort **(A–D)** and Polg$^{m/m}$ in the presence or absence of mCAT '**(E–H). (A, E)** Left ventricular mass index (LVMI = calculated LVM/body weight), **(B, F)** systolic function measured by percentage of fractional shortening (FS), **(C, G)** diastolic function measured by tissue Doppler imaging Ea/Aa, **(D, H)** the myocardial performance index (MPI) were analyzed. The linear trends across ages in WT mice were significant for all parameters ($p < 0.05$ for all, *left panels*). The beneficial effect of mCAT *versus* WT was analyzed by the interaction between genotype and the linear age trend, and was significant in all cases ($p < 0.01$ for all except fractional shortening, $p = 0.03$). $^{*}p < 0.05$ *versus* Polg$^{m/m}$ at 4–6 months old, $^{\#}p < 0.05$ *versus* Polg$^{m/m}$ at 13–14 months old (*right panels*). Data reanalyzed from Dai, *et al.* (67,71). LVMI, Left ventricular mass index; mCAT, catalase targeted to mitochondria.

C. Canines

Canines are among the most commonly used large animal models for cardiovascular research, as the bridge from rodent model to clinical research. The application of canine models include the study for cardiac arrhythmia (118), ischemia-reperfusion (IR) injury, and genetic models of heart failure such as in Duchene cardiomyopathy (156).

Canine hearts developed several aging changes: myocardial hypertrophy, with accumulation of lipofuscin and amyloid, which increased myocardial stiffness. Degenerative valvular heart diseases are common in dogs older than 16 years, the prevalence of which approached 75%. The most common is mitral valve myxomatous degeneration, followed by aortic valve calcification and stenosis. These myocardial and valvular aging changes increased cardiac failure in aged animals (112). The dog model has been widely used for the electrophysiological study, as the distribution of cardiac conduction system and activation sequence (electrophysiological properties) in dogs closely resembles that in the human heart (119). Aged dog hearts demonstrated prolonged action potential duration and decreased responsiveness to adrenergic stimulation as well as an increased risk of developing sick sinus syndrome and atrial fibrillation (14).

D. Nonhuman primates

Rodents are the most common model organisms used in mammalian aging research; however, models that are phylogenetically closer to humans, similar to nonhuman primates,

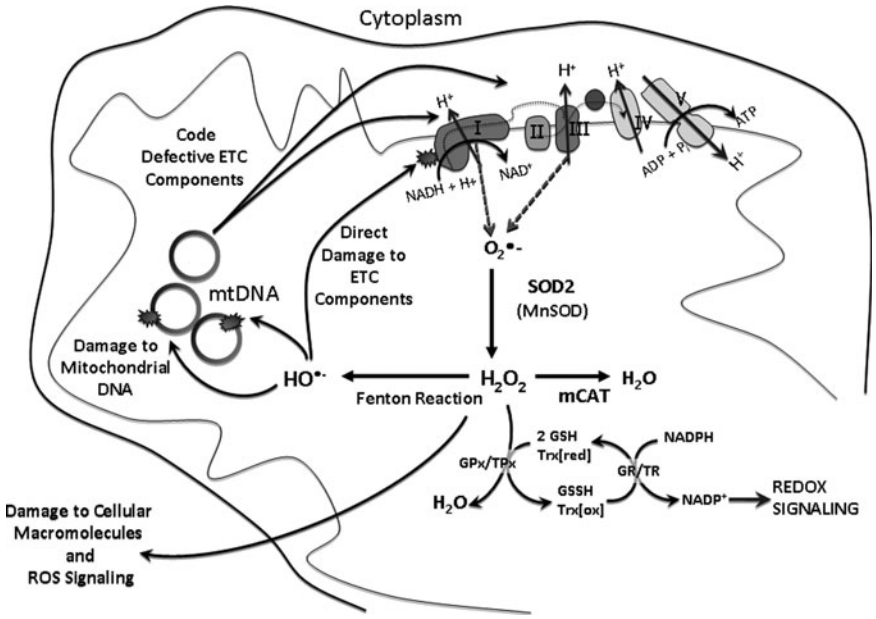

FIG. 4. Mitochondrial ROS theory of aging: ROS vicious cycle. Mitochondria are primary producers and targets of ROS. ROS production as a byproduct of escape of electrons from the electron transport chain (ETC) can directly damage ETC components or damage to mitochondrial DNA, which, in turn, results in altered levels or function of ETC components (complexes I-V are shown). In either case, the compromised ETC produces increased ROS, resulting in increased damage and a vicious cycle of mitochondrial ROS induced ROS. The end results are decreased ETC efficiency, damage to cellular macromolecules, and activation of cellular ROS and redox signaling. ROS, reactive oxygen species.

better recapitulate complex human physiology, pharmacokinetics, and electrophysiology. The National Institute of Aging's longitudinal study of aging in rhesus monkeys (Macaca mulatta) reported that monkeys fed with normal diets develop many age-related cardiac pathologies. These include aortic and mitral valves degenerative calcifications, loss or degeneration of myocardial fibers with hypertrophy of remaining cardiomyocytes, lipofuscin accumulation and variable degree of myocarditis, multifocal interstitial fibrosis, myocardial infarction, and congestive heart failure (175,176,201,279). Interestingly, monkeys did not develop spontaneous atherosclerotic plaques unless they were fed high fat diets. Research using the rhesus monkeys and other nonhuman primates will be valuable for study of the biology of human aging, as well as for allowing potential anti-aging interventions to be evaluated before actual clinical trials are performed in humans.

V. Mitochondria and the Free Radical Theory of Aging

A. ROS and aging

First formally proposed by Denham Harman in the 1950s, the free radical theory of aging suggests that aging is a degenerative process driven primarily by the accumulation of damage to cellular macromolecules as a result of their reaction with free radicals (121). Harman modified this theory in 1972 to specify mitochondria as both the primary producers and primary targets of harmful reactive oxygen species (ROS) (122). In his revised theory, Harman noted the failures of dietary antioxidants in extending the murine maximum lifespan and suggested that this observation could be explained by the inability of the exogenous antioxidants to effectively accumulate in the mitochondria. Harmon proposed that if researchers were able to target antioxidant therapies to the primary source and target of ROS, the mitochondria, then the result would be an increase in both the median and maximum lifespan of the organism treated. The mitochondrial ROS theory of aging is, in many ways, an attractive model. Implicating mitochondria as the primary source and target of ROS suggests a mechanism for the progressive nature of aging (ROS-induced damage of mitochondrial macromolecules leads to the development of dysfunctional mitochondria and a concomitant increase in ROS production), and provides a suggestive link between metabolic rate and the rate of aging (Fig. 4). Supportive evidence of the central role of mitochondrial ROS in aging was provided by the mice overexpressing catalase targeted to mitochondria (mCAT), which showed an 18% prolongation of lifespan, while mice overexpressing wild-type peroxisomal catalase (pCAT) did not (289).

Harman's theory, while a useful model for many years, has proved to be a greatly oversimplified view of the role of ROS and mitochondria in the biology of aging. Contrary to Harman's original theory, cellular organisms are now known to efficiently sense and neutralize ROS under normal conditions. Cellular antioxidant systems include the superoxide dismutases (SOD), catalase, and the glutathione peroxidases (GPxs), among others, and are both redundant and complementary in many cases. The complexity of antioxidant systems is surprising. Genetic manipulation of antioxidant system components has resulted in a plethora of seemingly inconsistent data. In the nematode *Caenorhabditis elegans*, for example, single or double SOD mutants have a normal lifespan, except in the case of the mitochondrial SOD2 mutants (single or double with cytoplasmic SOD1) where lifespan is actually *increased* (329). Fly researchers have been able to extend the lifespan of *Drosophila melanogaster* by overexpressing exogenous antioxidant proteins under specific conditions (213,250), but this is complicated by the observations that mitochondrial- targeted antioxidants Euk-8, Euk-134, and MitoQ (198), as well as ectopic expression of the hydrogen peroxide neutralizing enzyme catalase (212), fail to extend fly lifespan under normal conditions. This confusion is not limited to the invertebrate models; a series of lifespan studies using transgenic mice demonstrate that overexpression of several endogenous antioxidants do not prolong mouse lifespan, including CuZnSOD (cytoplasmic SOD1), MnSOD (mitochondrial SOD2), catalase, or a combination of CuZnSOD/catalase and CuZnSOD/MnSOD (132,137,253).

Furthermore, not only does antioxidant enzyme overexpression fail to extend murine lifespan, but also deletion of the same components (SOD's, GPx, *etc*), even in combination, typically does not decrease lifespan in the absence of additional perturbation (132,137,253,354).

B. Pleiotropy of ROS

During recent years, observations in multiple model systems have highlighted numerous biological roles for ROS in signaling and stress response [reviewed in (116,117,199)]. A decade-long perspective of ROS as a primary proximal cause of aging and disease is challenged by the realization that ROS are crucial mediators of normal cellular responses. Mitochondrial ROS are now known to be required for a proper response to hypoxia in the heart (44,49,113) through activation of hypoxia inducible factor 1 (HIF-1), are necessary for ischemic preconditioning (45,81), and play a fundamental role in the phenomena of hormesis (270), an idea which is validated by recent work in *Caenorabditis elegans* demonstrating that some conditions which increase mitochondrial ROS production also significantly increase lifespan *via* mitochondrial ROS-mediated activation of HIF-1 (179). Given these results, it is becoming increasingly clear that antioxidant therapies are unlikely to provide significant benefits to lifespan or healthspan without further characterization of the role of ROS in signaling and disease and refinement of drugs that specifically target the detrimental reactive species and/or specific compartment of cells (*e.g.*, mitochondria). This conclusion is supported by a large 2007 meta-analysis that examined the effects of nonspecific antioxidant therapies (beta carotene, vitamins A, C, and E, and selenium) on mortality over a total of 232,606 participants and found all but vitamin C and selenium to slightly but significantly *increase* mortality (34) (See Section VIIIB).

C. Mitochondrial hormesis in aging

Interestingly, recent evidence suggests that oxidative stress might promote longevity and metabolic health. Dietary restriction (DR), especially glucose restriction, has been shown to preferentially induce mitochondrial metabolism to extend lifespan in various model organisms, including *Saccharomyces cerevisae* (193), *Drosophila melanogaster* (359), and *C. elegans* (290), and increased mitochondrial respiration is expected to increase the production of ROS. For instance, glucose restriction in *C. elegans* extends lifespan by inducing mitochondrial respiration and increasing oxidative stress, and this adenosine monophosphate-activated protein kinase (AMPK)-dependent lifespan extension is abolished by pretreatment of antioxidant N-acetyl cysteine (NAC), indicating that oxidative stress is crucial in the signaling to induce stress resistance (290). This concept of mitochondrial hormesis (mitohormesis) hypothesizes that a low dose of oxidative stress, induced by caloric restriction, exercise (271), or other stimuli, may trigger an adaptive response that improves overall stress resistance (such as increased endogenous antioxidant defense) which may cause a long-term reduction of oxidative stress (270) and the extension of lifespan.

D. Mitochondrial turnover in aging

A recent understanding of the involvement of mitochondria and mitochondrial ROS in the aging of postreplicative tissues such as cardiac tissue has highlighted the role of mitochondrial turnover, mediated by fission, fusion, and mitophagy. This has in the past been termed the mitochondrial-lysosomal axis theory of aging [see reviews (42,108,318–322)]. According to this theory, proper regulation of mitochondrial turnover is vital to the maintenance of a functional pool of mitochondria, with removal of damaged mitochondria occurring primarily through fusion, fission, autophagy, and lysosomal degradation. When mitochondrial turnover is perturbed by changes in the rates of mitochondrial fission or fusion or alterations in autophagy/mitophagy, the result is an accumulation of damaged and dysfunctional mitochondria (Fig. 5). Dysfunctional mitochondria produce high levels of ROS, have reduced membrane potential and impaired ATP production capacity, and likely participate in aberrant signaling (218,300). This accumulation of damaged and dysfunctional mitochondria produces cells that have compromised respiratory capacity, increased oxidative stress, and aberrant mitochondrial signaling. Tissues become affected by senescence or apoptosis when a critical level of dysfunctional mitochondria have accumulated, or when the tissue is challenged with an external stress and is unable to properly respond because of the cellular or subcellular dysfunction that has accrued.

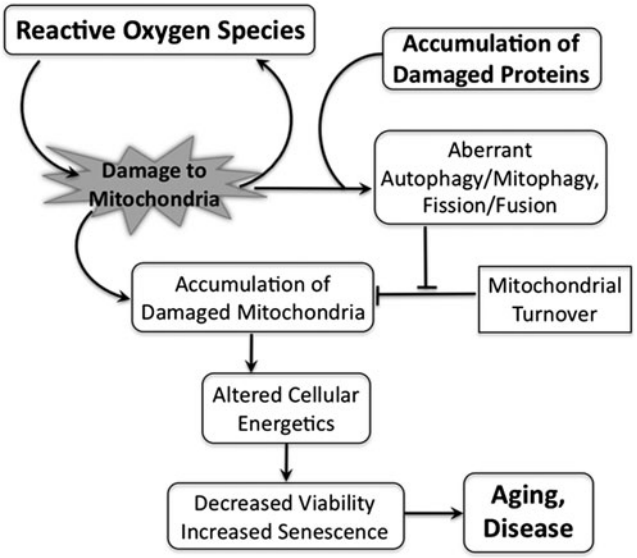

FIG. 5. Mitochondrial-lysosomal axis theory of aging. Damaged mitochondria accumulate when normal autophagy/mitophagy or mitochondrial fission/fusion are disrupted. According to the mitochondrial-lysosomal axis theory of aging, this accumulation of damaged mitochondria is a key step in the progression of aging and mitochondria-associated disease.

