The population in the Western world is aging at an unprecedented rate. The substantial increase in life expectancy is associated with significant age-related cardiac, arterial, and microvascular disease burden. In the United States, ischemic heart disease and stroke are the leading cause of death (see definition in Table 1), and their incidence exponentially increases with advanced age. Epidemiological studies clearly show that aging itself is the major risk factor for cardiovascular and cerebrovascular diseases. Yet, most of the research efforts on prevention of these diseases have ignored the mechanisms underlying cardiac and vascular effects of aging and have focused, instead, on the development of interventions that target conventional risk factors, including hypertension, hyperglycemia, hypercholesterolemia, and high circulating levels of triglycerides. However, recent preclinical studies have identified new approaches to combat cardiovascular disease. Calorie restriction has been reproducibly shown to prolong lifespan in various experimental model animals. This has led to the development of calorie restriction mimetics and other pharmacological interventions capable to delay age-related diseases. In this review, we will address the mechanistic effects of aging per se on the cardiovascular system and focus on the prolongevity benefits of various therapeutic strategies that support cardiovascular health. (Circ Res. 2016;118:1626-1642. DOI: 10.1161/CIRCRESAHA.116.307475.)

Key Words: aging ■ calorie restriction ■ cardiovascular diseases ■ pharmacological strategies ■ prevention

Mechanisms of Cardiovascular Aging: From Oxidative Stress and Chronic Low-Grade Inflammation to Structural and Functional Impairment

Cardiac Aging

A continuum of progressive cardiac structural and functional alterations occurs with age in humans and laboratory animals, including increases in collagen levels, cardiac hypertrophy, decreased heart rate and diastolic filling rate, and impaired left ventricle function (reviewed recently in Dai et al). The molecular and cellular mechanisms of cardiac benefits of therapeutic strategies that have the potential to improve cardiovascular function in the elderly and delay the onset of age-related cardiovascular diseases (CVD) are also discussed (see summary in Table 2 and Figure 1).
aging involve macromolecular damage and mitochondrial oxidative stress,48–50 perturbation of proteostasis,51 age-dependent declines in autophagy and ubiquitin proteasome degradation,52,53 stem cell dysfunction,54,55 extracellular matrix remodeling,56,57 increased apoptosis,58 impaired bioavailability of nitric oxide (NO),59 poly(ADP-ribose) polymerase 1 (PARP-1) activation and cellular energetic dysfunction,60 activation of the renin–angiotensin–aldosterone system, and age-related low-grade sterile inflammation.

Vascular Aging
Changes in the structure and function of the large arteries that occur throughout life include diffuse intimal and medial thickening and increased stiffness of wall components, a cause of reduced distensibility of central arteries.61,62 Age-related chronic inflammation in the large arteries promote the pathogenesis of atherosclerotic diseases (stroke, peripheral artery disease, and myocardial infarction), which are a leading cause for mortality and morbidity in the elderly.

The microcirculation, with a total length of ≈100,000 km, is the most ubiquitous organ system, which envelops virtually every cell in the human body and whose age-related alterations fundamentally impact the function of every organ. The mechanisms by which microvascular alterations contribute to age-related functional decline of multiple organ systems include microvascular endothelial dysfunction,63–65 microvascular rarefaction,66–68 dysfunction of local vasoregulatory mechanisms, including impaired shear stress–induced vasodilation,69 myogenic autoregulatory dysfunction,70–72 impaired microvascular functional adaptation to hypertension in the brain,73,74 disruption of microvascular barrier function (eg, blood brain barrier disruption70,71), neurovascular uncoupling,72 activation of inflammatory processes,73,74,75,76 impaired angiogenic capacity,71,72,76 and alterations in the secretory function of microvascular endothelial cells.77

Studies on aged laboratory rodents, nonhuman primates, and human subjects showed that cellular and molecular mechanisms underlying both arterial and microvascular aging include endothelial dysfunction,78 extracellular matrix remodeling, nicotinamide adenine dinucleotide (NAD) phosphate oxidase activation and mitochondrial oxidative stress,79–85 increased peroxynitrite production,86 nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) activation and upregulation of proinflammatory cytokines and chemokines,87–89 NF-E2-related factor 2 (Nrf2) dysfunction and impaired cellular stress resistance,89,90 increased susceptibility for vascular injury,91–94 mitochondrial dysregulation,2,91,95 and endothelial senescence95 and apoptosis.91,96

Table 1. List of Terms and Their Definitions

<table>
<thead>
<tr>
<th>List of Terms</th>
<th>Definitions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cause of death</td>
<td>The disease or injury, which initiated the train of morbid events leading directly to death, or the circumstances of the accident or violence which produced the fatal injury</td>
</tr>
<tr>
<td>Healthspan</td>
<td>Period of time of disease-free health</td>
</tr>
<tr>
<td>Lifespan</td>
<td>Amount of time that a person or animal actually lives</td>
</tr>
<tr>
<td>Morbidity</td>
<td>Incidence or prevalence of a disease or of all diseases</td>
</tr>
<tr>
<td>Mortality rate</td>
<td>Measure of the number of deaths in a given population</td>
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</table>

Potential Interventions That Retard Cardiovascular Aging
Although aging was historically believed to be an inevitable and intractable process, it is now well-appreciated that aging can be modulated through various environmental, lifestyle, genetic, and pharmacological interventions. Dietary regimens and drugs that can slow the aging process continue to raise interest among the general public as well as the scientific and medical communities.92 There are already a few antiaging interventions available that promote healthspan and lifespan extension and have been validated in at least 3 model organisms by 3 different laboratories. These interventions include fasting regimens, calorie restriction (CR; the reduction in the intake of calories without malnutrition), exercise, and the use of low molecular weight compounds, including metformin, resveratrol, and rapamycin.92

Cardiovascular Protective Effects of Calorie Restriction
To date, CR is the most robust intervention that has been reproducibly shown to prolong lifespan and delay the onset of age-associated diseases in both invertebrates and vertebrates, including mammals.93,94 Therefore, the successful use of pharmacological interventions that slow aging and prevent chronic disease requires an understanding of how CR delays cardiovascular aging and increases lifespan.

