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The Intersection Between Aging and Cardiovascular Disease

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Abstract

The average lifespan of humans is increasing, and with it the percentage of people entering the 65 and older age group is growing rapidly and will continue to do so in the next 20 years. Within this age group, cardiovascular disease will remain the leading cause of death, and the cost associated with treatment will continue to increase. Aging is an inevitable part of life and unfortunately poses the largest risk factor for cardiovascular disease. Although numerous studies in the cardiovascular field have considered both young and aged humans, there are still many unanswered questions as to how the genetic pathways that regulate aging in model organisms influence cardiovascular aging. Likewise, in the molecular biology of aging field, few studies fully assess the role of these aging pathways in cardiovascular health. Fortunately, this gap is beginning to close, and these two fields are merging together. We provide an overview of some of the key genes involved in regulating lifespan and health span, including sirtuins, AMP-activated protein kinase, mammalian target of rapamycin, and insulin-like growth factor 1 and their roles regulating cardiovascular health. We then discuss a series of review articles that will appear in succession and provide a more comprehensive analysis of studies carried out linking genes of aging and cardiovascular health, and perspectives of future directions of these two intimately linked fields.

Keywords

aging; cardiovascular disease; calorie restriction; longevity genes

The most important determinant of cardiovascular health is a person's age. By 2030, approximately 20% of the population will be aged 65 or older. In this age group, cardiovascular diseases (CVD) will result in 40% of all deaths and rank as the leading cause. Furthermore, the cost to treat cardiovascular disease will triple in that time.[1,2] Hence, it remains vital that we understand why age is such a critical component of CVD etiology. However, until recently, the fields of cardiovascular disease and molecular biology of aging have remained largely separate. Most rodent studies of atherosclerosis or cardiomyopathies were performed in young mice, whereas studies of genetic and pharmacological interventions that extend lifespan rarely assessed whether CVD or heart function are improved. Fortunately, the situation is changing. In this introductory review, we discuss genetic pathways that have been identified to regulate the process of aging. Furthermore, we provide an overview of changes that occur in the cardiovascular system with age and introduce a series of reviews that will highlight the recent breakthroughs that have been made bridging the gap between the aging and cardiovascular research fields.

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Measuring the Impact of Aging on the Heart and Vasculature

Aging is associated with a progressive decline in numerous physiological processes, leading to an increased risk of health complications and disease. By delivering oxygenated blood to all tissues in the body, the health of the cardiovascular system is vital for health of every tissue and longevity of the organism as a whole. Aging has a remarkable effect on the heart and arterial system, leading to an increase in CVD including atherosclerosis, hypertension, myocardial infarction, and stroke.[3] Aging cardiovascular tissues are exemplified by pathological alterations including hypertrophy, altered left ventricular (LV) diastolic function, and diminished LV systolic reverse capacity, increased arterial stiffness, and impaired endothelial function[3,4] (Figure 1). However, the health of the arterial and cardiac systems is not mutually exclusive, as each system greatly affects the other. For instance, an increase in arterial stiffness leads to compensatory mechanisms by the myocardium including LV hypertrophy and fibroblast proliferation, resulting in decreased cardiac output and increase in fibrotic tissue.[3,4] Heart rate modulation is also affected by age with a decrease in both rate variability and maximum heart rate.[5] Heart rate is influenced not only by the loss of cells in the sinoatrial node (responsible for controlling heart rate) but also by structural changes in the heart, including fibrosis and hypertrophy, which slow propagation of electric impulse throughout the heart.