A large body of experimental evidence supports this theory of aging in metabolically active, postreplicative tissues. Damaged, high-ROS producing mitochondria have been demonstrated to accumulate in aging cardiac tissue (218,300)(Fig. 6), and are thought to play a major role in pathologic cardiac hypertrophy by accumulating in the cell and forcing a compensatory increase in the total number of mitochondria in order to offset the decreased energy output of the damaged mitochondria (42). Inhibition of autophagy by 3-methyladenine, an inhibitor of autophagosome formation, results in an

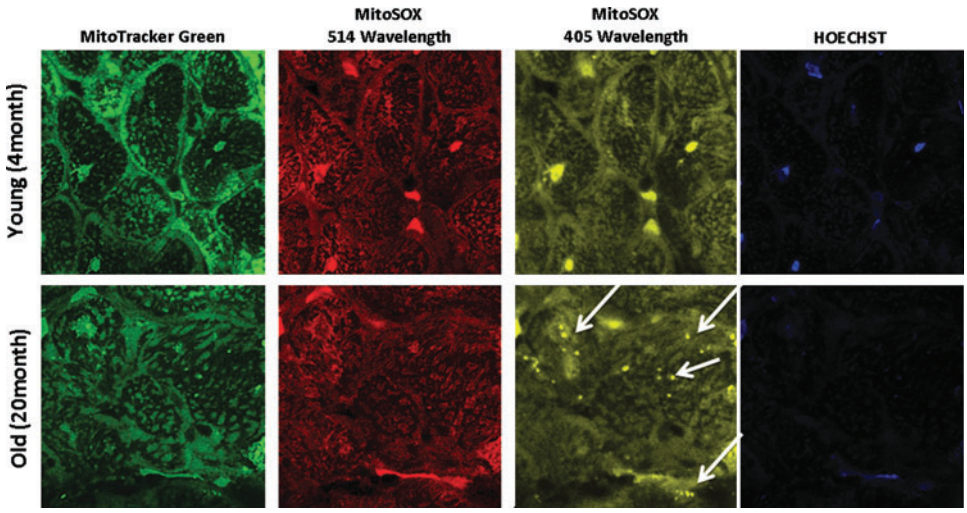

FIG. 6. Increased production of ROS in mitochondria of old mouse cardiac tissue. *Ex-vivo* live staining of cardiac tissue from 4 month (top row) and 20 month (bottom row) old male mice. MitoSOX has unique excitation/emission spectra for superoxide specific products (405 wavelength, shown in *yellow*) and nonspecific products of oxidation (514, shown in *red*). *White arrows* point to focal high-superoxide producing mitochondria. (To see this illustration in color the reader is referred to the web version of this article at www.liebertonline.com/ars).

accumulation of dysfunctional and oversized mitochondria (30,126,281). Furthermore, the two best-documented interventions in mammalian aging, DR and inhibition of mTOR by treatment with the drug rapamycin, have both been demonstrated to increase autophagy and prevent or delay mitochondrial dysfunction (although the mechanistic link between these two effects has not been fully explored) (130,204,244,263). A further discussion of the role of autophagy in DR and pharmaceutical interventions of aging is provided in subsequent sections.

The mitochondrial turnover/lysosomal axis theory of aging is also an attractive model of cardiac aging, because it is able to account for a number of observations that other models of aging cannot. For example, ROS production among mitochondria becomes highly heterogeneous in aged tissue (see above). The model of mitochondrial damage accumulation in aging cannot directly explain this heterogeneity. In the damage accumulation model, ROS production is uniform within the cell, and damage accumulation is stochastic. This model would predict that all mitochondria should be damaged in a relatively uniform (Poisson-distributed) pattern of damage accumulation among the mitochondria of a given cell. In contrast, the mitochondrial-lysosomal axis theory of aging is defined by the production of a heterogeneous population of mitochondria, with distinctly dysfunctional outliers. Furthermore, the mitochondrial-lysosomal axis theory of aging would predict that while ROS are important contributors to the aging process, increased ROS production is secondary to mitochondrial dysfunction. This prediction allows ROS to be separable from the process of aging under the proper conditions. As discussed, this separation is well supported by experimental results in systems where knockout of antioxidant system components fails to decrease lifespan or overexpression of antioxidant proteins fails to increase lifespan.

Mitochondrial homoplasmy is another phenomenon that is difficult to reconcile with the damage accumulation models of aging. Homoplasmy occurs when a mutant or variant form of mitochondrial DNA becomes the predominant form existing within a cell (338). Heteroplasmic cells that contain both mutant and wildtype mtDNA could accumulate 60%–90% pathogenic mtDNA molecules without showing any defect in respiratory activity (56,277). Damage accumulation models would lead us to expect aging cells to exhibit vast heterogeneity in mitochondrial DNA mutations, as mutations are stochastic and the model does not provide for a clear mechanism by which any single variant could become dominant. Under the mitochondrial-lysosomal theory of aging, mitochondrial homoplasmy is explained by the clonal expansion of variant mitochondria as a result of a mutation that allows the abnormal organelles to escape normal autophagosomal degradation or enables the dysfunctional mitochondria to expand through mitochondrial fission at an uncontrolled rate (42). Given the success of the mitochondrial-turnover/lysosomal theory in describing the biology of cardiac aging and the growing body of evidence detailing the role of autophagic dysfunction in aging, it seems clear that aberrant autophagy will prove to be a major component of the aging process in postreplicative tissues such as cardiac tissue.

Another important factor related to mitochondrial damage accumulation, mitochondrial repair, and turnover in aging is the phenomenon of mitochondrial dynamics—a dynamic network within which the mitochondria regularly exchange proteins, mtDNA, and lipids by rapid fusion and fission processes (47). Mitochondrial fusion allows two mitochondria to mix their contents, and this physiological process is crucial to control mitochondrial morphology and function. The mechanism of mitochondrial fusion requires three large GTPase: mitofusins 1 and 2 (MFN1 and MFN2) and optic atrophy 1 (OPA1) [see review (50)]. A recent study using mice with skeletal muscle specific deletion of the MFN 1 and MFN 2 demonstrates that mitochondrial fusion is required for mtDNA stability as well as tolerance to mtDNA mutations (51). Due to the mixing of mtDNA from mitochondria with different levels of heteroplasmy (different proportions of mutated mtDNA), mitochondrial fusion has been proposed to mediate the functional complementation of pathogenic mtDNA genes (222,223). On the other hand, mitochondrial fission is shown to separate dysfunctional mitochondria with a reduced membrane potential and a decreased level of mitochondrial fusion proteins, which are subsequently targeted for mitophagy (326,48). Although the mechanisms and the exact role of mitochondrial fusion and fission in aging remains unclear, due to the importance of mitochondrial dynamics in mitochondrial homeostasis and DNA stability, it has been postulated that mitochondrial dynamics play a crucial role in aging (161).

VI. Molecular Mechanisms of Cardiac Aging

A. Mitochondrial oxidative stress in cardiac aging

Several studies have documented that an increase in mitochondrial dysfunction with age is correlated with abnormal mtROS production and detoxification [reviewed in (199,323,324)]. An age-dependent reduction in mitochondrial oxidative phosphorylation function is related to the decline in mitochondrial state 3 respiration (maximal stimulated respiration) due to diminished activity of electron transport complexes I and IV; some parts of the complexes include mitochondrial DNA-encoded subunits (Fig. 4), while complexes II, III, and V are relatively unaffected [see review (228)]. Impaired electron transport function is likely to be directly related to elevated electron leakage and generation of mtROS.

As an organ with a high metabolic demand and rich in mitochondria, the heart is particularly vulnerable to mitochondrial oxidative damage. Deficiency of mitochondrial energetics has been documented in human and experimental animals with heart failure (333), the mechanisms of which may include mitochondrial biogenesis that does not keep up with the increasing demand [see review (105)], mitochondrial uncoupling and decreased substrate availability (217), and increased mitochondrial DNA deletions (69).

As shown in Figure 6, damaged mitochondria from aged mouse hearts produce more ROS than healthy mitochondria in young hearts. Consistent with this, studies from our laboratory displayed an age-dependent increase in mitochondrial protein carbonylation in mice (Fig. 7A), which is indicative of increased oxidative damage to mitochondrial proteins (67,71). Furthermore, we found that aged hearts had a 3–4-fold increase in mitochondrial DNA point mutation and deletion frequencies. This mitochondrial damage stimulates signaling for mitochondrial biogenesis, manifest in the aged heart by an increase in mtDNA copy number concomitant with significant upregulation of the master regulator peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α) and its downstream transcription factors (71).

The most direct evidence for the critical role of mitochondrial ROS in cardiac aging was shown by our experiments using mice overexpressing mCAT. We demonstrated that mCAT mice were significantly protected from the age-dependent increase in LVMI (Fig. 3A, $p < 0.01$ for WT *vs.* mCAT), a decline in diastolic function measured by Ea/Aa (Fig. 3C, $p < 0.01$) and an increased prevalence of diastolic dysfunction, as well as a decline in systolic function (FS%, Fig. 3B, $p = 0.03$) and impairment of myocardial performance (Fig. 3D, $p < 0.01$) (71). Consistent with this, we found that mCAT attenuates age-dependent mitochondrial oxidative damage, as displayed by significant reductions of mitochondrial protein carbonyls and mtDNA deletion frequencies (Fig. 7A, B).

Another line of evidence for the critical role of mitochondria in aging is demonstrated by mice with proofreading-deficient homozygous mutation of mitochondrial polymerase gamma ($Polg^{D257A/D257A}$ designated as $Polg^{m/m}$), which induces a substantial increase in mtDNA point mutations and deletions (166,325). The accumulation of mitochondrial DNA mutations has been shown to increase apoptosis (166). These mice were shown to have a shortened lifespan and "accelerated aging-like" phenotypes, such as kyphosis, graying and loss of hair, anemia, osteoporosis, and age-dependent cardiomyopathy (67,325), which include marked LV hypertophy (Fig. 3E), systolic and diastolic dysfunction (Fig. 3F, G, respectively), and impairment of myocardial performance (increased MPI, Fig. 3H). The observations that mitochondrial damage and cardiomyopathy in these mice can be partially rescued by mCAT (Fig. 3E–H) suggest that mitochondrial ROS and mitochondrial DNA damage are part of a vicious cycle of ROS-induced ROS release (Figs. 4, 7A, B) (67). Furthermore, it has been shown that accumulation of mtDNA deletions is better correlated with the "premature" aging-like phenotype in these mice than are mtDNA point mutations (335).

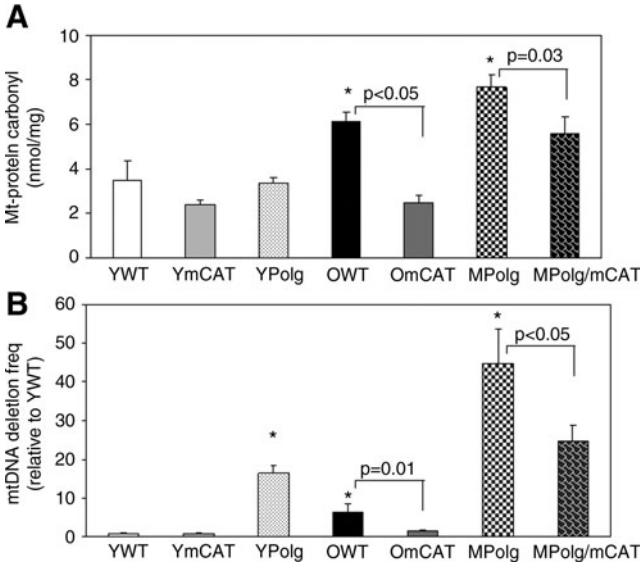

FIG. 7. Mitochondrial oxidative damage in cardiac aging. (A). Mitochondrial protein carbonyl (nmol/mg) significantly increased in old WT (OWT, > 24 months) and even more in middle-aged Polg (MPolg, 13.5 months) mouse hearts when compared with young WT mouse hearts. mCAT significantly reduced these age-dependent mitochondrial protein carbonylation. **(B)**. Mitochondrial DNA deletion frequency significantly increased in old WT (> 24 months) and young Polg (4 months) when compared with young WT, and this is dramatically increased in middle-aged Polg (13.5 months). mCAT overexpression significantly reduced the deletion frequency for both. $^*p < 0.05$ compared with YWT.

A recent paper reports the striking result that endurance exercise in $Polg^{D257A/D257A}$ mutant mice can prevent both their skeletal muscle and cardiac progeroid phenotypes (283). The authors suggest that the augmented level of mitochondrial biogenesis seen with exercise in these mice is a critical factor in maintaining mitochondrial and organ function. This is a highly relevant observation, as age-associated accumulation of mtDNA deletions have been documented in various tissues in man, including the heart (62,353).

