Autophagy in Cardiovascular Aging

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Abstract: **Cardiovascular diseases are the most prominent maladies in aging societies. Indeed, aging promotes the structural and functional declines of both the heart and the blood circulation system. In this review, we revise the contribution of known longevity pathways to cardiovascular health and delineate the possibilities to interfere with them. In particular, we evaluate autophagy, the intracellular catabolic recycling system associated with life- and health-span extension. We present genetic models, pharmacological interventions, and dietary strategies that block, reduce, or enhance autophagy upon age-related cardiovascular deterioration. Caloric restriction or caloric restriction mimetics like metformin, spermidine, and rapamycin (all of which trigger autophagy) are among the most promising cardioprotective interventions during aging. We conclude that autophagy is a fundamental process to ensure cardiac and vascular health during aging and outline its putative therapeutic importance. (*Circ Res*. 2018;123:803-824. DOI: 10.1161/CIRCRESAHA.118.312208.)**

Key Words: autophagy ■ caloric restriction ■ cardiovascular diseases ■ heart ■ longevity

Life expectancy has substantially increased during recent decades[1] and is projected to continue increasing in most countries.[2] However, with a growing aged population, diseases connected to aging are on the rise as well. Among them, cardiovascular pathologies are the leading cause of death in the elderly.[3] The increase in incidence and prevalence of age-associated cardiovascular diseases impose a grave global problem to human health worldwide and a major financial burden to healthcare systems.[4,5] In addition, conventional risk factors (eg, hypertension, obesity, dyslipidaemia, diabetes mellitus type 2) facilitate the development of cardiovascular diseases. Consequently, lifespan extension in our aging societies comes at the expense of poor health, chronic disability, and diminished quality of life.[6,7] The development of preventive and therapeutic treatments that directly target the mechanisms underlying cardiac and vascular effects of aging per se is urgently needed. A promising approach is to reinstate quality control processes that promote cardiovascular homeostasis upon aging and thus delay the onset of, or possibly even avoid, disease altogether in the elderly. One such fundamental homeostatic mechanism is macroautophagy (herein referred to as autophagy).

Autophagy is an evolutionary conserved process critical for cellular homeostasis and survival. Long-lived, damaged, dysfunctional, and potentially harmful cellular components are broken down for detoxification, energy production, and cellular renewal, providing building components and stimulating anabolic processes for effective cellular recycling.[8] During autophagy, cytoplasmic components are first sequestrated within de novo formed double-membraned vesicles (ie, autophagosomes) that fuse with lysosomes to form so-called autolysosomes. The autolysosomal content is subsequently enzymatically degraded by lysosomal hydrolases[9] (Figure 1). Autophagy can function nonselectively, meaning that any cytoplasmic content is targeted for catabolic recycling, which is the case upon limited nutrient supply (eg, starvation) to maintain cellular energy production. In addition, highly selective forms of autophagy exist that specifically target damaged organelles, as exemplified for mitophagy that results in the clearance of damaged mitochondria.[10] Of note, mitochondria are involved in both vital and lethal cellular functions, and mitochondrial dysfunction is a crucial determinant for lifespan across species.[11–14] Hence, besides preserving organismal homeostasis under baseline physiological conditions, autophagy also contributes to metabolic fitness and adaptation to stressful conditions, such as nutrient deprivation, hypoxia, oxidative stress, or physical exercise.[15,16] Accordingly, autophagy is subjected to a complex regulatory network.[8,17] In recent years, it has become evident that autophagy-driven homeostatic resolutions govern the lifespan of all eukaryotic cells, including those composing the cardiovascular system.[9]

A growing body of evidence suggests that reduced autophagy is implicated in cardiovascular decline and increased susceptibility to (cardiovascular) disease upon aging. The majority of cardiomyocytes in the adult heart are terminally differentiated cells and thus have limited ability to proliferate. In

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Nonstandard Abbreviations and Acronyms	
Akt2	serine/threonine-specific protein kinase 2
AMPK	AMP-activated protein kinase
CR	caloric restriction
FMD	fasting-mimicking diet
GH	growth hormone
Gsk3a	glycogen synthase kinase-3
IF	intermittent fasting
IGF-1	insulin-like growth factor-1
IP_3	inositol 1,4,5-trisphosphate
IP_3R	inositol 1,4,5-trisphosphate receptor
LV	left ventricle
mTOR	mammalian target of rapamycin
NO	nitric oxide
ROS	reactive oxygen species

fact, less than half of all cardiomyocytes are exchanged (ie, renewed) during a normal lifespan,[18] resulting in the accumulation of damaged cellular material. Hence, an intact autophagic machinery is indispensable for the maintenance of functional and structural homeostasis of the aging heart.

In this review, we discuss the current understanding of the functional role of autophagy in the regulation of cardiac and vascular aging. To focus our analysis, we have opted to exclude descriptive comparisons between young and midlife healthy animals, as well as nontargeted, mainly observational methodologies. Instead, we focus our discussion on those studies that (1) provide experimental evidence in the context of accelerated or genuine aging,[19] and (2) targeted autophagy by dietary, genetic, or pharmacological interventions.

Cardiovascular Aging

Biological aging undermines cardiovascular homeostasis by promoting slow and progressive structural and functional alterations of the heart and vasculature, increasing the vulnerability of individuals to develop various cardiovascular diseases (eg, coronary artery disease, stroke, hypertension, atherosclerosis, atrial fibrillation, and heart failure).

Cardiac Structural and Functional Decline During Aging

In otherwise healthy individuals, aging is associated with left ventricular (LV) wall thickening[20,21] and myocardial fibrosis characterized by increased accumulation and intermolecular crosslinking of collagen.[22,23] Cardiac hypertrophy and fibrosis are typical hallmarks of structural remodeling that promote myocardial stiffening. Although increased LV stiffness contributes to preserve LV ejection against the aged stiff vasculature at rest, it comes at the expense of a dramatic cardiac functional decline comprising impaired filling properties, namely a slower rate of early passive filling and the resulting augmented late active filling by the left atrium. This leads to left atrial enlargement and remodeling, as well as a characteristic decline in diastolic function, the hallmark of cardiac aging.[20,24] In addition, aging is associated with reduced maximum ejection fraction, decreased maximal heart rate,[25] (ie, decreased responsiveness to β-adrenergic stimulation), and so diminished maximal cardiac output. This culminates in a compromised cardiac reserve capacity,[26] which is translated into a reduction in peak oxygen consumption[26,27] and therefore into effort intolerance, frailty, and reduced life quality in the elderly (Figure 2).

Mechanistically, the age-related decline of mitochondrial function and structure characterized by swelling, loss of cristae, and matrix deformation is considered a major driver of cardiomyocyte senescence.[28] Aged mitochondria not only produce less ATP but they also generate increased amounts of reactive oxygen species (ROS), which may ignite cell death.[29] Thus, aged cardiomyocytes are not only steadily deprived from their major energy source, failing to meet their high energy demands, but are also exposed to high levels of oxidative stress. However, they cannot dilute damaged mitochondria as other cells do by proliferation because the majority of cardiomyocytes reside in a postmitotic state.[18] At the same time, their catabolic defensive mechanisms, namely autophagic and proteasomal degradations, decline with age.[30,31] Aged cardiomyocytes also accumulate large amounts of lipofuscin, which is composed of lipid-containing residues from lysosomal digestion,[32] and potentially inhibits mitochondrial function and precipitates cell death.[33] In addition, increased oxidative stress and mitochondrial dysfunction have been linked to other mechanisms of cardiac aging (eg, impaired calcium homeostasis[34] and sodium mishandling,[35] increased apoptosis,[36] and telomere dysfunction[37]). Bearing this in mind, it is important to emphasize the complexity of the mechanisms driving aging of the cardiovascular and other systems. These are not limited to cell-autonomous mechanisms but also involve systemic and coronary subclinical low-grade inflammation,[38,39] circulating factors, such as exosomes,[40] neurohumoral signaling (eg, the renin-angiotensin and sympathetic nervous systems),[28] as well as exhaustion of stem cell pool.[41]

Vascular Structural and Functional Decline During Aging

With age, large conduit arteries (eg, aorta and common carotid) develop enlarged lumina and thickened walls.[42] Aging is also associated with endothelial dysfunction manifested as compromised eNOS (endothelial nitric oxide [NO] synthase) activity with subsequent reduction in NO-dependent vasodilation.[43] In addition, aged endothelial cells show reduced postinjury proliferation and migration, as well as disruption of their barriers. This enables subendothelial migration of vascular smooth muscle cells, enhancing intimal thickening by extracellular matrix deposition.[44] In conjunction with greater elastin fractures and collagen deposition in vascular media,[45] these alterations contribute to increased stiffness of central arteries[46] and elevated pulse wave velocity. Arterial stiffness shifts the reflected pressure waves to an earlier stage during systole, consequently augmenting pulse pressure (increased systolic and reduced diastolic central pressures), a major predictor of adverse cardiovascular events.[42] Such remodeling of the arterial system accelerates the concurrent cardiac changes by imposing more afterload and further increases the risk for cardiovascular morbidity