At early ages, the LV diastolic filling rate begins to decline, which is compensated for by increasing arterial contraction to sustain stroke volume and workload, maintaining sufficient ejection fraction.[6,7] However, with age, the LV contractility and ejection fraction, as well as sympathetic modulation of heart rate, and response to β-adrenergic receptor activation all decrease.[4] A reduction in cardiac output due to decline in function with age stimulates the myocardium to compensate by increasing muscle mass by undergoing cardiac hypertrophy; although this may provide short-term enhancement of cardiac output, the long-term effect of hypertrophy diminishes cardiac function.[8] Ventricular hypertrophy is the result of an increase in size of individual cardiomyocytes and can be either physiological, which is reversible (eg, exercise-induced), or pathological which is irreversible (disease-based).[9] Hypertrophy in mammalian models such as mouse and rat can be measured with a variety of techniques including echocardiography to measure LV size, histological analysis of heart tissue, or isolation of cardiomyocytes for cell size measurements. The noninvasive nature of echocardiography allows for the unique opportunity to measure alterations in cardiac size and functional parameters longitudinally in an aging study cohort.

Aging of the vasculature results in increased arterial thickening and stiffness as well as dysfunctional endothelium. Clinically, these changes result in increased systolic pressure and present major risk factors for development of atherosclerosis, hypertension and stroke, and arterial fibrillation.[4] Vascular dysfunction associated with aging leads to a variety of age-related pathologies, including loss of adequate tissue perfusion (resulting in ischemia), insufficient vascular growth or regression (resulting in hypertension), or excessive growth and remodeling (resulting in age-related macular degeneration). The vasculature undergoes structure and function alterations with age that are well documented, such as luminal enlargement with wall thickening and a decline in endothelial cell function negatively affecting endothelium-dependent dilation and promoting vascular stiffness.[10] In addition, endothelial cells lose their ability to proliferate and migrate after tissue injury.[11] Furthermore, endothelial barriers become porous and vascular smooth muscle cells migrate into subendothelial spaces and deposit extracellular matrix proteins that result in intimal thickening. At the molecular level, as endothelial cells age, they exhibit a reduction in endothelial nitric oxide synthetase (eNOS) activity, reducing the abundance of NO.[12] NO is a critical vasodilator produced by endothelial cells, regulating vascular tone, in addition to inhibiting vascular inflammation, thrombotic events, and aberrant cellular proliferation.[13]

Loss of NO also promotes endothelial cell senescence.[14] Numerous mechanisms can modulate eNOS activity. However, hemodynamic shear stress, the frictional force acting on endothelial cell surface as a result of blood flow, is one of the most potent inducers of eNOS activity.[12] As vessels age, they are exposed to less hemodynamic stress due to reduced blood flow caused by decline in heart function; in addition, endothelial cells become less responsive to shear stress, resulting in a decline in the protective NO.[15] Therefore, measuring arterial thickness and stiffness in aged models can lead to further understanding of the role various longevity genes influence vasculature aging. Elastic properties of arteries can be directly measured, as well as histological analysis of luminal enlargement, arterial thickness, and deposition of vascular smooth muscle cells. In addition, measuring eNOS activity in vessels, as well as endothelial senescence, can also serve as a marker of vascular aging.

The heart undergoes complex changes during aging that affect the cellular composition, marked by a decrease in absolute number of cardiomyocytes due to increased apoptosis and necrosis and a decrease in repopulation of cardiomyocytes from cardiac stem cell reserves.[16,17] With age, cardiomyocytes become more susceptible to stress, including oxidative stress. Therefore the increase in oxidative stress due to the increase in reactive oxygen species (ROS) production with age results in an overall enhancement in the rate of cardiomyocyte death with age. In cases when cardiomyocytes undergo necrosis, the release of cellular components can affect survival of neighboring cardiomyocytes, in addition to promoting the development of proinflammatory and profibrotic environments in the aging heart. Cardiomyocyte senescence, defined by the increased expression of senescence markers and decreased telomere length, also increases with age.[18] However, a recent study suggests that removal of senescence cells alone may not be sufficient to improve cardiac defects observed in some aging models.[19] Measuring cardiac-specific senescence, DNA damage, as well as levels of apoptosis and necrosis, coupled with fibrosis measurements in animal models of aging, will lead to a better understanding of the link between aging and CVD. Senescence is coupled with expression of such factors as p53, p21, p16, and senescence-associated β-galactosidase activity, which can be measured by immunoblotting or histological techniques. In addition, the levels of damaged nuclear and mitochondrial DNA can be assessed by quantifying levels of 8-oxoguanine, a common DNA lesion associated with oxidative stress, and thus a marker for fundamental damaged DNA pathways in the heart.[20] DNA damage and can also be assessed by determining phosphorylation status of γ-H2Ax, a histone variant that is phosphorylated near DNA break sites and thus forms readily detectable foci in cells.[21] Fibrosis is measured primarily by histological methods, using staining techniques such as Masson trichrome. These biomarkers of aging can be used in cardiac tissue to assess how modulation of longevity genes influences the rate and degree of cardiovascular aging at the cellular level.

