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Views From Within and Beyond

Narratives of Cardiac Contractile Dysfunction Under Senescence

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Senesecence is associated with enhanced risk of cardiovascular diseases. It is generally considered that decline in growth hormones (such as insulin-like growth factor I), intrinsic myocardial and endothelial functions, as well as accumuation of reactive oxygen species with increased age may contribute to cardiovascular senescence. It is believed that heart function, especially cardiac reserve declines with advanced age. However, most experimental and clinical investigations on ventricluar function only included young or adult subjects and failed to address this important age issue in heart pathophysiology. Although senescent but otherwise healthy hearts may possess normal pumping function at the resting or non-stressed state, some aging-associated factors such as accumulation of reactive oxygen species and activation of selective stress signaling pathways may interact with certain risk factors and compromise overall cardiac function. The precise cause and progression of compromised cardiac function in the elderly remain controversial. This review will focus on senescene-related alterations in cardiac contractile function with a special emphasis on oxidative stress and activation of stress signaling.

Key Words: Age; stress signaling; cardiac, contractile function.

Introduction

With the advance in medical science and improvement in health care, the average life-span of human beings is increasing. In 1900, about 1 in every 25 Americans were considered elderly (individuals aged 65 and older) in comparison to 1 in every 8 in 2000 *(1,2)*. This ratio is predicted to reach 1 in every 6 by year 2020 *(1,2)*. Advancing age or senescence, *per se,* is a major risk factor for cardiovascular disorders such as coronary artery disease, hypertension, obesity, insulin resistance (metabolic syndrome), congestive heart failure, atherosclerosis, and stroke. The overall incidence of these cardiovascular problems is likely to rise steeply with increased age. Cardiovascular diseases continue to be the leading cause of death despite the progressively relative reduction in deaths from cardiovascular diseases with aggressive medical management *(3)*. The age profile of the population appears to switch from a pyramidal distribution in which the young are predominant, to a columnar structure in which the adults and the elderly gradually become prevalent with prolonged life expectancy *(1,2,4)*. This change in demographic distribution of the population, which adds to increased life-span, enhances the duration and propensity of exposure to "extrinsic" cardiovascular risk factors. This may be one main reason why cardiovascular disease such as coronary heart disease, myocardial infarction, hypertension, and stroke still remains the leading cause of mortality. If the satiety life-style factor is considered due to the 21st century industrialization and urbanization, this synergy between aging and cardiovascular risk becomes much more pronounced. The foundation for the cardiovascular disease epidemic in middle-aged and elderly adults is largely based on both the amount and duration of exposure to cardiovascular disease risk factors and changes in intrinsic cardiovascular components due to the aging process *(1,4)*.

Several scenarios have been put forward depicting the possible mechanism of senescence. However, at present, no single theory may adequately explain the host of complex biochemical and pathological facets of aging. One so-called "evolutionary theory of aging" envisioned that human longevity is related to the cost of impaired reproductive success *(5)*. The advanced vision of this theory, "antagonistic pleiotropy theory of aging," is based on facts that natural selection has favored genes conferring short-term benefits to the organism at the cost of deterioration in qualify of health condition in later life *(6)*. The most widely accepted theory, the predominant one discussed in our review, is the "free radical theory of aging," in which the progressive accumulation of oxidant insult during life span results in oxidative stress leading to ultimate biological events of senescence *(7)*. This theory has paved the way to the modern "mitochondrial theory of aging" *(8),* which has been fur-

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ther developed as the new concept of "mitochondrial–lysosomal axis theory of aging" *(9,10)*. The "glycation theory of aging" implicates advanced glycation endproducts (AGEs) formed by reaction between reducing sugar and biological amines (Millard reaction) as the major cause of complications of aging *(11)*. Age-related increases in oxidative stress and protein glycation either individually, or more probably, in a synergistic manner could account for aging-induced organ damage including hearts. This review will focus on senescene-related alteration in cardiac contractile function with a special focus on oxidative stress and activation of specific stress signaling.

Senescence and Myocardial Electromechanical Function

There is a growing body of evidence showing alterations of cardiac structure and function emerging with advancing age *(12)*. Observable increases of left ventricular hypertrophy, heart failure, and atrial fibrillation with advanced age are common characteristics of cardiac senescence *(12)*. The prevalence of left ventricular hypertrophy increases with blood pressure and body mass index *(12,13)*. In another study, it was shown that aging causes cardiac myocyte enlargement without an overt increase in left ventricular mass *(14)*. In these studies, however, an increase in collagen was observed in the aging myocardium *(14)*. It has been increasingly evident that the development of heart failure with apparently preserved systolic function (as evidenced by a normal ejection fraction), occurs in 30–50% of older patients with heart failure *(15)*. However, this apparently preserved cardiac function may be attributed to a compensatory increase in sympathetic outflow to the heart. Reduced intrinsic contractile function and structural changes within left ventricular myocardium has been demonstrated in senescence, which results in reduced early diastolic filling in the left ventricle *(16)*. Nevertheless, ventricular ejection fraction, the essential clinical measure of left ventricular systolic performance, is often preserved during the aging process *(17)*, probably due to enlarged end diastolic volume of left ventricle. Although resting heart rate in the supine position does not change with age *(17)*, heart rate increases less significantly in older than in younger individuals when they switch from supine to seated position *(18)*. Atrial fibrillation is detected in approx 3–4% of healthy volunteers over the age of 60 who are rigorously screened to exclude clinical coronary artery disease. This is a rate 10-fold higher than that observed in the general adult population *(19)*. Impaired systolic and diastolic ventricular contractile functions are both characteristic of aging-related cardiomyopathy *(20)*. Myocardium and ventricular myocytes from aging hearts exhibit reduced contractility, prolonged duration, and slowed rate of contraction and relaxation *(20)*. These observations are correlated with prolonged action potentials, depressed cytosolic Ca^{2+} reuptake by sarcoplasmic reticulum (SR), isozyme switch from α- to β-myosin heavy chain *(21,22)*, alteration in cardiac troponin T expression, and troponin I phosphorylation *(4),* all related to oxidative stress. Consistent with the hypothesis that insulin/IGF-1 are important regulators of biology and physiology of the aging process *(23)*, myocytes from cardiac IGF-1 overexpressing mice showed higher contractility and velocity of contraction and relaxation in all ages than those from age-matched wild-type mice *(24)*.