There is increasing epidemiological and experimental evidence that CR confers multifaceted cardiovascular protective effects (Figure 2) in aging and in pathological conditions associated with accelerated vascular aging.95 In laboratory animals, CR
### Table 2. Pharmacological Strategies to Retard Cardiovascular Aging

<table>
<thead>
<tr>
<th>Intervention or Compound</th>
<th>Main Mechanism of Action</th>
<th>Lifespan Extension</th>
<th>Effects on the Cardiovascular System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calorie restriction</td>
<td>• Sirt1 activator</td>
<td>Yeast, flies, worms, mice</td>
<td>• Reduction of body weight, body fat, and blood pressure; increase in insulin sensitivity; improved lipid profile and adipocyte dysfunction; and improvement of endothelial function&lt;sup&gt;2–5&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• AMPK activator</td>
<td>Healthspan and possibly average life span in primates and humans.</td>
<td>• Prevention of atherosclerosis and arterial stiffening&lt;sup&gt;6&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• mTOR inhibition</td>
<td></td>
<td>• Reduction of myocardial interstitial fibrosis, cardiac apoptosis, and improvement of cardiac function&lt;sup&gt;7&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• NRF2 activator</td>
<td></td>
<td>• Confers microvascular protection by improving endothelial angiogenic capacity and increasing cortical microvascular density&lt;sup&gt;8&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• Others?</td>
<td></td>
<td>• Restoring microvascular NO synthesis, enhancement of metabolism of parenchymal tissues&lt;sup&gt;9&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rapamycin</td>
<td>• mTOR inhibitor</td>
<td>Yeast, flies, worms, mice</td>
<td>• Attenuation of load-induced cardiac hypertrophy, restraint in the increase in myocyte cell size&lt;sup&gt;10,11&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Reduction of ischemic injury after myocardial infarction&lt;sup&gt;12,13&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Decrease in inflammation and hypertrophy&lt;sup&gt;14&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td></td>
<td>• Higher metabolism&lt;sup&gt;14&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>• Preservation of cardiac stem cell pool&lt;sup&gt;15&lt;/sup&gt;</td>
</tr>
<tr>
<td>Metformin</td>
<td>• Increase in AMPK activity</td>
<td>Yeast, flies, worms, mice</td>
<td>• Improvement in endothelial function&lt;sup&gt;16&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>• Diabetic humans (under clinical trials)</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td>• Increase in blood flow, reduction in systolic BP, and improvement in vasodilation&lt;sup&gt;17–19&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Regulation of endothelial progenitor cell differentiation&lt;sup&gt;20&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>• Stimulation of ischemia-induced revascularization&lt;sup&gt;21&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Improvement in vascular anti-inflammatory properties and decrease of serum levels of high-sensitivity C-reactive protein&lt;sup&gt;22&lt;/sup&gt;</td>
</tr>
<tr>
<td>Resveratrol</td>
<td>• Sirt1 activator</td>
<td>Yeast, worms</td>
<td>• Lowering of blood pressure, increase in flow-mediated dilatation of the brachial artery, improvement of endothelial function, decrease in plasma inflammatory biomarkers (in humans, depending on the metabolic state of patients)&lt;sup&gt;23&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• AMPK activator</td>
<td>Controversial results in flies, mice and humans</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Others?</td>
<td></td>
<td></td>
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<tr>
<td>SRT1720/SRT2104</td>
<td>• Sirt1 activators</td>
<td>Mice and rats</td>
<td>• Improvement in endothelial function and attenuation in vascular oxidative stress and inflammation&lt;sup&gt;24&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Under clinical trial for humans</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Antiatherogenic activity&lt;sup&gt;25&lt;/sup&gt;</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>• Angiotensin-converting enzyme inhibition</td>
<td>Worms and rats</td>
<td>• Increase of NO production&lt;sup&gt;26&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Improvement in cardiac function and metabolism and endothelial function&lt;sup&gt;27&lt;/sup&gt;</td>
</tr>
<tr>
<td>Aspirin</td>
<td>• Irreversible inactivation of cyclooxygenases</td>
<td>Male mice</td>
<td>• Decreased expression of inducible nitric oxide synthase (iNOS) and Cox-2&lt;sup&gt;28&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>• AMPK activator</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Statins</td>
<td>• Inhibition of HMG-CoA reductase</td>
<td>Flies</td>
<td>• Reduction in ROS levels in cardiac muscle&lt;sup&gt;22&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Increased NO synthesis and neoangiogenesis in endothelial cells and the central nervous system&lt;sup&gt;29&lt;/sup&gt;</td>
</tr>
<tr>
<td>β-blockers</td>
<td>• β-adrenergic receptor antagonists</td>
<td>Flies</td>
<td>• Use in treatment of hypertension and ischemic heart disease, prevention of the transition to heart failure via NO-dependent mechanisms (celiprolol)&lt;sup&gt;30&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Only mean lifespan in mice</td>
<td></td>
</tr>
<tr>
<td>AT1 blockers</td>
<td>• Angiotensin II receptor antagonists</td>
<td>Hypertensive mammals</td>
<td>• Improvement in cardiac function and metabolism and enhanced endothelial function&lt;sup&gt;31&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Increase in eNOS expression in the heart and carotid artery and marked reduction in tissue ACE expression/activities&lt;sup&gt;31&lt;/sup&gt;</td>
</tr>
<tr>
<td>Omecamtiv mecarbil</td>
<td>• Enhances myosin and actin cross-bridge formation</td>
<td>Unknown</td>
<td>• Prolonged systolic ejection time and increased ejection fraction</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Disparate effects on myocardial oxygen consumption</td>
</tr>
</tbody>
</table>

