

Cardiovascular Disease in Aging and the Role of Oxidative Stress

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List of Abbreviations

3-NPA 3-nitropropionic acid
 ADP adenosine diphosphate
 AKT protein kinase B
 AMP adenosine monophosphate
 AMPK adenosine monophosphate-activated protein kinase
 AP-1 activator protein 1
 ATP adenosine triphosphate
 CAT catalase
 ERK extracellular signal regulated kinase
 FADH flavin adenine dinucleotide
 FOX forkhead box
 FOXO O class forkhead box
 GPx glutathione peroxidase
 GST glutathione S-transferase
 HIF-1 hypoxia-inducible factor 1
 IGF1 insulin-like growth factor 1
 LDLs low density lipoproteins
 LKB1 liver kinase B1
 mCLK1 mammalian CLOCK1
 MiR1 microRNA 1
 miRNA microribonucleic acid
 Mn SOD manganese superoxide dismutase
 mTOR mammalian target of rapamycin
 mTORC1 mammalian target of rapamycin complex 1
 mTORC2 mammalian target of rapamycin complex 2
 NADH nicotinamide adenine dinucleotide
 NADPH nicotinamide adenine dinucleotide phosphate
 p66^{Shc} 66 kDa splice variant of the Shc locus
 PI3K phosphoinositide 3-kinase

PIT1 pituitary transcription factor 1
 PKCB protein kinase C-β
 PRO1 homeobox protein prophet of pituitary transcription factor 1
 PTP permeability transition pore
 RNA ribonucleic acid
 ROS reactive oxygen species
 SIR2 sirtuin
 siRNA small interfering ribonucleic acid
 SIRT sirtuin family member
 TSC1 tuberous sclerosis complex 1
 TSC2 tuberous sclerosis complex 2
 UCP2 mitochondrial uncoupling protein 2
 VEGF vascular endothelial growth factor

INTRODUCTION

Aging is an inevitable fate for any biologic system, and unfortunately the deterioration that occurs with aging predisposes individuals to numerous conditions. Cardiovascular diseases represent a large group of conditions with a dramatically increased occurrence with age. As well as the emotive impact on the family unit and communities, cardiovascular diseases claim a large number of lives and cause a significant detriment to the economy through healthcare costs and productivity losses. In Europe this amounts to 1.9 million deaths and a cost of €196 billion per year¹. One-third of all deaths

in the USA are caused by cardiovascular disorders, with more than 83 million Americans currently living with one or more of these fatal conditions. Furthermore, cardiovascular disorders cost the American economy \$444 billion a year, equating to one sixth of the country's spending on health.²

Biologic aging predisposes individuals to cardiovascular disease through age-related perturbation of systemic and/or cellular oxidative balance, i.e. discord between the rates of generation and clearance of reactive oxygen species (ROS). Due to this increased susceptibility, death related to cardiovascular events rapidly becomes more common with aging. For example, in the United Kingdom in 2009 there were approximately 180 000 deaths due to cardiovascular disease, 72% of which were in populations over the age of 75³ (see Figs 3.1 and 3.2 for representative statistics related to cardiovascular diseases).

Oxidative stress has long been known to increase with the biologic aging process, and it independently causes a greater risk of cardiovascular disease. Biologic systems are equipped to neutralize endogenous oxidative stress and respond appropriately to oxidative challenges. Throughout biologic aging, these protective mechanisms decline and thus the oxidative theory of aging extends to pathophysiologic developments that predispose individuals to a much higher risk of conditions such as cardiovascular disease. The cardiovascular system is particularly sensitive to endogenous oxidative stress as the myocardium is particularly rich in mitochondria,

and mitochondrial metabolism is the main source of cellular free radical generation.⁴

The genes that regulate cardiovascular physiologic function and responses to oxidative challenge in aging have been identified as *longevity genes*, a subset of which interacts extensively as the *longevity network*.⁵ Biologic aging is also heavily attributed to the *telomere theory*. The telomere theory states that telomere shortening, which is accelerated by oxidative stress, is responsible for much of the age-related deterioration.⁶ The implication of oxidative stress in age-related physiologic decline is well established and supported by data showing a marked increase in plasma potential for oxidative damage with age (see Fig. 3.3).⁷ This increased oxidant potential is indicative of the increasing imbalance between the generation and clearance of ROS throughout the biologic aging process, contributing to the pathogenesis of age-related cardiovascular disease. Furthermore, as age increases, the antioxidant activity of several antioxidant enzymes in the cardiovascular system decreases (see Fig. 3.4a). The oxidative balance of the cardiovascular system is further disturbed by the increased oxidative stress from mitochondria and lipid peroxidation that occurs with aging (see Fig. 3.4b).

In this chapter we describe the physiologic changes that accompany aging and which predispose individuals to a decline in vascular function and the risk of cardiovascular diseases. We focus specifically on the effects of oxidative stress and the dual role it plays in aging and in the pathogenesis of cardiovascular disease.

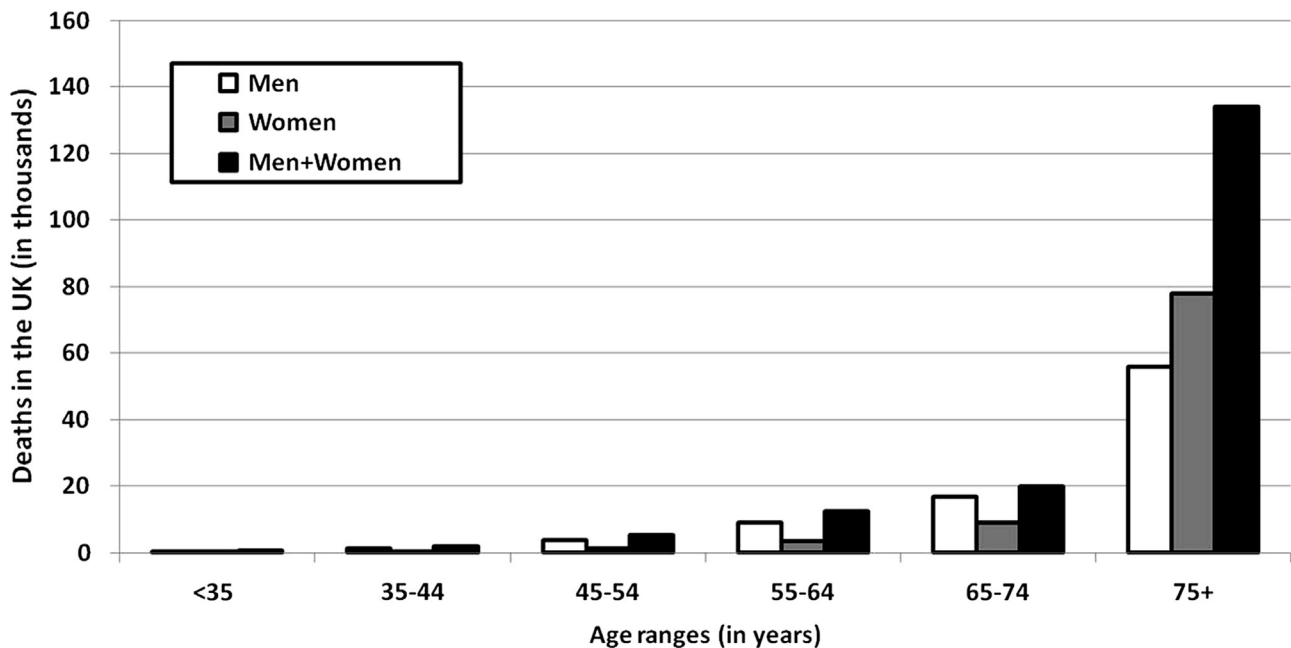


FIGURE 3.1 Rates of death from cardiovascular disease increase with age in the United Kingdom. Cardiovascular diseases represent the most common causes of death in the UK. This figure shows the deaths in the UK in 2009 that are due to cardiovascular disease. The risk of death from cardiovascular disease increases with age, with the highest risk group being those above 75 years of age; this trend is in both men and women. Data adapted from Nichols et al 2012.¹

PHYSIOLOGY OF THE AGING CARDIOVASCULAR SYSTEM

The cardiovascular system is the most widely reaching system within the human body, delivering oxygenated blood and nutrients to all tissues. However, like any system, aging alone will cause a physiologic and functional decline that increases susceptibility to complications.

In the cardiovascular system, the decline in functional capacity greatly increases the risk of hypertension and atherosclerosis, leading to life-threatening events such as myocardial infarction or stroke. Aging and the associated functional decline are greatly accelerated by systemic and local oxidative stress.