Using young (10- or 12-wk-old) and older (25- or 36-wk-old) Wistar–Kyoto rats (WKY) and spontaneously hypertensive rats (SHR), we found that advancing age did not affect blood pressure whereas it significantly reduced the size of liver and kidney when normalized to respective body weight *(25)*. Advanced age seems to facilitate the intracellular Ca^{2+} clearing measured by Ca^{2+} fluorescence decay time probably due to increased intracellular Ca^{2+} load at rest or Ca^{2+} overload (i.e., rise in resting intracellular Ca^{2+} level) with advancing age *(25)*. Aging did not affect myocardial force generating capacity, although myocardium tended to develop less tension when the peak tension developed was normalized to papillary muscle weight *(25)*. Duration of both contraction and relaxation was prolonged by advanced age as comparable to those of young animals *(25)*. Neither advanced age nor hypertension altered maximal velocity of contraction and relaxation *(25)*. Myocytes from 36-wk-old WKY rats exhibited an enhanced shortening capacity (peak shortening amplitude) associated with significantly reduced duration of shortening (systole) but not relaxation (diastole) compared to younger (12-wk-old) animals *(25)*. Advanced age displays striking similarities to the myocardial alterations associated with hypertension, including a thickened left ventricular wall and prolonged contraction duration. Both advanced age and hypertension may predispose to increased risk of hemorrhagic stroke and myocardial infarction and may be accompanied by cardiac hypertrophy, subnormal cardiac output, and an elevated total peripheral resistance *(25)*.

Ventricular myocytes isolated from 36-wk-old but not 12-wk-old SHR rat hearts exhibit considerably larger dimension (either cell length or surface area) compared to those of the age-matched WKY rats *(26,27)*. Advanced age alone has little effect on cell dimension. Neither cell length nor surface area is affected by IGF-1 exposure *(26,27)*. Myocytes isolated from 12-wk-old animals display similar extent of shortening capacity as indicated by peak contraction amplitude *(26,27)*. However, peak contraction amplitude is significantly greater in myocytes from 36-wk-old SHR compared to those from the age-matched WKY. The shortened duration of contraction/relaxation in myocytes from older SHR rats exhibits increased maximal velocities of contraction and relaxation. Interestingly, the myocyte shortening capacity decreases with advanced age *(26,27)*. The contraction

Table 1
Effect of Aging on Intrinsic Ventricular Contractile Properties in Myocardium and Isolated Myocytes[a]

Species	Age	Preparation	Maximal contractility	Velocity of contraction	Velocity of relaxation	Duration of contraction	Duration of relaxation
WKY rats	25 wk	Papillary muscle	N.C.	N.C.	N.C.	Prolonged	Prolonged
WKY rats	36 wk	Papillary muscle	N.C.	N.C.	N.C.	Prolonged	Prolonged
WKY rats	36 wk	Myocytes	Increased	Increased	Increased	Shortened	N.C.
WKY rats	36 wk	Myocytes	Decreased	N.C.	N.C.	Shortened	N.C.
FVB	24 mo	Myocytes	N.C.	N.C.	N.C.	N.C.	Prolonged

[a]Wk = weeks of age; mo = months of age; N.C. = no (significant) change; number in parenthesis indicates the source of reference citation. The apparent discrepancies between different studies may be associated with strain of animals (sometimes even different vendors) and experimental conditions such as isotonic contraction versus isometric contraction.

and relaxation durations were not affected by early stage of hypertension and were reduced with advanced age *(26)*. Moreover, myocytes from older SHR rats exhibit a prolonged contraction duration along with a normal relaxation duration as compared to the age-matched WKY rats *(26, 27)*. Alterations in nitric oxide modulation of IGF-1–induced contraction may underlie resistance to this inotropic peptide with advancing age and/or hypertension *(26,27)*. Our recent studies indicated that senescent myocytes displayed a larger cell cross-sectional area, prolonged shortening duration, normal peak contraction, maximal velocity of contraction and relaxation, as well as relaxation duration compared with young myocytes *(28)*. In addition, senescent myocytes did not tolerate well high stimulus frequency (from 0.1 to 5 Hz) compared to young myocytes, an indicative of reduced cardiac reserve during stress such as exercise *(28)*. A summary table has been provided to recapitulate the essential findings of cardiac intrinsic mechanical properties among different studies using rats or mice mentioned above (Table 1).