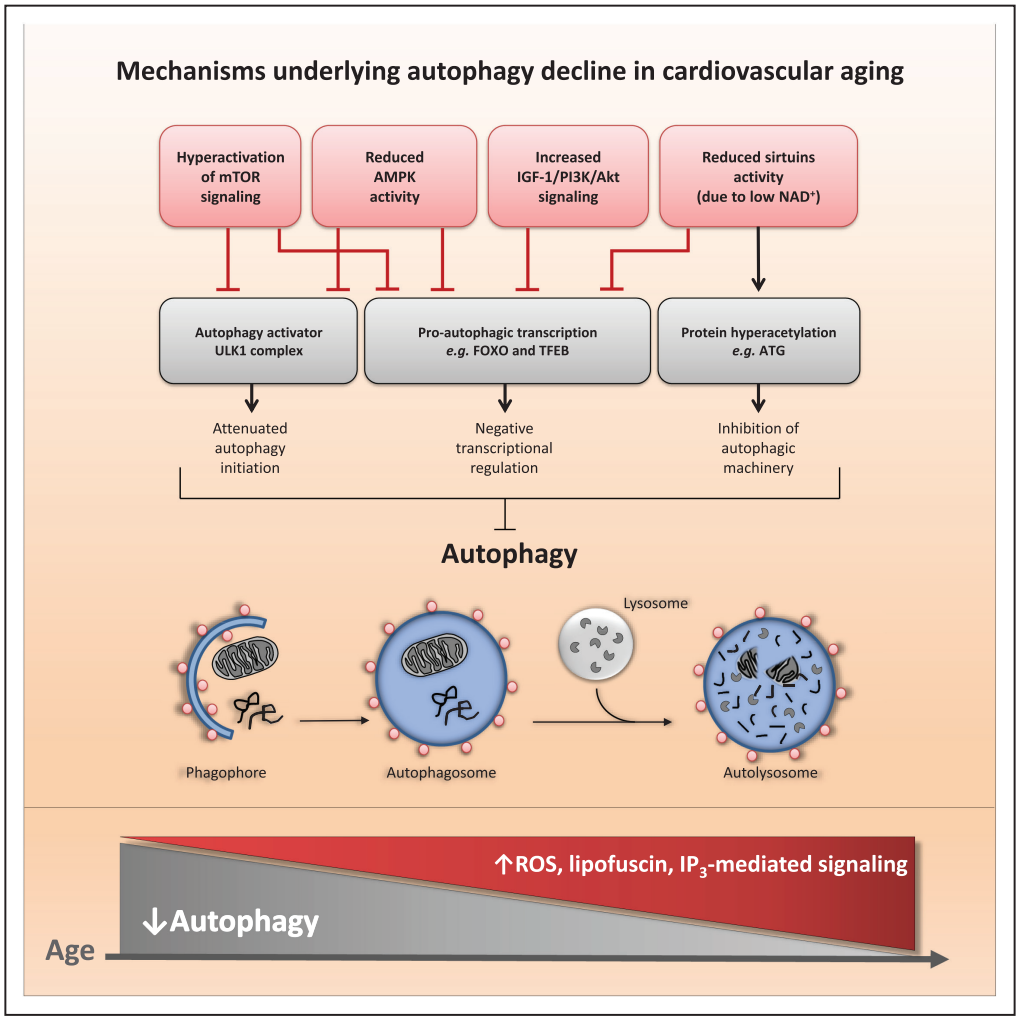

Figure 1. Mechanisms underlying autophagy decline in cardiovascular aging. The majority of cardiomyocytes in the adult heart are terminally differentiated cells and thus cannot proliferate. Long-lived, damaged, and dysfunctional cellular components are broken down for detoxification, energy production, or cellular renewal, providing building components and stimulating anabolic processes for effective cellular recycling. Thereby, cytoplasmic components designated for autophagy are first sequestrated within de novo formed double-membraned vesicles (ie, autophagosomes) that fuse with the lysosomes to form autolysosomes. The autolysosomal content is subsequently enzymatically degraded by lysosomal hydrolases. Autophagy can function nonselectively, meaning that any cytoplasmic content may be targeted for catabolic recycling. In addition, highly selective forms of autophagy exist that specifically target damaged organelles, for example, mitochondria, which are cleared by mitophagy. However, autophagy experience reduced activity during the course of aging under the influence of longevity signaling pathways and other age-related mechanisms, for example, increased reactive oxygen species (ROS), lipofuscin, and inositol 1,4,5-trisphosphate (IP_3)–mediated signaling. AMPK indicates AMP-activated protein kinase; IGF-1, insulin-like growth factor-1; mTOR, mammalian target of rapamycin; NAD^+, nicotinamide adenine dinucleotide; and PI3K, phosphoinositide 3-kinase.

and mortality. Another important functional deficit in the elderly is ventricular-vascular uncoupling. Efficient coupling between the ventricular and arterial systems is a fundamental prerequisite for a normal cardiovascular performance. At rest, the rise in vascular stiffness is compensated for by the parallel increase in cardiac stiffness. However, when the elderly are effort-challenged, ventricular-vascular uncoupling is unmasked, explaining the relative exercise intolerance and compromised functional reserve of the whole cardiovascular system (Figure 2).[26]

From a mechanistic point of view, age-related vascular remodeling is driven by increased ROS accumulation,[47] which in turn leads to peroxynitrite ($ONOO^-$) production,[48] limiting NO bioavailability, and increasing mitochondrial DNA instability.[49] Oxidative stress also enhances nuclear factor-κB activation and promotes proinflammatory cytokine secretion, causing vascular inflammation.[39]

Aging Instigates Cardiovascular Disease in the Elderly

The cardiovascular system in old individuals is highly susceptible to the development of manifest disease with ongoing age-associated transformation and decline of other organ systems. For instance, combined with traditional risk factors, vascular intimal thickening, along with inflammation, favors the development of atherosclerotic lesions and ischemic heart disease, especially in the presence of LV hypertrophy.[20,50,51] Also, age-associated vascular stiffening promotes isolated systolic hypertension, the major form of hypertension in the elderly.[42] Myocardial stiffness, hypertrophy, and diastolic dysfunction can give rise to heart failure with preserved ejection fraction,[52,53] the foremost cause of hospitalization in the elderly >65 years that currently affects more than half of all patients with heart failure.[54] Atrial enlargement and remodeling in response to increased filling are implicated in the development

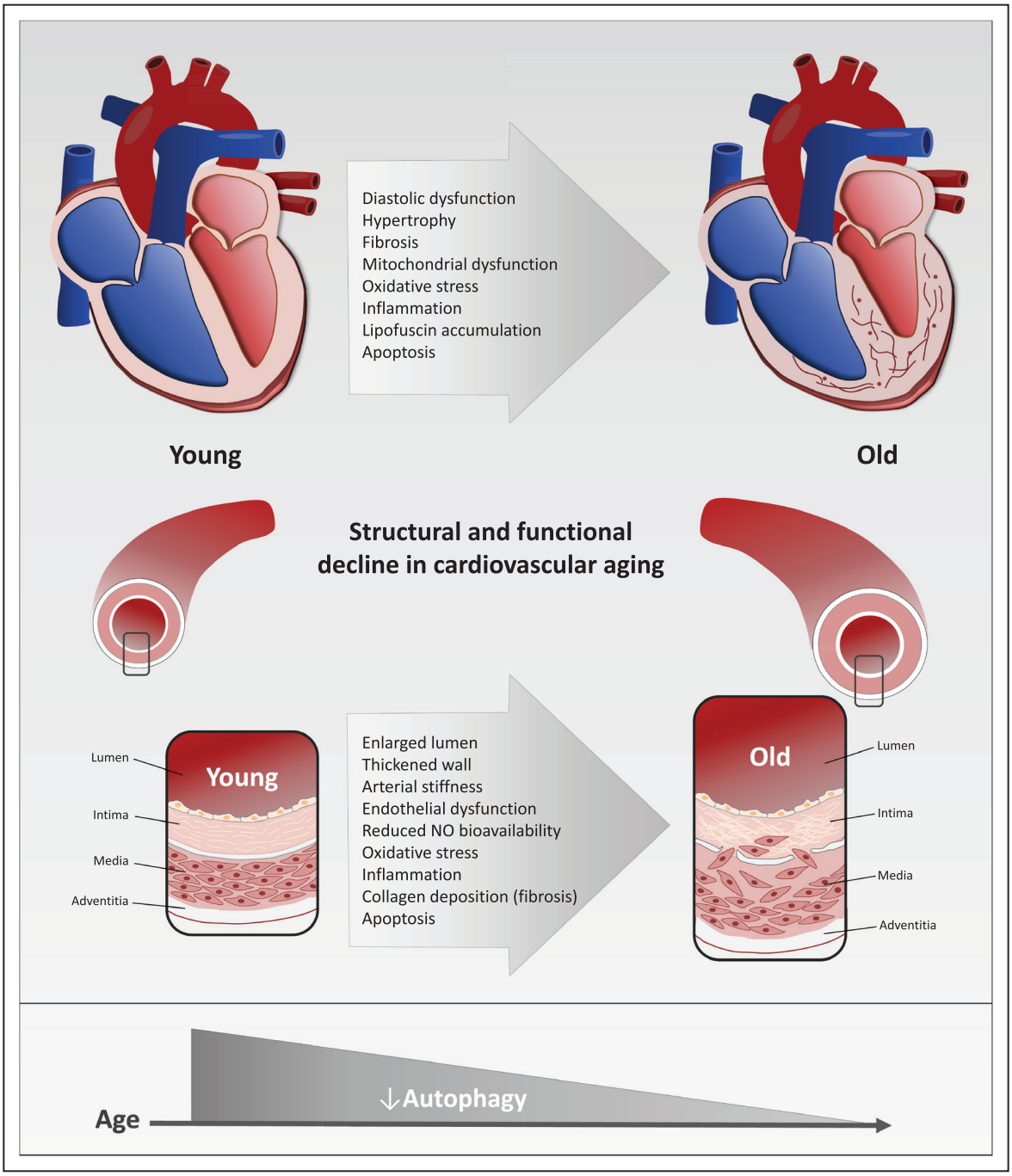

Figure 2. The hallmarks of cardiovascular aging. Age-related decline in autophagy stimulates detrimental cellular processes that coincide with adverse remodeling, stiffening, and functional decline of the heart (**top**) and the arterial system (**bottom**). Note that the aged myocardium shows ventricular hypertrophy, fibrosis, and apoptosis, as well as atrial remodeling, whereas the vascular endothelial cells display reduced postinjury proliferation and migration, as well as disruption of their barriers. In turn, this enables subendothelial migration of vascular smooth muscle cells, subsequently enhancing intimal thickening by extracellular matrix deposition. In conjunction with endothelial dysfunction, greater elastin fractures, and collagen deposition in vascular media, these alterations contribute to increased stiffness of central arteries. NO indicates nitric oxide.

of atrial fibrillation,[55] which is the most prevalent, clinically significant cardiac arrhythmia.