The success of targeted, specific ROS scavenging intervention by mCAT suggests that a key to successful intervention lies in specificity. Given the complexity of the systems involved, it seems possible that mitochondrial dysfunction and aberrant ROS production may contribute to aging through interference with normal signaling and energetics as much, or more than by the direct damaging effect to cellular

macromolecules. Age-dependent decline in the rate of mitochondrial electron transfer also favors mitochondrial superoxide production, leading to a positive feedback between complex I inhibition and mitochondrial ROS production, as well as the more classical viscous cycle of mitochondrial DNA mutation and protein damage amplifying ROS. When viewed in the light of alterations in both signaling and energetics, this may be a critical factor in cardiac (and other organ system) aging.

Finally, there are potential shared mechanisms between intrinsic cardiac aging and cardiac hypertrophy and failure. These mechanisms are discussed in Section VII. A. Oxidative stress and mitochondria in CVDs are discussed next.

B. Neurohormonal regulation of cardiac aging

1. Renin-angiotensin system in cardiac aging. As the key neurohormonal system that regulates hypertension and stress induced cardiac hypertrophy, the renin-angiotensin aldosterone system (RAAS) has been linked to cardiovascular disease and age-related declines in cardiac function. We and others have found that intracardiac Ang II concentrations significantly increased with age, and many structural, functional, and molecular changes found in aged hearts are consistent with the effects of Ang II (71,111). Direct evidence for the role of Ang II in cardiac aging came in 2007, when Basso, *et al* reported that inhibition of Ang II signaling by either angiotensin converting enzyme inhibitor enalapril or angiotensin receptor type I inhibitor losartan extended the lifespan of normal male Wistar rats and slowed the onset of age-related cardiovascular pathologies (22). These drugs have also been shown to reduce myocardial fibrosis and fibrosis-related arrhythmias in aged mice (304). This was confirmed by a study which demonstrated that mice with disruption of angiotensin receptor type I prolong mouse survival (29). The RAAS appears to be involved not only in disease but also in tissue aging in multiple angiotensin responsive tissues, and angiotensin blockade has also proved to be beneficial in the aging kidney (133).

Mechanistically, angiotensin has been shown to induce an increase in total cellular and mitochondrial ROS, and the increased ROS appears to be a clear proximal cause of cardiac disease in this case (69,125). A part of the pathologic increase in ROS is likely mediated by an ROS-induced ROS feedback loop of signaling and damage, in which cardiac mitochondria play a key role by virtue of the mitochondrial localization of the NOX4 isozyme of NADPH oxidase (NOX) (See section VII.A.). Support for this model is provided by the success of mitochondria targeted antioxidants that attenuate cardiac hypertrophy and failure (see below).

2. B-adrenergic signaling. The detrimental effects of β-adrenergic signaling on cardiovascular health are well understood. These effects are mediated by the demand for augmented cardiac output that results from sympathetic signaling for increased heart rate and blood pressure. Clinical trials of beta-blockers (β-adrenergic receptor inhibitors) have demonstrated a clear benefit for survival in patients with heart failure [reviewed in (95)]. β-adrenergic signaling has been implicated in aging because of the observation that adenylyl cyclase type 5 (AC5- an enzyme involved in β-adrenergic downstream signaling) disruption leads to mice that are protected against age-dependent cardiac hypertrophy, systolic dysfunction, apoptosis, and fibrosis and which have an increased lifespan (240,241,351).

There is mounting evidence that important effects of β-adrenergic signaling are mediated by mitochondrial ROS. Chronic β-adrenergic stimulation induces mitochondrial membrane depolarization and apoptosis in adult rat cardiomyocytes that is inhibited by Mn-SOD/catalase mimetics and by overexpression of catalase (267). Furthermore, it has recently been shown that acute β-adrenergic stimulation induces a cAMP and PKA-dependent increase in mitochondrial ROS in ventricular mouse cardiomyocytes *in vitro*. That this increase in ROS plays a significant role in the β-adrenergic inotropic effect was indicated by observations that the β-adrenergic induced increase in $Ca2+$ transient amplitude was diminished in the presence of the antioxidant NAC as well as the mitochondria-targeted antioxidant SS31 (11).

Although beta-adrenergic antagonists are well tolerated and commonly used in the treatment of heart disease (82), their effects on lifespan are yet to be determined. It will be also important to determine safe and effective ways to combine such inhibitors with those of other signaling pathways, and potentially, with inhibitors of mitochondrial ROS, in order to obtain the maximal positive effects.

3. Insulin/insulin-like growth factor 1/PI3K signaling. Insulin-like growth factor signaling is one of the best-characterized determinants of longevity in model systems. Nematodes deficient in the insulin-like receptor DAF-2 have greatly increased median and maximum lifespans (15,77). Insulin-like signaling also regulates lifespan in flies (317) and mammals, the latter demonstrated by the increased lifespans of the Ames and Snell dwarf mice [deficiency in growth hormone and prolactin, reviewed in (189)] as well as Klotho mice with overexpression of the insulin/insulin-like growth factor signaling inhibitor (169). Drosophila and mouse models have also demonstrated the specific benefits of reduced insulin-like growth factor 1 (IGF1) signaling on cardiac aging (188,345). Unfortunately, the role of IGF1 signaling in human cardiac aging is complicated by the observations that IGF1 levels significantly decrease with age (61), and low serum IGF1 correlates with an *increased* risk of heart failure among elderly patients with no previous history of heart disease (332), and that growth hormone therapy, which increases signaling through IGF1 pathways, may actually be beneficial in some patients with heart failure (40). These correlations between lowered IGF1 and an increased risk of disease complicate our understanding of the role of IGF1 in aging and lifespan, and as a result, the role of IGF1 in human cardiac aging remains unclear. Careful studies involving well-controlled participant groups will be needed to address the discrepancies and determine the role of IGF1 signaling in human aging and disease. This is further discussed next in the context of nutrient signaling in cardiac aging.

Insulin receptor signaling activates the phosphoinositide-3 kinase (PI3K) signaling cascade, which phosphorylates and activates AKT. Activated Akt translocates into nucleus and inactivates the Forkhead box O (FOXO) by phosphorylation. The FOXO transcription factors have an anti-aging role, as supported by the evidence of increased lifespan in *C. elegans* with activated DAF-16 (the homolog of FOXO in worms) (152,192) and in Drosophila with constitutively active dFOXO

(the homolog of FOXO in flies) (103). In Drosophila, suppression of insulin signaling by creating insulin receptor mutation or overexpressing dFOXO prevents the decline in cardiac performance with age (345). In the heart, the FOXO family is thought to function in response to oxidative stress (157), regulation of metabolism (260), and apoptosis (303) [see review (276)]. The mechanism of FOXO in preventing cardiac aging involves activation of endogenous antioxidants and sirtuin-1 (SIRT1) (103), which has been shown to prevent aging phenotypes in several tissues (see section VI.C on nutrient signaling). Mice with cardiac-specific moderate overexpression of SIRT1 are protected against oxidative stress through *FOXO*-dependent upregulation of endogenous antioxidants, including catalase (8), SOD, and peroxiredoxin (53). Suppression of insulin/PI3K signaling activates transcription factor FOXO, which transcribes target genes including atrogin-1, a ubiquitin ligase that promotes calcineurin degradation and prevents NFAT activation (185,186), thereby attenuating cardiac aging, as the calcineurin-NFAT pathway activation is associated with LVH in cardiac aging (71).

4. Natriuretic peptides signaling. Although the role of natriuretic peptides in cardiac aging remains unclear, it has been shown that plasma levels of atrial natriuretic peptide (ANP) and brain natriuretic peptide (BNP) increase with age in populations without CVDs (180,266) as well as in rodent models (352). ANP and BNP bind to membrane-bound guanylase cyclase receptors to exert the physiological effect of maintaining hemodynamics through body fluid and electrolyte homeostasis. Most circulating ANP and BNP in healthy subjects are secreted by atrial cardiomyocytes at the basal levels. Stimulated by cardiac wall stretch, BNP is secreted by ventricular cardiomyocytes in the setting of volume or pressure overload and ischemia, as well as in response to various neuroendocrine or cytokine stimuli, with endothelin I and Ang II being the most powerful of these stimuli. In a small clinical study, human ANP (carperitide) has been shown to suppress thioredoxin (TRX), reduce oxidative stress, and improve the symptoms and hemodynamics of patients with heart failure (297). A recent study demonstrated that C-natriuretic peptide (CNP) has an antiproliferative effect on cardiac fibroblasts *in vitro*. The concentration of circulating CNP progressively declines with age in Fischer rats, and this is reciprocally associated with LV fibrosis and precedes the impairment of diastolic as well as systolic function (284).

C. Nutrient signaling in cardiac aging

DR is an extremely well-established intervention that has been clearly demonstrated as increasing lifespan and reducing the onset and severity of a variety of age-related pathologies. This intervention has been demonstrated in a wide array of model organisms, from yeast and nematodes to mice, rats, and, most recently, rhesus monkeys (59,64,92,146,203). DR has been shown to be a powerful preventative for cardiovascular disease in rodents and nonhuman primates (64,230,294). Similar protective effects have also been observed in human and animal trials of alternate-day fasting (330), demonstrating that even transient activation of the pathways involved in the DR response can be beneficial. These studies have shown that DR reduces risk factors associated with heart disease: resting heart rate and blood pressure are decreased, insulin sensitivity is enhanced, lipid profiles are improved, and inflammatory processes that likely contribute to atherosclerosis are reduced [reviewed by Mattson and Wan, 2005 (202)]. However, the evidence that DR improves intrinsic cardiac aging is less abundant. Taffett *et al.* found that DR of mice had a large positive effect on age-related impaired diastolic function (313). A recent study suggested that in humans undertaking DR for a mean of 6.5 years, there is lower blood pressure, lower systemic oxidative stress, and improved diastolic function (206). In another recent study, the Dahl salt-sensitive rat, which develops gradual, hypertension-associated diastolic dysfunction, was compared in DR *versus ad libitum* animals. Moderate DR markedly attenuated changes in heart weight, left ventricular mass, and wall thickness in these rats, and echocardiography demonstrated that DR reduced cardiac diastolic dysfunction in this model (291).

Recently, there has been growing interest in the role of the mTOR pathway in nutrient signaling, bolstered by data implicating mTOR activity in aging. Target of rapamycin (TOR) kinase is an evolutionarily conserved gatekeeper that integrates nutrient and hormonal cues to modulate growth and longevity (151). When nutrients are abundant, TOR activity is high, which favors faster growth and cell division; when nutrients become limiting, TOR activity is decreased, leading to reduced growth, enhanced resistance to stress, and increased life span. The nutrient-sensitive target of rapamycin complex 1 (TORC1) branch of the mTOR pathway is illustrated in Figure 8. The TORC2 branch (not shown) controls the organization of the actin cytoskeleton, for which there is much less evidence for implication in aging. TORC1 is a complex of mTOR and raptor (regulatory associated protein of mTOR), and activity of this complex is inhibited by rapamycin. Active TORC1 phosphorylates p70S6K, which accelerates ribosome biogenesis. TORC1 also phosphorylates 4E binding protein 1 (4EBP1), which results in its release from the inactive 4EBP1/eukaryotic initiation factor 4E (eIF4E) complex, allowing the mRNA cap binding protein eIF4E to associate with eIF4G and eIF4A to form the active eIF4F complex. This complex is required for cap-dependent translation initiation, the major translation initiation pathway in eukaryotes.

The relevance of the mTOR pathway to cardiac health is indicated by data showing that rapamycin is protective in models of cardiac hypertrophy and heart failure. Administration of rapamycin inhibits Ang II-induced increases in protein synthesis in cardiac myocytes (282) and markedly (67%) suppresses transverse aortic constriction (TAC) induced cardiac hypertrophy (295). Even more strikingly, rapamycin treatment can cause regression of established TAC pressure overload induced LVH: rapamycin improved left ventricular end-systolic dimensions, fractional shortening, ejection fraction, and regressing left ventricular fibrosis in mice with hypertrophy and heart failure (100,204). This is accompanied by suppression of activated phosphorylated ribosomal S6 protein and eIF4E due to pressure overload (100). Rapamycin also attenuates hypoxia-induced pulmonary vascular remodeling and right ventricular hypertrophy in mice (247).