A newly emerged experimental model reminiscent of the aging effect on myocardial function due to oxidative stress is cardiac ischemia/reperfusion *(29,30)*. It is well known that the production of oxygen radicals increases in the myocardium as a result of ischemia/reperfusion although recent studies have shown that enhanced ischemia/reperfusion injury in aged rat hearts may be related to reduced antioxidative capacity, rather than increased reactive oxygen species production *(31)*. Aging is associated with an increase in myocardial dysfunction during reperfusion. It is expected that manifestation of worsened myocardial function during reperfusion should be more evident in aged animals when compared to their adult counterparts if mitochondria and free radicals play a significant role in alteration of function during cardiac ischemia/reperfusion *(29)*. Results from an isolated rat heart model indicate that ischemia–reperfusion induced decline in NADH-linked ADP-dependent respiratory rate is more pronounced in senescent rats. Preexisting age-related alterations in electron transport chain components, oxygen radical generation, pro-tein conformation and susceptibility, and/or antioxidant and stress response elements likely predispose senescent animals to greater loss in mitochondrial and enzyme activities *(29)*. Brief episodes of ischemia in young hearts caused early changes in expression of genes related to remodeling, which primarily represented a down-regulation phenomenon *(30)*. Young ischemic hearts demonstrated down-regulation of gene expression associated with early-remodeling including down-regulation of tissue inhibitor of metalloproteinase 1, decorin, collagen, tropoelastin, and fibulin, as well as decreases in hypertrophy-related transcripts *(30)*. In contrast to the changes in young hearts, old hearts showed a unique injury-related response that included up-regulation of numerous hypertrophy and apoptosis-related genes. Ischemia/reperfusion, which is thought to be otherwise protective in nature, may have opposite effects in the aging heart by promoting expression of deleterious genes *(30)*. Recently, this model has been used for studying the effect of *p66(ShcA)* gene on aging *(32)*.

Although only a few studies concerning the influence of age on chronic hypoxia-induced structural and functional alterations in the cardiovascular system have been carried out, there is an interesting paper that investigates the effects of aging on the cardiac remodeling induced by chronic high-altitude hypoxia in rats *(33)*. In this study, the extent of right ventricular hypertrophy diminished with age. Right ventricular cell size and pericellular fibrosis showed a significant increase in the 2- and 6-mo-old rats exposed to hypoxia compared to control *(33)*. The remodeling at morphological and electrophysiological levels induced by chronic hypoxia in the right ventricle can be decreased by the natural aging process *(33)*.

Myocardial aging could depend on attenuation of cell growth with accumulation of old cells *(34)*. At the cellular level, imbalance between myocyte growth and death can result in a premature increase in number of senescent myo-

cytes with depressed contractile performance *(34)*. Aged diseased hearts had moderate hypertrophy and dilation, accumulation of $p16^{INK4a}$ (a tumor suppressor protein and a key biomarker for cellular senescence), positive primitive cells and myocytes, but no structural damage *(34)*. Cell death markedly increased and occurred only in cells expressing $p16^{INK4a}$ *(34–36)*.

The Aging Endocrine System and Cardiac Dysfunction

Hormonal Aging

The endocrine system is one of the most sensitive organ systems to reflect advanced age. The function of many hormones such as growth hormone and sex hormones decreases drastically with increasing age *(37–39)*. Aging is believed to affect the endocrine system in several ways including the abundance of endocrine cells, the amount of hormones secreted, and the level and/or affinity of hormonal receptors or post-receptor components in target cells. Insulin receptor /IGF-1 receptor (IR/IGF1R) play an important role in the endocrine circuit of human and other mammals *(40)*. It has been shown that $Prop1^{df}$ (Ames Dwarf mice) and Pit^{dw} (Snell mice) hamper pituitary production of growth hormone, thyroid stimulating hormone, and prolactin (PRL) while the adult life span was increased by 40–60% in these mice *(41,42)*, suggesting a link between reduction in growth hormone (and IGF-1) and life span *(40,42–45)*. In another study, reduced thyroid-releasing hormone or diminished overall thyroid function was observed in Ames Dwarf or Snell mice *(46)*. Growth-hormone-receptor knock-out mice, which show extended life span, also display reduced levels of insulin, glucose, and body core temperature associated with hypothyroidism *(47)*. Reduced synthesis of thyroid hormone is a characteristic of pituitary mutant with retarded aging *(40)*. It is believed that this hormone regulates energy homeostasis and fatty acid metabolism *(48)*. Growth hormone/IGF-1–deficient Ames dwarf mice live longer than their wild-type counterparts. Nevertheless, data from our laboratory revealed that the cardiomyocyte contractile function is significantly reduced in Ames dwarf mice compared to their age-matched control mice *(49)*. It is worthy pointing out that the multiple endocrine deficiencies and developmental anomalies inherent in these models may have confounded the interpretation of aging and endocrine as well as cardiac function *(50,51)*. The effect of aging on endocrine function appears to be complex involving many endocrine systems such as the hypothalamic neuroendocrinal function *(52)* and is beyond the scope of the present review.