Molecular Pathways of Longevity and Cardiovascular Health During Aging

Studies in aged animal models show that highly evolutionary conserved pathways underlying lifespan regulation also play a fundamental role in the aging-related functional decline of different organs, including the cardiovascular system.

Mammalian Target of Rapamycin

Mammalian target of rapamycin (mTOR), also known as mechanistic target of rapamycin, is a serine-threonine protein kinase that is inhibited by rapamycin, a Food and Drug Administration–approved immunosuppressant. This nutrient sensor kinase is involved in regulating a plethora of cellular processes, including growth, proliferation, motility, survival, protein synthesis, autophagy, and metabolism,[56] many of which are relevant for maintaining normal function in the

adult heart. Downregulation or inhibition of mTOR promotes longevity of different model organisms.[57] In mice, reduced expression of mTOR[58] or its inhibition by rapamycin early[59] or even late in life[60] is sufficient to extend lifespan.

In the heart, aged mice exhibit higher mTOR activity than younger mice.[61] While reduction of mTOR signaling by caloric restriction (CR)[62] or rapamycin supplementation[63] provides beneficial cardiac effects during aging, its chronic activation by cardiac-specific overexpression of the serine/threonine kinase Akt (serine/threonine-specific protein kinase 2) has deleterious consequences.[61] Similarly, reversing the increased mTOR activity in aged arteries by rapamycin prevents from age-related arterial remodeling.[64] Hence, it is widely accepted that the mTOR pathway is an important regulatory mechanism of cardiac as well as vascular aging, regardless of the recent reports of potential side effects or limited health-span promotion by rapamycin,[65,66] which might be ascribed to mTOR-independent effects of rapamycin.

Sirtuins

Another group of evolutionary conserved energy sensors are the nicotinamide adenine dinucleotide–dependent deacetylases (sirtuins). The mammalian sirtuin family comprises 7 members (SIRT 1 through 7) that are localized in different cellular compartments (nucleus, cytoplasm, and mitochondria) and constitute major regulators of cellular metabolism.[67] In addition, sirtuins are intimately linked to the aging process. Lifespan extension in response to CR, for instance, is associated with upregulation of SIRT1[68] while this effect is abolished in mice lacking SIRT1.[69] Furthermore, late-in-life dietary supplementation of nicotinamide riboside, which activates sirtuins by replenishing nicotinamide adenine dinucleotide stores, prolongs mouse lifespan.[70] However, the role of sirtuins per se in lifespan extension is matter of discussion because SIRT1 transgenic mice, despite being healthier at old age, do not live longer.[71] Also, sirtuin-activating compounds (eg, resveratrol and SRT172037) fail to extend lifespan of healthy mice but rather prevent early mortality induced by high-caloric diets.[72–74]

Irrespectively, the effects of sirtuins in promoting healthspan are widely accepted. In the cardiovascular system, for instance, SIRT1 levels decline with aging,[75] and low-to-moderate cardiac-specific SIRT1 overexpression attenuates cardiac aging by reducing hypertrophy, fibrosis, apoptosis, and senescence markers.[76] Conversely, the absence of SIRT3 or SIRT6 causes early mortality and a progeroid cardiac phenotype characterized by hypertrophy and fibrosis at young age.[77,78] In line with these findings, vascular expression of SIRT1 is lowered in aged humans and mice with the level of reduction being in linear correlation with the degree of endothelial vasodilatory dysfunction.[79] In addition, inhibition of SIRT1 deprives young mice from any advantage on vascular functionality as compared with their aged counterparts.[79] In human vascular endothelial cells, inhibition of SIRT1 hastens a senescence-like phenotype.[80] Altogether, these findings argue for a mechanistic role of sirtuins in vascular and cardiac aging as well.

Growth Hormone/IGF-1

The growth hormone (GH)/insulin-like growth factor 1 (IGF-1) pathway, involving the secretion of GH from the anterior pituitary gland, and consequently that of IGF-1 from the liver, constitutes a well-studied longevity-related route.[81] Inhibition of GH/IGF-1 signaling has been shown to promote lifespan in multiple model organisms.[82] It seems evident that this pathway plays an imperative role in mammalian aging. For instance, an inverse correlation between circulating IGF-1 levels and lifespan was identified in an extensive study including 31 genetically different mouse strains.[83] In line, 3 independent epidemiological studies recently linked higher survival chances in exceptionally long-lived human populations to lower levels of IGF-1[84] or GH/IGF-1 pathway activity.[85,86] Moreover, CR is associated with reduced circulating levels of IGF-1 in mice,[87] an effect that is proposed to be causally involved in its lifespan-extending effect.[88] Similarly, voluntary CR reduces IGF-1 bioavailability in humans by increasing IGFBP-1 (IGF-binding protein 1) levels.[89]

Cardiomyocyte-specific deletion of the IGF-1 receptor has been recently shown to attenuate cardiac aging by reducing fibrosis, hypertrophy, and cardiac proinflammatory cytokines in mice.[90] Enhanced expression of IGF-1, locally in the heart, induces premature cardiac aging by increased collagen production, maladaptive hypertrophy, and impaired systolic function despite seemingly improved cardiac performance at younger age.[91] In stark contrast, cardiac-specific IGF-1 overexpression alleviated aging-associated cardiomyocyte dysfunction[92] and extended the lifespan of these transgenic mice.[93] Later on, however, the same group showed a similar beneficial effect upon induction of local IGF-1 deficiency.[94] Thus, the effects of IGF-1 on cardiac function remain ambiguous. Regarding aged vasculature, the influence of the IGF-1 pathway also remains unclear. Reduced GH and IGF-1 levels in long-lived *Ames*[95] and *Little* mice[96] were connected to signs of vascular aging, including increased arterial stiffness, endothelial dysfunction, and oxidative stress. Strikingly though, unlike their wild-type controls, *Little* mice did not further deteriorate their vascular function with advanced age.[96] Young mice lacking endothelial cell–specific IGF-1 receptor expression showed enhanced endothelial function[97] and regeneration,[98] but thus far, no data has been published to confirm these observations in aged mice as well. Future studies will need to fully elucidate the exact impact of GH/IGF-1 pathway on cardiovascular aging.

AMP-Activated Protein Kinase

The AMP-activated protein kinase (AMPK) is a master regulator of the cellular energy status. AMPK is activated by elevated AMP or reduced ATP intracellular levels (ie, increased AMP versus ATP ratio), initiating a cascade of cellular processes, whereby more energy is produced and ATP utilization is spared in a highly organized manner.[99] Activation of AMPK by the biguanide antidiabetic drug metformin promotes healthy aging and significantly extends lifespan in mice.[100,101] AMPK is also activated by CR[102–104] although it remains unclear whether this is causally required for the health and lifespan benefits of such dietary regimens in mammals.

In the myocardium and vasculature of mice, AMPK activity declines with age.[105–107] Further reduction in the enzyme activity by an induced genetic mutation leads to a more pronounced cardiac aging phenotype, including hypertrophy, contractile dysfunction, impaired calcium homeostasis, and disrupted mitochondrial structure along with increased oxidative stress.[105]

Short-term in vivo treatment of old mice with metformin enhanced cardiomyocyte contractile function[105] and mitigated cardiac fibrosis promoted by β-adrenergic stimulation.[108] Similarly, metformin treatment is associated with a reduced likelihood of developing age-related cardiovascular disease in old type 2 diabetic men.[109] In the vasculature, pharmacological activation of AMPK by aminoimidazole carboxamide ribonucleotide restored vascular functionality in aged mice by enhancing endothelium-dependent vasodilation and reducing oxidative stress.[106] Similarly, curcumin-induced vascular protection through enhanced NO bioavailability and reduced oxidative stress in otherwise healthy middle-aged and older humans[110] as well as in aged rats[107] seems to depend on AMPK activation.[107] Altogether, these findings support the notion that reduced AMPK activity contributes to cardiovascular aging.

Crosstalk Between Longevity Pathways and Autophagy

The molecular pathways governing longevity encompass nutrient sensors and cellular energy regulators that affect and modulate the activity of each other.[24] Interestingly, autophagy is at the intersection of these pathways, and impairment of autophagic flux can jeopardize the protective role of longevity pathways. For instance, lifespan extension by attenuating insulin-like signaling in *daf-2* mutant *Caenorhabditis elegans* nematodes is abolished when the autophagy-related gene *bec-1* (orthologue of mammalian Beclin-1) is inhibited.[111] Similarly, CR fails to promote longevity in nematodes when autophagy-related genes are inhibited.[112] Intriguingly, targeted tissue-specific inhibition of autophagy (eg, in the intestine) is sufficient to eliminate CR-induced longevity.[113] Furthermore, lifespan extension in *C elegans* by the SIRT1 activator resveratrol occurs only under conditions of intact autophagy.[114] In line with these findings, lifespan extension in the fruit fly *Drosophila melanogaster*—whether induced by rapamycin[115] or the AMPK activator β-guanidinopropionic acid[116]—is completely abrogated when autophagy induction is repressed by reducing *Atg5* expression. These data argue for a causal mechanistic involvement of autophagy underlying, at least in part, the effect of longevity pathways. In fact, autophagy induction per se is sufficient to extend the lifespan in diverse species ranging from yeast to mammals.[117,118] Lifespan extension does not necessarily imply a proportional promotion of healthspan, that is, disease-free life period (reviewed elsewhere [119,120]). However, an increasing body of evidence supports the notion that induction of autophagy does also improve healthspan as we will discuss below in the context of cardiovascular aging.

Autophagy in Cardiac Aging

Reduced Autophagy Accelerates Cardiac Aging

For most tissues, aging is associated with reduced autophagic activity.[121–125] Although autophagy in general and mitophagy in particular have been reported to decline in the hearts of aged flies[126] and aged C57BL/6 mice (20–26 months old),[30,61,75,127,128] higher levels of lipidated microtubule-associated protein light chain 3 (LC3-II), a marker of autophagosome formation, were observed in 18-month-old C57BL/6J mice,[129] and normal LC3-II levels were found in 20-month-old FVB mice.[130] One reason for this discrepancy is that these latter studies considered LC3-II levels as a genuine marker for autophagy, instead of measuring actual autophagic flux.[131] Increased LC3-II levels merely mirror an increased number of autophagosomes, which can result either from (1) accumulation because of blocked autophagic lysosomal degradation or from (2) increased autophagosome formation because of augmented autophagic flux (Figure 1).[132] Hence, future studies need to rely on standardized guidelines for evaluating the effects of age on autophagy.[131] Other reasons that may account for this variance are differences between strains, animal age, and the general housing conditions between laboratories. Irrespectively, the majority of existing data points toward a direct connection between reduced autophagy and cardiac aging.