Figure 3.5 summarizes the physiologic changes that occur in an aging cardiovascular system. These effects

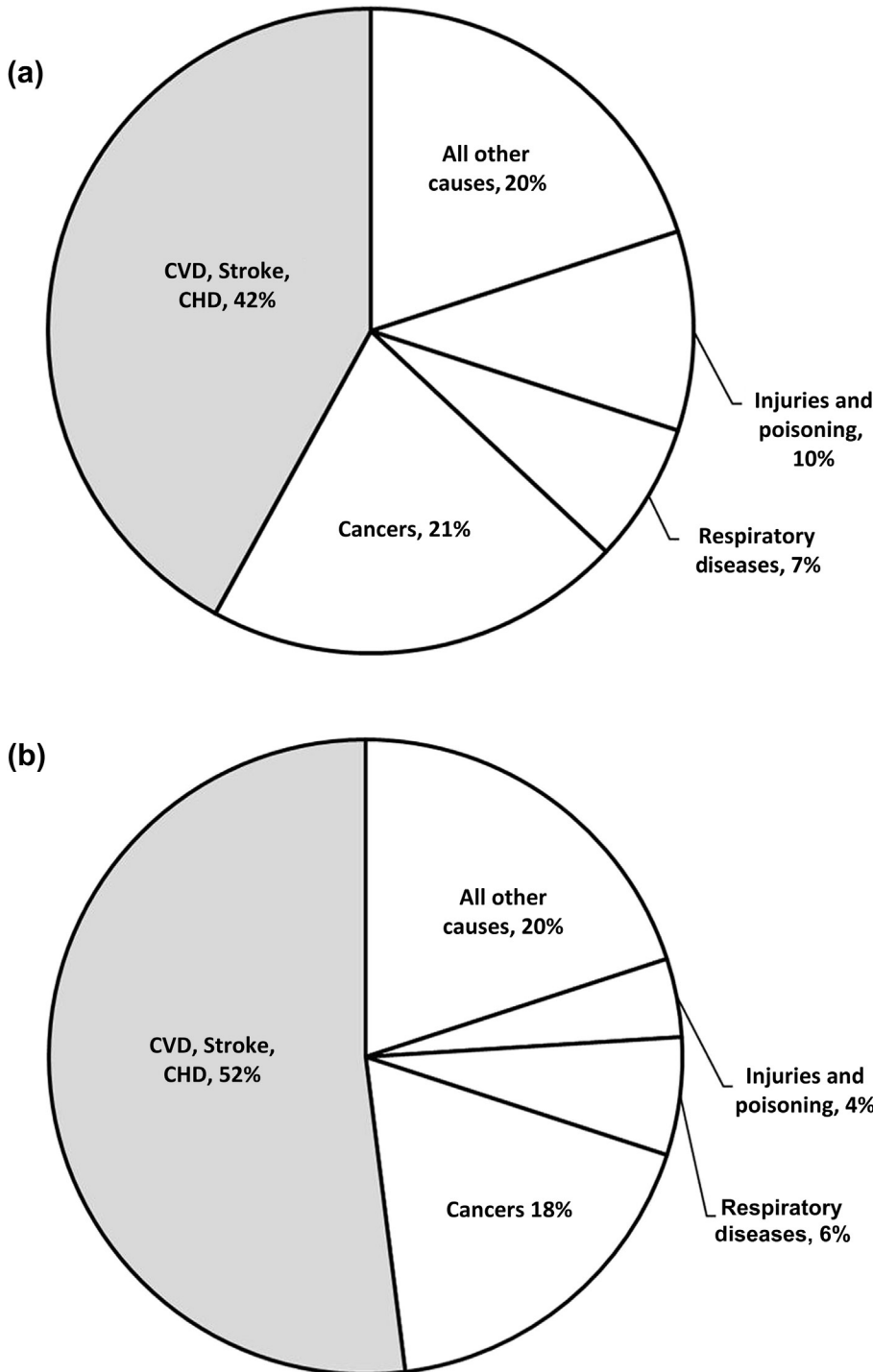
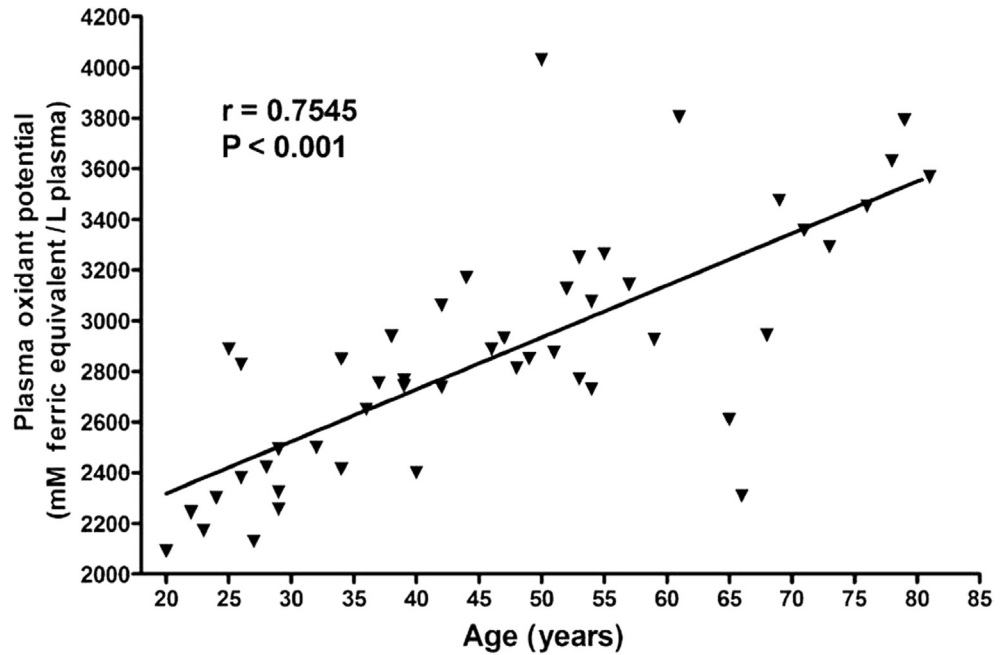


FIGURE 3.2 Impact of cardiovascular disease in Europe. (a) This shows the leading causes of death of men in Europe in 2012; cardiovascular disease, stroke and coronary heart disease are the leading causes of death. (b) This shows the leading causes of death of women in Europe in 2012; cardiovascular disease, stroke and coronary heart disease cause more than half of the deaths in Europe. *Data adapted from Scarborough et al 2011.³ CVD: cardiovascular disease; CHD: coronary heart disease.*

FIGURE 3.3 The potential for oxidative damage increases with age. Oxidative stress contributes to the biologic aging process and is a central part of the pathogenesis of cardiovascular disease. This figure shows the clear relationship between aging and the increased potential for oxidative stress (plasma oxidant potential). An increased potential for oxidative stress increases the risk of developing age-related pathologies, including cardiovascular disease. Reprinted with permission from Mehdi & Rizvi (2013).⁷



mainly cause a decline in endothelial function, left ventricle systolic reverse capacity and left ventricular diastolic function.⁵ Interestingly, the effects of aging in either the vascular or cardiac system will induce a compensatory and potentially pathologic change in the other. For example, increased arterial stiffness causes accumulation of fibrotic tissues or hypertrophy in the myocardium.⁵

The main functional changes that occur with aging are: (1) a decline in heart rate, and (2) a decline in cardiac output. The decline in heart rate is due to cell loss in the sinoatrial node as well as impedance of electrical impulses from structural changes such as hypertrophy and fibrosis. These changes are due largely to an age-related decline in stress-response mechanisms.^{5,8} The decline in cardiac output begins with hemodynamics adapting to meet changing requirements with age. The cardiovascular system's immediate response stimulates myocardial hypertrophy – which increases cardiac output accordingly. These adaptations subsequently lead to a decline in cardiac output and overall cardiovascular function. Myocardial hypertrophy, independently or as part of age-related compensation, is a risk factor for morbidity and mortality associated with cardiovascular disease.^{5,8}