Studies in Rolf Bodmer's laboratory using a Drosophila model of aging-related attenuation of cardiac function confirmed that inhibition of the mTOR pathway in flies could attenuate the aging-related decline (197). Subsequently, they

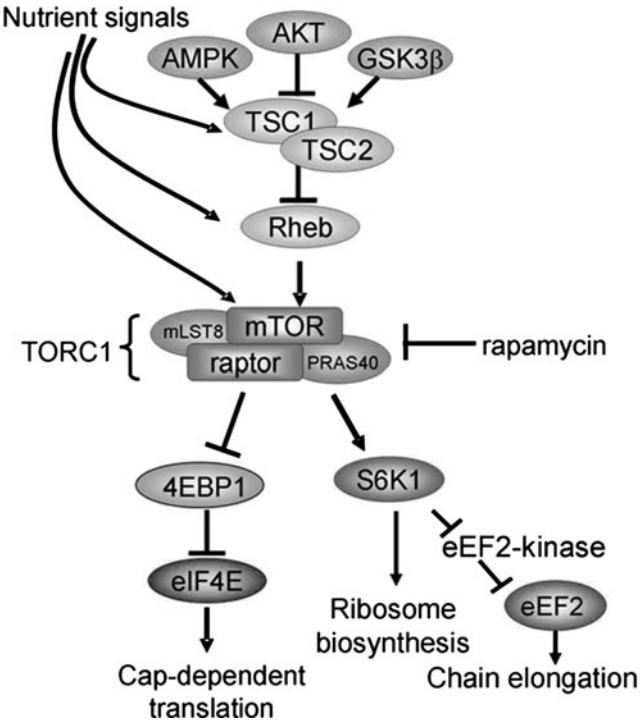

FIG. 8. mTOR pathway in aging. A simplified diagram of key nutrient signaling inputs to mTOR and key targets of TORC1 phosphorylation (4EBP1 and S6K1 (also known as p70S6K). mTOR, mammalian target of rapamycin; TORC, target of rapamycin complex.

determined that overexpression of 4EBP prevented the aging cardiac decline to the same extent as overexpression of the TOR antagonist tuberous sclerosis complex (TSC), and that, conversely, overexpression of eIF4E leads to a more rapid decline of myocardial function with age (344). They concluded that the level of 4EBP activity regulated cardiomyocyte growth in the fly heart by controlling the initiation of translation. Consistent with this model, work from George Thomas's laboratory using transgenic mouse models showed that deletion of both p70S6K11 and p70S6K12 had no impact on the development of cardiac hypertrophy after TAC, exercise-induced hypertrophy, or cardiac hypertrophy in IGF1 receptor or PI3K mutants (205). Thus, while activation of P70S6K1 was previously conjectured to play a role in cardiomyocyte hypertrophy, these data indicate that P70S6K signaling alone is not critical for the induction of cardiac hypertrophy.

While there are still many unanswered questions with regard to the mechanisms of DR, the robust effects of DR in preventing cardiovascular disease and cardiac aging are evident. However, the use of DR as a clinical intervention in humans is problematic; hence, the search for DR "mimetics" is a very active discipline with the potential to yield important clinical interventions for the treatment and prevention of heart disease and cardiac aging.

SIRTs are a conserved family of NAD+-dependent deacetylases (class III histone deacetylases). In *Saccharomyces cerevisiae, Caenorhabditis elegans,* and Drosophila, extra copies of the Sirt genes are associated with an extended lifespan (21,24,170). In yeast, *Sir2* has been previously reported to mediate the extension of replicative lifespan in response to DR (reduced glucose) (10,191), although later studies have shown controversial results (141,143). Mammals have 7 isoforms of SIRTs, SIRT1-7, of which SIRT1 has the closest amino acid sequence homology to yeast *SIR2*. Mice deficient in SIRT1 displayed several developmental defects and shortened lifespan compared with wild-type mice. Mice with an extra copy of Sirt1 have been created by transgenesis, and these mice demonstrated a metabolic phenotype similar to wild-type mice that are dietary restricted (37). Although lifespan extension in these mice has never been reported, it is possible that Sirt1 could mediate some of the effects of DR. In the cardiomyocyte mitochondrion, we have previously noted the potential importance of nicotinamide adenine dinucleotide metabolism and SIRTs. Sirt3 expression was reported to be reduced with age in sedentary individuals, but elevated by endurance training in both young and old persons (177). In transgenic mice, low to moderate overexpression of Sirt1 in hearts attenuated age-dependent increases in cardiac hypertrophy, apoptosis/fibrosis, cardiac dysfunction, and senescence markers; whereas a high level (12.5-fold) of Sirt1 increased apoptosis and hypertrophy and decreased cardiac function (8). It has recently been reported that SIRT3 deacetylates the regulatory component of the mPTP, cyclophilin D, preventing the mitochondrial permeability transition in cardiac tissue during aging, and that Sirt3 knockout mice show accelerated signs of aging in the heart, including cardiac hypertrophy and accelerated fibrosis (115). Perhaps most intriguing, increased expression of SIRT3 due to polymorphism in the Sirt3 promoter was found to be associated with an extended lifespan of man (27). Resveratrol was also shown to extend the lifespan of *S. cerevisiae*, *C.elegans,* and Drosophila through an Sirt1-dependent mechanism (131,349), but this effect was not reproducible in later studies (21,144). In mammals, resveratrol has been reported to ameliorate shortened lifespan and metabolic derangement in mice fed with a high fat diet, probably through activation of SIRT1 and PGC-1α, hence improving mitochondrial function (23,171).

The involvement of SIRTs in nutrient signaling and cardiac aging directly implicates histone deacetylation and epigenetic regulation of DNA expression in these processes. This is consistent with an increasing awareness of the potential role of epigenetic modification in aging (110) and cardiovascular disease (104). DNA methylation is the second of these two most-cited mechanisms of epigenetic modification. DNA hypomethylation has been associated with conditions related to cardiovascular risk (18), but while such observations have been made in smooth-muscle cells, atherosclerotic lesions, and peripheral blood leukocytes, they have not yet been documented directly in the heart. It is easy to predict, however, that this will be an area of vigorous study in the future.

D. Cardiac stem cell aging and telomeres

Although an attractive target for therapeutic interventions in cardiac aging, the existence and role of adult cardiac stem cells in cardiac physiology remains under debate. There is evidence suggesting the existence of multipotent populations of cells in the heart capable of differentiating into cardiomyocytes after isolation and culture (28,140); however, these cells are clearly incapable of preventing the progression of cardiovascular disease during cardiac aging and after acute ischemic events in humans. A high impact report by

Bergmann *et al.* in 2009 attempted to address the conflicting opinions in the adult cardiac stem cell field by addressing the question of whether human hearts experience cell turnover. Using data from environmental exposure to ^{14}C in human tissues (caused by increases in atmospheric ^{14}C after the atomic bomb testing of the mid-20th century) and mathematical modeling of the rate of DNA turnover, they estimated that cardiomyocyte turnover decreases from 1% per year at the age of 25 to 0.45% per year at the age of 75 in adult human hearts (31). Unfortunately, this approach has limitations, including numerous assumptions made during the modeling process, and the rate reported does not account for DNA repair mechanisms. Furthermore, there remains the inability of cardiac tissue to mount a significant regenerative response after acute ischemic injury or during the aging process in man.

Telomere length data and genetic models of telomere deficiency have provided some intriguing data concerning the role of cardiac stem cells in aging. Endomyocardial biopsies of hearts from old patients with dilated cardiomyopathy show decreased telomere length, increased levels of senescence markers, and increased cell death compared with age-matched control hearts (52). While the shortened telomeres could be a secondary result of an increase in cellular ROS, the increased expression of senescence markers indicates that senescence may play a role in heart disease and cardiac aging. Telomere shortening may be an initiating factor in the progression of cardiac progenitor senescence and a contributor to cardiac aging. Cardiac progenitor cells were described in rats after BrdU labeling of the adult rat myocardium, and the myocardial cells thst incorporated BrdU were found to be telomerase positive (106). Mouse genetic models of telomere deficiency support this observation, with telomere reverse transcriptase (280) or telomerase RNA (35) showing an increase in age-related cardiac hypertrophy, while mice overexpressing telomerase present a non-pathologic cardiac hypertrophy and a resistance to the acute ischemic insult of myocardial infarction (238). Additional evidence for the importance of cardiac stem cells in heart aging comes from a study in which the knockout of the pro-senescent gene p66shc (involved in senescence and apoptosis after oxidative stress and in response to telomere shortening) was shown to prevent age-related cardiac pathologies in a diabetic mouse model (278). Given these data, it seems reasonable that cardiac stem cells may someday play an important role in the treatment and prevention of cardiac dysfunction and cardiac aging. At the present time, however, a much larger research effort is underway to define a therapeutic role for other sources of induced pluripotent stems cells in cardiac regeneration (163).

VII. Aging, Oxidative Stress, and CVDs

A. Oxidative stress and mitochondria in CVDs

Increased oxidative stress is well known to play an important role in the pathogenesis of CVDs, such as hypertension, atherosclerosis, cardiac hypertrophy related to aging or pressure overload, cardiac IR injury, and cardiac failure. Several important sources of ROS have been reported in the cardiovascular system, including NOXs, mitochondria, xanthine oxidase, monoamine oxidase, and nitric oxide synthase. Free radicals generated by these sources are usually maintained at the physiological levels by several endogenous antioxidant systems, including SOD, catalase, GPxs, and glutathione reductase (GR). Other antioxidant systems involving thiol-disulphide oxidoreductase systems include the cytosolic proteins TRX and glutaredoxin. At physiological levels, ROS acts as a signaling mediator, but at pathological levels in response to noxious stimuli, an acute increase of ROS may activate autophagy (part of the cellular defense mechanism that prevents propagation of ROS through damaged mitochondria) or incite opening of the mitochondrial permeability transition pore (mPTP), which will cause cytochrome c release and activation of apoptosis.

1. The central role of mitochondrial oxidative stress and redox status in hypertension and heart failure. As discussed in section III, the prevalence of hypertension increases dramatically with age, with up to 70% of the population older than 70 years having hypertensive cardiovascular disease. Hypertension is the most common cause of LVH, which predisposes to chamber dilatation, heart failure, and sudden cardiac death (101). As a key molecule in the Renin-Angiotensin System, which plays a pivotal role in hypertension, Ang II is well known to cause LVH and fibrosis (190). Inhibition of the Renin-Angiotensin System by angiotensin converting enzyme inhibitor or angiotensin receptor blocker is a standard clinical practice that improves the survival of patients with congestive heart failure.

Mutations of genes encoding mitochondrial enzymes have been shown to be associated with various forms of idiopathic hypertrophic and dilated cardiomyopathies, as seen in various mitochondrial diseases (76). Furthermore, mitochondrial DNA deletions have been found in experimental models of heart failure (200). We have demonstrated that Ang II delivered for 4 weeks using an osmotic minipump increased cardiac mitochondrial protein carbonyl content and the prevalence of mitochondrial DNA deletions (Fig. 9A, B) (69), both of which indicate oxidative damage to mitochondria. Accumulation of this damage activated mitophagy, as evident by an increased number of autophagosomes having the characteristic double-membrane structure seen by electron microscopy (Fig. 9C arrows) as well as by a significant increase in the LC-3 II/I ratio (Fig. 9D). As a homeostic mechanism, ROS-induced mitochondrial damage and turnover can result in increased signaling for mitochondrial biogenesis through activation of PGC-1α and its target genes. This is consistent with the report that PGC-1α is transcriptionally upregulated by ROS (302).

The central role of mitochondrial ROS in Ang II-induced cardiomyopathy is shown by the observation that mice which overexpress mCAT, but not mice that overexpress wild-type pCAT, are resistant to cardiac hypertrophy, fibrosis, and diastolic dysfunction induced by Ang II (69). As shown in Figure 9, mCAT mice have attenuated AngII induction of protein carbonyls, mtDNA deletions, and autophagy (69). As shown in Figure 10, Ang II exposure for 4 weeks significantly increased normalized heart weights, which was attenuated by mCAT but not pCAT overexpression (Fig. 10A). Echocardiographic analysis demonstrated significant thickening of both interventricular septum and left ventricular posterior wall thickness, which was significantly attenuated by mCAT but not pCAT (Fig. 10B). Likewise, Ang II decreased Ea/Aa in WT mice, indicating diastolic dysfunction, which was again ameliorated by mCAT but not pCAT (Fig. 10B).