Mechanisms Underlying Reduced Autophagy in Aging

Although the mechanisms underlying the age-related decline in autophagy have not been fully elucidated, it is conceivable that the longevity pathways (discussed above) contribute to such reduction in autophagy. For instance, hyperactivation of mTOR,[57,61] as well as reduced AMPK activity[105–107] in old age can directly inhibit autophagy via inactivating the proautophagic ULK1 (Unc-51 like autophagy activating kinase-1) complex.[133] Moreover, age-associated alterations in the longevity signaling pathways contribute to the transcriptional regulation of autophagy[134] as exemplified by the proautophagic transcription factor EB family, which is negatively regulated by mTOR-mediated phosphorylation, resulting in the cytosolic retention and inactivation of transcription factor EB.[135] Similarly, the forkhead box O transcription factor family can be negatively regulated[135–137] during aging because of (1) reduced AMPK activity,[105–107] (2) Akt-mediated phosphorylation,[61] and (3) lysine acetylation resulting from SIRT1 deactivation,[75] which occurs because of age-induced nicotinamide adenine dinucleotide decrease.[138] This negative transcriptional regulation can, in turn, result in reduced expression of autophagy genes in the heart, similar to the low levels of *Atg5* and *Atg7* reported in other aged tissues, such as the brain (Figure 1).[125]

Moreover, experimental evidence indicates that ROS accumulation in conjunction with damaged and dysfunctional mitochondria profoundly contributes to the inhibition of general autophagy and specifically mitophagy.[122,139] On the one hand, ROS promote accumulation of oxidized proteins that are prone to form aggregates and further stimulate ROS production, causing membrane lipid peroxidation, mitochondrial DNA mutations, and protein misfolding.[28] This vicious circle may cause reduced autophagy because of exhaustion of the aged autophagic machinery.[140] On the other hand, upon exposure to high levels of ROS-induced mitochondrial damage, several mitochondria-located proteins (eg, MFN1 [mitofusin 1], DRP1 [dynamin-related protein 1], FIS1 [fission 1]), fundamental for the regulation of mitophagy, become dysfunctional. This contributes to the abnormal mitochondrial turnover and removal of the damaged mitochondria.[141] Excessive ROS may also cause similar consequences to cytosolic proteins involved in autophagy regulation eventually deriving in autophagy inhibition. Also, the age-associated accumulation of lipofuscin granules,[142] specifically in lysosomes, can impede lysosomal function[143] and hence can likely inhibit autophagy.

In recent years, the role of intracellular calcium as a key regulator of both basal and induced autophagy in

cardiomyocytes has become evident.[144] Calcium release activity of inositol 1,4,5-trisphosphate receptors (IP_3Rs) is necessary for autophagy suppression through the formation of the IP_3R/beclin-1 complex.[145] Conversely, inositol 1,4,5-trisphosphate (IP_3) depletion or IP_3R antagonists increase autophagy in neonatal rat ventricular cardiomyocytes.[145] In addition, the IP_3R-mediated control of autophagy has been coupled to the regulation of cellular bioenergetics, whereby the increased IP_3-mediated calcium transfer to mitochondria exerts an inhibitory effect on autophagy by suppressing AMPK activation.[146] Interestingly, expression of IP_3Rs is increased in aged, hypertrophied, and failing myocardium of rodents[147,148] and humans,[149,150] suggesting that increased IP_3R-mediated calcium signaling possibly decreases autophagy in these conditions.[151] Of note, IP_3-mediated calcium signaling forms part of a larger signaling network, namely the IGF-1 longevity signaling pathway,[152] which also regulates autophagy.

Finally, the age-associated increase in cytosolic protein acetylation[153,154] because of increased availability of specific endogenous metabolites, such as acetyl-coenzyme A,[155] may inversely correlate with the rate of autophagy in the aged cardiovascular system. This negative regulation can occur via epigenetic control of autophagy-related genes, direct post-translational inactivation of proteins engaged in the autophagic machinery, or through the modulation of nutrient-sensing kinase pathways.[156]

Complete Deactivation of Cardiac Autophagy

Because of the vital role of autophagy during embryogenesis, fetal development, and neonatal metabolic adaptation to extrauterine life (reviewed elsewhere [157–159]), conventional/embryonic global deletion of nonredundant autophagy-related genes causes perinatal lethality.[157–159] However, partial inactivation or conditional tissue-specific knockouts are viable and have shed light on the physiological role of autophagy in the structural and functional homeostasis of different tissues and organs.[160] In the heart, mutations that interrupt autophagy lead to accelerated cardiac aging or premature heart failure and death (Table 1). For example, cardiomyocyte-specific *Atg5* ablation in mice causes a dramatic acceleration of cardiac decline, including myocardial hypertrophy, fibrosis, impaired contractile function, disrupted cardiomyocyte sarcomere and mitochondrial structure, decreased mitochondrial respiration, as well as enhanced oxidative stress, altogether culminating in dilated cardiomyopathy by the age of 10 months, resulting in a shortened lifespan.[30] In another study, mice with a global deficiency in LAMP-2 (lysosome-associated membrane protein-2), resulting in an interruption of autophagic flux because of impaired autophagosome-lysosome fusion, exhibited premature mortality, usually between 20 and 40 days.[161] Mice that survived until adulthood exhibited accumulation of nonhydrolyzed autophagic vacuoles, cardiac hypertrophy, and severe contractile dysfunction by the age of 19 months.[161] Interestingly, a similar mutation in humans is associated with Danon disease, a lysosomal and glycogen storage disorder associated with cardiomyopathy.[168,169] Despite the fact that cardiac autophagy abrogation is not lethal, it may result in early-life detrimental effects, including defective cardiac morphogenesis. Indeed, *Atg5*$^{-/-}$ mice experience impaired valve development and chamber septation.[170] Similarly, knockdown of essential autophagy genes in zebrafish results in flawed cardiac looping, atypical chamber morphology, and aberrant valve development.[170] Given such pivotal role of autophagy in early development and also in postnatal metabolic maturation, especially in the heart,[170–172] it cannot be ruled out that premature heart failure and the associated mortality in animal models of complete cardiac autophagy inactivation occur primarily because of developmental rather than aging-related defects. Therefore, inactivation of autophagy after birth,[173] during maturation,[174] or ideally late-in-life may be more appropriate to study the physiological role of autophagy in the context of (cardiac) aging.

Attenuated Cardiac Autophagy

An alternative to study the involvement of autophagy in these settings is to interfere with longevity pathways so that autophagic activity is reduced rather than completely blocked (Table 1). For example, global *Gsk3a* (glycogen synthase kinase-3a) knockout mice, which have enhanced mTORC1 activity and thus reduced autophagy, show exacerbated aging in the heart.[164] This manifests in hypertrophic and fibrotic remodeling, severe cardiac dysfunction (diastolic and systolic) combined with cardiomyocyte loss, vacuole accumulation, sarcomere disarray, as well as swollen and disrupted mitochondrial structure associated with increased superoxide production. These hallmarks of aging develop already during middle age (12 months of age), further deteriorate as the animals grow older and are associated with shortened lifespan.[164] Nevertheless, the lifespan of *Gsk3a*-deficient mice is longer than that of *Atg5* and LAMP-2 knockout mice, which have a complete loss/block of autophagy. Strikingly, the cardiac phenotype of *Gsk3a* knockout mice could be, at least partially, rescued by treatment with the mTOR inhibitor and autophagy inducer everolimus, which reduced hypertrophy and enhanced contractility.[164] Although no data is available on whether everolimus might also promote lifespan in *Gsk3a* knockout mice, these data causally link mTOR-mediated autophagy inhibition to the development of the cardiac phenotype in these animals. Similarly, accentuated cardiac aging in mice overexpressing Akt in the heart is causally linked to reduced autophagy. This is suggested by in vitro restoration of cardiomyocyte function upon autophagy activation via rapamycin-mediated mTOR inhibition.[61] Moreover, cardiac-specific overexpression of the miRNA miR-199a promoted mTOR signaling, reducing autophagy, and causing premature hypertrophy and impaired contractility by the age of 7 months. Reactivation of autophagy either by short-term rapamycin treatment or by overexpression of *Atg5* in neonatal cardiomyocytes could attenuate hypertrophy.[165] Similar deleterious effects (ie, premature hypertrophy and contractile dysfunction by 4 months of age) were observed in tuberous sclerosis complex 2-deficient mice, which show reduced autophagy because of constitutively increased mTORC1 activity. Reactivation of autophagy by trehalose treatment attenuated cardiac dysfunction and structural abnormalities of mitochondria in tuberous sclerosis complex 2-deficient hearts.[166] These results suggest that autophagy via the tuberous sclerosis complex 2-mTORC1 signaling pathway

Table 1. List of Genetic Mutations That Reduce Autophagy and Cause Accelerated Cardiac Deterioration