(Continued)
Table 2. Continued

<table>
<thead>
<tr>
<th>Intervention or Compound</th>
<th>Main Mechanism of Action</th>
<th>Lifespan Extension</th>
<th>Effects on the Cardiovascular System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Berberine</td>
<td>• AMPK activator</td>
<td>• Flies</td>
<td>• Decrease in the expression of iNOS and Cox-2 as well as increase the (AMP+ADP)/ATP ratio by impeding the efficiency of mitochondrial electron transport32</td>
</tr>
<tr>
<td>PUFAs</td>
<td>• Peroxidation</td>
<td>• Low amounts increase lifespan in worms</td>
<td>• Lowering of triglyceride levels in the blood32</td>
</tr>
<tr>
<td></td>
<td>• Membrane modification</td>
<td>• High amounts decrease lifespan in worms and mice</td>
<td></td>
</tr>
<tr>
<td>Nrf2 (Nfe2l2) activators</td>
<td>• Activate Nrf2-antioxidant response</td>
<td>• Flies and worms</td>
<td>• Regulation of cellular antioxidant defenses and maintenance of redox homeostasis33-39</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Regulation of the proteasome and removal of oxidized proteins43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Maintenance of the functional integrity of the heart and vasculature41,42</td>
</tr>
<tr>
<td>Mito-targeted antioxidants</td>
<td>• Antioxidant</td>
<td>• Controversial results</td>
<td>• Reduction of ischemia reperfusion injury and reperfusion arrhythmia and preservation of myocardial function42,44</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Antiatherogenic effects45</td>
</tr>
</tbody>
</table>

Mechanism of action of various interventions and their effects on lifespan extension, if any, and on the cardiovascular system are described. ACE indicates angiotensin-converting enzyme; AMPK, adenosine monophosphate–activated protein kinase; AT1, angiotensin II receptor, type 1; BP, blood pressure; eNOS, endothelial NO synthase; HMG-CoA, 3-hydroxy-3-methylglutaryl-coenzyme A; iNOS, inducible NO synthase; mTOR, mechanistic target of rapamycin; NO, nitric oxide; Nrf2, NF-E2-related factor 2; PUFAs, polyunsaturated fatty acids; and ROS, reactive oxygen species.

has been shown to improve endothelial function,2-5 prevent atherosclerosis and arterial stiffening,6 reduce myocardial interstitial fibrosis and cardiac apoptosis, and improve cardiac function.7 CR also confers significant microvascular protection by improving endothelial angiogenic capacity, increasing cortical microvascular density,8 and restoring microvascular NO synthesis, all of which enhance the metabolism of parenchymal tissues.9

Insight into the beneficial effect of CR on several CVD and stroke risk factors in humans emanates from studies in which obese individuals were treated with some form of relatively short-term dietary restriction to lose weight. Nearly 70% of American adults are either overweight or obese, and obesity dramatically increases the risk for health problems, such as heart disease, stroke, high blood pressure (BP), type 2 diabetes mellitus, and more.1 In fact, >2150 Americans die from CVD each day, claiming more lives than cancer and chronic lower respiratory diseases combined.1 Therefore, weight loss offers significant improvement in the incidence of cardiovascular and metabolic disease in these individuals through reduction in body mass index, body fat, total cholesterol, serum triglyceride, inflammation, and endothelial and adipocyte dysfunction.96-98 Of significance, a diet enriched in multiple functional ingredients and concepts, that is, natural antioxidant-rich foods, omega-3 fatty acids, prebiotics and probiotics, low-glycemic-impact foods/meals, and blood cholesterol–normalizing ingredients, reduces blood lipids and improves other cardiometabolic risk markers in healthy overweight/obese subjects in a manner that is independent of body weight reduction.99 Moreover, results from a 6-month clinical trial show that 25% CR is able to reduce the estimated 10-year CVD risk100 in nonobese individuals, based on total and high-density lipoprotein cholesterol (expressed as their ratio), systolic BP, age, and sex.100 The Comprehensive Assessment of Long-Term Effects of Reducing Intake of Energy (CALERIE) Study Group has recently published a 2-year study of 25% CR in nonobese individuals that shows significant decreases in body weight, serum cholesterol, triglycerides, and mean BP without adverse events.102 The risk of coronary heart disease deaths in Asian nonobese individuals of both sexes was found to be reduced with lower energy intake.103 Although high heart rate variability is associated with improved cardiovascular function, low heart rate variability has been linked to poor cardiovascular function.63,104 Long-term 30% CR (7 years on average) increases heart rate variability to a level comparable with published norms for healthy individuals 20 years younger, indicating a systemic effect that counteracts the expected age-associated changes in autonomic function.105

As seen in humans, studies of the effects of CR in rhesus monkeys have shown a reduction in body weight, body fat, BP, and triglyceride levels that was accompanied by improvement in glucoregulation and lipoprotein profile.54 A 50% reduction in the incidence of CVD among CR-fed monkeys has been reported by Colman et al106; however, this observation could not be replicated in a second CR study in monkeys,43 probably because of differences in diet composition and feeding protocols.