THE MOLECULAR BASIS OF OXIDATIVE STRESS AS APPLIED TO THE CARDIOVASCULAR SYSTEM

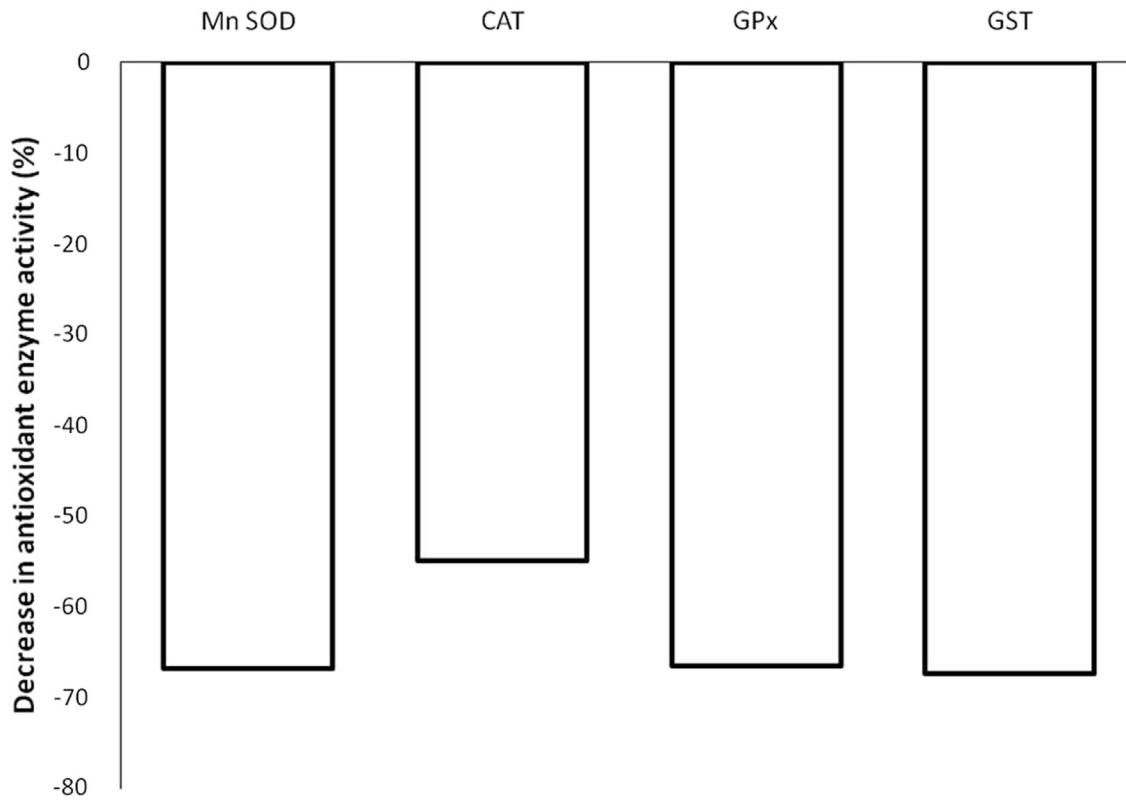
In any cell, a multitude of reactions require the transfer of electrons. Whenever electrons are exchanged, there is a change in oxidative state of the molecules involved in these reactions. Oxidation or reduction constitutes the gain or loss of electrons, respectively. These processes occur simultaneously and are termed redox reactions, where a reductant will be oxidized by donating an electron to an oxidant.⁹

Reactive oxygen species (ROS), generated as by-products of the above redox reactions, contain unpaired electrons, making them highly reactive and potentially dangerous sources of oxidative stress. The main endogenous process that generates ROS is mitochondrial oxidative phosphorylation.⁹ These mitochondrial reactions are particularly relevant to the cardiovascular system, as 45% of the heart's cellular volume is taken up by mitochondria.⁴

During oxidative phosphorylation, electrons from nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide (FADH) are processed via four mitochondrial enzyme complexes. The end product of

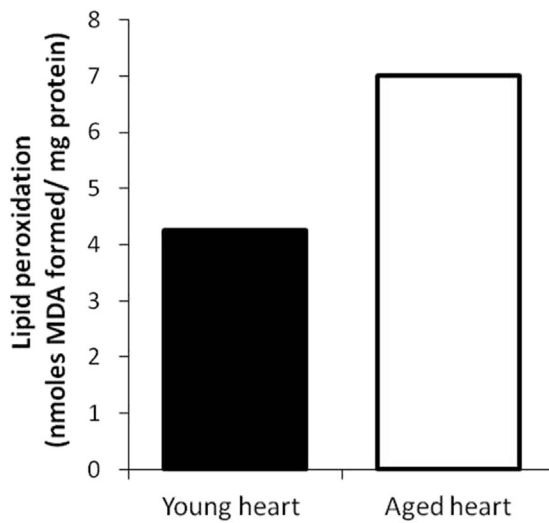
FIGURE 3.4 Decrease in antioxidant enzyme activity and increase in oxidative stress in the aging cardiovascular system; there is decreased activity of several antioxidant enzymes, namely, manganese superoxide dismutase (Mn SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione S-transferase (GST). (a) This shows the percentage decrease in activity of these enzymes between young and aged mouse hearts. As well as the decrease in these antioxidant enzymes, there is increased oxidative stress that occurs through increased lipid peroxidation and the generation of mitochondrial ROS. (b) This shows the increased production of ROS in hearts from aged versus young mice. Mn SOD: manganese superoxide dismutase; CAT: catalase; GPx: glutathione peroxidase; GST: glutathione S-transferase; MDA: malondialdehyde; RFU: relative fluorescence units; ROS: reactive oxygen species. Data adapted from Sudheesh et al 2010.⁶⁹

(a) **Percentage decrease in antioxidant enzyme activity
between young and aged mouse hearts**

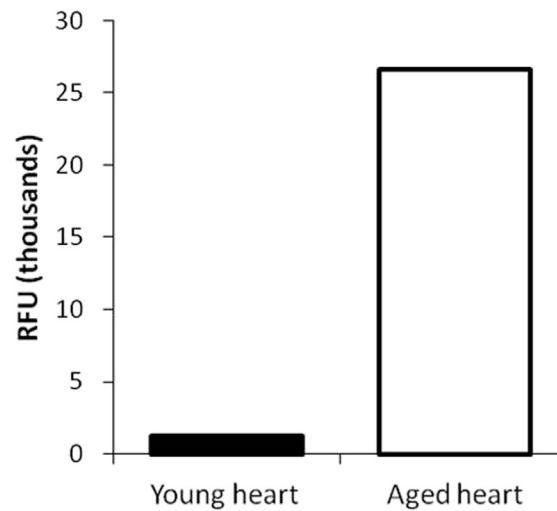


(b)

Lipid peroxidation in young and aged mouse hearts



Reactive oxygen species in heart mitochondria of young and aged mice



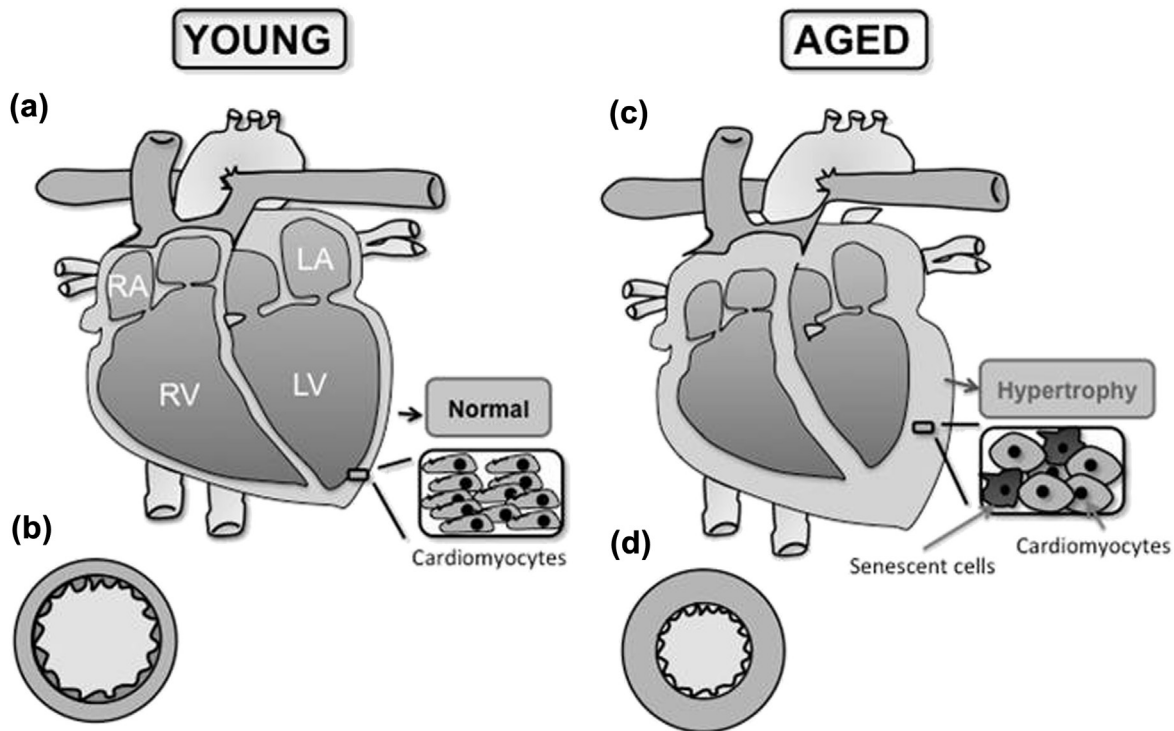


FIGURE 3.5 Age-related changes in the cardiovascular system. In the heart, several functional and structural changes occur with the aging process. Panel (a): Young heart with normal thickness of the myocardium and no compromised function. Panel (b): Young artery with normal sized lumen and vessel wall; adaptive contractility and relaxation is maintained. Panel (c): Aged heart with myocardial hypertrophy; cardiomyocytes are undergoing senescence and thus have compromised function. Hypertrophy and fibrotic tissues will lead to impeded signaling from the sinoatrial node. Panel (d): Aged artery with narrower lumen, increased wall thickness; adaptive contractility and relaxation is lost. RA: right atrium; LA: left atrium; RV: right ventricle; LV: left ventricle. Reprinted with permission from Shaik et al 2013.⁶⁸ Available from: <http://www.intechopen.com/books/senescence-and-senescence-related-disorders/endothelium-aging-and-vascular-diseases>.