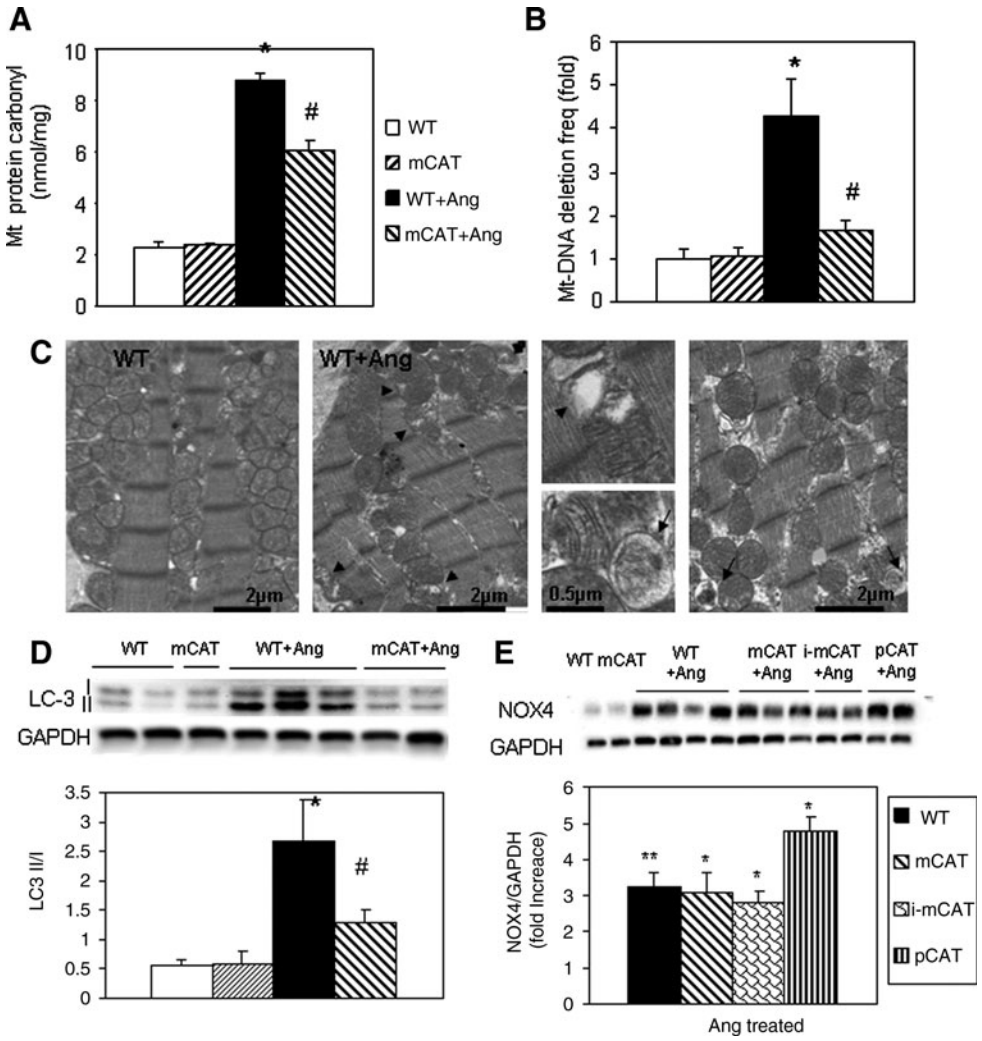

FIG. 9. Mitochondrial oxidative damage after Ang II. Ang II delivered for 4 weeks in an osmotic ominipump (1.1 mg/kg/d) significantly increased **(A)** mitochondrial protein carbonyl (nmol/mg) and **(B)** mitochondrial DNA deletion frequency in WT hearts, both of which are significantly ameliorated in mCAT hearts. **(C)**. Electron micrographs demonstrate damaged/vacuolated mitochondria (*arrowheads*) and autophagosomes (*arrow*) after Ang II in WT hearts. **(D)** Western blots of LC-3 showed that LC-3 II/I ratio, an indicator of autophagosomes, significantly increased in WT after Ang II, and this was attenuated by mCAT. **(E)** NADPH oxidase isoform 4 (NOX4) significantly increased after Ang II for 4 weeks. $^{*}p<0.05$, $^{**}p<0.01$ compared with saline-treated WT controls, $^{\#}p<0.05$ compared with Ang II-treated WT. Ang, Angiotensin II; NADPH, nicotineamide adenine dinucleotide phosphate.

At the molecular level, Ang II binds to Angiotensin II receptor 1, a Gαq coupled-receptor, then activates NOX through a protein kinase C (PKC)-dependent manner to produce ROS (214). ROS from NOX might increase mitochondrial ROS production, as previously shown in endothelial and vascular smooth muscle cells (79,153). The data from our laboratory provide direct evidence that amplification of ROS within mitochondria is a key mediator of Ang- and Gαq-induced cardiac hypertrophy and failure (Fig. 10) (69). Mechanisms of ROS amplification might include ROS-induced ROS release as well as an ROS-mtDNA damage vicious cycle (Fig. 11). ROS production from NOX2/p47 phox at the cell membrane, and more specifically, NOX4 at the mitochondrial membrane can increase electron leakage from the electron transports chain, further stimulating ROS production. NOX4 expression is itself increased by AngII treatment (Fig. 9E) (69). This mechanism is also consistent with observations that primary damage to mtDNA is sufficient to elevate ROS, cause cardiac hypertrophy and accentuate Ang II effect to induce heart failure (69,71). Thus, breaking the ROS vicious cycle within mitochondria by mCAT or mitochondrial-targeted antioxidants (see Section VIII.B) is effective in attenuating both cardiac hypertrophy and failure (Fig. 11).

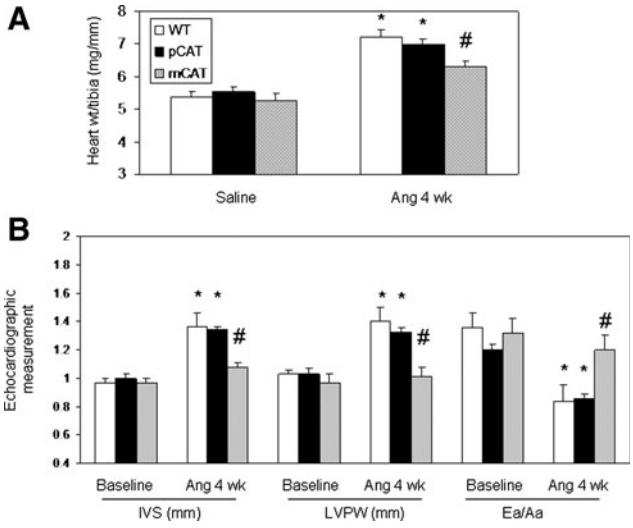

FIG. 10. Mitochondrial oxidative stress in Ang II-induced cardiac hypertrophy. (A) Normalized heart weight, **(B)** Echocardiography at baseline and after 4-week Ang II. $^{*}p<0.05$, compared with saline-treated WT controls, $^{\#}p<0.05$ compared with Ang II-treated WT.

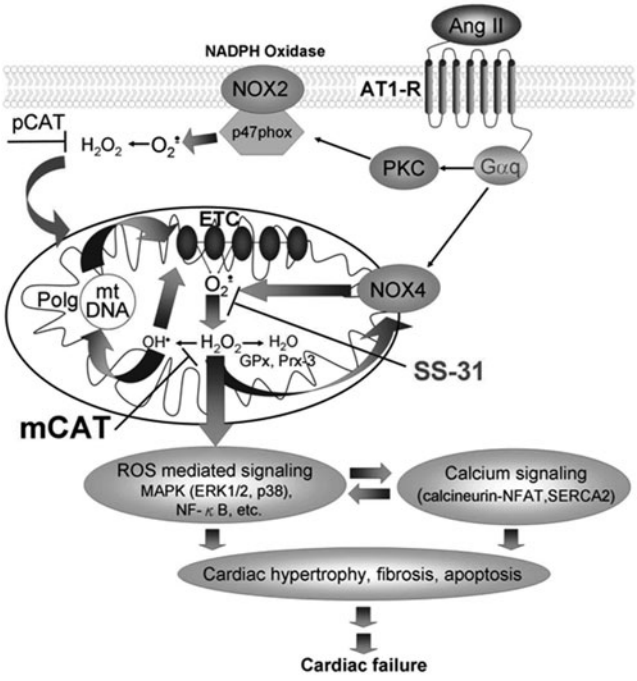

FIG. 11. Mitochondria amplify ROS signaling in cardiac hypertrophy and failure. Ang II binds to ATR1, a Gαq coupled-receptor, then activates NADPH oxidases (NOX2 and NOX4). ROS from NOXs may contribute to mitochondrial ROS, which will then be amplified within mitochondria through ROS-induced ROS release and an ROS-mitochondrial DNA damage-increased ROS vicious cycles. Primary mtDNA damage in mice with homozygous mutation of polymerase gamma induces cardiac hypertrophy and failure through increase in mitochondrial ROS secondary to these vicious cycles. Breaking the ROS vicious cycles within mitochondria by mCAT or mitochondrial targeted SS-31 antioxidant is effective in attenuating both cardiac hypertrophy and failure. ATR1, Angiotensin II receptor 1.

The understanding that NOX4 is localized to the mitochondrial membrane increases the importance of considering mitochondrial ROS and nicotinamide adenine dinucleotide metabolism in Ang II-mediated cardiac hypertrophy and failure. Activation of NOX4 consumes nicotineamide adenine dinucleotide phosphate (NADPH) and directly contributes superoxide anions and ROS damage to mitochondria (3,168). Detoxification of hydrogen peroxide derived by dismutation of superoxide is usually performed in mitochondria by Peroxiredoxin-3 (Prx-3) and GPx. After their oxidation by hydrogen peroxide, these enzymes are regenerated using the ultimate reductive power of NADPH. However, the consumption of NADPH by NOX4 establishes another potential mitochondrial vicious cycle. Note that mitochondrial catalase or other mitochondrial antioxidants (see section VIII.C) can bypass this vicious cycle by removing superoxide or hydrogen peroxide without consuming glutathione (GSH) or NADPH. NADPH can itself be regenerated from NADP+by electron exchange with nicotineamide adenine dinucleotide (NADH), catalyzed by nicotinamide nucleotide transhydrogenase. Thus, cardiomyocyte mitochondrial redox status is intimately bound with nicotinamide adenine dinucleotide metabolism. This further implicates SIRTs (sensors of the ratio of NAD+/NADH), particularly SIRT3, in an epigenetic cardiac response to stress (see section VIII.C).

Recently, it has been reported that mitochondrial nitric oxide synthase (mtNOS) is also activated by Ang II subsequent to binding of Ang II to an AT2R receptor located in the mitochondrial inner membrane (mtAT2R) (1). The mtNOS uses NADPH and arginine as substrates to generate the nitric oxide radical (NO·). NO·has been shown to regulate oxygen consumption by inhibition of Complex IV of the electron transport chain (ETC) (86,258). Thus, while consuming NADPH, production of nitric oxide (NO) could directly compromise respiratory function. In addition, formation of peroxynitrite by reaction of NO·with $O2^{\cdot-}$ would lead to increase nitrosative stress in the mitochondrial compartment, including damage to respiratory complexes and opening of the MPTP (262).

It is clear from the discussion just conducted that mitochondrial oxidative and nitrosative stress can have a direct impact on glutathione redox status (GSH:GSSG ratio) and nicotinamide nucleotide redox potential (NAD/NADH and NADP/NADPH) and that these are closely interrelated within mitochondrial metabolism. GSH:GSSG ratios have been reported to be lower in hearts of aged mice compared with young mice (264) and rats (167) and senescence-accelerated *versus* wild-type mice (265). Similar effects have been seen in NAD+/NADH ratios in aging rat hearts (39). These interconnections between oxidative stress, GSH and nicotinamide nucleotide redox potential, and Sirt activity motivate the desire to enhance redox balance in aging and disease by pharmacologic treatment. Past and future strategies for such intervention are discussed in section IX, given next.

2. The role of mitochondria and oxidative stress in IR injury. Reperfusion therapy, by either primary percutaneous coronary intervention or thrombolytic therapy to reopen the blocked coronary artery within the "golden period" after ischemia, has become a standard therapy for acute myocardial infarction. Reperfusion therapy is necessary and beneficial to salvage the ischemic myocardium; however, it also exposes the heart to IR injury, which may induce reperfusion arrhythmia, stunned myocardium (transient myocardial dysfunction), and massive myocardial damage that can eventually lead to congestive heart failure, despite the successful revascularization of the coronary artery. Coronary revascularization using coronary artery bypass grafting may also be associated with myocardial stunning and cell necrosis due to IR injury.

At the molecular level, ROS have been well known to be primary mediators in IR injury. ROS begin to accumulate during ischemia (25), causing mitochondrial respiratory complex dysfunction, which, in turn, produces a burst of ROS after reperfusion. Furthermore, the marked acidosis associated with IR may facilitate the conversion of the less potent oxidants superoxide and hydrogen peroxide to the highly reactive oxidants peroxynitrite and the hydroxyl free radical. It has been shown that several conditions associated with post-IR, such as ROS accumulation, acidic pH, and a rise in $[Ca^{2+}{}_i]$, open the mPTP, which, in turn, triggers more mitochondrial ROS generation. This is one of the mechanisms involved in mitochondrial ROS-induced ROS release (360).

The mPTP is a high conductance channel located in the inner mitochondrial membrane (IMM). The physiological

roles of the mPTP include regulation of mitochondrial matrix volume and pH as well as redox reaction, regulation of Ca2+ release, and pyridine nucleotides rapid exchange across the IMM [see review (74)]. Several factors have been reported as facilitating mPTP opening: increasing matrix [Ca2+], mitochondrial depolarization (drop in $\Delta\psi_m$), which is related to increased oxidative stress or a decline in NADPH/NADP+ and thiol/disulfide ratios, slight acidic pH (around 7.3), and the loss of the buffering effect of mitochondrial inorganic phosphate (74). The pathological roles have been widely studied in IR injury, during which the mPTP is in the fully open state and allows passive diffusion of solutes with molecular masses up to 1.5 kDa. The mPTP opening may cause mitochondrial swelling, further collapse of mitochondrial membrane potential ($\Delta\psi_m$), ATP depletion, and eventually trigger apoptosis (73).

The aged myocardium is more susceptible to both ischemia and hemodynamic stress than is young myocardium (134). Aged cardiomyocytes have a lower threshold for ROS-induced ROS release and increased susceptibility to mPTP opening (142). Furthermore, ischemic preconditioning, the endogenous cardioprotective mechanism incited by repetitive ischemia that reduced the area of myocardial infarction, is also impaired in the aged myocardium [reviewed by (142)]. This loss of endogenous protective mechanisms of ischemic preconditioning in the aged heart might include a decrease in mitochondrial heat shock protein-70 (232), decline in NO bioavailability (57), damaged mitochondria that are vulnerable to stress, and diminished PKC translocation, all of which are believed to be required for the protective effect of ischemic preconditioning (158,316).