Intermittent fasting is a well-established intervention that exerts beneficial effects on many biomarkers of cardiovascular aging and risk factors for CVD in humans, including a decrease in circulating C-reactive protein.107 Intermittent fasting entails to fast on some days and feed on others and, in doing so, reduces cardiovascular risk.108 This CR-like regimen also improves physiological cardiovascular parameters,109-111 facilitates weight loss, prevents the progression of type 2 diabetes mellitus, and seems to be cardioprotective by providing resistance to ischemic injury in rodents.112,113

Although the cellular and molecular mechanisms underlying the cardiovascular protective effects of CR regimes
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are still not completely understood,95 the molecular basis of cardiovascular protection relies on its beneficial effects on different hallmarks of aging, such as metabolism, cellular oxidative stress, inflammation, autophagy, mitochondrial activity, and stem cell function.114 The existing evidence suggests that CR may improve vascular health by eliciting changes in circulating neuroendocrine factors.115 Indeed, studies show that circulating factors present in the sera of CR-fed rats and nonhuman primates confer significant antioxidative, anti-inflammatory, and proangiogenic effects in cultured endothelial cells.3,75 In addition, there is also evidence that CR activates endogenous antioxidative, proangiogenic, anti-apoptotic, and anti-inflammatory mechanisms in cell-autonomous manner, retaining a youthful phenotype in vascular cells.2

Previous studies suggest that sirtuins (see below) are key mediators of the antiaging effects of CR,116 including its antioxidant and anti-inflammatory vascular effects.3 There is also important evidence that activation of Nrf2, an evolutionarily conserved transcription factor with cytoprotective and prosurvival functions, contributes to the beneficial effects of CR.2,34 The activation of adenosine monophosphate–activated protein kinase (AMPK) by mechanistic target of rapamycin (mTOR) is another key signaling pathway implicated in CR-mediated cardiovascular protection.117 Potentially, the aforementioned mechanisms that contribute to the effects of CR can be harnessed for the development of new pharmacological approaches to prevent and treat cardiovascular and cerebrovascular diseases in elderly patients.118

In response to 25% to 30% CR, which usually leads to a 10% decrease in body weight and to intermittent fasting, improvement in CVD markers is observed in humans.101,102 However, more studies are needed to understand the dynamic interplay between the degree of CR and the frequency of food consumption in the modulation of metabolic and molecular pathways and prevention of cardiovascular diseases.

Pleiotropic Cardiovascular Protective Effects of Growth Hormone/Insulin-Like Growth Factor 1 Axis

Growth hormone (GH) is involved in the regulation of somatic growth and development119 and in the regulation of metabolism120 by acting directly via the GH receptor and subsequent

Figure 1. Pharmacological strategies to combat cardiovascular (CV) aging. Age-associated changes in cardiac and vascular properties (depicted in the inner red circle) can be delayed by targeting the related pathways (in the middle yellow circle) with small molecules (represented in the outer blue circle). Some of the pharmacological strategies highlighted in the diagram (bold and underlined) have been shown to improve longevity in healthy mammals. AMPK indicates 5’ adenosine monophosphate–activated protein kinase; Ang-II, angiotensin II; AT1, angiotensin II receptor, type 1; Chol, cholesterol; GH, growth hormone; iACE, inhibitors of angiotensin-converting enzyme; IGF-1, insulin-like growth factor-1; mTOR, mechanistic target of rapamycin; NO, nitric oxide; NOS, nitric oxide synthase; Nrf2, NF-E2-related factor 2; PARP-1, poly(ADP-ribose) polymerase 1; PUFAs, polyunsaturated fatty acids; ROS, reactive oxygen species; and SIRT-1, sirtuin (silent mating type information regulation 2 homolog) 1.

Figure 2. Antiaging effects of caloric restriction in the cardiovascular system. The up arrow notation indicates an improvement or increase, whereas the down arrow shows decrease or impairment of cardiovascular functions and pathologies. NO indicates nitric oxide.
production of insulin-like growth factor 1 (IGF-1) from the liver. The local production of IGF-1 in the cardiovascular system promotes paracrine signaling and is associated with cardiovascular protection in humans and laboratory animals.122–124 The systemic GH and IGF-1 levels decline progressively during aging,125 and although controversial, GH and IGF-1 deficiency seem to be involved in the increased CVD risk and endothelial dysfunction.126–128 The CR-mediated increase in cardiac-specific IGF-1 expression could contribute to the paracrine cardiovascular protection.129,130 The mammalian heart has a limited amount of cardiomyocyte stem cells, and this number tends to decrease with aging.131 IGF-1 overexpression is able to prevent this loss by mounting an effective response on several fronts: delay in cellular aging and death via enhanced nuclear localization of phosphoautokinase B (AKT) and increased telomerase activity,132 protection against apoptosis133 and oxidative damage, and lower replicative senescence rate of resident stem cells.132 The use of IGF-1 as a adjuvant in stem cell therapy has been demonstrated through exposure of old animals to youthful circulation—rich in circulating IGF-1 levels—by heterochronic parabiosis.134 It is well documented that GH deficiency and low circulating levels of IGF-1 significantly increase the risk for cardiovascular and cerebrovascular diseases in humans (for a review, see Ungvari and Csiszar135). In addition to its effect on stem cell function, significant microvascular protection is conferred by endocrine and paracrine IGF-1 signaling.136 Microvascular dysfunction because of age-related IGF-1 deficiency has been causally linked to the pathogenesis of vascular cognitive impairment and has also implications for the pathophysiology of cardiac failure.137 Despite evidence that treatment with low doses of GH may exert beneficial effects in the cardiovascular system, the administration of supraphysiological levels of IGF-1 is accompanied with side effects (eg, potential diabetogenic and protumorigenic action of IGF-1) that should be carefully monitored.