this pathway is the production of adenosine triphosphate (ATP) from adenosine diphosphate (ADP). Harmful ROS are generated when electrons lost from mitochondrial complexes I and II form superoxide radicals. Furthermore, NADH and FADH are free to react with other redox compounds throughout the mitochondrial pathway.⁹

As vessels age, they produce more ROS, leading to functional impairment. Increased production of the superoxide anion with aging leads to increased reaction of this ROS with nitric oxide. This reaction will not only inhibit the biological activity of nitric oxide as a vasodilator, but also results in the formation of peroxynitrite. Peroxynitrite is a powerful membrane-permeable oxidant that inactivates several enzymes, including free radical scavengers, through substrate nitration. Age-dependent increases in nitric oxide synthase expression are associated with the peroxynitrite formation and are therefore implicated in age-related oxidative damage to vasculature.^{9,10}

Age-related oxidative stress is also central to the pathologic changes leading to atherosclerosis, a starting point of more serious cardiovascular conditions. Endothelial cells and vascular smooth muscle cells produce reactive oxygen and nitrogen species which oxidize low-density lipoproteins (LDL). Oxidized LDL enter subendothelial

spaces where they initiate atherosclerosis. The oxidation process will increase mitochondrial rupture and the release of proapoptotic molecules to the cytosol, increasing plaque cell apoptosis. Furthermore, the scavenging process for reactive nitrogen species increases endothelial dysfunction, smooth muscle cell proliferation, leukocyte adhesion and inflammatory responses. Thus, the generation and clearance of oxidative stress in vasculature is an extremely significant regulator of age-related cardiovascular decline on many levels.^{5,9}

Alongside gross physiologic adaptation of the cardiovascular system, minute molecular and genetic mechanisms are known to mediate cardiovascular responses to oxidative stress and age-related decline. The three leading molecular mediators are the involvement of longevity genes and the longevity network (which are discussed later) and the telomere theory of aging that is also strongly associated with cardiovascular decline.^{5,6}

Telomeres are structures of repeating nucleotides at the end of eukaryotic chromatids that are shortened during replication. This mechanism exists to protect valuable genetic data from being lost by incomplete replication. Telomere length, and thus protective capacity, are determined genetically, are decreased by oxidative stress and

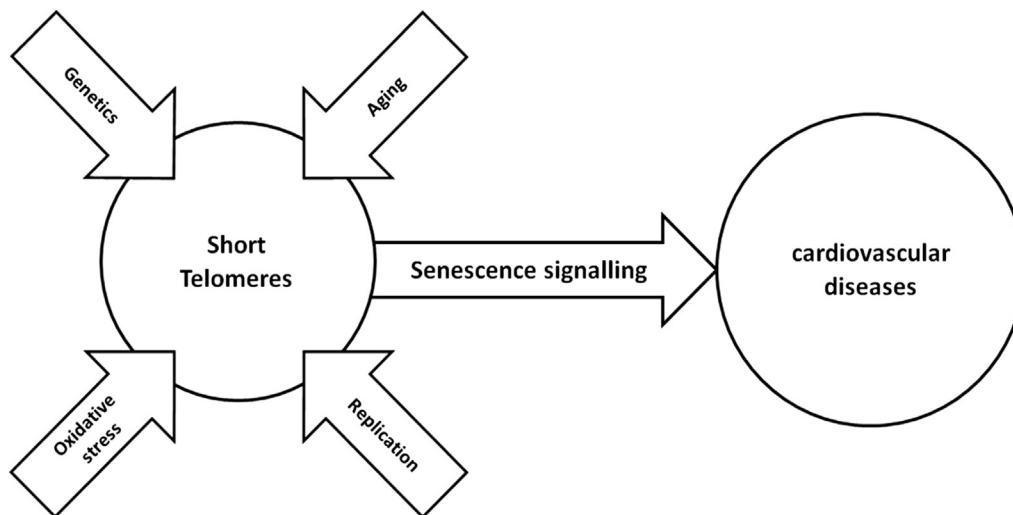


FIGURE 3.6 Factors that lead to telomere attrition and a higher risk of cardiovascular disease. The factors that affect telomere length help to explain interindividual variation in the risk of developing cardiovascular disease. Telomere length will vary genetically at birth and will shorten with age and exposure to oxidative stress (e.g. smoking or metabolic conditions such as obesity). Cell replication also shortens telomere length; when a critical length has been reached telomeres activate senescence signaling which, in turn, increases the risk of cardiovascular disease. *Adapted from Samani & van der Harst (2008).*⁶

vary with lifespan.⁶ In conditions such as cardiovascular disease, with a significant multifactorial etiology involving both aging and metabolic oxidative stress, it is important to consider the role of telomere attrition. This is because the telomere theory is a candidate for distinguishing individual variability of risk and susceptibility, a factor that remains largely unknown, unlike population variability, which is attributed to known factors such as smoking or ethnicity.

When shortened to a critical length, telomeres will drive senescence signaling mechanisms. Dysregulation in the pathways of cell senescence are a strong underlying mechanism leading to cardiovascular decline. There have also been strong associations between systemic cell-type-specific telomere shortening and the development of cardiovascular disease. For example, the presence of shortened telomeres in circulating leukocytes is associated with the development of coronary artery disease. In observational studies, the presence of shortened telomeres precedes the development of clinically relevant disease, indicating a more causal role rather than a consequential effect of cardiovascular decline.^{11,12}

Many interesting studies have investigated this hypothesis. For example, Cawthon et al¹³ found that in subjects aged over 60, a shorter telomere length was strongly associated with a high cardiac mortality rate within a decade. In this study, the presence of other classic cardiovascular risk factors did not explain the higher mortality rate. Furthermore, as telomeres shorten with aging, the average loss of telomeres can be equated to the number of years. Those with coronary artery disease are, on average, 8–12 years older, in terms of telomere loss (i.e. similar average telomere length to healthy subjects actually 8–12 years

older). Telomere attrition has been specifically associated with a number of cardiovascular pathologic events, including: congestive heart failure, peripheral vascular disease and carotid artery atherosclerosis.^{6,11–13}

It is not surprising that there are continuously strengthening links between aging, cardiovascular disease and telomere shortening. Oxidative stress is certainly well known to be implicated in negatively affecting all three processes: accelerating telomere loss, accelerating aging and acting as a significant pathologic component of cardiovascular disease.⁶ Whether a common causal pathway is responsible for the cardiovascular aging phenotype, for telomere attrition and for sensitivity to oxidative stress, is an extremely exciting area of current research. [Figure 3.6](#) summarizes the factors that increase telomere attrition, senescence signaling and, subsequently, a higher risk of cardiovascular disease.

LONGEVITY GENES AND THE LONGEVITY NETWORK

Longevity genes regulate oxidative stress response pathways and biologic aging. These factors are major parts of cardiovascular disease pathology⁵. The list of genes includes the Sirtuins (SIR2), insulin-like growth factor-1 (IGF1), CLOCK1 (mCLK1), adenosine monophosphate-activated protein kinases (AMPK), p66^{Shc}, catalase and klotho; see [Table 3.1](#) for a complete list of the genes discussed in the following section. A subset of the longevity genes interacts extensively and constitutes the longevity network, the functions and interactions of which are addressed first.