Mutation; Specificity	Mechanism of Autophagy Regulation	Effect on Cardiac Phenotype	Age; Model	Cardiac Phenotype Rescued by	Refs.
Cardiomyocyte-specific *Atg5*$^{-/-}$	Interrupted autophagosome formation	Early onset hypertrophy and fibrosis; contractile dysfunction; dilated cardiomyopathy (later by 10 mo of age); early mortality Structural and functional mitochondrial abnormalities, increased oxidative stress	6 mo; mice	Not reported	30
Global *Lamp2*$^{-/-}$	Obstructed autophagosome-lysosome fusion	Accentuated hypertrophy; contractile dysfunction; premature mortality	19 mo; mice	Not reported	161
Global *Atrogin-1*$^{-/-}$	Compromised autophagosome-lysosome fusion because of impaired endosomal sorting	Accentuated hypertrophy, fibrosis, impaired intraventricular conductance, diastolic dysfunction and exercise intolerance; shortened lifespan Increased apoptosis, sarcomeric disarray and endoplasmic reticulum stress	16 mo; mice	Not reported	162
BNip3 (global) and *BNip3L* (cardiomyocyte-specific) double knockout Global *BNip3L*$^{-/-}$	Hampered mitophagy and autophagic vesicle formation	Increased LV mass; depressed contractility Mitochondrial abnormalities (impaired subcellular organization, size heterogeneity, abnormal morphology and degeneration)	30 wk; mice 60 wk; mice	Not reported	163
Global *Gsk3a*$^{-/-}$	Enhanced mTORC1 activity	Exaggerated hypertrophy and fibrosis; systolic and diastolic dysfunctions; shortened lifespan Cardiomyocyte loss, vacuole accumulation, sarcomere disarray, disrupted mitochondria, and increased oxidative stress	12 mo (deteriorates henceforth); mice	Reactivation of autophagy by TOR-inhibitor everolimus reduces hypertrophy and enhanced contractility	164
Cardiac-specific *Akt*^{tg}	mTOR activation	Augmented hypertrophy and fibrosis; impaired contractility and prolonged relaxation Cardiomyocyte calcium mishandling	24–26 mo; mice	Autophagy activation by TOR-inhibitor rapamycin, restores cardiomyocyte function in vitro	61
Cardiac-specific miR-199a^{tg}	Gsk3β/mTOR complex signaling	Early onset of hypertrophy and impaired contractility	7 mo; mice	Reactivation of autophagy by short-term rapamycin treatment in vivo or by overexpression of atg5 in neonatal cardiomyocytes could attenuate hypertrophy	165
Cardiac-specific *TSC2*$^{-/-}$	mTORC1 hyperactivity	Early onset of hypertrophy and contractile dysfunction; shortened lifespan (10 mo) Mitochondrial abnormalities (misalignment, aggregation, reduced size, and increased number)	4 mo; mice	Autophagy inducer trehalose attenuates cardiac dysfunction and mitochondrial structural abnormalities	166
Global *Parkin*$^{-/-}$	Impaired Parkin-mediated mitophagy	Accelerated decline of cardiac functional reserve; shortened lifespan Mitochondrial integrity and function; increased mitochondrial DNA mutations, oxidative stress and aging biomarkers Exaggerated injury and reduced survival upon myocardial infarction despite the normal cardiac performance at baseline (confirmed up to 12 mo of age)	15 mo; mice 3 mo; mice	Not reported	128 167

$^{-/-}$ indicates knockout; Akt, serine/threonine-specific protein kinase; Atg5, autophagy-related 5; BNip3, BCL2 interacting protein 3; BNip3L, BCL2 interacting protein 3 like; Gsk3a, glycogen synthase kinase-3a; LAMP-2, lysosome-associated membrane protein-2; LV, left ventricle; mTOR, mammalian target of rapamycin; tg, transgenic overexpression; and TSC2, tuberous sclerosis complex 2 .

plays an important role in the maintenance of cardiac function and mitochondrial homeostasis.

Impaired mitophagy in *Parkin*-knockout mice is associated with accelerated decline of mitochondrial integrity and function, as well as enhanced mitochondrial DNA mutations in the heart. This is coupled to prematurely compromised cardiac functional reserve and ATP synthesis, increased oxidative stress, and the accelerated manifestation of aging biomarkers,[128] resulting in shortened lifespan.[175] Similarly, impaired mitophagy in mice with global and cardiomyocyte-specific knockouts of the Bcl2 family members BCL2-interacting protein 3 and BCL2-interacting protein 3 like (known as Nix), respectively, is associated with premature hypertrophy and impaired contractility already at 30 weeks of age.[163] Also, mice lacking the muscle-specific ubiquitin ligase atrogin-1 (also known as MAFbx), which show impaired autophagy because of defective endosomal sorting, display accelerated cardiac aging.[162] This phenotype is characterized by exaggerated hypertrophy and fibrosis, intraventricular conductance defects, as well as by diastolic dysfunction and exercise intolerance. The characteristics of atrogin-1 knockouts are driven by compromised autophagosome-lysosome fusion, which results in increased cardiomyocyte apoptosis, sarcomeric disarray, and endoplasmic reticulum stress.[162] Altogether, these findings underscore a causal relationship between impaired autophagy and accelerated cardiac aging.

Enhanced Autophagy Decelerates Cardiac Aging

The inverse relationship between autophagy and cardiac aging implies that, in turn, autophagy induction might promote cardioprotective effects. In fact, studies in aged animals have provided strong evidence that activation of autophagy by nutritional, genetic, or pharmacological means (summarized in Figure 3; Table 2) attenuates age-related structural remodeling and functional decline in the heart.

Dietary Activation of Autophagy

Caloric Restriction

CR, defined as the reduction of calorie intake without malnutrition, is the most reproducible dietary intervention known to promote health and lifespan in various model organisms.[190,191] Indeed, CR is the most potent physiological stimulus of autophagy that ameliorates cardiac dysfunction (systolic and diastolic) and attenuates myocardial hypertrophy and fibrosis in different rodent models of aging,[62,178,179] whether applied for long[62,179] or short duration late in life,[178,180,181] as well as in humans.[182] At the cardiomyocyte level, CR reduces mitochondrial damage, lipid accumulation, oxidative stress, apoptosis, telomere shortening, senescence markers, and the levels of circulating proinflammatory cytokines.[178] Strikingly, these beneficial cardiac effects in middle-aged or old C57BL/6 mice are lost, or even reversed, if CR is initiated early in life. Young C57BL/6 mice subjected to a CR diet display reduced cardiac autophagy associated with a deteriorated cardiac phenotype,[178] which can be further accentuated upon additional

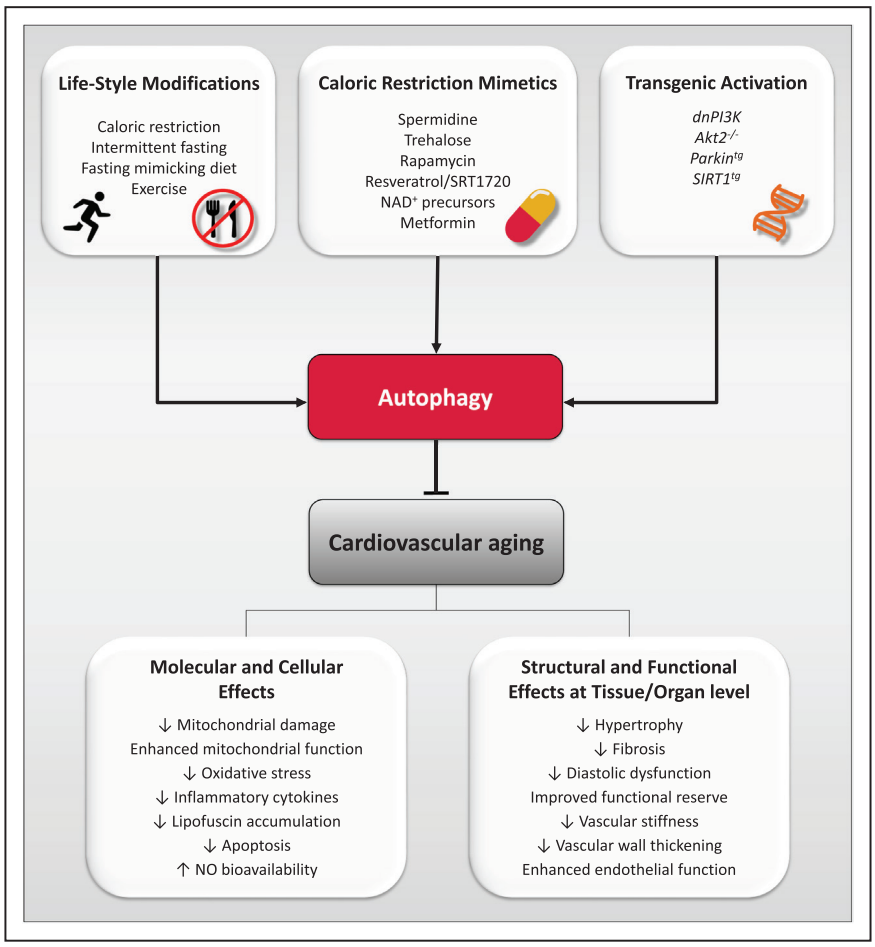

Figure 3. Interventions that induce autophagy and retard cardiovascular aging. $^{-/-}$ indicates knockout; Akt2, serine/threonine-specific protein kinase 2; dnPI3K, dominant negative phosphoinositide 3-kinase; NAD^+, nicotinamide adenine dinucleotide; SIRT1, sirtuin 1; and tg, transgenic overexpression.