Sex Hormones and Heart Dysfunction

Similar to decrease in growth hormone and IGF-I, both male and female sex hormones, testosterone and estradiol, decline with aging *(39,53–55)*. Sex hormones, which are associated with the leuteinizing-hormone/follicle-stimulating-hormone (LH/FSH) system, have been implicated to play a role in cardiac function and onset of heart diseases *(56, 57)*. The levels of estrogen undergo a precipitous decline with aging in women *(58,59)*. Estrogen was demonstrated to attenuate myocardial hypertrophy and left ventricular remodeling *(60,61)*. Our studies indicated that 17β-estradiol (but not phytoestrogen α-zearalanol) has a direct cardiac stimulatory action in ventricular myocytes, likely through enhanced intracellular Ca^{2+} release *(54)*. Mechanical and protein functions of ventricular myocytes are directly regulated by 17β-estradiol *(55)*. Cardiac myocytes from rats exhibited myocardial dysfunctions when exposed to hyperglycemic media as compared to the ones exposed to euglycemic media *(62,63)*. These hyperglycemia-induced cardiac dysfunctions were reversed or abolished by phytoestrogens such as daidzein or genistein, indicating that phytoestrogenic isoflavones may protect against glucose toxicity-induced cardiac mechanical dysfunction *(63)*. Estrogen has been shown to directly affect the cardiac function in certain disease conditions under aging. For example, estrogen, similar to the superoxide dismutase mimetic EUK-8 was shown to markedly improve cardiac functional recovery after ischemia/reperfusion injury in the aged female rats *(64)*.

Both total and bioavailable levels of testicular androgen production decrease steeply in men due to increase in circulating sex hormone binding globulin with age *(65)*, as serum testosterone concentration declines *(66)*. However, sole testosterone exposure does not shorten life span in men and women *(67)*. One of the models used for cardiomyopathy and heart failure is the transgenic mouse with cardiac-specific overexpression of β2-adrenergic receptors. In this model system female mice had less cardiac remodeling, dysfunction, and pathology and a marked survival advantage over male mice, indicating a contribution by testicular hormones to the progression of the cardiomyopathic phenotype *(56)*. Deprivation of testicular hormones by castration during 3–15 mo of age improved survival and significantly ameliorated left ventricular dysfunction, remodeling, and hypertrophy compared with intact cardiac overexpression of β2-adrenergic receptors transgenic male mice *(56)*.

Growth hormone regulates cardiovascular function and its deficiency has been postulated to be one of factors responsible for cardiovascular diseases *(68,69)*. There are several lines of evidence suggesting that IGF-1 and growth hormone improve cardiac function in experimental heart failure *(70–73)*. Long-term growth hormone administration restores the impaired contractile reserve in myocytes from rats with postinfarction heart failure *(72)* and prolongs survival of rats with postinfarction heart failure *(73)*. Upregulation of ventricular protein levels of SERCA2 in myocytes *(72)* or marked attenuation of myocyte apoptosis *(73)* may be potential molecular mechanisms involved in this process. Gender difference exerts impact on basal and IGF-1–regulated nitric oxide synthase activity in adult rat ventricular myocytes *(74)*. Overexpression of IGF-1 prevents streptozo-

tocin-induced cardiac contractile dysfunction and β-adrenergic response in ventricular myocytes *(75)*. This is consistent with the observation that exogenous IGF-1 supplementation attenuates diabetes-induced cardiac contractile dysfunction in ventricular myocytes *(76)*. The loss of IGF-1 and its binding protein, insulin-like binding protein-3, has also been implicated in growth defects and retardation in nephrotic children *(77)*.

Lipoprotein (a), which is associated with hormone metabolism, is an independently atherogenic lipoprotein that can be thrombogenic and may be used as plasmatic marker for individuals at risk for cardiovascular events *(78)*. Insulin treatment of patients with type 2 diabetes increased lipoprotein (a) *(79,80)*, whereas estrogens reduced lipoprotein (a) levels by 10% in menopausal women *(81,82)*. Growth hormone–treated adolescents had lower concentrations of lipoproteins (a) in their plasma compared to untreated group *(83)*. Growth hormone–deficient adolescents had elevated lipoproteins (a) levels, compared with healthy counterparts *(83)*. Although these authors failed to detect any difference in cardiac mass or function or early atherosclerotic changes in those adolescence patients, cardiovascular risk factors such as dyslipidemia does exist in these patients, which may contribute to increased cardiovascular morbidity at an early age. An increased circulating level of lipoprotein (a) is a well-recognized risk factor for coronary artery disease. A dose-dependent relationship between IGF-1 administration and lipoprotein (a) reduction in patients with Laron syndrome was observed *(84)*. Long-term growth hormone treatment increases and IGF-1 decreases circulating levels of lipoprotein (a), which may have clinical relevance in view of the increasing use of human GH in children and adults and the role of lipoprotein (a) as a cardiovascular disease risk factor *(85,86)*.