TABLE 3.1 Longevity Genes^a

Official Gene Symbol	Name of Gene	Sequence ID (Known Human Transcript Variants)
SIRT1	Sirtuin 1	NM_001142498.1; NM_012238.4
SIRT3	Sirtuin 3	NM_00107524.2; NM_012239.5
SIRT6	Sirtuin 6	NM_001193285.1; NM_016539.2
SIRT7	Sirtuin 7	NM_016538.2
IGF1	Insulin-like growth factor 1	NM_000618.3; NM_001111283.1; NM_001111284.1; NM_00111285.1
FOXO1	Forkhead box O1	NM_002015.3
FOXO3	Forkhead box O3	NM_001455.3; NM_201559.2
PIT1	Pituitary-specific positive class 1 homeobox	NM_000306.2; NM_001122757.1
AMPK	Protein kinase, AMP-activated, alpha 1 catalytic subunit	NM_006251.1; NM_206907.3
mTOR	Mechanistic target of rapamycin	NM_004958.3
p66 ^{Shc}	Src homology 2 domain-containing transforming protein C1	NM_001113331.2; NM_011368.5
KL	Klotho	NM_004795
CAT	Catalase	NM_001752
CLK1	Circadian locomotor output cycles kaput	NM_001254772

^aThese genes have been found to have a substantial link with age-related physiologic decline in cardiovascular function and with cellular and systemic responses to oxidative stress. A number of these genes (SIRT1, AMPK, IGF-1 and mTOR) form the longevity network, a network that regulates biologic aging and therefore the development of cardiovascular disease.

Genes of the Longevity Network: SIR2, IGF-1, AMPK and mTOR

The Sirtuins

Sirtuins (SIR2) are a class of evolutionarily conserved enzymes. In mammals there are seven members of the SIR2 family with diverse cellular localizations. These proteins have a wide range of functions, namely, ribosyl-transferase and deacetylase activity, as well as functions in aging, transcription, apoptosis, inflammation, DNA damage and repair, cell-cycle regulation, stress resistance and mitochondrial function.¹⁴

The Cardiovascular System and SIR2 Proteins

SIR2 family members 1, 3 and 7 (SIRT1, SIRT3 and SIRT7, respectively) have proven roles in the cardiovascular system. Cardiac-specific overexpression of SIRT1 in mice delays age-dependent cardiomyopathy and decreases stress-induced apoptosis. Knockout and constitutive overexpression is detrimental, increasing ischemia-reperfusion injury, cardiomyopathy, apoptosis and oxidative stress. SIRT1 also effects vasculature, inhibiting angiotensin-II-mediated vascular smooth muscle hypertrophy. It regulates angiogenesis and prevents arterial calcification and stiffness.^{15–17} Knockout of mitochondrial SIRT3 and nuclear SIRT7 causes age-dependent, pressure-induced cardiac hypertrophy and inflammatory cardiomyopathy. Thus, SIRT3 and SIRT7 protect from oxidative damage as well as prevent cardiovascular remodeling and inflammation.^{18,19}

Oxidative Stress and SIR2 Proteins

The roles of SIR2 proteins in oxidative stress are complex and are member-, site- and species-specific. SIRT1, SIRT6 and SIRT7 proteins have been strongly associated with mediating oxidative stress responses in mammals. Expression of SIRT1 ameliorates the aggravated oxidative stress seen in cardiovascular disease.²⁰ SIRT6 is expressed in a number of tissues, including the heart; its overexpression promotes resistance to oxidative stress and associated DNA damage. SIRT6 substrate specificity is very high, exerting deacetylase activity to maintain chromatin function, in turn promoting telomere stability. Therefore, SIRT6 prevents cell senescence through mediating resistance to oxidative stress, ameliorating DNA damage and preserving telomere length.²¹ As for SIRT7, diminished expression in cardiomyocytes decreases oxidative stress resistance and increases apoptosis by up to 200%. This is mediated via hyperacetylation of the p53 protein and has been confirmed in haplotype mice with diminished SIRT7 expression.¹⁹

Insulin-Like Growth Factor 1

One of the initial genes to be identified as a longevity gene, insulin-like growth factor 1 (IGF1), encodes a peptide structurally similar to insulin with an important function in early growth and an anabolic effect in adults. IGF1 exerts its effects by binding to its receptor that is expressed on many cell types and tissues. From this binding site, IGF1 inhibits apoptosis and acts as a stimulator of cell growth and proliferation.^{5,22} In mammals, the loss of this protein's function causes dwarfism, deficient growth or postnatal death from respiratory failure.²³

The Cardiovascular System and IGF1

Several studies have implicated IGF1 signaling in the maintenance of cardiovascular health. In mice, cardiac-specific overexpression of IGF1 prevents cell death after myocardial infarction and reduces hypertrophy, ventricular dilation and diabetic cardiomyopathy. However,

other overexpression studies have shown negative effects, including hypertrophy and diminished recovery after ischemia. Live knockout of mammalian IGF1 increases cardiomyocyte cell death under oxidative stress.²⁴⁻²⁶ In simple organisms, with a primitive cardiovascular system, the induction of IGF1 delays age-related degeneration. Some studies report opposite effects in mammals. This difference is possibly explained by the complex autocrine and paracrine signaling pathways that are targeted by IGF1 and are not present in simpler organisms.^{5,22}

Oxidative Stress and IGF1

Oxidative stress is associated with decreased plasma IGF1 in humans, leading to cardiac and mitochondrial dysfunction. When mice have cardiac-specific overexpression of IGF1 – or have IGF1 administered exogenously under conditions that induce cardiovascular disease – there is decreased apoptosis and mitochondrial dysfunction as well as lower systemic and myocardial oxidative stress. Furthermore, IGF1 promotes protective regulation of other mediators of aging and cardiovascular risk (e.g. FOXO and mTOR). Despite protective effects of IGF1 on cardiovascular function, remodeling and hypertrophy still occur due to preserved mitochondrial function and increased cell survival under pro-oxidant conditions.^{25,26}

The interaction between IGF1 and its receptor regulates cell growth, transformation and survival under oxidative stress. When this interaction is perturbed (e.g. in haplotype mice and in myoblasts cultured from them) there is increased resistance to oxidative stress.²⁷ Studies in other types of cell show opposite effects; for example, increased IGF1 signaling confers protection from oxidative stress in microglial cells and in induced pluripotent stem cells.²⁸ Studies applying small interfering RNA (siRNA) have shown the complexity of these mechanisms. The effects of perturbing these mechanisms will vary upstream or downstream of the IGF1 signaling system²⁸. Through the aforementioned studies, microRNA-1 (MiR1) targeting of the 3'-UTR region of the IGF1 gene has been shown to regulate the cytoprotective properties and prevent oxidative stress-induced apoptosis signaling.²⁸

Forkhead Box Transcription Factors

Forkhead box (FOX) proteins are a large family of transcription factors regulating expression of genes that control cell growth, proliferation, differentiation and aging. Of particular interest to cardiovascular health and aging are the O class FOX proteins (FOXO) that also regulate metabolism and stress tolerance. They are post-translationally controlled by ubiquitination, phosphorylation and acetylation.²⁹

The Cardiovascular System and FOXO Proteins

Maintaining finely tuned function of FOXO proteins is necessary for life. Deletion of the FOXO1 member is

embryonically lethal and causes deficient cardiac and vascular growth. Interestingly, cardiac-specific overexpression of FOXO1 is also lethal and causes reduced heart size and myocardium thickness as well as heart failure and impaired cardiomyocyte proliferation.^{29,30} A variant of FOXO3 is commonly found in centenarians, and is thought to delay aging and preserve cardiovascular health. Overexpression of the FOXO3a variant decreases cardiomyocyte size, whereas deficiency increases endothelial nitric oxide synthase expression and promotes postnatal angiogenesis and vessel formation.^{31,32}

Oxidative Stress and FOXO Proteins

FOXO signaling maintains homeostasis and mediates responses to environmental pathologic changes, such as oxidative stress. FOXO3a is inhibited by the protein kinase B (AKT) pathway and upregulated by hypoxic conditions. FOXO3a is known to inhibit MYC-mediated adaptive mitochondrial metabolism. Under cellular oxidative stress, AKT is inhibited, which increases FOXO3a signaling. FOXO3a, via inhibition of MYC signaling, prevents increased mitochondrial metabolism under hypoxia. In this cascade, the knockdown of FOXO3a allows MYC signaling to increase mitochondrial oxidative stress generation.^{32,33} Furthermore, under metabolic oxidative stress, FOXO3a signaling disrupts vascular function by facilitating degradation of vascular calcium/potassium channels. In mice, suppression of FOXO3a preserves cardiovascular function under metabolic oxidative stress.³⁴

Adenosine Monophosphate-Activated Protein Kinase

Adenosine monophosphate (AMP)-activated protein kinase (AMPK) is involved in cellular energy homeostasis, glucose metabolism, lipid metabolism, cell growth, polarity, gene expression and autophagy. AMPK regulates cellular energy metabolism by mitophagy and mitogenesis: destruction of defective mitochondria and activation of mitochondrial biogenesis, respectively. AMPK is also a metabolic energy sensor for the AMP:ADP ratio, stimulating ketogenesis and hepatic fatty acid oxidation as well as inhibiting lipogenesis, triglyceride and cholesterol synthesis.³⁵