Table 2. List of Interventions That Induce Autophagy and Delay Cardiac Aging

Intervention; Specificity	Mechanism of Autophagy Regulation	Effect on Cardiac Aging Phenotype	Age; Model	Cardiac Effect Abolished by	Refs.
Caloric restriction	SIRT1 activation mTOR inhibition AMPK activation	Attenuated hypertrophy and fibrosis; ameliorated systolic and diastolic functions, as well as cardiac reserve; prolonged lifespan Reduced mitochondrial damage, lipid accumulation, oxidative stress, apoptosis, telomere shortening, senescence, and inflammatory markers	22–28 mo; mice 24–30 mo; rats 52.7±11.9 y (M±SD); humans	Initiating the intervention in young animals (3-mo-old) and *Prkaa* ablation[176,177]	62,178–183
Intermittent fasting	SIRT1 activation mTOR inhibition AMPK activation	Reduced hypertrophy and fibrosis; prolonged lifespan Decreased myocardial collagen deposition, oxidative stress, inflammatory markers, and B-type natriuretic peptide levels	24 mo; rats	Not reported	184–186
Cardiomyocyte-specific *dnPI3K*	mTOR activation	Reduced hypertrophy and fibrosis; enhanced cardiac functional reserve; improved survival Reduced oxidative stress, lipofuscin accumulation, senescence, and inflammatory biomarkers, as well as rejuvenated genetic profile	20–24 mo; mice	Not reported	130
Global *Akt2*$^{-/-}$	mTOR activation Forkhead box O inhibition	Enhanced contractile function despite accentuated hypertrophy; prolonged lifespan Improved cardiomyocyte mechanical properties, calcium handling, and mitochondrial integrity	24 mo; mice	Autophagy inhibitor 3-methyladenine attenuates the effects at the cardiomyocyte level	75
Cardiomyocyte-specific *Parkin*^{tg}	Enhanced Parkin-mediated mitophagy	Increased cardiac functional reserve Enhanced mitochondrial structure and function; reduced oxidative stress, senescence, and inflammatory markers	20 mo; mice	Not reported	128
Low-to-moderate cardiomyocyte-specific *Sirt1*^{tg}	SIRT1 activation	Reduced hypertrophy, fibrosis; Enhanced contractility Reduced apoptosis and aging markers	18 mo; mice	Not reported	76,137
Spermidine	Acetyltransferase EP300 inhibition[187]	Attenuated hypertrophy; improved diastolic function and ventricular-vascular coupling; lifespan extension Enhanced mitochondrial and myofibrillar relative volumes, mitochondrial function, and titin phosphorylation; reduced inflammation	24 mo; mice	Cardiomyocyte-specific ATG5 ablation	188
Rapamycin	mTOR inhibition	Reduced hypertrophy; enhanced contractile function; extended lifespan Promoted mitochondrial biogenesis and restored fatty acid oxidation; reduced systemic and cardiac inflammation	24–27 mo; mice	Not reported	63,189

(*Continued*)

Table 2. Continued

Intervention; Specificity	Mechanism of Autophagy Regulation	Effect on Cardiac Aging Phenotype	Age; Model	Cardiac Effect Abolished by	Refs.
Resveratrol	SIRT1 and AMPK activation	Restored myocardial performance index Rejuvenated genetic signature	25 mo; mice	Not reported	179
SRT1720	SIRT1 activation	Rescued cardiomyocyte contractility in vitro	24 mo; mice	Parkin deficiency or insulin coincubation inhibited the induced autophagy/mitophagy and contractility enhancement in vitro	75

$^{-/-}$ indicates knockout; Akt2, serine/threonine-specific protein kinase 2; AMPK, AMP-activated protein kinase; dnPI3K, dominant negative phosphoinositide 3-kinase; M, mean; mTOR, mammalian target of rapamycin; SIRT1, sirtuin 1; and tg, transgenic overexpression.

autophagy reduction by AMPK ablation.[176,177] Hence, the positive effects of CR seem to depend on the induction of autophagy and to be of benefit for cardiac remodeling in the late phase of life.

Intermittent Fasting

Because of the difficulty of calorie counting every day, recent years have seen an increasing interest in the identification of dietary interventions that have better compliance and long-term adherence than CR.[192,193] As we discuss below, various health-promoting dietary regimens that target nutrient-sensitive pathways and mimic beneficial biochemical changes of CR have been proposed as an alternative remedy for the prevention and treatment of cardiovascular and other age-related diseases. One such regimen is intermittent fasting (IF), whereby individuals go through regular cycles, composed of time periods with no or minimal caloric intake interrupted by periods of normal food consumption. One form of IF is alternate-day fasting, that is, feeding every other day, which—similar to CR—extends lifespan[184] and delays cardiac aging in rats as determined by reduced hypertrophy[185] and fibrosis.[186] Life-long alternate-day fasting reduces myocardial collagen deposition, oxidative stress, inflammatory markers, and B-type natriuretic peptide levels.[185,186] These effects were attributed to reduced phosphoinositide 3-kinase signaling,[185] known to induce autophagy.[130] Although, as observed for CR,[178] alternate-day fasting beneficial effects were challenged in young rats,[194] aging-related benefits of IF seem to be evolutionarily conserved. Time-restricted feeding, a specific form of IF where food access is allowed during a restricted time window every day, can attenuate age-related decline of cardiac function in fruit flies.[195] At present, there is no direct evidence that autophagy activation by IF[196,197] is responsible for such cardioprotection in aging. At least in the setting of myocardial ischemia-reperfusion injury, the cardioprotective effect of IF observed in wild-type mice is absent in LAMP2-deficient, and thus autophagy-incompetent, animals.[196] This suggests that an intact autophagy-lysosome machinery is indispensable for myocardial homeostasis and cardioprotection induced by IF.

Metabolism-Modulating Diets

CR-induced metabolic shifts have been also achieved by changing the composition—rather than amount—of dietary intake. Recently, it has been shown that cyclic ketogenic diet (consisting of high-fat and low- or no-carbohydrate) administered in weekly intervals to mice promotes healthier aging and reduces midlife mortality.[198] Despite the lack of comprehensive cardiac evaluation, ketogenic diet-fed aged mice seemed to have an improved cardiac phenotype based on a composite cardiac score generated from heart rate, LV mass, aortic valve pressure gradient, and fractional shortening.[198] Although the mechanisms underlying beneficial cardiac response to increased levels of circulating ketone bodies are as yet elusive, it is reasonable to speculate that the inhibition of mTORC1 by high levels of ketone bodies,[198] thereof the most abundant β-hydroxybutyrate, stimulate autophagy in the heart and vasculature. Mechanistically, autophagy may improve lipostasis (essentially regulated by lipogenesis and lipolysis) through several independent, not mutually exclusive mechanisms, including such involved in metabolic capacity or lipid metabolism. For instance, autophagy may improve mitochondrial function of respiring tissues or impact lipid homeostasis by lipid degradation through lipophagy or by affecting lipogenesis (eg, degradation of lipogenic enzymes).[199] Nevertheless, further studies focusing on the heart are warranted to confirm the role of autophagy in ketogenic diet effects in aging.

Another dietary intervention that recapitulates the benefits of CR is fasting-mimicking diet (FMD), which contains reduced amounts of calories, sugars, and proteins but high levels of unsaturated fats, and so it can be consumed ad libitum.[200] In mice, FMD promotes lifespan and healthspan by reducing cancer incidence, obesity, and inflammation, improving cognitive function and rejuvenating the immune system.[200] Most importantly, 3 months of FMD consumptions in humans is sufficient to lower age-related cardiovascular disease risk factors, including reduced blood pressures, body mass index, fasting glucose, and inflammation, as well as improved lipid profile.[201] From a mechanistic point of view, FMD lowers IGF-1 while increasing IGFBP-1 levels and ketone bodies in the circulation,[200] all of which are linked to autophagy. In fact, FMD have been shown to decelerate the age-dependent decline of autophagy.[200] Nonetheless, it remains to be experimentally tested whether FMD can delay cardiovascular aging and whether this depends on autophagy.

Transgenic Activation of Autophagy

The understanding of mechanisms underlying the protective effects of autophagy in aged hearts has greatly advanced using transgenic animal models with enhanced autophagic

activity. For example, cardiomyocyte-specific dominant negative phosphoinositide 3-kinase mice showed improved survival, reduced hypertrophy and fibrosis, as well as enhanced cardiac functional reserve coupled to enhanced cardiac autophagy.[130] Cardiomyocytes of dominant negative phosphoinositide 3-kinase mice displayed attenuated senescence, reduced levels of inflammatory biomarkers, reduced oxidative stress, diminished lipofuscin accumulation, and rejuvenated genetic profile.[130] Similarly, enhanced autophagy and mitophagy upon ablation of Akt2 is associated with enhanced contractile function and improved cardiomyocyte mechanical properties, calcium handling, and mitochondrial integrity in aged mice.[75] Nevertheless, the associated increase in myocardial mass seems to be rather adaptive because these mice have also prolonged lifespan.[75] Because treatment of aged *Akt2*$^{-/-}$ cardiomyocytes with the autophagy inhibitor 3-methyladenine attenuated the cardiac improvements, these effects seem to be essentially driven by autophagy.[75] Cardiomyocyte-specific Parkin overexpression results in enhanced cardiac functional reserve and reduced senescence biomarkers in aging mice.[128] The absence of age-related cardiac alterations was attributed to the observed increase in (Parkin-mediated) mitophagy, which was associated with enhanced integrity and respiratory function of cardiac mitochondria, as well as with reduced oxidative stress and proinflammatory cytokines.[128] Unfortunately, this study did not specifically test causality, that is, whether autophagy inactivation would blunt these beneficial effects. Future studies using relevant experimental models that enhance cardiac autophagy, such as mice overexpressing SIRT1[76,137] or long-lived *Atg5* transgenic mice,[202] are warranted to test for the causal relationship between enhanced autophagy and delayed cardiac aging. Collectively, transgenic activation of autophagy fairly recapitulates CR effects against cardiac aging (Table 2). This is true regardless of the observed differences between distinct transgenic models. Such differences are to be expected given that the molecular targets of these mutations are involved in multiple cellular functions, and so their regulatory effect is not limited only to autophagy but also includes other intracellular processes.