In support of a critical role for mPTP opening and apoptosis in human cardiac disease, a small pilot clinical study applying cyclosporine, a mitoprotective agent that blocks the opening of the mitochondrial permeability-transition pore, has shown very promising results in reducing infarct size in patients with acute myocardial infarction receiving reperfusion therapy (256).

B. Mechanisms of progression to heart failure in the aged hypertrophic heart

In response to chronic stress, the aged myocardium remodels, by a complex of events that includes myocyte growth or hypertrophy, re-expression of a fetal gene program and remodeling of extracellular matrix (ECM). These changes reduce functional reserve and predispose the aged hearts to the development of heart failure. Molecular mechanisms underlying the progression to heart failure are discussed below and in ref. (239).

1. Increased cardiomyocyte death. Increased cardiomyocytes death is observed in various types of cardiomyopathy, such as ischemic and dilated cardiomyopathy, hypertensive cardiomyopathy, and cardiac aging (221). The increase in cardiomyocyte death has been reported to contribute to impaired contractility and induce ventricular remodeling, which may eventually cause symptomatic heart failure (90). Noxious stimuli incite signaling molecules in favor of cell death rather than cell survival, and this perturbation will result in cardiomyocytes death and subsequent cell loss, as is frequently seen in heart failure. Various types of cardiomyocytes cell death, including necrosis, apoptosis, and autophagy have all been reported in heart failure.

Necrosis could be caused by ischemia or IR through increased burst of ROS and augmentation of Ca^{2+} entry, which triggers the opening of mPTPs by activating cyclophilin D (226). Apoptosis can be elicited by several different stimuli that are also mediated by increased ROS, such as Ang II, sympathetic stimulation, and cytokines. Increased cardiomyocyte apoptosis has been widely documented as contributing to the phenotype of heart failure (90,343). Autophagy is characterized by recycling of proteins within organelles. A study using mice overexpressing cardiac specific diphtheria toxin receptors demonstrated that diphtheria toxin-induced autophagy is associated with cardiomyocyte death and heart failure (6). Decreased cardiac autophagy in mice with heterozygous disruption of the gene coding for beclin 1 was associated with diminished pathological remodeling when challenged by severe pressure overload (358). Conversely, beclin 1 overexpression increased autophagic activity and accentuated pathological remodeling (358). Consistent with this, increased autophagic activity has been observed in human heart failure (159). Interestingly, impairment of autophagy in mice with cardiac specific disruption of Atg5 was also found to have cardiac hypertrophy, left ventricular dilatation, and systolic heart failure, accompanied by increased levels of ubiquitination (225). Collectively, these studies suggest that constitutive autophagy is a homeostatic mechanism in the heart that is required to maintain normal cardiac structure and function, and that genetic models of increased autophagy or upregulation of autophagy as a maladaptive response to hemodynamic stress will cause heart failure. While the term "autophagic cell death" has been used in previous literature, recent studies have shown that autophagy can be a pro-survival rather than a pro-death mechanism. Hence, cell death may often be associated with, but not caused by, the accumulation of autophagosomes (165,183).[see review (72)].

2. ECM remodeling. In response to various injury stimuli, the myocardium can undergo compensatory or pathologic remodeling, with cardiomyoctye death and hypertrophy of the remaining cells, as well as alterations in the composition and structure of the ECM. ECM remodeling might affect cardiac size, structure, and function and directly contribute to the development of heart failure (80,194,195,274) and cardiac aging (196). The ECM is always in an active and continuous turnover process involving the synthesis of ECM by myofibroblasts and the degradation of collagens by MMPs through TGF-β dependent signaling (178). Pathological ECM remodeling, especially interstitial fibrosis, increases cardiac stiffness and reduces cardiac compliance as well as disrupts the coordination of myocardial excitation-contraction coupling [reviewed by Berk (32) and Spinale (301)]. Several studies using animal models of heart failure have shown that inhibition of pathological ECM remodeling by MMP inhibitors ameliorates cardiac dysfunction (80,178,194,195,274).

3. Alteration of calcium handling proteins. Ca^{2+} is the signaling molecule of excitation-contraction coupling. This coupling is initiated by opening of the L-type calcium channels during depolarization (action potential), which allows entry of a small current of Ca^{2+} that subsequently triggers the ryanodine receptor to release a large scale Ca^{2+} current from

sarcoplasmic reticulum Ca^{2+} storage ($SRCa^{2+}$). This increase in cytosolic Ca^{2+} binds and activates Troponin C within the myofilaments and induces myocyte contraction. Relaxation is initiated by reuptake of cytoplasmic Ca^{2+} into the SR through phospholamban-regulated Calcium-ATPase (SERCA2a) and subsequent trans-sarcolemmal Ca^{2+} removal through the sodium calcium exchanger. Thus, in normal hearts, electrical propagation (action potential) precedes calcium transients, which triggers the cardiomyocytes contraction-relaxation cycle, and the whole process is called excitation-contraction coupling.

It has been reported that Ca^{2+} reuptake into the SR is impaired in cardiac aging and congestive heart failure, and consequently, the SR Ca^{2+} storage is also decreased (71,127,234). The decline in Ca^{2+} reuptake in heart failure could be explained by decreased SERCA2 protein concentration [as seen in murine cardiac aging (71)], oxidative/nitrative modification that impairs SERCA2 protein function, reduced levels of phospholamban phosphorylation, and the depletion of SR Ca^{2+} through leaky ryanodine receptor channels (33). Genetic manipulation of mice with improvement of Ca^{2+} reuptake, such as overexpression of SERCA2 (210) or disruption of phospholamban, has been reported to attenuate heart failure in experimental animals (129,208).

4. Hypoxic response and angiogenesis. Cardiac hypertrophy results in increased myocardial oxygen demand and decreased coronary perfusion pressure; the latter is due to compression of the coronary microcirculation. The mismatch in oxygen/nutrient supply-demand induces a relative ischemia in the hypertrophic heart, which may eventually cause energetic failure. Previous studies in mice with Akt-induced cardiac hypertrophy showed a concomitant increase in angiogenic growth factors such as vascular endothelial growth factor (VEGF) and angiopoeitin 2 during the hypertrophic phase, and that inhibition of VEGF signaling in hypertrophic hearts would induce relative ischemia and progress to heart failure (135,296). Furthermore, the cardiac transcription factor GATA4, a fetal gene reactivated during hypertrophy, is shown to pro-angiogenic to help maintaining the balance between growth of new muscle and capillaries in hypertrophic hearts (123). However, in murine hearts, activation (phosphorylation) of GATA4 is reduced in aging (71).

5. Mitochondrial dysfunction and abnormalities in energetics. Cardiac hypertrophy is associated with upregulation of the signaling for mitochondrial biogenesis, including PGC1α and its downstream target genes (69). However, mitochondrial proliferation often does not keep pace with the increasing energy demand of the hypertrophic hearts (261). Our recent study demonstrates that Ang II-induced cardiac hypertrophy is associated with depletion of mitochondrial DNA copy number and impairment of mitochondrial respiratory function. Mice with homozygous mutation of the exonuclease domain of mitochondrial polymerase gamma ($Polg^{D257A/D257A}$), which, as just noted, have a progeroid phenotype accompanied by increased mitochondrial DNA mutations, are susceptible to the development of heart failure at middle age or at a young age after challenge with 4 weeks of Ang II (69,71). This suggests that primary damage to mitochondrial DNA contributes directly to the phenotype of systolic heart failure. Studies on human hearts using ^{31}P NMR spectroscopy indicated that the ATP content of failing hearts is generally 20%–30% lower than that of normal hearts (26). Furthermore, phosphocreatine, an important short-term reserve energy source that maintains a high phosphorylation potential to cope with acute increases in energy demand (*e.g.*, exercise), significantly declined by up to 60% in elderly heart failure patients (341). The magnitude of this reduction is related to the severity of heart failure (224) and is shown to predict mortality in patients with dilated cardiomyopathy (229).

VIII. Exercise, Cardiovascular Risks, and Cardiac Aging

Exercise is broadly accepted as reducing the risk of cardiovascular disease. Several epidemiological and clinical studies have shown an inverse correlation between exercise and the components of metabolic syndrome (68,147,249), which include abdominal obesity, high serum triglyceride and low serum high density lipoprotein (HDL) cholesterol, high blood pressure, and impaired fasting glucose. Lifestyle modification including exercise significantly reduced obesity (337). The beneficial effect of exercise on improvement of serum atherogenic lipoprotein profiles mainly include reduction of serum triglycerides, increase in HDL cholesterol, and increase in average size of low density lipoprotein (LDL) particles (155,162,181) [see review (4)]. The effect of exercise to decrease total cholesterol and LDL cholesterol usually occurs in association with significant weight loss (215). Meta-analyses of exercise programs demonstrate that exercise is effective in reducing blood pressure in hypertensive patients (75,84) and in better controlling blood glucose in diabetic patients (58,327). In addition, higher physical activity has also been shown to reduce carotid artery intima media thickness, a surrogate marker of atherosclerotic burden, which is an independent predictor of cardiovascular events, as shown in the Framingham Offspring Study (259).

Cardiovascular risks, including hypertension and diabetes, have been shown to induce LVH, impairment of cardiac function, and several molecular changes mimicking "premature cardiac aging," including telomere shortening (331), and expression of senescent associated proteins p53, p21 and p16INK4a (160,278). A recent study on patients undergoing cardiac surgery demonstrated that obesity resulted in disturbed mitochondrial biogenesis and respiratory function, increased oxidative stress, and shortening of telomere in cardiomyocytes isolated from young obese patients, at levels comparable to those found in old nonobese patients (231). This study suggests that obesity, similar to hypertension and diabetes, also induces phenotypes of premature cardiac aging in young patients.

Regular aerobic exercise is effective in reducing cardiovascular risks, and it is also expected to attenuate cardiovascular aging. Previous cross-sectional studies revealed that subjects with higher levels of self-reported physical activity are associated with a reduction of age-dependent large artery stiffness, diastolic dysfunction (102,315), and LVH (68). Small longitudinal studies comparing 12 elderly athletes/runners with 12 age-matched healthy sedentary seniors reported that athletes have significantly better LV compliance and diastolic function (16) as well as better aerobic capacity (VO_2 max), independent of the exercise effect on body mass index and lipid profiles (219). An interventional study ($n = 12$, age = 50–60 years, nonobese, 1 year duration) demonstrated that both

caloric restriction and exercise improved diastolic function, in association with significant weight loss (269). In contrast, progressive and vigorous exercise training of previously sedentary elderly ($n = 12$, mean age = 70, 1 year duration) failed to reverse preexisting cardiac stiffness and diastolic dysfunction, although exercise increased maximal exercise capacity and improved arterial elastance (reduced pressure-overload) (96,235).

Since exercise is an inexpensive way to retard aging changes and to reduce the risk of CVDs and cancer, the World Health Organization recommends at least 150 min of moderate-intensity aerobic physical activity throughout the week, or 30 min of aerobic exercise per day, 5 days per week (WHO 2010 Global Recommendations on Physical Activity for Health). For individuals who cannot reach the target recommended by WHO, lower amounts of exercise are also beneficial, as shown by a recent prospective cohort study of more than 400,000 individuals which demonstrated that self-reported low-volume physical activity (exercise $\sim$90 min per week or 15 min per day) reduced all-cause mortality by 14%, and every additional 15 min increase in exercise beyond the minimum amount of 15 min/day further reduced all-cause mortality by 4% (342). Since exercise has been shown to improve aerobic capacity and the phenotypes of cardiac aging, this study is consistent with previous animal studies showing the relationship between cardiac aging, aerobic capacity, and lifespan. In rodent models, lifespan was independently predicted by the degree of cardiac aging in the mouse model (71) and by the difference in genetically determined aerobic capacity (treadmill running capacity), which was created by selective breeding of rats with diverse genetic background (154).

In experimental animals, exercise training ameliorated age-dependent impairment of adrenergic signaling as well as enhanced responsiveness to sympathetic stimulation in the aged rat heart (182) and in the rabbit model of pacing-induced heart failure (99). The mechanism is mainly through the activation of endogenous antioxidant mechanisms (99,220). As just discussed, endurance exercise induced systemic mitochondrial biogenesis, prevented mtDNA depletion and mutations, and improved mitochondrial oxidative capacity in skeletal muscles of mtDNA mutator mice ($Polg^{m/m}$), thereby rescuing the "premature aging" phenotypes of these mice (283). The beneficial effect of exercise on improvement of insulin sensitivity, mitochondrial biogenesis, and upregulation of endogenous antioxidant defense is also dependent on ROS, as these effects are blocked by oral vitamin C and vitamin E in humans (271), indicating the mitohormesis effect (see section V.C). Aging is also associated with reductions in AMPK activity and decreased mitochondrial biogenesis in skeletal muscles (268). These aging changes are counteracted by exercise training, which upregulates AMPK. Activation of AMPK phosphorylates and directly activates PGC-1α (136). In addition, AMPK activation might also increase mitochondrial biogenesis indirectly through SIRT1 and SIRT3 dependent PGC-1α deacetylation (187,248).