The Cardiovascular System and AMPK

In humans, hereditary syndromes due to AMPK mutations have a pathologic component in cardiovascular decline. AMPK mutations cause hypertrophic cardiomyopathy and ventricular pre-excitation. Diminished expression of AMPK exacerbates the effects of myocardial infarction, whereas overexpression is cardioprotective, ameliorating damage from ischemia-reperfusion injury and preventing pressure-overload hypertrophy.^{36,37}

Oxidative Stress and AMPK

AMPK is a target of pharmaceutical regulation for its role in defense against mitochondrial oxidative stress. Endothelial mitochondrial ROS are implicated in age-related cardiovascular decline. Endothelial oxidative stress and AMPK expression increase during impaired vasodilation and hypertension from coronary artery disease and diabetes. Diabetes is strongly associated with systemic oxidative stress and is a leading co-morbidity with aging cardiovascular disease.³⁸

Further observations of mitochondrial oxidative stress and AMPK regulation have been made in human saphenous vein endothelial cells. When these cells, under oxidative stress, are incubated with a mitochondrial-targeted antioxidant, AMPK expression decreases, providing evidence for the role of AMPK in mitochondrial oxidative stress defence.³⁹ It is necessary to maintain the delicate balance in production and clearance of such reactive species given their functions as signaling molecules in normal cardiovascular function and adaptability. Cardiovascular dysfunction occurs when their rate of production overtakes endogenous free radical scavenging processes. In this regard, AMPK's role as an energy sensor is clear as the major sources of oxidative stress are mitochondrial nicotinamide adenine dinucleotide phosphate (NADPH) oxidases. There is currently strong evidence to support AMPK's role as a suppressor of NADPH oxidase activity and therefore reactive species generation.^{38,39}

AMPK also has a role in regulating adaptive angiogenesis under oxidative stress. This has been shown by rotenone treatment in coronary artery endothelial cells (rotenone increases oxidative stress and inhibits mitochondrial complex I). Vascular tube formation induced by vascular endothelial growth factor (VEGF) is inhibited by rotenone treatment. In the same process, the rotenone induction of oxidative stress increases expression of AMPK. Interestingly, knockdown of AMPK in rotenone treatment preserves VEGF-induced tube formation.³⁹

Mammalian Target of Rapamycin (mTOR)

Rapamycin is an antifungal compound originally discovered in soil samples from Easter Island. It is clinically used as an immunosuppressant after organ transplantation.⁴⁰ Rapamycin inhibits the activity of the mammalian target of rapamycin (mTOR). The product of mTOR is a serine/threonine protein kinase that also integrates products of upstream pathways, including insulin and IGF1. These functions are mediated via two complexes (mTORC1 and mTORC2). The mTOR pathway is dysregulated in a number of human metabolic pathologies that induce oxidative stress, such as diabetes and obesity.⁶

The Cardiovascular System and mTOR

Cardiac inhibition of mTOR activity reverses pressure-overload hypertrophy, via inhibition of mTOR's control

over cell size and protein translation.⁴¹ The mTOR protein also interacts with AKT and phosphatidylinositol 3-kinase (PI3K) in the PI3K/AKT/mTOR pathway, which mediates hypoxia-induced angiogenesis. Rapamycin perturbation of the PI3K/AKT/mTOR pathway inhibits vessel growth, normally mediated by hypoxia-inducible factor 1 (HIF1) and VEGF.⁴² The PI3K/AKT/mTOR pathway is central to many other signaling pathways, making these genes and their products vital to the understanding of oxidative damage, cardiovascular health and aging.

Oxidative Stress and mTOR

Oxidative stress influences targets upstream and downstream of the mTOR pathway. Sustained increased activity of the mTOR pathway is a strong causal candidate for cardiovascular decline with age and oxidative stress. Furthermore, mTOR's response to oxidative stress is associated with the inflammatory pathways that progress cardiovascular disease.⁴³ In human coronary artery endothelial cells, inducing oxidative stress by rotenone inhibits mTOR activity.³⁹ Furthermore, mTOR regulates arterial responses to oxidative stress alongside AMPK. Under oxidative stress, arterial contraction and compliance are compromised. When rapamycin is applied to mouse tissues, arterial contraction is preserved, indicating that mTOR mediates adaptive loss of contractile function under oxidative stress.⁴⁴ The effect of mTOR on cardiac remodeling is associated with, and possibly governed by, the body's renin-angiotensin-aldosterone axis, which is central to the oxidative stress response, maintaining blood pressure and maintaining arterial vasoconstriction. When renin is overexpressed in rats, there is a concomitant increase in mTOR activity, as well as metabolic, biochemical and physical manifestations of oxidative stress and of cardiovascular system decline.⁴⁵

The Longevity Network: SIR2, IGF1, AMPK and mTOR

The longevity network comprises the interactions between SIRT1, IGF1, mTOR and AMPK (see Fig. 3.7).⁵ SIRT1 is cardioprotective and increases stress resistance when mildly overexpressed. It regulates the hepatic AMPK pathway via the upstream liver kinase B1 (LKB1). IGF1 is regulated both directly by SIRT1 and via the mitochondrial uncoupling protein 2 (UCP2). SIRT1 also inhibits the mTOR pathway through the complexes formed by tuberous sclerosis 1 and 2 (TSC1, TSC2).⁴⁶

IGF1 interacts with both mTOR and SIRT1 pathways. FOXO is a downstream effector of SIRT1's action on IGF1.⁵ The mTOR pathway is stimulated by AKT signaling via IGF1 and it is inhibited by AMPK and phosphorylation of the TSC1 and TSC2 complex.⁴⁷ AMPK, in turn, activates SIRT1 and IGF1 signaling. SIRT1 activity increases with levels of nicotinamide