Sexual Dimorphism in Cardiac Senescence

It is commonly recognized that female life expectancy is longer than males probably due to a lower cardiovascular risk *(87)*. The female sex hormone estrogen is considered to be responsible for the reduced cardiovascular risk because women exhibit a dramatic rise in coronary heart disease during the post-menopausal period compared to age-matched men. This idea was further supported by the fact that postmenopausal women become more resistant to coronary heart disease when they receive estrogen-replacement therapy *(88)*. Because the female reproducitve organs exhibit a much faster rate of aging compared to other organ systems *(89)*, it is natural that sex hormones or reproductive factors may be treated as important contributors to senecence according to evolutionary theory of aging *(5)*. In addition to hormonal factors, recent evidence suggested that myogenic as well as autonomic regulation of heart function may also play a significant role in the sexual dimorphism of cardiac senescence. It has been demonstrated that both cardiac contractile and peripheral vascular dynamic responses to β-adrenergic stimulation are preserved in old female but not old male monkeys *(90)*, which may explain, in part, the reduced cardiovascular risk in the older female population. Observations from our lab did not reveal any significant gender-related differences in myocardial contractile function and intracellular Ca^{2+} handling under senescence *(91)*. Interestingly, myocytes from young (premenopasual) female diabetic animals possess better-preserved contractile function compared to age-matched male diabetic mice. However, this "female advantage" disappeared in older (presumably postmenopausal stage) female diabetic mice *(91)*, indicating the significance of sex hormone to intrinsic myocardial function. The gender difference in IGF-1/IGF-1 receptor system is also believed to contribute to sexual dimorphism of cardiac senescence. Leri and colleagues measured expression of IGF-1, extracellular α- and transmembrane β-subunits of IGF-1R in left ventricular myocytes isolated from male and female Fischer 344 rats at different ages. They found aging triggered an 83%, 84% decrease, and complete disappearance of IGF-1, IGF-1R-α and IGF-1R-β, respectively, in myocytes from males. In contrast, the corresponding changes in the levels of IGF-1, IGF-1R-α, and IGF-1R-β in myocytes from females were only 40%, 28%, and 43%, respectively. These molecular changes in single myocytes reflected those found in ventricular tissue injury involving multiple foci, indicating that enhanced IGF-1-IGF-1R system may favorably condition the myocytes isolated against female rats form aging *(92)*.

Senescence Difference in Myofilament Ca^{2+} Responsiveness

Down-regulation in the expression of sarco (endo) plasmic reticulum Ca^{2+}-ATPase (SERCA) has been associated with the aging myocardium *(4)*. In addition, aging-related decline in function of SERCA and other SR Ca^{2+} transport proteins such as SR Ca^{2+} release channel and phospholamban has also been reported, which was attributed to post-translational modification (tyrosine phosphorylation) of these proteins *(4,93)*. The notion of alteration in protein expression and function of cardiac contractile or Ca^{2+} regulatory proteins in senescent hearts received support from observation from prolonged action potential duration in aged myocytes *(94)*. However, it remains unclear whether changes in action potential duration affect intracellular Ca^{2+} regulation or simply a phenomenon resulted from intracellular Ca^{2+} regulation in senescent ventricular myocytes *(94)*. Developed cytosolic Ca^{2+} was higher in IGF-1 cardiac overexpressing mice than wild-type myocytes at a high rate of stimulation. L-type Ca^{2+} current differed in amplitude, being higher in young and old transgenic mice than in wild-type mice, indicating that transgenic myocytes have a more efficient SR Ca^{2+} reuptake and improved diastolic relaxation *(24)*.

The age-related changes of the Ca^{2+} transients are thought to cause systolic and diastolic failure if senescent mouse hearts beat at high frequencies *(95)*. Ca^{2+}-binding activity of heart mitochondria does not change with aging of rats *(96,97)*. The neuronal Ca^{2+} channels from older rats are more susceptible to blockade than those from younger rats *(98)*. The diminished ability to efficiently generate cellular ATP may ultimately underlie the loss of calcium homeostasis and cellular function during aging *(99)*. Modest but sustained increase in Ca^{2+} influx triggers a coordinated remodeling of Ca^{2+} handling to maintain Ca^{2+} homeostasis *(99)*.

Senescence Difference in Neuroregulatory System of the Heart

Aging has been shown to upregulate the activity of several neuroendocrinal systems including renin–angiotensin, adrenergic, endothelin, and nitric oxide systems *(100)*. The autocrine/paracrine action has been demonstrated with the sympathetic nervous stimulation in human hypertension and heart failure *(101)*. Deficits in myocardial β-adrenergic receptor signaling cascade occur with aging. A reduced myocardial contractile response to either β_1-adrenergic receptor or β_2-adrenergic receptor stimulation is observed with aging *(102–104)*. The major limiting modification of this signaling pathway that occurs with advanced age seems to be the coupling of the β-adrenergic receptor to adenylyl cyclase via the G_s protein and changes in adenylyl cyclase protein, which lead to a reduction in the ability to sufficiently augment cellular cAMP and to activate protein kinase A to drive the phosphorylation of key proteins that are required to augment contractility *(104)*. In contrast, the apparent desensitization of β-adrenergic receptor signaling that occurs with aging does not seem to be mediated via increased β-adrenergic receptor kinase (βARK) or increased G_i activity *(104)*. A blunted response to β-adrenergic receptor stimulation of the cells within older myocardium can, in one sense, be viewed as adaptive with respect to its effect to limit the risk of Ca^{2+} overload and cell death in these cells.