IX. Emerging "Anti-Aging" Interventional Strategies for Cardiac Aging and CVDs

A. Dietary restriction

DR is an extremely well-established intervention that has been clearly demonstrated as increasing lifespan and reducing the onset and severity of a variety of age-related pathologies. Whether the effects of DR in multicellular organisms are cell autonomous or neurohormonal is largely still open to discussion. This intervention has been demonstrated in a wide array of model organisms, from yeast and nematodes to mice, rats, and, most recently, rhesus monkeys (59,64,92,146,203). Specific to cardiac aging, DR has been shown to be a powerful preventative for cardiovascular disease in rodents and non-human primates (64,230,294). Similar protective effects have also been observed in human and animal trials of alternate-day fasting (330), demonstrating that even transient activation of the pathways involved in the DR response can be beneficial. In addition to the role of mTOR in nutrient signaling just discussed (Section VI.C), both DR and exercise signal to some extent through the NAD(+) dependent deacetylase SIRT3 (248) (the Sirt that is most prevalent in the heart) demonstrating a functional overlap between DR, exercise, and nutrient signaling. DR has also been shown to activate SIRT1, probably dependent on NO signaling (292,293).

DR is mediated primarily through nutrient signaling pathways, but the beneficial effects of DR are difficult to assign to individual processes or pathways downstream of nutrient signaling. A flood of recent work on DR in cardiac aging indicate that cardiac stem cells (294), mitochondrial function (124,230,275), and ROS production (60,209) have all been demonstrated to be beneficially affected by DR, and have all been implicated in the beneficial affects provided by restricting dietary intake.

Adding to the list of potential benefits provided by DR, increased autophagy (see the mitochondrial-lysosomal theory of aging, above) is a well-documented result of DR. Autophagy, though often associated with cardiac disease, is upregulated in both DR and inhibition of mTOR signaling by the drug rapamycin (107,347), and is thought to be a key component of the beneficial effects of DR. While there are still many unanswered questions with regard to the mechanisms of DR, and the use of DR as a clinical intervention is problematic, the robust effects of DR on preventing cardiovascular disease and cardiac aging are clear. The cardiac aging field will undoubtedly continue to benefit from and contribute to the scientific understanding of DR. The search for DR mimetics is an active discipline and may provide important clinical interventions for the treatment and prevention of heart disease and cardiac aging.

B. Antioxidant interventions

1. Nontargeted antioxidants. Since many animal and clinical observational studies suggest a crucial role for increased ROS in many diseases, including CVDs, the therapeutic application of antioxidant therapies have been evaluated in several placebo-controlled trials involving tens of thousands of patients with the endpoint of cardiovascular events as well as mortality (164,336). The results from these studies are either disappointing or equivocal. Only a few smaller studies with short-term follow-up show the beneficial effects of vitamin E or C for secondary prevention of cardiovascular events, while other larger trials with longer follow-up (up to 12 years, as in the Physicians' Health Study) fail to show any effect [see review by Kris-Etherton, *et al.* (164)]. A few large-scale meta-analyses of randomized trials have been performed in recent years. In a meta-analysis of 68

randomized trials including 232,606 participants from the general population or patients with heterogeneous diseases, Bjelakovic *et al.* reported that antioxidant supplements had no significant effect on overall mortality. When including only the trials with high methodological quality (180,938 participants), there was a significant increase in mortality in subjects receiving beta carotene, vitamin A, and vitamin E, either singly or combined (34). In an earlier meta-analysis of 12 randomized trials involving 138,113 participants, there was no effect of vitamin A on all-cause mortality, cardiovascular death, or stroke. Again, beta-carotene was shown to significantly increase all-cause mortality and cardiovascular death (336). This is very disappointing, given the abundance of epidemiologic, clinical pathophysiologic studies, and mechanistic data from animal studies supporting the application of antioxidant therapies in the treatment and prevention of cardiovascular disease. It might, however, be too early to claim that antioxidants as a class are ineffective. Indeed, another recent meta-analysis of 44 observational clinical studies measuring endogenous antioxidant activity in cells or biological fluids demonstrated that there was a significant inverse association between circulating levels of GPx, SOD, and catalase activities with coronary heart disease (89). Given these controversies, it is, therefore, very important to evaluate why most clinical trials have failed to show beneficial effects.

There are many potential explanations for the failure of antioxidants clinical trials for prevention and treatment of CVDs (305): First, the wrong drugs may have been studied. Most studies have used supplementary vitamins as "antioxidants," not because they have better efficacy as antioxidants but rather because they are readily available. Some agents, such as vitamin E, have subsequently been shown to have pro-oxidant effects, consistent with the fact that vitamin E supplement significantly increases mortality in the meta-analyses just noted.

The endogenous antioxidant enzymes, SOD, catalase, and GPx, are not feasible drug molecules because of their size, rapid degradation, and potential antigenicity. Over the past few years, there have been a number of attempts to develop synthetic molecules that can mimic the activities of these scavenging enzymes. Several major classes of synthetic SOD, catalase, and GPx mimetics have been reported and were recently summarized in a review article (252). The most extensively investigated have been the Mn (III) porphyrins, and these SOD mimetics have been reported to reduce oxidative stress, inflammation, and tissue damage in animal models of human disease. AEOL10150 is currently in phase 2 clinical trial for radiation injury; while M40403 is being developed by Metaphore for inflammatory pain. These metalloporphyrins can also possess varying extents of catalase activity due to their extensive conjugated ring system that can undergo reversible one-electron transfers in addition to the one-electron transfer on the metal center. Another class of synthetic catalytic antioxidants, the Mn (III) salen complexes, are both SOD and catalase mimetics. The EUK series of drugs have demonstrated efficacy in several animal models of human disease, and EUK-8 and EUK-134 were reported to attenuate pressure-overload induced cardiac failure (272,328). Ebselen was one of the first selenium-based GPx mimetics shown to scavenge peroxides in the presence of reducing equivalents such as GSH, NAC, and dihydrolipoate. Ebselen can stimulate the decomposition of several ROS species, including singlet oxygen, hypochlorous acid, and peroxynitrite. Ebselen has been shown to be effective in several models of ischemic injury and neurodegenerative disease, but its effectiveness is complicated by its lack of specificity due to its binding to thiol groups on proteins, as many cellular proteins have reactive thiols in their catalytic domains. Despite the promising animal data, there is no evidence that these synthetic catalytic antioxidants can scavenge mitochondrial ROS and protect mitochondria against oxidative stress. Furthermore, since mitochondria are the primary sites of ROS generation in cardiac aging, hypertensive, and ischemic heart diseases, the nontargeted antioxidants might not be as effective as targeted antioxidants (see below).

Second, the wrong patient population may often have been selected. Secondary prevention might have failed, because antioxidants were ineffective to reverse advanced diseases and even in primary prevention, antioxidants might not be effective to reverse established subclinical diseases. Third, inappropriate dosing may often have been used. It is noteworthy that the meta-analyses just mentioned have included multiple different regimens with different dosing. Finally, these studies have included very heterogeneous participants ranging from the general population to patients with myocardial infarction or cancers. Taken together, further high-quality prospective studies using well-established antioxidant regimens are required.

2. Mitochondrial-targeted antioxidants. Given the disappointing results from clinical studies of nontargeted antioxidants just noted (34), coupled with the generally negative data from model organisms, also as just noted, it is evident that untargeted antioxidant therapies have as yet failed to provide effective interventions in cardiac aging and, in most cases, disease. This finding supports the idea that antioxidant therapies that are targeted to the mitochondria—the major source of pathogenic ROS and the damaged target of ROS in many diseases—may be a much more effective strategy. There has been some success in the development of therapies targeted to the mitochondria, such as the Szeto-Schiller synthetic antioxidant peptides (54,310–312,356) and the drugs MitoQ and Euk (309,328,334). These therapies have demonstrated some potential effectiveness in models of cardiac stress (see below), although their effectiveness in preventing the progressive functional decline associated with cardiac aging is yet to be determined.

Mitochondria are complex organelles, and it is important to consider the specific mitochondrial compartment that should be targeted for effective antioxidant therapies. Mitochondria are composed of two membranes, an outer mitochondrial membrane (OMM) and an IMM. The protein complexes of the ETC reside on the IMM, and the proton gradient generated across the IMM as a result of electron transfer from NADH to oxygen *via* Complexes I, III, and IV drives the production of ATP by the F_0F_1 ATP synthase. In fact, many approved drugs can penetrate the OMM and inhibit mitochondrial function, by either inhibiting the protein complexes of the ETC, uncoupling the proton gradient, or directly inhibiting the ATP synthase [for review, see (41)]. The IMM is unique in that it has a very high concentration of proteins and is rich in cardiolipin, a very anionic phospholipid that has four fatty acid chains rather than two. This special composition makes the IMM highly impermeable to most molecules. Thus, it is much more

difficult for therapeutic molecules to penetrate the IMM and reach the mitochondrial matrix.

Excessive electrons in the ETC results in electron leakage and the generation of superoxide anion, especially at Complex I and Complex III. Superoxide from complex I is released into the matrix, while complex III can release superoxide into the matrix as well as the intermembrane space. The proteins and cardiolipin of the IMM are, therefore, most vulnerable to oxidative damage. Cardiolipin peroxidation will result in loss of cytochrome c and inhibition of electron transfer, thereby setting up a feed-forward cycle of ROS-induced ROS production. Thus, effective antioxidant therapy would need to be targeted to the IMM or mitochondrial matrix to be successful in reducing mitochondrial oxidative damage.

a. TPP^+ conjugated antioxidants. The most common method for targeting compounds to the mitochondrial matrix takes advantage of the potential gradient (150–180 mV) that is generated as a result of the proton gradient across the IMM. Conjugating lipophilic antioxidants to a cation can result in 100–1000-fold accumulation in the mitochondrial matrix (251). Triphenylalkylphosphonium ion (TPP+) has successfully been used to deliver a number of redox-active compounds such as coenzyme Q (MitoQ) and plastoquinone (SkQ1) (298), into the mitochondrial matrix. MitoQ is taken up rapidly by energized isolated mitochondria, and addition of the uncoupler FCCP caused its immediate efflux (150). Likewise, the cellular uptake of both [^{3}H]MitoQ and SkQR1 (a rhodamine analog of SkQ1) is dramatically reduced in the presence of FCCP (12,150). MitoQ and SkQ1were shown to inhibit lipid peroxidation in isolated mitochondria, with SkQ1 being almost 1000-fold more potent than MitoQ (12). Both MitoQ and SkQ1 protected against oxidative cell death in nM concentrations, with SkQ1 being more potent (12). This lipophilic-cation approach has been used to generate mitochondria-targeted antioxidants that are designed to decrease superoxide (MitoSOD), hydrogen peroxide (MitoPeroxidase), ferrous iron (MitoTEMPO), and lipid peroxidation (MitoE2) [see review (216)].

The use of this lipophilic cation approach has some limitations. The dependence on mitochondrial potential for their uptake restricts their uptake by mitochondria with compromised potential gradient, which is often seen in disease conditions such as ischemia. Furthermore, MitoQ and SkQ1 have been reported to disrupt mitochondrial potential and inhibit respiration at concentrations above 25 μM (12,150). The reduction in mitochondrial potential effectively limits further uptake of these lipophilic cations (150). Another potential limitation of MitoQ is its prooxidant action as it is reduced to a semiquinone radical at the level of complex I (216,233,288). The radical formed reacts with O_2 to form superoxide radicals. MitoQ only serves as an antioxidant when complex I is inhibited. Thus, the antioxidant activity of MitoQ should be interpreted with caution. The "window" between antioxidant and prooxidant activity of SkQ1 is higher than that of MitoQ (12).

MitoQ and SkQ1have been evaluated in a number of *in vivo* animal models. Pretreatment of rats with MitoQ for 2 weeks reduced ischemia-induced cardiac dysfunction in the isolated perfused heart (2). Likewise, 3 week pretreatment with SkQ1 reduced infarct size after ligation of the coronary artery *in vivo* (19). Interestingly, treatment of young spontaneously hypertensive rats with MitoQ for 8 weeks produced a significant, but modest, reduction in systolic blood pressure, improved the availability of endothelial NO, and reduced cardiac mass (150). Since only a single dose of MitoQ was examined in this study, it is not known whether higher doses might provide more protection. It is also not possible to determine whether the slight reduction in cardiac mass index in mitoQ treated rats is a direct effect of mitoQ or a secondary response to the decrease in blood pressure. Similarly, daily treatment with SkQ1 for 3 weeks significantly reduced postischemic cardiac arrhythmias and infarct size in rats, although the dose-response curves were biphasic with loss of protection at higher doses (19).

The effectiveness of these TPP^+-conjugated antioxidants appear to require a substantial period of pretreatment before the induction of cardiac stress. When a single dose of SkQ1 was administered 1 day before the disruption of renal blood flow, there was no significant protection of renal function 24 h after ischemia as measured by blood creatinine concentration (19), The reason for this necessity of a substantial period of pretreatment is not clear, but these potential-dependent antioxidants are unlikely to be effective if administered after ischemia due to mitochondrial depolarization. Thus, these compounds may be more suitable for the treatment of chronic disease than the prevention of acute disease.