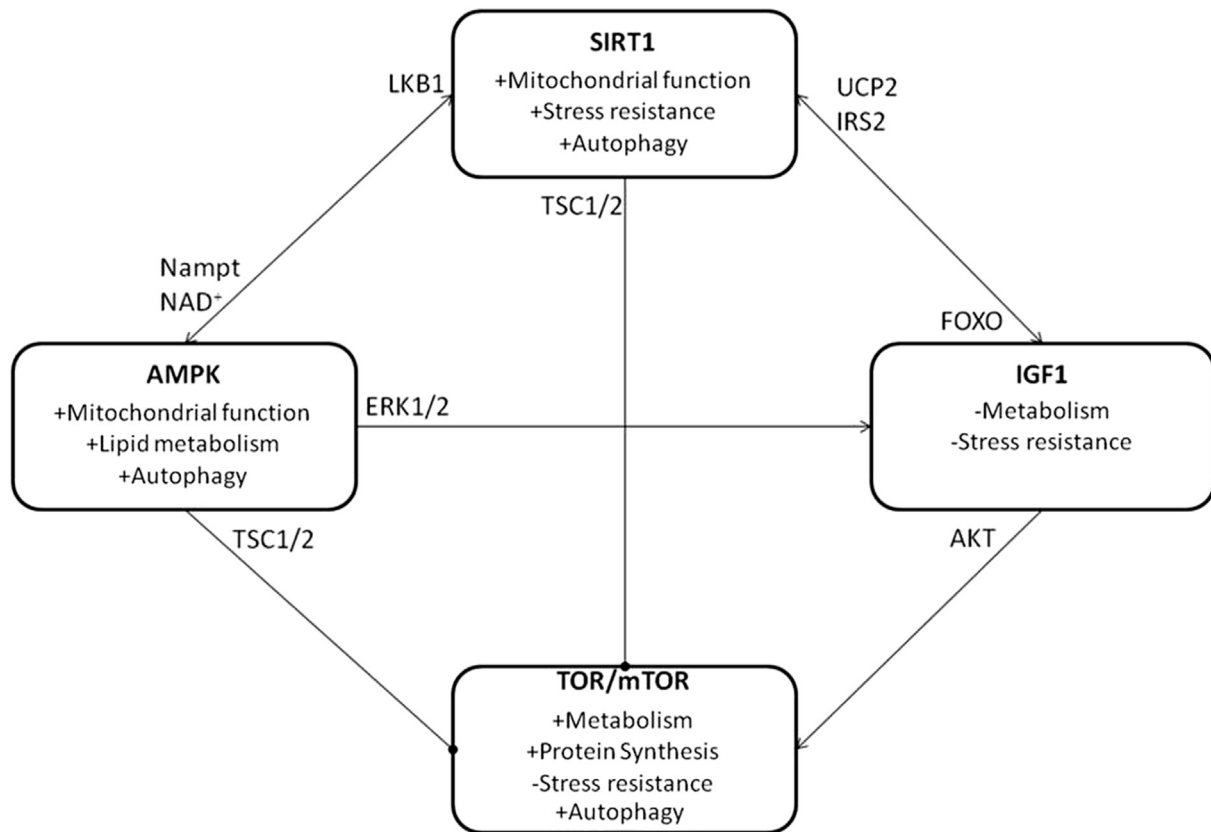


FIGURE 3.7 Interactions of the longevity network. The longevity network principally refers to positive and negative feedback mechanisms between four genes: SIRT1, IGF1, AMPK and mTOR. The signaling between these genes regulates biologic aging and numerous age-related diseases, including cardiovascular diseases. At the end of each line, an arrowhead indicates positive regulation/stimulation, and a round end indicates negative regulation or inhibition. SIRT1: Sirtuin1; TSC1/2: tuberous sclerosis 1/2; UCP2: mitochondrial uncoupling protein 2; IRS2: insulin receptor substrate 2; FOXO: O class forkhead box transcription factor; IGF1: insulin-like growth factor 1; AKT; protein kinase B; TOR/mTOR: target of rapamycin/mammalian target of rapamycin; ERK1/2: extracellular signal-regulated kinases 1/2; AMPK: adenosine monophosphate-activated protein kinase; Nampt: nicotinamide phosphoribosyltransferase; NADH: nicotinamide adenine dinucleotide; LKB: liver kinase B. *Adapted from North & Sinclair (2012).*⁵

phosphoribosyltransferase (Nampt) and nicotinamide adenine dinucleotide (NAD). In contrast, IGF1 is stimulated through the extracellular signal-regulated kinase cascade (ERK1/2).^{5,47}

The longevity network has strong links to the oxidative stress response and aims to preserve both cellular and organ function throughout aging. In the longevity network mitochondrial function, metabolism and protein synthesis are modulated to preserve cellular function, whereas stress resistance and autophagy are stimulated to promote cellular senescence or proliferation to preserve organ function above cell fate.^{5,46,47}

OTHER GENES AND PATHWAYS IN OXIDATIVE STRESS AND AGE-RELATED CARDIOVASCULAR DISEASES

CLOCK1

CLOCK1 (mCLK1) is a mitochondrial hydroxylase necessary for biosynthesis of ubiquinone, an endogenous

antioxidant and cofactor in cellular redox pathways. Ubiquinone is present in all membranes and is responsible for the hydroxylation of 5-demethoxyubiquinone to 5-hydroxyubiquinone in the mitochondrial electron transport chain.⁴⁸

The Cardiovascular System and mCLK1

Mutations causing partial or complete inactivation of mCLK1 are associated with delayed aging. Mice lacking mCLK1 are also protected from ischemia–reperfusion injuries, suggesting a role in vascular function and ischemic response. Studies show that partial loss of function impedes but does not stop certain developmental processes, including embryogenesis and the cell cycle (<http://emboj.embopress.org/content/18/7/1783>).⁴⁸

Oxidative Stress and mCLK

In mCLK haplotype mice, aging is delayed, despite increased generation of mitochondrial ROS. As well as protection from this oxidative stress, haplotype mice also have enhanced immunity and suffer limited damage from other challenges. Biomarkers of oxidative stress

in aging are also attenuated in the haplotype mouse. Seemingly, there is an initial increase in oxidative stress, with a subsequent higher protective capacity. A similar phenotype, without the longer lifespan, is observed in mice lacking antioxidant enzymes such as superoxide dismutase.^{48,49} This marks mCLK-modulated mitochondrial oxidative stress as a 'safe' process to alter within the biologic aging framework. Given the enhanced immunity and oxidative stress resistance, mCLK is currently a valuable target for investigation.^{48,49} In light of the above, the mCLK knockout mouse provides a genetic model to study mitochondrial oxidative stress in age-related cardiovascular decline. Work thus far is indicative of an increasingly important role for mitochondrial metabolism in cardiovascular aging.

Catalase

Catalase contains four porphyrin heme groups to facilitate degradation of hydrogen peroxide. Because of a high specific reactivity and easily detectable outcome, catalase is used analytically and industrially (e.g. in classifying bacteria).⁵⁰ Physiologically, catalase is a very common redox enzyme in nearly all aerobic organisms which protects the organism from oxidative stress. Despite this important function, catalase deficiency produces a largely normal phenotype. Animals and humans with acatalasia are only slightly more sensitive to oxidative stress.⁵¹

The Cardiovascular System and Catalase

When catalase is overexpressed in mitochondria, age-related cardiac damage is reduced and lifespan is increased by 20%.⁵² Transgenic mice are resistant to hypertrophy, fibrosis, angiotensin II-mediated mitochondrial damage and G_q- α subunit-mediated heart failure.^{52,53} In an *ex vivo* study of rat hearts, cardiac dysfunction occurs with ischemia and ischemia-reperfusion. When treated with catalase (and co-functioning superoxide dismutase), the ischemia-reperfusion damage is ameliorated. Other abnormalities from ischemia alone were also ameliorated (e.g. disruption of sodium/potassium ATPase, sodium-calcium exchange, and calcium uptake and release).⁵⁴

Oxidative Stress and Catalase

Typically catalase is a large contributor to neutralizing metabolically by-produced hydrogen peroxide to water and oxygen, thus preventing accumulation of hydroxyl radicals. It is theorized that other redox proteins, such as superoxide dismutase, will compensate when catalase levels are low.⁵⁴ However, catalase also interacts with many important pathways related to oxidative balance (e.g. host and pathogen defense and alcohol metabolism). Catalase also forms part of myocardial local redox defense systems against systemic oxidative stress (e.g. in obesity or insulin resistance). Under such

conditions, blocked angiotensin receptors increase antioxidant enzyme activity by 50–70%, with catalase having a marked increase.⁵⁵

A significant role for catalase has also been demonstrated in oxidative stress-induced cardiac remodeling. Mice with cardiac-specific G_q- α subunit overexpression provide a model for structural changes in the cardiovascular system. In their system, oxidative stress also increases at a faster than normal rate. The increase in oxidative stress causes dilated cardiomyopathy, which progresses to heart failure. When G_q- α subunit overexpressing mice are cross-bred with cardiac-specific catalase overexpressing mice there is a reduction in age-related and oxidative stress-related structural changes. Myocyte hypertrophy, apoptosis and heart failure were all prevented in cross-bred mice overexpressing both the G_q- α subunit and catalase in the heart. This occurred without affecting the initial oxidative stress phenotype of G_q- α subunit overexpressing mice.⁵⁶

Klotho

Klotho is a transmembrane protein, related to β -glucuronidases, that is highly expressed in specific kidney and brain regions. Klotho hydrolyzes steroid β -glucuronides and partially regulates systemic glucose metabolism and insulin sensitivity.⁵⁷ Overexpression of klotho delays aging, whereas deficiency results in a phenotype resembling human accelerated aging.^{58,59}

The Cardiovascular System and Klotho

Klotho expression maintains cardiovascular health. In fact, low klotho expression is thought to be a leading cause of vascular degeneration in chronic renal failure patients. Deficiency is also associated with arteriosclerosis, impaired angiogenesis and impaired endothelium-dependent vasodilation.⁵

Soluble klotho is formed when the extracellular domain is shed into the circulation; its levels decline with age. Cell surface binding sites with which soluble klotho interacts remain unknown. It is, however, known that klotho binding results in perturbed intracellular insulin/IGF1 signalling. This is proposed as a mechanism for klotho's implication in healthy aging, as the aging phenotype of klotho-deficient mice is averted by perturbing the insulin/IGF1 signaling cascade. This explains the association of klotho single nucleotide polymorphisms (SNPs) with age-related cardiovascular decline.^{5,60}

Oxidative Stress and Klotho

In vitro and *in vivo* research has provided evidence of klotho's potential to increase the endothelial layer's resistance to oxidative stress. This is achieved by maintaining signals for nitric oxide production. Defective

klotho signaling also affects the heart, leading to fatal sinoatrial node dysfunction under stress.^{6,61}

Pituitary Transcription Factor 1 and Prophet of Pituitary Transcription Factor 1

Pituitary transcription factor 1 (PIT1) is a pituitary-specific transcription factor responsible for normal pituitary development and for expression of hormones regulating mammalian growth.⁶² Homeobox protein prophet of PIT1 (PROP1), another pituitary transcription factor, possesses transcriptional activation properties as well as DNA-binding properties. PROP1 expression leads to the development of specialized pituitary cells. The knockout of PIT1 in Snell/Ames dwarf mice inhibits the development of such anterior pituitary cells, in turn disturbing expression of other signaling peptides and contributing to the risk of diseases.⁶²