Aging increases human sympathetic nervous activity at rest *(100,105)*. Although the increase in total norepinephrine spillover to plasma with mental stress is unaffected by age, the increase in cardiac norepinephrine spillover is two to three times higher in the older subjects *(100,105)*. With advancing age the sympathetically mediated stress on the body, specifically the cardiovascular system, may outweigh the benefits an intact sympathetic nervous system conveys for short-term survival *(106)*. β-Blockers temper the effects of the sympathetic nervous system by slowing heart rate and decreasing blood pressure. Recently, β-blockers have been shown to improve outcome and survival following surgery and myocardial infarction and have beneficial effects in patients with heart failure, and may have an antiatherosclerotic effect *(106)*. Instituting β-receptor blockade pharmacotherapy at an early age will increase longevity by countering the adverse effects of sympathetically mediated stress *(106)*.

The effects of norepinephrine on protein phosphorylation in rat cardiac ventricular myocytes were determined by autoradiography of ^{32}P-labeled proteins separated by electrophoresis *(102,107)*. Cells from young adult rats (6-mo-old) exhibited a marked increase in the norepinephrine-mediated incorporation of ^{32}P into proteins identified as troponin I and C-protein; in cells from senescent rats, this increase is much attenuated *(102,107)*. Age-associated decrements in protein phosphorylation are much diminished when maximally effective concentrations of the adenylate cyclase activator forskolin and the cAMP analog 8-(4-chlorophenylthio)-cAMP are used instead of norepinephrine *(102,107)*. Moreover, age-associated differences are abolished if the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine was present in addition to norepinephrine, or alone *(102,107)*. The development of increased levels of reactive nitrogen species and antioxidative enzymes with the onset of β-amyloid plaque deposition provides further evidence that developmentally and aging-induced alterations in brain oxidative status exhibit a major factor in triggering enhanced production and deposition of β-amyloid, and potentially predispose to Alzheimer's disease *(108)*. Aging increases the susceptibility of cerebral and peripheral blood vessels to β-amyloid–related dysfunction and that functional change precedes structural change *(108,109)*.

The peripheral sympathetic nervous system activation is intended to increase β-adrenergic thermogenesis in order to expend excess energy as heat rather than by storage of fat. Recent evidence, however, indicates that these adjustments are not effective in augmenting energy expenditure with aging *(110)*. Indeed, older sedentary adults demonstrate reduced, not increased, β-adrenergic stimulation of metabolic rate because of reduced tissue responsiveness, presumably mediated by sympathetic nervous system–induced impairment of β-adrenergic signaling *(110)*. As a result, age-associated peripheral sympathetic nervous system activation that initiates as a consequence of accumulating adiposity with the intent of preventing further fat storage, ironically, may in time evolve into a potential mechanism contributing to the development of obesity with aging *(110)*. With aging, sympathetic activation was evident in the heart, and the gut and liver at rest *(111)*. The mechanism appears to be mediated via activation of sympathoexcitatory noradrenergic suprabulbar projections from brainstem resulting augmented sympathetic nervous responses *(111)*. Conversely, adrenal medullary release of epinephrine is subnormal in the elderly, at rest and during exercise stress *(111)*. A disparate sympathetic nervous activation is also present in older men *(101)*. Overall, levels of both cardiac and hepatomesenteric norepinephrine spillover are directly correlated with subcortical norepinephrine turnover *(101)*. These findings suggest that sympathetic nervous system overactivation usually accompanies aging, similar to enhanced sympathetic nervous stimulation in hypertension and heart failure. Although the mechanism of action responsible for

such enhanced sympathetic tone under aging is not fully elucidated, influence of sympathoexcitation from the noradrenergic projections to suprabulbar subcortical regions was suggested to play a role *(101)*.

Ag(e)ing vs AGEs

In addition to alteration in cardiac contractile proteins and intracellular Ca^{2+} homeostasis due to aging, several additional factors have also been implicated to play a role in the aging-related compromised ventricular function. Proteins can be damaged by accumulation of free radicals and/or by inappropriate glycation. Glycation or the Maillard reaction is non-enzymatic glycosylation in which sugar molecules become anchored onto functional proteins *(112)*. This reaction starts with the formation of a double bond between glucose aldehyde group (the carbonyl group) and primary amino group of proteins with the elimination of a water molecule. The double bond formed between the glucose carbon and the nitrogen is usually an imine (a Schiff base). The Schiff base can quickly re-arrange atoms such that the 2-carbon of the glucose loses its two hydrogen ions, resulting in a carbonyl and in hydrogen saturation of the carbon and nitrogen that formerly constituted the imine. The re-rearranged structure is called an Amadori product, which is further modified by other insults such oxidative reaction. The final product is called advanced glycation end-products (AGEs). Both glycation and Amadori product formation are completely reversible reactions, but the formation of AGEs by oxidation or other reactions of Amadori products is irreversible. AGE cross-linking of peptides and proteins is protease-resistant and may cause irreversible oxidative or lipid peroxidative damage to different cells, such as macrophages/monocytes, cardiac myocytes, fibroblasts, and vascular smooth muscle cells. Increasing evidence suggests a role for AGE-induced oxidative stress in the pathogenesis of normal aging and age-related diseases, including diabetes *(112)*. Accumulation of AGEs during the aging process occurs in long-lived proteins, such as collagens, lens crystalline, and cartilage. Therefore, AGE in essence is a sugar-derived post-translational modification of functional proteins *(113)*. AGE deposits have been immunolocalized in skin, lung, kidney, intestine, intervertebral disks, as well as in heart and arteries of aged and diabetic patients *(114)*. Furthermore, the pathophysiological significance of AGE-induced oxidative stress is supported by animal and clinical studies showing an improvement in arterial and cardiac compliance after treatment with ALT-711, a breaker for AGE-cross-linking *(115)*.