There are to date two reports of MitoQ in human trials. In patients with Parkinson's Disease, MitoQ showed no difference from placebo in any measure of disease progression (299). In chronic hepatitic C virus patients, a double-blind, randomized, parallel-design trial of two different doses of MitoQ over a 4 week treatment demonstrated that there was no effect of MitoQ on viral load compared with placebo; however, both MitoQ treatment groups showed significant decreases in serum alanine transaminase from baseline (98). This study was the first report of a potential clinical benefit from the use of mitochondria-targeted antioxidants in humans.

b. Szeto-schiller peptides. The Szeto-Schiller (SS) peptides are the first compounds that selectively target and concentrate in the IMM where ATP and free radical production takes place. These synthetic compounds are tetrapeptides with an alternating aromatic-cationic motif. They are cell-permeable and selectively concentrate in the IMM as demonstrated by confocal laser scanning microscopy and isolated mitochondria studies (19,78,356). The mitochondrial uptake of these SS peptides is not dependent on mitochondrial potential, and they are rapidly taken up even by depolarized mitochondria. Potential-independent uptake is a significant advantage when dealing with diseased mitochondria that are likely to have a reduced mitochondrial potential. SS-02 (H-Dmt-D-Arg-Phe-Lys-NH_2; Dmt = 2'6'dimethylTyr) and SS-31 (H-D-Arg-Dmt-Lys-Phe-NH_2) have been estimated to concentrate > 1000-fold in the IMM when compared with extracellular concentrations (78,356). Unlike MitoQ and SkQ1, these SS peptides do not cause mitochondrial depolarization even at high concentrations, presumably because they are not distributed into the matrix.

The SS peptides are multi-functional compounds in mitochondria. The Dmt- or Tyr-containing analogs have intrinsic antioxidant activity, because Tyr is known to scavenge oxyradicals, forming relatively unreactive tyrosyl radicals that

can be followed by radical-radical coupling to give dityrosine, or react with superoxide to form tyrosine hydroperoxide (109). Unlike MitoQ and SkQ1, which are reduced by the respiratory chain and can be recharged, the SS peptides are not rechargeable antioxidants. SS-02 and SS-31 were shown to dose-dependently scavenge hydrogen peroxide, hydroxyl radical, and peroxynitrite *in vitro* (311,356). Since these peptides are targeted directly to the IMM, SS-02 and SS-31 are positioned to scavenge H_2O_2 from both the matrix and the intermembrane space. Both SS-02 and SS-31 reduced spontaneous H_2O_2 emission from isolated mitochondria under basal conditions (311,356). SS-02 and SS-31 can also inhibit lipid peroxidation and are, therefore, ideally situated to prevent cardiolipin peroxidation from hydroxyl radicals.

The selective targeting and concentration of SS-02 and SS-31 in the IMM makes them extremely potent in reducing mitochondrial oxidative stress in intact cells. An extracellular concentration of 1 nM can produce a concentration of > 1 μM in the IMM, which is well within the concentration determined to be sufficient to protect mitochondria in isolated mitochondrial studies (356). SS-02 and SS-31 have been shown to potently inhibit cell death caused by prooxidants including *tert*-butylhydroperoxid, H_2O_2,and hypochlorous acid in cell cultures (78,128,355).

In addition to their antioxidant actions, the SS peptides can also promote mitochondrial respiration and inhibit mitochondrial permeability transition and swelling (257,356). These properties make the SS peptides highly effective in prevention and treatment of IR injury (311). The Tyr-containing SS peptides can significantly reduce reperfusion arrhythmias, myocardial contractile function, and infarct size after myocardial ischemia (54); attenuate infarct size after cerebral injury (254); reduce acute kidney injury after renal IR (346); protect skeletal muscles; and preserve normal cellular architecture after 3 h of hind limb ischemia (350). Most importantly, these SS peptides are effective even when administered on reperfusion and require no pretreatment. Pretreatment with these peptides protects mitochondrial structure and respiration in early reperfusion and accelerates ATP recovery on reperfusion (346). These preclinical studies support mitochondrial protection as an upstream target for pharmacological intervention in IR injury.

Other preclinical studies support a beneficial role for these SS peptides in age-related problems, including Parkinson's Disease (257), Alzheimer's Disease (131), muscle weakness (55), heart failure (66), and insulin resistance (9). Reduced mitochondrial ETC function in skeletal muscles is associated with insulin resistance, and type 2 diabetes (349). A high fat diet leads to mitochondrial dysfunction and elevated ROS production in skeletal muscle (9). Administration of SS-31 was able to inhibit the increase in skeletal muscle mitochondrial H_2O_2 production following a high fat diet in rats, and this prevented the development of insulin resistance (9). Similar findings were seen in mCAT mice, demonstrating that SS-31 can mimic the protective effect of mitochondrial catalase overexpression.

Finally, and most relevant to cardiac aging, SS-31 was able to provide the same protection as the mCAT mice against angiotensin and Gαq-induced cardiomyopathy (69). Using the same angiotensin model described earlier for inducing cardiac failure in mice, it was found that treatment with SS-31 significantly prevented LVH and diastolic failure, while treatment with the untargeted antioxidant, NAC, provided no protection (Fig. 12) (66). These results highlight the importance of antioxidant delivery to mitochondria in order to provide protection against mitochondrial oxidative stress.

These preclinical studies support the therapeutic potential of the SS peptides against age-related decline in physiological function, especially for cardiac aging. Results from a completed first-in-human Phase 1 clinical trial demonstrated linear pharmacokinetics and good tolerability in normal human subjects. Phase 2 clinical trial with a modified form of SS-31 in patients with first-time onset of acute ST elevation myocardial infarction is now ongoing.

C. Resveratrol and SIRTs activators

The results in invertebrate models, early data from mammals, and the potential of SIRTs as a DR-mimetic have provoked strong research interest to search for Sirt activators. Among several small molecules that activate SIRTs, resveratrol has been identified as one of the most potent SIRT1 activators (131). Resveratrol (3,5,4′-trihydroxystilbene) is a natural compound found in grapes and red wine. It was widely believed to be the active compound that explains the cardioprotective effects of red wine. Resveratrol was reported to have anti-inflammatory effects, protect against cardiac IR injury and brain ischemia. This protection might be attributed to the effect of resveratrol to prevent platelet aggregations (339), attenuate atherosclerosis (340), promote vasodilation, enhance endothelial NO signaling (184,243), induce antioxidant activity, and reduce LDL oxidation (88,93,211)[see review (24)].

Dietary resveratrol was found to retard several aging-related physiological changes in aged mice, including cardiac diastolic dysfunction, impaired myocardial performance, and insulin resistance (20). Furthermore, resveratrol effects on global transcriptional profiles overlap strikingly with those

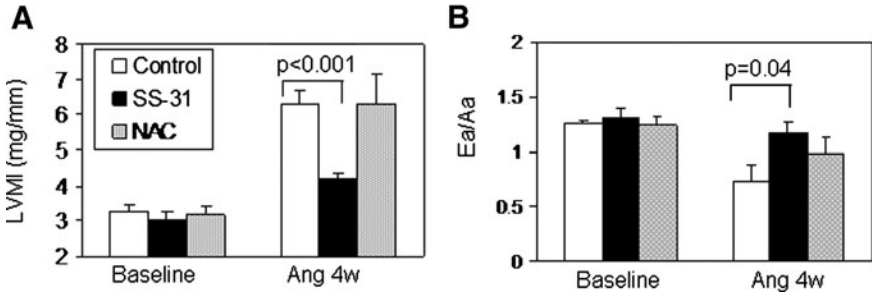

FIG. 12. Mitochondrial-targeted antioxidants SS-31 in Ang II-treated mice. (A). Left ventricular mass index (LVMI), **(B)**. Diastolic function measured by Ea/Aa at baseline and after a 4 week exposure to Ang II. SS-31, but not NAC is protective of cardiac hypertrophy (LVMI) and diastolic function (Ea/Aa). NAC, N-acetyl cysteine.

found after DR in heart, skeletal muscle, and brain (20). To translate these encouraging experimental data into clinical practice, several phase 1 and phase 2 clinical trials are being performed. These trials investigate the safety and adverse effect, pharmacokinetics and pharmacodynamics, as well as efficacy of resveratrol in apparently healthy adults, with the clinical outcomes of type 2 diabetes, obesity, Alzheimer disease, and cancer [See review in (251,252)].

Sirt activators promise to provide benefits similar to DR by activating the protein deacetylases that are involved in metabolic remodeling (46,148,149,349). However, the efficacy of these drugs *in vitro* has been controversial following the discovery that Fleur de Lys assay used to screen these drugs might be prone to artifacts. While the importance of SIRTs in metabolic responses and cardiac aging and disease is clearly supported by a number of reports (83,115,120,255,285–287,307,308,357), the ability of currently recognized Sirt activators to modulate these processes remains to be determined (207).

X. Conclusion and Future Directions

CVDs are the leading causes of death in North America and most of the western hemisphere. As just discussed, old age is a significant risk factor for CVDs. Since the number of the elderly in the United States is predicted to double in the next 25 years, by the year 2030, there will be more than 70 million elderly persons or about 20% of the population. This tremendous increase in the aging population underscores the need to develop therapeutic strategies that prevent myocardial dysfunction in the elderly, especially LVH and diastolic dysfunction. Hypertension and old age are the most common causes of LVH, which increases the risk of coronary heart disease, congestive heart failure, stroke, and sudden death. Understanding the molecular mechanism of hypertrophy in aging and pressure-overload hearts will assist the development of strategies to prevent or ameliorate cardiac hypertrophy and failure, or even to delay cardiac aging changes. The roles of mitochondrial ROS, insulin-IGF-PI3K, catecholamine, and nutrient signaling have been just discussed. Further studies are needed to elucidate the complex interactions between mitochondrial ROS, SIRTs, mTOR, Ca^{2+}, and other cellular signaling.

Although clinical trials applying antioxidants to attenuate the progression of CVDs have been disappointing (305), these may not be the optimal therapeutic agents. As just discussed, nontargeted antioxidants are not effective in the prevention of Ang II-induced cardiac hypertrophy or Gαq overexpression-induced heart failure. However, there are now several promising mitochondrial-targeted small molecule antioxidants, including mitochondrial-targeted ubiquinone (MitoQ), and SS-31 peptide antioxidants (2,9,328). Other mitochondrial targeted mechanisms, such as cyclosporine to block the opening of mitochondrial permeability-transition pore (256), are also attractive treatment strategies. Further clinical trials are necessary to study the potential application of mitochondrial-targeted therapeutics in the treatment or prevention of cardiac aging, hypertensive cardiomyopathy, and heart failure.

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Author Disclosure Statement

H.H. Szeto is the inventor of SS-31 and the Cornell Research Foundation (CRF) holds several patents covering the SS peptides and a patent application has been filed for the findings described in this article, with H.H. Szeto and P.S. Rabinovitch as inventors. CRF has licensed the SS peptide technology for further research and development to a commercial enterprise in which CRF and H.H. Szeto have financial interests.

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Abbreviations Used

AMPK = adenosine monophosphate-activated protein kinase
Ang = angiotensin II
ANP = atrial natriuretic peptide
ATR1 = angiotensin II receptor 1
BNP = brain natriuretic peptide
CNP = C-natriuretic peptide
CVD = cardiovascular diseases
DR = dietary restriction
ECM = extracellular matrix
ETC = electron transport chain
FoxO = forkhead box O
GPX = glutathione peroxidase
GR = glutathione reductase
GSH = glutathione
HIF-1 = hypoxia inducible factor 1
HDL = high density lipoprotein
IGF = insulin-like growth factor
IMM = inner mitochondrial membrane
IR = ischemia-reperfusion
LDL = low density lipoprotein
LV = left ventricular
LVH = left ventricular hypertrophy
LVMI = left ventricular mass index
MAC = mitral annular calcification
mCAT = catalase targeted to mitochondria
MitoQ = ubiquinonyldecyl triphenylphosphonium
MMP = matrix metalloproteinase
MPI = myocardial performance index
mPTP = mitochondrial permeability transition pore
mTOR = mammalian target of rapamycin
NAC = N-acetyl cysteine
NADH = nicotineamide adenine dinucleotide
NADPH = nicotineamide adenine dinucleotide phosphate
NCHS = National Center for Health Statistics
NHLBI = National Heart Lung and Blood Institute
NO = nitric oxide
NOX = NADPH oxidase
OMM = outer mitochondrial membrane
pCAT = peroxisomal catalase
PGC-1α = peroxisome proliferator-activated receptor gamma coactivator-1 alpha
PKC = protein kinase C
Polg = mitochondrial polymerase gamma
RAAS = renin angiotensin aldosterone system
ROS = reactive oxygen species
SERCA2a = sarcoplasmic reticulum calcium-ATPase
Sirt = sirtuins
SkQ = plastoquinonyldecyl triphenylphosphonium
SOD = superoxide dismutase
SS = peptides szeto-schiller peptides
TAC = transverse aortic constriction
TOR = target of rapamycin
TORC = target of rapamycin complex
TRX = thioredoxin
VEGF = vascular endothelial growth factor