Pharmacological Activation: CR Mimetics

Recent years have seen an increasing interest in the search for pharmacological agents that can promote autophagy and slow cardiac aging.[203,204] We have shown that the natural polyamine and potent autophagy inducer spermidine exert cardioprotection in aged mice.[188,205] Late-in-life spermidine supplementation attenuates age-associated hypertrophy, substantially reduces myocardial passive stiffness and diastolic dysfunction, and improves ventricular-vascular coupling, thus promoting cardiovascular efficiency. The protective effects of spermidine are associated with increased cardiomyocyte autophagy and mitophagy. Thereby, cardiomyocyte rejuvenation is attained, at least partially, by reversing age-associated reduction in relative mitochondrial and myofibrillar volumes, restoring mitochondrial respiratory function, and enhancing titin phosphorylation (reduction of myocyte stiffness). Spermidine also ameliorates typical age-related low-grade inflammation. Interestingly, inactivation of autophagy does not only block the cardiac beneficial effects of spermidine, but similar to CR, lack of autophagy actually reverses these effects. Cardiomyocyte-specific *Atg5*-deficient mice develop hypertrophy and diastolic dysfunction upon spermidine treatment, as opposed to their wild-type counterparts, which display enhanced contractility along with reduced myocardial mass, denoting extensive cardiovascular efficiency.[188] This supports an essential role of autophagy in cardioprotection and healthspan extension in response to spermidine supplementation[205,206] and also supports the notion that autophagy induction in old or even young age (using suitable triggers) can exert beneficial effects.[188]

Rapamycin, which induces autophagy by inhibition of mTOR, can reverse the age-associated deterioration of cardiac structure and function in old female mice.[207] After 3 months of treatment, rapamycin significantly reduced age-related hypertrophy and ventricular dysfunction, as well as systemic and cardiac inflammation.[63] Recently, another report showed that only 2 weeks of rapamycin treatment is sufficient to attenuate aging-related heart remodeling. Rapamycin induced autophagy followed by increased mitochondrial biogenesis that was associated with restored fatty acid oxidation and energy metabolism homeostasis in the heart.[189] Interestingly, no additional reduction in cardiac remodeling (eg, hypertrophy) was observed after this period of transient autophagy activation, suggesting that reversed cardiac aging depended on rapamycin-induced autophagy.

Another autophagy inducer is resveratrol,[208,209] a SIRT1 activator that confers cardiac rejuvenation in aged mice both at the functional and transcriptional levels by phenocopying most of the molecular signatures of CR.[179] It has not been directly examined whether autophagy mediates resveratrol-induced deceleration of cardiac aging. Another SIRT1 activator, SRT1720, rescues age-induced mechanical impairment of aged cardiomyocytes, and insulin-mediated autophagy inhibition can abolish these beneficial effects.[75] Nonetheless, direct testing for causality is warranted, not only for resveratrol and SRT1720 but also for other autophagy inducers like metformin[210] and caffeine,[211,212] which reportedly retard cardiac aging.[105,213,214] Similarly, other candidate autophagy inducers[215] remain to be tested in the setting of biological aging, in particular those that have been already shown to induce cardiac autophagy (eg, trehalose).[216,217]

CR mimetics hold a great translational promise because of the fact that they sufficiently delay cardiac aging despite lacking some of the effects induced by CR (eg, reduction of cardiac fibrosis has not been reported by any mimetic to date). This is reasonable as these pharmacological agents typically have a few, if not single, targets for autophagy induction, unlike CR-mediated autophagy activation (Table 2) and age-related decline of autophagy (Figure 1) that are both of multifactorial nature.

Exercise-Mediated Activation of Autophagy

Multiple studies have shown that regular physical activity effectively improves cardiovascular function not only in aged, otherwise healthy individuals,[218–220] but also in patients with heart failure.[221] Like CR, aerobic exercise training increases effort tolerance (ie, peak oxygen consumption), and a combination of exercise and CR exerts additive beneficial effects in

obese older patients with heart failure with preserved ejection fraction.[221] Although the benefits of exercise on the cardiovascular system are well recognized and broadly accepted,[222] current knowledge of the pivotal molecular mechanisms responsible for exercise-induced cardiac protection in aging is limited. However, it has been shown in an elegant study[16] that acute exercise increases the number of autophagosomes via disruption of the BCL-2-beclin-1 complex in skeletal and cardiac muscles in vivo. Furthermore, induction of autophagy by exercise was shown to be necessary for its beneficial effects against obesity as demonstrated by BCL2 AAA mice that did not metabolically profit from exercise training because of defective stimulus-inducible autophagy.[16] In line with these findings, a recent study pointed out that exercise re-establishes autophagic flux and mitochondrial quality control in the failing hearts of rats upon myocardial infarction.[223] This further supports a critical role of autophagy in exercise-mediated cardioprotection. That being said, more research effort is warranted to understand the crosstalk between exercise and autophagy not only in cardiovascular aging but also in biological aging in general.

Autophagy in Vascular Aging

Reduced Autophagy Accelerates Vascular Aging

Although vascular aging has been less intensively studied than cardiac aging, significant advances have been made in recent years toward understanding the connection to autophagy. Indeed, like in many other tissues, autophagic activity is diminished in vascular tissues derived from aged mice and humans.[224,225] This decay has been linked to arterial functional decline, which is primarily linked to increased levels of oxidative stress and proinflammatory cytokine expression, reduced NO bioavailability, and impaired vasodilation as typical hallmarks of vascular endothelial dysfunction.[224]

Complete Deactivation of Vascular Autophagy

Studies in genetically modified mice show that lack of essential autophagy genes in vascular endothelial or smooth muscle cells impedes normal vascular physiology (Table 3). For example, mice with vascular smooth muscle cell–specific *Atg7* deficiency display premature defects in calcium mobilization, as well as abnormal vascular reactivity and smooth muscle cell contractility at young age.[226] This is in agreement with in vitro data demonstrating an inverse correlation between autophagy and stress-induced vascular smooth muscle senescence.[233,234] Similarly, vascular endothelial cell–specific *Atg5* or *Atg7* knockout mice ($Atg5^{VE\text{-}KO}$ and $Atg7^{VE\text{-}KO}$, respectively) show impaired synthesis and secretion of von Willebrand factor. Thus, these animals have higher bleeding tendency already at young age despite the lack of apparent defects in vessel structure.[227] Even though $Atg5^{VE\text{-}KO}$ and $Atg7^{VE\text{-}KO}$ mice survive at least for up to 1 year,[227] it remains unknown whether they develop more obvious vascular defects late-in-life. At least under stressful conditions, they do: $Atg5^{VE\text{-}KO}$ mice exhibit reduced vessel maturation, low perfusion, and impaired endothelial cell lining in induced tumors.[235] Also, $Atg7^{VE\text{-}KO}$ mice are more susceptible to bleomycin-induced pulmonary fibrosis and collagen accumulation.[236] Collectively, these findings show that manipulation of autophagy interferes with vascular homeostasis and may impair vascular functions early in life.

Attenuated Vascular Autophagy

Other gene mutations that reduce (rather than fully block) autophagy support the idea that autophagy attenuates vascular aging (Table 3). Reduced autophagy in global and endothelial cell–specific *Prkaa* (protein kinase AMP-activated α catalytic subunit) knockout mice is sufficient to cause aortic endothelial dysfunction and mitochondrial fragmentation, essentially because of reduced autophagy-dependent dynamin 1-like degradation.[228] Interestingly, this phenotype can be reversed by dynamin 1-like depletion using genetic or pharmacological interventions, including the autophagy inducer rapamycin.[228] This argues for an essential role of autophagy in the maintenance of vascular function and mitochondrial integrity. Similarly, endothelial cell–specific ablation of the transcriptional regulator of autophagy Kruppel-like transcription factor 4 completely blocks acetylcholine-induced vasodilation in vivo, thus denoting endothelial dysfunction.[229] However, activation of autophagy in middle-aged mice overexpressing Kruppel-like transcription factor 4 in endothelial cells enhances vascular dispensability, which is eliminated upon autophagy inhibition by chloroquine.[229] A loss-of-function mutation in *Krit1* that compromises autophagic flux causes cerebral cavernous malformations with enlarged and leaky capillaries predisposing to seizures and intracerebral hemorrhages, in mice and humans.[230] Importantly, the hallmarks of this disease, namely endothelial-to-mesenchymal transition and oxidative stress, are attenuated upon autophagy induction by rapamycin or ATP-competitor Torin-1.[230] Rapamycin also prevents vascular malformations associated with gene mutations affecting RAS p21 protein activator 1[232] although autophagic flux was not quantified in this setting. Altogether, these studies provide compelling evidence that normal vascular dispensability requires intact autophagic responses.

Enhanced Autophagy Decelerates Vascular Aging

Mounting evidence proves that vascular aging can be improved by induction of autophagy through a variety of nutritional or pharmacological strategies (Figure 3; Table 4).

Dietary Activation: CR

The most feasible dietary approach to promote autophagy-mediated vascular health is CR, which exerts a multitude of vasoprotective effects during aging. Lifelong CR substantially attenuates wall thickening, reduces stiffness, and reinstates endothelial function of conduit arteries in aged mice.[237] CR also neutralizes age-related arterial collagen accumulation and elastin remodeling.[237] These effects are mediated through reduced oxidative stress and inflammation, as well as by increased eNOS expression that boosts NO bioavailability.[237] Even short-term CR initiated late in life is capable of restoring endothelial functionality and reducing oxidative stress in aged rodents.[238,239] Along the same lines, treatment of cultured endothelial cells with sera obtained from aged CR-treated rats is sufficient to reduce oxidative stress and inflammatory markers.[240] Given that these antiaging effects depend on the activity of mTOR and SIRT1,[237,238,240] it is tempting to speculate

Table 3. List of Genetic Mutations That Reduce Autophagy and Accelerate Vascular Deterioration

Mutation; Specificity	Mechanism of Autophagy Regulation	Effect on Vascular Aging Phenotype	Age; Model	Vascular Phenotype Rescued by	Refs.
Vascular smooth muscle cell–specific *Atg7*$^{-/-}$	Interrupted autophagosome formation	Premature defects in calcium mobilization; impaired smooth muscle cells contractility; abnormal vascular reactivity	3.5 mo; mice	Not reported	226
Vascular endothelial cell–specific *Atg7*$^{-/-}$ or *Atg5*$^{-/-}$ Global *Atg7*$^{-/-}$	Interrupted autophagosome formation	Abnormal stimulated secretion of von Willebrand factor; prolonged bleeding times Reduced basal plasma levels of von Willebrand factor; postnatal mortality	2 mo; mice Postnatal; mice	Not reported	227
Global or endothelial cell–specific *Prkaa1*$^{-/-}$ or *Prkaa2*$^{-/-}$	AMPK inhibition	Aortic endothelial dysfunction; abnormal mitochondrial fragmentation	3.5 mo; mice	ATG7 overexpression or rapamycin, mTOR inhibitor, promotes autophagy-dependent DNM1L degradation and attenuates mitochondrial fragmentation	228
Endothelial cell–specific ablation of *Klp4*$^{-/-}$	Autophagy modulation at the transcriptional level	Endothelial dysfunction manifested by lack of in vivo acetylcholine-induced vasodilation	5–7 mo; mice	Not reported	229
Endothelial-specific *Ccm1*$^{-/-}$ or *Ccm3*$^{-/-}$	mTOR-ULK1 activation	Venous malformations resembling human cavernous angiomas (clustered, abnormally dilated, and leaky capillary channels)	Postnatal day 14; mice	Rapamycin or ATP-competitor Torin-1 attenuate the phenotype (less endothelial-to-mesenchymal transition and oxidative stress) in vitro	230,231
Ephb4 or *Rasa1a/b* knockdown	Enhanced mTOR1 activity	Impaired vascular development, including arteriovenous endothelial cell defects (more venous connections at the expense of arterial ones); caudal vessel deformity	48 h post-fertilization; zebrafish	Rapamycin restored vascular development and re-established normal blood vessel structure and function	232