A number of receptors that bind AGEs, including AGE-R1, AGE-R2, AGE-R3, the scavenger receptor II, and the receptor for AGEs (RAGE), were identified in aging human heart *(116–118)*. In a recent study, Western blot and RT-PCR analysis of the AGE receptors from the cardiac auricles in senescent and adult patients were compared. While the expressions of AGE-R3 as well as RAGE protein were significantly upregulated in senescence, only the upregulation of RAGE was associated with reduced heart function *(116–118)*. These results support a pathophysiological function for RAGE in cardiac senescence. Among all AGE receptors, RAGE is believed to be the most important with its involvement not only in aging but also in inflammation *(119)*. The observation that interruption of the AGE–RAGE interaction reduces vascular lesion size and plaque formation suggests that limiting RAGE expression may be a novel strategy for vascular anomalies in aging *(120)*. These and other results clearly indicate that RAGE plays a key pathophysiological role in aging-associated diseases *(116)*.

Diet is considered as a major environmental source of proinflammatory H-AGEs (heat-generated advanced glycation end products) *(121)*. Vlasarra and colleagues showed that serum AGEs increases by 64.5% after 2 wk on H-AGE. In contrast, treatment with low-heat-generated AGE (L-AGE) resulted in a decrease of serum AGE by 30% *(121)*. Environmental (dietary) AGEs promote inflammatory mediators, leading to tissue injury. Restriction of dietary AGEs suppresses these effects *(121)*. There is a growing body of evidence showing that in healthy older Fisher 344 rats without diabetes, long-term treatment with aminoguanidine, a blocker of AGEs, may improve both arterial and ventricular function and optimize ventricular–arterial coupling *(115, 122–125)*. Moreover, existing accumulation of AGE was reduced by a novel agent, the AGE cross-link breaker ALT-711 *(126–132)*. Our recent studies demonstrated that cardiac AGE level is approx 2.5-fold higher in senescent hearts than young ones *(28)*. These data suggest that cardiac diastolic dysfunction and reduced stress tolerance in senescent cardiac myocytes may be associated with enhanced AGE levels and proteins modification by AGEs.

Stress Signaling Behind Senescence-Related Cardiac Defect

$p66^{Shc}$ *and Aging*

$p66^{Shc}$ is recognized as a longevity gene and the level of which responds directly to oxidative stress status *(133)*. It was suggested that the production of oxygen free radicals may be regulated by the expression of *$p66^{Shc}$* gene *(133)*. ShcA is expressed in the cardiovascular system and participates in the regulation of heart development *(134)*. It sensitizes cells in culture and in vivo for mitogen-activated protein kinase (MAPK) activation and controls cytoskeletal structure *(134)*, indicating that MAPK pathway is closely linked to oxidative stress. Histone deacetylation and cytosine methylation are probably the mechanisms underlying *$p66^{Shc}$* silencing in *$p66^{Shc}$* nonexpressing cells *(135)*. Examination of apoptotic cell death in the pacing canine heart showed that accumulation of reactive oxygen species potentiates the oxidative stress response by *$p66^{Shc}$* expression *(136)*. In agreement with these findings, resistance to apoptosis in-

duced by simulated ischemia in vitro satellite muscle cells from $p66^{ShcA-/-}$ silencing mice also consolidated the important role in senescence of this gene *(32)*.

Insulin Signaling Pathway and Aging

It is well known that aging induces insulin resistance although the precise mechanisms have not been elucidated. The major role of Ras in mammalian IGF-1 signaling raises the possibility that homologs of yeast Ras2 might accelerate aging in mammals *(137)*. Recently, Torella and colleagues hypothesized that IGF-1 promotes cell growth and survival and delays myocyte aging. They found that IGF-1 overexpression in transgenic mice attenuated all of aging markers, such as $p27^{Kip1}$, $p53$, $p16^{INK4a}$, and $p19^{ARF}$ shown in wild-type mice *(24)*. Among the main stress signaling pathways and/or central mediators activated in response to oxidant injury are the extracellular signal-regulated kinase (ERK) *(137,138)*, c-Jun amino-terminal kinase (JNK) and p38 MAPK signaling cascades *(139)*, the phosphoinositide 3-kinase (PI-3K) /Akt pathway *(24)*, the nuclear factor (NF)-κB signaling system, p53 activation, and the heat shock response *(140,141)*. Although not unique to aging, these pathways play a major role in cellular responses to injury and also regulate cell growth and metabolism. ERK, PI-3K/Akt, and NF-κB are thought to have a pro-survival effect on cells *(139)*. p53, JNK, and p38 are thought to have the opposing effect (i.e., apoptosis). Nonetheless, exceptions to this rule have been reported *(141)*.