mTOR Signaling Is an Important Modulator of the Cardiovascular Aging Phenotype

A leading target for antiaging interventions is the nutrient response pathway controlled by mTOR signaling.138,139 Inhibition of this pathway by CR extends lifespan and confers healthspan increase in various animal models.138–142 mTOR is a serine/threonine kinase that activates cell anabolism, especially increasing protein synthesis and cell growth, while inhibiting catabolic mechanisms, notably autophagy. mTOR associates with specific adaptor proteins to form 2 distinct complexes, termed mTORC1 and mTORC2. mTORC1 phosphorylates S6K1 or 4EBP1 to promote messenger RNA translation, and AKT and AMPK are the main mTORC1 regulators. Increase in nutrient and growth factor availability stimulates AKT-mediated activation of mTOR, but suppresses AMPK function. Activation of AMPK occurs during stress or energy deprivation, thereby inhibiting mTOR.138,139 The fundamental role of mTOR signaling in metabolic regulation contributes to the biogenesis and proper functioning of the cardiovascular system.140 In fact, embryos lacking mTORC1 or mTORC2 have failed to develop.141 Genetic disruption of mTORC1 in mouse myocardium has been implicated in dilated cardiomyopathy, through activation of autophagy and apoptosis, and accumulation of 4EBP1 associated with an increase in heart failure.146 Mice deficient in raptor (component of the mTORC1 complex) had impaired metabolism at first, followed by high mortality a few weeks later because of dilated cardiomyopathy caused by increase in autophagy and apoptosis and reduction in cardiomyocyte growth.147 Similarly, deletion of Rictor (mTORC2 complex member) is lethal for most embryos, but the surviving mice display cardiovascular abnormalities.148 However, a down-modulation of mTOR signaling confers cardiovascular benefits in the aging animals as evidenced by the fact that the mTORC1 inhibitor rapamycin has been reported to attenuate load-induced cardiac hypertrophy, dampen the increase in myocyte cell size,10,11 and reduce ischemic injury after myocardial infarction.12,13 Furthermore, female mice supplemented with rapamycin late in life showed improved cardiovascular aging through the decrease in inflammation and hypertrophy and higher metabolism.14

There is a progressive incidence of cardiac hypertrophy and diastolic dysfunction with advancing age as well as accumulation of protein damage mediated by oxidation and ubiquitination. Of significance, these age-associated conditions are hampered by short-term CR and rapamycin treatment.137 Inhibition of mTORC1 by rapamycin confers protection against these age-related CVD, especially in the presence of metabolic disorders. In fact, mTOR seems to be dysregulated with aging, and therefore, a partial inhibition of this pathway allows for better control of mTOR activity in cardiovascular aging.144 By acting as a regulator of cell growth and proliferation, mTOR is also responsible for stem cell exhaustion and dysfunction. So, mTOR inhibition is beneficial also for the preservation of cardiac stem cell pool that normally decreases during aging and disease.15

Sirtuin Activation Confers Diverse Antiaging Cardiovascular Protective Effects

Members of the sirtuin family of protein deacetylases are among the best-studied mediators of CR, and the contribution of silent information regulator 1 (SIRT1, after the yeast Sir2) has been the most extensively examined. The NAD+-dependent deacetylase SIRT1 is involved in several key cellular functions, including chromatin remodeling—through histone deacetylation—and gene expression, and also in cellular energy metabolism.116 The deletion of SIRT1 interferes with CR-mediated lifespan extension in yeast, worms, and flies.149–151 There is strong evidence that SIRT1 exerts multifaceted antiatherogenic,152 anti-inflammatory,153 endothelial protective,154 and cardioprotective155 effects. These findings have led to the search of small molecule activators of SIRT1 as therapeutics to improve cardiovascular health. Earlier studies have established that the natural polyphenol resveratrol was able to activate Sir2 in yeast and SIRT1 in humans and increase cell survival through acetylation of p53.156 In rodents, resveratrol promotes transcriptional responses comparable to CR-mediated SIRT1 activation,156 improves health and survival of mice on a high-calorie diet,157 and confers multifaceted antiaging vascular effects (including potent mitochondrial protective and anti-inflammatory effects) and protection against atherosclerosis, hypertension, ischemia/reperfusion...
injury, and heart failure. Resveratrol improves cerebrovascular function, increases cerebrovascular density and prevents cerebral microhemorrhages, all of which likely contribute to resveratrol-mediated improvement of cognitive function in aged mice. Preclinical studies also indicate that resveratrol supplementation reduces platelet aggregation and improves lipid metabolism, while inhibiting atherosclerotic plaque formation and markers of oxidative stress and inflammation. It is within this context that resveratrol improves arterial stiffness in nonhuman primates fed high-fat, high-sugar diet through decreased levels of caspase 3 and lipid peroxidation. However, resveratrol also elicits off-target cellular effects, whereby AMPK is activated in a SIRT1-independent fashion and phosphodiesterases inhibited nonselectively, causing a rise in intracellular cAMP levels with concomitant, sirtuin activation and improvement in age-related phenotypes. The redox-sensitive transcription factor Nrf2 is potently activated by resveratrol. The limited number of randomized clinical trials has generated controversial results on the effect of resveratrol supplementation in humans. It would seem that resveratrol is associated with lower CVD marker levels and reduced obesity at least when studies were conducted in subjects with metabolic syndrome (reviewed in Novelle et al). SRT1720 is a specific, synthetic SIRT1 activator that has demonstrated health and lifespan benefits in models of accelerated aging. There is improvement in endothelial function and attenuation in vascular oxidative stress and inflammation in SRT1720-treated mice as they age. SRT1720 possesses also antiatherogenic activity. The polyphenol S17834, which upregulates SIRT1, has similar anti-inflammatory and antiatherogenic actions and exerts cardioprotection in mice with accelerated cardiovascular aging phenotypes.

There are several other natural polyphenols with antioxidant, anti-inflammatory, antiapoptotic, and anti-senescent properties, including quercetin, kaempferol, and epicatechin, which may also potentially exert beneficial effects in cardiovascular aging either alone or in combination with existing drugs. However, rigorous preclinical and clinical studies are needed.