$^{-/-}$ indicates knockout; AMPK, AMP-activated protein kinase; Atg5, autophagy-related 5; Atg7, autophagy-related 7; Ccm1, cerebral cavernous malformation disease *KRIT1* gene mutation; Ccm3, cerebral cavernous malformation disease *PDCD10* gene mutation; DNM1L, dynamin 1-like; Klp4, Kruppel-like transcription factor 4; mTOR, mammalian target of rapamycin; Prkaa, protein kinase AMP-activated α catalytic subunit; and Rasa1a/b, homologues of human RAS p21 protein activator 1 in zebrafish.

that CR requires autophagy to slow vascular aging, in analogy with CR-mediated longevity and cardioprotection during aging (see above). Similarly, it is still to be tested whether autophagy is essential for the protective effects mediated by alternate-day fasting (eg, reduced aortic fibrosis and oxidative stress)[241] and by exercise (eg, reduced arterial stiffness and endothelial dysfunction)[246] in the setting of vascular aging.

Pharmacological Activation: CR Mimetics

CR cannot be easily implemented in everyday life and is not recommended for every individual, especially for elderly patients and those with severely compromised cardiovascular function. Thus, CR mimetics may provide a more feasible alternative. Induction of autophagy by spermidine rescues endothelium-dependent vasodilation and neutralizes aortic pulse wave velocity, resulting in reversed age-related endothelial dysfunction and arterial stiffness, respectively. Spermidine also reduces aortic collagen deposition and blunts accumulation of advanced glycation end products.[225] Mechanistically, spermidine enhances NO bioavailability by reducing oxidative stress[225] and possibly by increasing the bioavailability of arginine,[247] the only source for NO production. Most importantly, in vitro incubation of spermidine-treated aortic rings together with the autophagy inhibitor chloroquine abolishes these effects,[225] supporting the notion that spermidine delays vascular aging in an autophagy-dependent manner. Induction of autophagy by oral supplementation of the disaccharide trehalose exerts similar positive effects. Trehalose restores endothelial function in aged mice by reducing oxidative stress and enhancing eNOS expression, which leads to recovery of NO levels.[224] Trehalose also reduces vascular proinflammatory cytokines.[224] In endothelial cell culture, trehalose reduces oxidative stress and enhances eNOS expression and NO bioavailability, but in the presence of the autophagy blocker 3-methyladenine, these effects are prevented.[224] Recently, trehalose has been tested in humans: it conferred similar effects although rather in resistance arteries than in large conduit arteries.[243] In older adults, trehalose enhanced vascular function by increasing microvascular NO bioavailability and improving vascular smooth muscle sensitivity to NO.[243]

SIRT1 activators (resveratrol, SRT1720 and nicotinamide mononucleotide) also rescue age-related vascular dysfunction.

Table 4. List of Interventions That Induce Autophagy and Decelerate Vascular Aging

Intervention	Mechanism of Autophagy Regulation	Effect on Vascular Aging Phenotype	Age; Model	Vascular Effect Abolished by	Refs.
Caloric restriction	mTOR activation SIRT1 activation AMPK activation	Attenuated wall thickening, vascular stiffness, and restored endothelial function of conduit arteries; neutralized arterial collagen accumulation and elastin remodeling Reduced oxidative stress and inflammation; increased eNOS expression and bioavailability	29–31 mo; mice 24–28 mo; rats	Not reported	237–240,183
Intermittent fasting	mTOR activation SIRT1 activation AMPK activation	Attenuated fibrosis and vascular wall alterations in the aorta Reduced oxidative stress	24 mo; rats	Not reported	241
Spermidine	Acetyltransferase EP300 inhibition[187]	Rescued endothelial function; reduced arterial stiffness and collagen deposition Enhanced NO bioavailability; reduced oxidative stress and advanced glycation end-products accumulation	28 mo; mice	In vitro incubation of together with the autophagy inhibitor chloroquine abolishes the antioxidant effect	225
Trehalose	AMPK activation by inhibiting glucose transport[242]	Reduced vascular stiffness and restored vascular endothelial function Reduced oxidative stress and enhanced eNOS expressing and NO levels; reduced vascular inflammation. Enhanced vascular function in resistance arteries Increased microvascular NO bioavailability; improving vascular smooth muscles NO sensitivity	27–28 mo; mice Middle-aged to elderly humans	Autophagy blocker 3-MA abolishes trehalose induced reduction in oxidative stress and enhancement in eNOS expression and NO bioavailability in endothelial cell culture	224,243
Resveratrol	SIRT1 and AMPK activation	Attenuated arterial stiffness and vascular endothelial dysfunction Enhanced NO-mediated vasorelaxation; reduced vascular oxidative stress, inflammation, and endothelial apoptosis	18 mo; mice	Not reported	72
SRT1720	SIRT1 activation	Restored endothelial function Reduced oxidative stress and inflammatory markers; enhanced cyclooxygenase-2–dependent vasorelaxation	29–32 mo; mice	Not reported	244
NMN	SIRT1 activation by replenishing cellular NAD^+	Restored endothelial function; restored large elastic arteries stiffness; neutralized collagen deposition and partially limited elastin damage Reduced oxidative stress; restored NO bioavailability	26–28 mo; mice	Not reported	245

3-MA indicates 3-methyladenine; AMPK, AMP-activated protein kinase; eNOS, endothelial nitric oxide synthase; mTOR, mammalian target of rapamycin; NAD^+, nicotinamide adenine dinucleotide; NMN, nicotinamide mononucleotide; NO, nitric oxide; and SIRT1, sirtuin 1.

Resveratrol attenuates arterial stiffness and vascular endothelial dysfunction in aged mice by enhancing NO-mediated vasorelaxation, secondary to reduced vascular oxidative stress and inflammation, as well as to suppressed endothelial apoptosis.[72] Intriguingly, resveratrol is efficient against arterial stiffness in nonhuman primates fed a high-fat/sucrose diet[248] and in obese humans,[249] suggesting that its beneficial effects should be tested in aged, otherwise healthy individuals. Moreover, SRT1720, which directly activates SIRT1, reverses vascular endothelial dysfunction in mice by enhancing cyclooxygenase-2 signaling and reducing oxidative stress as well as inflammatory markers but not via NO-dependent vasorelaxation.[244] Furthermore, nicotinamide mononucleotide, which indirectly activates SIRT1 by replenishing cellular

nicotinamide adenine dinucleotide, reverses arterial dysfunction in aged mice.[245] Nicotinamide mononucleotide reverses large elastic artery stiffness both in vivo and in vitro as assessed by aortic pulse wave velocity and elastic modulus, respectively. Nicotinamide mononucleotide also restores endothelial function and NO-dependent vasodilation, reduces oxidative stress, neutralizes collagen deposition, and partially limits elastin damage in aged aortae.[245] Although SIRT1 activation by these compounds entails autophagy activation, it remains to be elucidated whether this is causative for the described vasoprotective effects.

Concluding Remarks and Perspectives

Accumulating evidence suggests that aging is a pliable process and that autophagy is a fundamental (and inducible) mechanism for the maintenance of cardiovascular homeostasis during aging. To further elucidate the potential of autophagy induction against age-related cardiovascular diseases, future efforts should consider focusing on aged animals (at least 18 months of age upon the cardiac or vascular evaluation), as well as mechanistic aspects (to truly establish a cause-effect relationship between enhanced autophagic flux and reduced aging). This is true for (1) pharmacological interventions designed to induce cardiac- and vascular-specific effects and for (2) genetic manipulation for autophagy induction or inhibition in adult mice throughout the body or in defined cell types. In addition, transgenic mouse models that have shortage, rather than lack, of basal autophagy (eg, *Gsk3a* knockout mice[164]) or mouse model with intact basal autophagy but a defective exercise-inducible autophagy (eg, BCL2 AAA mice[16]) may avoid confounding issues.[250] Thus, reduced autophagy mimics the age-related decline of cytoplasmic turnover more accurately than complete autophagy blockage that results in excessive (and perhaps nonphysiological) accumulation of cellular toxic material. Another aspect that thus far has been ignored concerns the specific modulation of autophagic responses in other cells of the heart than cardiomyocytes (eg, cardiac endothelium, fibroblasts, and inflammatory cells), knowing that the interaction between different cell types within the cardiovascular system affects aging. Moreover, given that mitochondrial degradation can take place via multiple mechanisms and it has been shown to occur even in the absence of Parkin and other identified mitophagy-mediating molecules, for example, MNF2 (mitofusin 2) and DRP1 (dynamin-related protein 1; reviewed in ref. 251), the precise distinction between the functional significance of mitophagy and that of nonselective autophagy is far from resolved. Indeed, more research efforts are needed to delineate that conundrum and to help scrutinize the general assumption that mitochondrial damage is a major underlying mechanism of biological aging, in general, and cardiac aging, in particular. In addition, it will be important to develop novel noninvasive in vivo methods to evaluate autophagy (eg, by blood sampling[252]) and hence to repeatedly and accurately monitor autophagic flux during pharmacological interventions on aging. We surmise that such improved methodologies might clarify many of the pending issues with respect to the age-related deregulation of autophagy and adequate nutritional and pharmacological interventions on this homeostatic system.

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Disclosures

None.

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