Cardiovascular Disease and PIT1/PROP1

Mostly what is known about PIT1 and PROP1 is their effects on biologic aging and growth. Patients deficient in PIT1 and PROP1 typically have growth hormone deficiency. PROP1 deficiency also causes hypogonadism and deficiency of prolactin and thyroid-stimulating hormone.⁶² Interestingly, Snell/Ames dwarf mice with PROP1 deficiency and delayed aging also exhibit lower cardiac collagen content and smaller cardiomyocytes. These properties may be beneficial with regard to preserving cardiovascular health in aging.⁶³

Oxidative Stress and PIT1/PROP1

In Snell/Ames dwarf mice, where the PIT1/PROP1 signaling is perturbed, there is an altered response to oxidative stress. This altered response is thought to increase resistance to oxidative damage and contribute to healthy aging in this mouse.⁶⁴

The dwarf mouse is known to be particularly resistant to mitochondrial oxidative stress. This was investigated by inducing mitochondrial oxidative stress through inhibition of mitochondrial complex II with 3-nitropropionic acid (3-NPA) treatment. The activator protein 1 (AP-1) transcription factor, which regulates transcriptional responses to numerous stimuli, contains c-Jun family proteins. Phosphorylation of Ser⁶³ and Ser⁷³ residues on the c-Jun protein is necessary to mediate apoptotic and/or proliferative responses to oxidative stress. In the dwarf mouse, after generation of mitochondrial oxidative stress by 3-NPA treatment, there is a lack of c-Jun Ser⁶³ phosphorylation. This contrasts with the wild type rapid and robust phosphorylation of Ser⁶³ and Ser⁷³ residues. This mechanism allows cell survival in the face of mitochondrial oxidative stress in the dwarf mouse.⁶⁴

p66^{Shc}

The Shc locus regulates several metabolic processes in mammals. Three splice variants are coded with molecular masses of 46, 52 and 66 kDa; they each carry a Src-homology 2 domain, a collagen-homology region and a phosphotyrosine-binding domain. The p66 splice variant has a unique N-terminal region with redox enzyme properties. It is actively involved in generating mitochondrial ROS. This N-terminal region is also part of the signaling cascade that translates oxidative signals to apoptosis.^{5,65}

The Cardiovascular System and p66^{Shc}

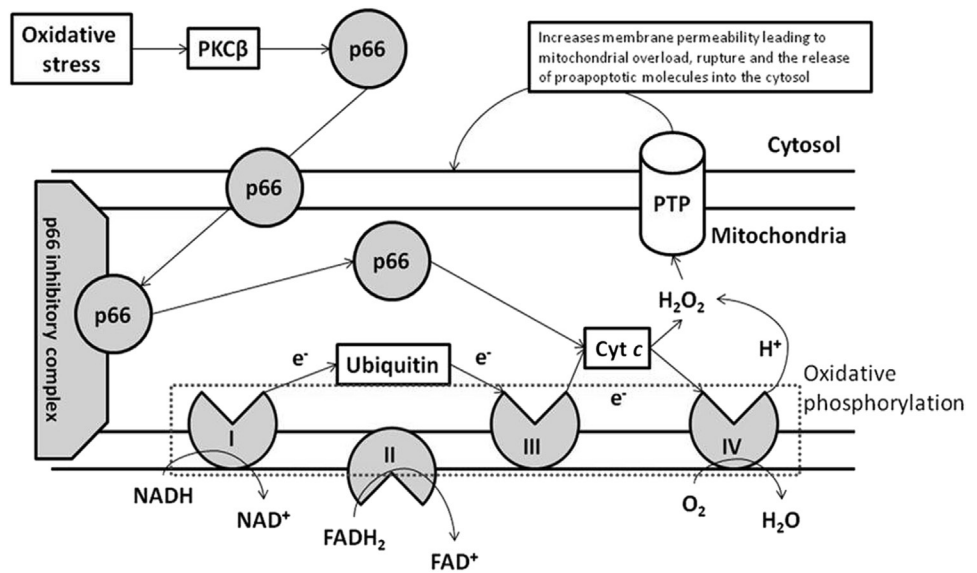
Knockout of p66^{Shc} delays aging, decreases generation of ROS, cardiac progenitor cell senescence, necrosis and DNA damage; vascular endothelial cell resistance to oxidative stress is increased.⁶⁶ Loss of p66^{Shc} maintains left ventricular volume, reduces heart failure, and protects high-fat fed mice from atherogenesis.⁶⁶ In humans, p66^{Shc} expression increases with age, under basal and disease-related conditions.⁶⁷ Generally speaking, p66^{Shc} is a mediator of many cellular processes, namely apoptosis and stress response to maintain cardiovascular function.

Oxidative Stress and p66^{Shc}

A mechanistic model of p66^{Shc} is that it oxidizes cytochrome *c*, preventing it from reducing oxygen radicals to water. Cytochrome *c* is at the final steps of mitochondrial oxidative phosphorylation. The presence of p66^{Shc} at this point diverts electron flow to produce hydrogen peroxide rather than water. Hydrogen peroxide then opens the mitochondrial permeability transition pore (PTP) which increases mitochondrial membrane permeability to ions, solutes and water. This increased influx swells and ruptures mitochondria, releasing molecules such as cytochrome *c* into the cytosol. Cytochrome *c* acts as a proapoptotic factor in the cytosol. Therefore, p66^{Shc} plays a vital role in mediating cellular generation of oxidants (e.g. hydrogen peroxide) as well as cellular apoptotic responses through downstream signaling.⁹ See [Figure 3.8](#) for the process through which p66^{Shc} induces mitochondrial rupture and cellular apoptosis.

What remains unclear is the signaling between extracellular, intracellular, exogenous and endogenous oxidative stress and p66^{Shc}. It is hypothesized that p66^{Shc} traffics between cytosolic and mitochondrial compartments following phosphorylation by protein kinase C-β (PKCB) in response to oxidative stress. Entry of p66^{Shc} disrupts calcium signaling, thus rearranging and fragmenting the mitochondrial matrix, allowing the pathway of oxidative stress and apoptosis to continue. This provides a convenient explanation for p66^{Shc} knockout animals having similar resistance to apoptotic signals responding to exogenous as well as endogenous

FIGURE 3.8 The role of p66^{Shc} in apoptosis. Oxidative stress stimulates protein kinase C- β which phosphorylates p66^{Shc}. p66^{Shc} can then traffic into the mitochondria where it is bound to an inhibitory complex. Pro-apoptotic stimuli destabilize p66^{Shc}, allowing it to oxidize cytochrome *c* between the third and fourth steps of oxidative phosphorylation. This catalyses the production of hydrogen peroxide from oxygen. Hydrogen peroxide induces the opening of the mitochondrial permeability transition pore, leading to an increase in mitochondrial membrane permeability to ions, solutes and water. Mitochondria then swell and rupture, releasing pro-apoptotic factors into the cytosol. Adapted from Cosentino et al (2008).⁹



oxidative stress. This creates a third role for p66^{Shc} as an intermediary signaling molecule, as well as generating oxidative stress and regulating apoptosis.⁹

SUMMARY POINTS

- Cardiovascular disease is a large burden on the population, in terms of lives lost and economic loss.
- The aging portion of the population is increasing and is more likely to suffer the effects of cardiovascular decline leading to clinically relevant cardiovascular disease.
- Reduced cardiac antioxidant enzyme activity and increased generation of oxidative stress with aging contributes significantly to the risk of cardiovascular disease.
- The telomere theory states that telomere attrition, which is increased with oxidative stress and with age, significantly contributes to cardiovascular decline.
- Cardiomyocytes are particularly rich in mitochondria to meet the continuous and lifelong demand for energy and oxygen. Therefore the cardiovascular system is especially sensitive to mitochondrial generation of ROS.
- On the cellular level, mitochondrial oxidative stress is a significant mediator of age-related cardiovascular decline.
- SIR2, IGF1, mTOR and AMPK are key genes in the longevity network that regulate oxidative stress and associated changes to cardiovascular function.
- p66^{Shc} is a powerful mediator of cellular oxidative balance. It functions as a signaling peptide for oxidative stress, as a contributor to mitochondrial generation of reactive oxygen species, and as an intermediary signaling molecule in apoptosis.

- PIT1 and PROP1 are pituitary-specific transcription factors that mediate important apoptosis signaling mechanisms in the cells that they target.
- Catalase is an important ubiquitously expressed antioxidant enzyme that controls apoptosis and hypertrophy responses in the myocardium.
- Klotho and mCLK regulate vascular health under oxidative stress and the generation of mitochondrial ROS, respectively.

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