Cardiovascular Protective Effects of PARP-1 Inhibitors in Aging Pharmacological inhibition of the PARP pathway has emerged as a potentially important therapeutic target for aging and age-associated diseases. PARP-1 is a member of the DNA damage surveillance network. The catalytic activity of PARP-1 was reported to increase in old age because of the age-related increases in peroxynitrite-mediated DNA strand interruptions. On activation, PARP-1 ADP-ribosylates various nuclear proteins, including transcription factors and histones, and as a consequence, it regulates a range of cellular pathways at the transcriptional level. PARP-1 activation upregulates NF-kB-dependent inflammatory gene expression, which is highly relevant in cardiovascular aging. PARP-1 is a NAD+-consuming enzyme that competes with SIRT1 for the same pool of NAD+. An increase in PARP-1 activity results in SIRT1 inhibition because of lower substrate availability. This antagonistic crosstalk between PARP-1 and SIRT1 represents a potentially important mechanism by which PARP-1 overactivation promotes age-related cardiac and vascular dysfunction. Indeed, there is evidence suggesting that inhibition of PARP-1 may confer protection against cardiovascular aging.

Activation of AMPK Pathway in Cardiovascular Aging Studies in invertebrates have indicated a link between increase in AMPK activity and lifespan extension. However, the role of AMPK in the health-protective effects of CR in mammals is under debate. AMPK has been traditionally viewed as an intracellular energy switch, but is now described as a key player in maintaining physiological processes in both the heart and the vasculature. Expression of constitutively active AMPK mutations produces extensive remodeling of the metabolic network to maintain energetic homeostasis at the expense of developing glycogen-storage cardiomyopathy. Several cellular processes that either decrease ATP levels or increase AMP concentrations promote activation of mammalian AMPK. Moreover, pharmacological interventions that include metformin, aspirin, 5-aminoimidazole-4-carboxamide riboside, statins, thiazolidinediones, and the phytochemicals berberine, quercetin, and resveratrol have the ability to activate AMPK signaling and rising the (AMP+ADP)/ATP ratio as a consequence of mitochondrial electron transport and glycolysis inhibition. Notably, the antidiabetic drug metformin provides protection against the development of hyperglycemia-induced vascular disease through improvement in endothelial function. This biguanide exerts vasoprotection via activation of AMPK, even though some cellular actions could be mediated in an AMPK-independent pathway. Resveratrol lowers BP in spontaneously hypertensive rats and reduces cardiac hypertrophy through AMPK signaling. Aspirin, also known as acetylsalicylic acid, is used at low doses as an antiplatelet drug in the prevention of vascular ischemic events and has been shown to increase lifespan in genetically heterogeneous male mice. This nonsteroidal anti-inflammatory drug activates AMPK to decrease the expression of inducible NO synthase and Cox-2 and, therefore, lowers inflammation and oxidative stress. Similar protective effects have been observed with berberine. These results have shed light on how metformin, aspirin, and other compounds promote lifespan extension.

Antiaging Effects of Interventions That Reduce Oxidative Stress and Improve NO Bioavailability NO is a crucial factor for the health and function of the aged cardiovascular system. One of the consequences of increased oxidative stress in aging is a functional inactivation of NO, resulting in significant vasomotor dysfunction and contributing to vascular inflammation, atherogenesis, and cellular energetic imbalance. Studies on genetically NO-deficient mice have linked the impaired NO bioavailability with increased mortality and reduced lifespan potential. Several experimental antiaging interventions exist (eg, CR, SIRT1 activators, resveratrol, pamycin, tumor necrosis factor-α antibodies and treatment with NAD phosphate oxidase inhibitors or antioxidant...
compounds\textsuperscript{213}) that improve NO bioavailability by means of increased production and lower NO degradation caused by oxidative stress.

The antidiabetic drug metformin has been shown to have favorable hemodynamic and rheological effects in elderly patients with cardiovascular risk factors. Infusion of the endothelial NO synthase (eNOS) substrate l-arginine enhances the hemodynamic effects of metformin in type 2 diabetic patients\textsuperscript{214} through increased blood flow in muscle and adipose tissue, reduction in systolic BP in response to vasoconstrictors, and improvement in acetylcholine-mediated vasodilatation.\textsuperscript{17,18} Although activation of AMPK partly mediates the pleiotropic effects of metformin, studies have shown that the biguanide improves NO-mediated endothelial-dependent vasodilatation under insulin-resistant conditions\textsuperscript{197} by mechanisms linked to increased phosphorylation of eNOS and AKT via SIRT1- and AMPK-independent pathways.\textsuperscript{19} However, the ability of metformin to regulate endothelial progenitor cell differentiation\textsuperscript{210} and stimulate ischemia-induced revascularization\textsuperscript{20} depends on AMPK/eNOS signaling cascade. Metformin also has vascular anti-inflammatory properties by downregulating NF-κB activation, caused by phosphorylation of its inhibitor IkB in the vessel wall of experimental atherosclerosis in rabbits and decreasing serum levels of high-sensitivity C-reactive protein.\textsuperscript{21}

The most commonly used classes of drugs to treat obese patients have pleiotropic antioxidant properties that contribute to their beneficial effects. Studies show that statins reduce reactive oxygen species (ROS) production in cardiac muscle, which leads to an increase in mitochondrial biogenesis and phase II antioxidant enzyme system via the PGC-1 (peroxisome proliferator-activated receptor gamma coactivator 1) signaling pathway.\textsuperscript{22} In endothelial cells, the activation of AKT by statins results in stimulation of eNOS activity, leading to increased NO synthesis and neoangiogenesis, whereas the increased production of endothelial NO in the central nervous system points to a role for statins in regulating sympathetic and vagal outflow and inhibiting central angiotensin-II mechanisms.\textsuperscript{23} Clinical trial results show that statin use has been associated with lower mortality in elderly people from age 85 to 90 years by providing total cholesterol-independent benefits.\textsuperscript{27}