Pro-inflammatory Signaling Pathway and Aging

Inflammation, measured as high levels of interleukin-6, C-reactive proteins, and IL-1RA, is significantly associated with poor physical performance and muscle strength in older persons *(142–144)*. The initiating events leading to activation of pathways responsive to oxidants are incompletely understood *(145,146)*. In the case of p53 activation, oxidative stress may be recognized as a consequence of the DNA damage. However, in some cells, elevated p53 expression results in increased oxidative stress *(147)*, suggesting that an important consequence of oxidant-induced p53 activation is a further increase in the level of oxidative stress. This positive feedback loop may be important in committing to an apoptotic response.

Oxidants seem to activate the ERK and the PI-3K/Akt pathways largely through stimulation of growth-factor receptors *(26)*, mimicking the action of natural ligands. Many growth-factor receptors have been shown to undergo enhanced phosphorylation in response to direct treatment with oxidants, and agents or conditions that prevent receptor phosphorylation likewise inhibit the activation of ERK and Akt by oxidants *(26,148,149)*. One mechanism proposed to explain this effect is oxidant-mediated inactivation of critical phosphatases necessary for dephosphorylation (turning off) of the growth-factor receptors. Support for such a mechanism has come from the finding that hydrogen peroxide, either derived exogenously or produced endogenously after growth-factor stimulation, can reversibly inactivate protein-tyrosine phosphatase 1B in cells *(150)*.

Cardiovagal Baroreflex Sensitivity

Aging is related to a reduction in cardiovagal baroreflex sensitivity *(151)* and increased levels of oxidative stress *(152–155)*. Other evidence for the effect of antioxidants is the finding that ascorbic acid increases cardiovagal baroreflex sensitivity in healthy old men *(156)*. Baseline cardiovagal baroreflex sensitivity was 56% lower in older men compared to younger subjects. Ascorbic acid infusion increased cardiovagal baroreflex sensitivity by 58% in older men, but had no effect in young men. After ascorbic acid infusion, the age-associated difference in cardiovagal baroreflex sensitivity was no longer statistically significant. Many cardiovascular disease states are associated with baroreflex impairment with oxidative stress playing a possible mechanistic role *(152–155)*.

$p16^{INK4a}$ and Telomeric Shortening: Lesson from Stem Cells

$p16^{INK4a}$ *(35,157)* and telomeric shortening *(158,159)* are efficient biomarkers of cellular senescence. Telomere uncapping has been demonstrated to lead to senescence *(160)*. The telomerase enzyme was deemed as the cap protector and plays an important role in senescence *(161,162)*. Taking advantage of this index, Chimenti and co-workers have established a valid model to assess the effect of senescence and primitive cell death on cardiac aging *(34)*. This model was applied to the study on the effect of aging on cardiac stem cells *(24)* and is considered as an important breakthrough due to the discovery of cardiac stem cells *(163)*. For decades, heart is considered as a terminally differentiated organ. These investigators found that 59% of c-kitPOS cells are labeled by $p16^{INK4a}$ in aged diseased hearts, whereas only 14% of cells express the kinase inhibitor in aged control hearts *(34)*. In aging-associated cardiomyopathy, 48% of cells were found with $p16^{INK4a}$, whereas in aged control hearts, only 16% of myocytes are $p16^{INK4a}$ positive *(34)*. Telomeric length, measured by confocal microscopy revealed a 39% reduction in average telomere length in aged diseased hearts compared to aged control hearts. Aged diseased hearts had moderate hypertrophy and dilation, accumulation of $p16^{INK4a}$ positive primitive cells and myocytes, and no structural damage. Cell death markedly increased and occurred only in cells expressing $p16^{INK4a}$ that had significant telomeric shortening. Cell multiplication, mitotic index, and telomerase activity increased but did not compensate for cell death nor prevented telomeric shortening. Idiopathic dilated cardiomyopathy had severe hypertrophy and dilation, tissue injury, and minimal level of $p16^{INK4a}$ labeling. These findings suggest that telomere erosion, cellular senescence, and cell death characterize aged diseased hearts and the development of cardiac failure in humans *(34)*. It has

also been reported that cardiac survival factor IGF-1 may interfere with aging-dependent increases in telomeric shortening and telomere length decreased more in myocytes in old wild-type mice than in myocytes in IGF overexpressing transgenic mice compared to young cells *(24)*.

Conclusions

Although the scenario behind senescene-associated increase in the propensity and clincal manifestation of cardiac dysfunction and overall heart disease is far from clear, cardiac senescence has been demonstrated to be related to intrinsic myocardial electromechanical properties, reduction in essential cardiac survival factor IGF-1, accumulation of reactive oxygen species, activation of stress signaling, and other neurohormonal alterations with advanced age. Many of these factors have undoubtly participated in the "on-and-off" switch of normal cardiac functional proteins, leading to ultimate onset of "senescent cardiomyopathy." With the ever increasing aging population in the 21st century, investigation and management of aging-related heart diseases and other non-cardiac problems deserve intensive effort.

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