**Antiaging Effects of Mitochondria-Targeted Antioxidants**

There is strong evidence that with advanced age, mitochondrial production of ROS significantly increases in the heart\textsuperscript{234} and vasculature.\textsuperscript{72} Direct evidence supporting a critical role of mitochondrial ROS in cardiac aging was demonstrated by studies in mice that overexpress catalase targeted to the mitochondria. These mice show 18\% extension of lifespan associated with protection against cardiac aging phenotypes.\textsuperscript{219,220} These observations have led to the development and testing of mitochondria-targeted antioxidants, including Mito-Q, MitoTEMPO, mito vitamn E, mitophenylterbutyline, and SkQ1, for their potential antiaging cardiovascular protective effects. The Szeto-Schiller compounds represent a novel class of potent mitochondria-targeted antioxidants capable of preserving mitochondrial function by scavenging H\textsubscript{2}O\textsubscript{2}, hydroxyl radical, and peroxynitrite.\textsuperscript{221,222} The tetrapeptide Szeto-Schiller-31 has been shown to reduce ischemia reperfusion injury and reperfusion arrhythmia and better preserve myocardial function in various infarct models.\textsuperscript{81,84} Although studies on aged ApoE\textsuperscript{−/−} mice show that treatment with MitoTEMPO exerts antiatherogenic effects,\textsuperscript{45} further research is needed to test the therapeutic benefits of mitochondria-targeted antioxidants on a range of age-related cardiovascular and cerebrovascular phenotypes both in animal models of aging and elderly humans.

**Antiaging Effects of Polyunsaturated Fatty Acids**

There are 2 dietary classes of essential polyunsaturated fatty acids (PUFAs), the n=6 PUFAs found primarily in vegetable oils and n=3 PUFAs mainly present in marine animals or plants. Commonly referred to as omega-3 fish oils or omega-3 fatty acids, n=3 PUFAs have been shown to be beneficial in CVD as a secondary prevention and are commonly used to lower high triglyceride levels in the blood. Experimental evidences have revealed multiple underlying molecular mechanisms of action for omega-3s, which include membrane modification, ion channel attenuation, regulation of proinflammatory gene expression, and production of lipid mediators.\textsuperscript{33} However, the mechanism(s) that contributes the most to the cardioprotective effects of PUFAs remains to be clarified. It is imperative that further testing be performed regarding the use of omega-3 supplementation (above the accepted minimum requirement) as a mean to slow aging and reduce diseases. Indeed, preclinical studies have shown that long-term intake of fish oil decreases lifespan in senescence-accelerated mice,\textsuperscript{223} long-lived F1 mice,\textsuperscript{224} and Caenorhabditis elegans.\textsuperscript{225}

**Antiaging Effects of Nrf2 Activators**

The redox-sensitive transcription factor Nrf2 plays an evolutionarily conserved role in orchestrating cellular antioxidant defenses and maintaining redox homeostasis, ultimately impacting on health span and lifespan.\textsuperscript{34-39} Recent evidence suggests that Nrf2 also regulates the proteasome and removal of oxidized proteins.\textsuperscript{40} Nrf2 has a critical role in preserving a youthful cardiovascular phenotype and maintaining the functional integrity of the heart and the vasculature.\textsuperscript{31,42} Accumulating evidence suggests that an age-related decline in cellular Nrf2 activity results in increased cellular sensitivity to the harmful effects of ROS in the aged cardiovascular system.\textsuperscript{89,90} Age-associated impairment of homeostatic responses that depends on Nrf2 has been linked to exacerbation of vascular oxidative stress\textsuperscript{89,90} and inflammation,\textsuperscript{33,228} impairment of angiogenesis,\textsuperscript{41} and increased atherogenesis.\textsuperscript{42} Importantly, activation of Nrf2 is thought to contribute significantly to the beneficial effects of CR,\textsuperscript{2,34} rendering Nrf2 an attractive drug target for antiaging interventions. Accordingly, an increasing number of experimental and clinical studies focus on the beneficial effects of compounds that activate Nrf2, such as sulforaphane, found in broccoli, and isoflavones, in animal models of age-related cardiovascular and cerebrovascular diseases.\textsuperscript{227,228} The CR-mimetic resveratrol is also a potent activator of Nrf2,\textsuperscript{31,163,165} suggesting that Nrf2 activation may also contribute to the potent antiaging vasoprotective effects of this polyphenol.\textsuperscript{64,88}

**Disruption of Angiotensin II Signaling Offers Antiaging Effects**

Angiotensin-converting enzyme (ACE) inhibitors and nonpeptide blockers of angiotensin II type 1 receptor are currently...
used widely to treat hypertension and cardiac heart failure. The ACE inhibitor, enalapril, does not improve longevity in healthy mice, despite the increase in heart mitochondria number and decrease in myocardial sclerosis. Enalapril increases rat lifespan and promotes NO production through activation of mitochondrial NO synthase activity. Ramipril, another ACE inhibitor, doubles the lifespan of hypertensive rats by improving cardiac function and metabolism as well as enhancing eNOS-mediated increase in endothelial function. Impairment in NO-dependent endothelial function in patients with Type II diabetes mellitus is aggravated by dyslipidemia and hypertension, which can be restored by ACE inhibition and weight loss. The generation of pro-oxidant molecules in response to angiotensin II contributes to cell oxidation and tissue damage both in normal aging and in cardiovascular and metabolic diseases. As predicted, targeted disruption of the Agtr1a gene that encodes angiotensin II type I receptor A has led to a marked increase of lifespan in mice. Long-term pharmacological inhibition of angiotensin II type I receptor with fosfomycin results in the doubling of lifespan in hypertensive rats, together with improvement in cardiac function and metabolism and enhanced endothelial function. The clinical benefits of angiotensin II type 1 receptor blockers can be explained by the increase in eNOS expression in the heart and carotid artery and marked reduction in tissue ACE expression/activities.

Nonselective β-adrenergic blockers, widely used to treat hypertension and ischemic heart disease, have been proposed as antiaging drugs. Metoprolol and nebivolol increase mean arterial pressure and decrease in myocardial sclerosis. Enalapril increases rat lifespan by marked reduction in tissue ACE expression/activities. Nonselective β-adrenergic blockers, widely used to treat hypertension and ischemic heart disease, have been proposed as antiaging drugs. Metoprolol and nebivolol increase mean arterial pressure and decrease in myocardial sclerosis. Enalapril increases rat lifespan by marked reduction in tissue ACE expression/activities.

The incidence of heart failure increases progressively with advanced aging. There are many treatment modalities available for heart failure associated with reduced contractile function of the myocardium. In addition to vasodilators and diuretics, which relieve cardiac workload, therapeutic approaches for heart failure include inotropic agents that increase cardiac contractility by working either through increasing the influx of calcium or modulating adrenoreceptor signaling in cardiac myocytes. Myofilament calcium sensitizers (such as omecamtiv mecarbil) represent a new class of inotropic agents that may be used in the treatment of heart failure. Omecamtiv mecarbil facilitates actin–myosin cross bridge formation, increases the number of myosin heads involved into the force generation, and stimulates myosin ATPase activity, which result in prolonged systolic ejection time and increased ejection fraction. The apparently disparate effects of omecamtiv mecarbil on myocardial oxygen consumption in animal models warrants further studies. Other emerging new treatments capable of restoring systolic function include the potentiation of cardiomyocyte contractility, increase in cardiomyocyte survival and adaptive hypertrophy, and promoting vascularization (for an excellent overview, see Tarone et al).

The lack of effective treatment options for patients with heart failure associated with age-related diastolic dysfunction is a growing clinical problem. To design effective therapeutic interventions, it is important to understand the various age-related pathophysiological factors contributing to diastolic stiffness. Our current understanding is that age-related diastolic stiffness is because of cardiac remodeling, cardiomyocyte hypertrophy, interstitial fibrosis with increased deposition of collagen and other extracellular matrix components, decreased elastin content, matrix metalloproteinase activation, redox imbalance, and increased inflammation and impairment in active diastolic relaxation. Phosphorylation of the myocardial protein titin is also an important molecular determinant of cardiomyocyte stiffness, which can be potentially modulated by therapeutic interventions.

**Progeria and Cellular Senescence in Cardiovascular Aging**

The dynamic organization of the cell nucleus is profoundly modified during growth, development, and senescence. Three different diseases of accelerated aging have been associated with defects of the nuclear lamina, including Hutchinson–Gilford progeria syndrome, mandibuloacral dysplasia, and atypical Werner syndrome. Treatment with the mTOR inhibitor rapamycin favors recruitment of p53-binding protein 1 or 53BP1, a key player in the DNA damage response, to the nuclear envelope and affects the levels of prelamin A in a pattern reminiscent of that observed in cells from centenarians. The link between mTOR pathway and nuclear lamina defects deserves further study.

Cell senescence has been proposed to have a role in cardiovascular aging because cells positive for the cyclin-dependent kinase inhibitor p16INK4a are key drivers of an age-related cardiac phenotype that leads to lifespan shortening in mice. In patients with their first acute myocardial infarction, tight glycemic control reduces senescent myocyte precursor cells, thus increasing the regenerative potential of the ischemic myocardium. Moreover, the secretory phenotype of p16INK4a-positive cells includes many proinflammatory cytokines and chemokines and matrix metalloproteinases (MMP), which are involved in tissue remodeling. It is known that MMP-9 increases with age, and its deletion in aged mice alleviates cardiac fibrosis and preserves LV diastolic function by modifying the extracellular matrix response and angiogenesis. Some drugs reduce MMP-9 expression, such as atorvastatin, Rosa hybrida extracts, or memantine. Also, inhibition of chymase, an angiotensin II–forming enzyme that activates MMP-9, has been proposed as a potential target to prevent cardiovascular diseases. Therefore, the therapeutic removal of senescent cells and reduction of MMP and chymase activities may be an attractive approach to improve cardiovascular aging and extend healthy lifespan.

**Perspectives**

Although significant progress has been achieved in describing age-related alterations in cardiac and vascular function and phenotypes, the specific roles for cell-autonomous and noncell-autonomous mechanisms involved in cardiovascular aging processes need to be elucidated further. It is critical to understand the interactions of age-related molecular mechanisms in vascular cells with both CVD pathogenesis and systemic aging processes and to develop interventions targeting these mechanisms to retard cardiovascular aging. Several examples of such potential therapies include CR mimetics,
mitochondrial protective agents, and mTOR inhibitors. There is reasonable consensus that oxidative stress and inflammation play a critical role in the pathogenesis of a range of age-related cardiovascular and cerebrovascular diseases. The concept that the same evolutionarily conserved pathways (such as sirtuins and Nrf2) controlling the aging process in mammals also determine cardiovascular health through changes in ROS production, cellular and organismal sensitivity to oxidative stress, and inflammatory processes raises the question of whether pharmacological or nutritional modulation of these pathways is effective both in retarding aging and delaying the onset of age-related CVD. Compelling evidence for circulating factors that alter aging phenotypes comes from studies using heterochronic parabiosis (eg, reversal of age-related cerebrovascular rarefaction\(^\text{255}\)). Further understanding of the circulating factors responsible for the transposition of the aging phenotypes in young mice and the induction of youthful phenotypes in aged mice in heterochronic parabiotic pairs will guide future experimental and translational studies on novel therapeutics to treat age-related CVD and to improve healthy cardiovascular aging. Significant advances have been made in recent years toward understanding the association between cellular senescence, aging, and age-related pathologies. Studies in genetically modified mice that express a drug-activated suicide gene specifically in senescent cells suggest that senescent cell clearance can ameliorate age-related organ dysfunction.\(^\text{256,257}\) These findings led to the recent development of small molecule senolytic agents to decrease senescent cell burden in aging.\(^\text{256,257}\) Research efforts should also persist in these directions to fully elucidate the specific relationship between cellular senescence in development of age-related CVD and, ultimately, to determine whether senolytic agents can reduce cardiovascular morbidity and mortality in the elderly.

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Disclosures
None.

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Approaches to Combat Cardiovascular Aging


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