Although long thought to be postmitotic, cardiomyocytes undergo division and regeneration, and recent work has discovered that cardiac regeneration is a vital mechanism of maintaining cardiovascular health. However, the rate of regeneration in the aged may not be adequate to maintain cardiomyocyte numbers in response to cardiomyocyte loss.[22] Regenerated cardiac tissue is thought to involve a small pool of cardiac stem cells and a subset of small incompletely differentiated cardiomyocytes that can reenter the cell cycle.[23,24] Regenerative capacity can be measured by assessing the proliferative index, using markers of proliferation such as Ki67 or proliferating cell nuclear antigen. Therefore, long-lived or short-lived progeroid animal models can be tested for alterations in their regenerative capacity to determine how regulatory mechanisms controlling regeneration are involved in the aging cardiovascular system.

Other less understood changes in the heart with age are a decrease in the number and function of sinoatrial nodal pacemaker cells and a concomitant increase in conduction abnormalities. The aging heart undergoes a decrease in heart rate variability and maximum heart rate.[5] Consistent with this notion, the aging models budding uninhibited by benzimidazoles related 1 (BubR1) and Klotho have both been described to contain abnormalities leading to arrhythmias and altered conduction system, respectively.[19,25] Very few aging models have been assessed for alterations in cardiac conduction. Therefore the use of electrophysiology (EP) studies as well as testing aging models for alterations in EP parameters is a future area of research that could lead to a prominent understanding for the increased incidence of cardiovascular disease with age. EP studies can be carried out in cultured cardiomyocytes and in vivo by intracardiac and surface ECG analysis. Like echocardiography, the noninvasive nature of measuring surface ECGs can allow for longitudinal studies in aging cohorts.

Aging Research Leads to a New View of CVD

In both the aging and cardiovascular fields, it is generally accepted that a low calorie diet combined with exercise will increase the health span of mammals and that adiposity and a sedentary lifestyle have the opposite effect. The traditional view is that CVD results from the accumulation of cholesterol and fatty acids in tissues, which compromise tissue function and stimulate the production of inflammatory cytokines as well as ROS. In the past decade, however, the aging field has proposed that there is another fundamental process at work: a diet high in calories without exercise may be harmful because it suppresses the expression of "longevity genes" that promote cellular defenses against aging and age-related diseases.[26]

The new concept has its roots in the seminal observation by McCay et al in 1935 that reducing a rat's caloric intake by 20% to 40% dramatically extends its lifespan.[27] Since then, the lifespan-extending properties of calorie restriction (CR) have been observed in yeast, fish, some rodents, dogs, and macaque monkeys, although there are exceptions, including conflicting results in various inbred and outbred strains of mice and wild mice.[28–30] In mammals, CR reduces the incidence of most age-related diseases, including cancer, sarcopenia, kidney failure, and CVD.[31]

Whether or not CR increases the lifespan of primates is debated, in part because of conflicting data from 2 different sites and questions about which types of death should be censored. Data from human studies indicate that Okinawans eat a moderately restricted diet (≈ 1785 kcal/d) and have lower rates of coronary heart disease and a relatively high frequency of centenarians. Consistent with this, individuals on CR diets (0.5–8 years) have a physiology consistent with protection from CVD, including reduced triglycerides, lower blood pressure, reduced inflammatory markers, and decreased oxidative stress.[32–34]

Many of the fundamental molecular processes involved in CR-mediated protection of the cardiovascular system are known. CR increases mitochondrial function while reducing oxidative stress in the vasculature, in part by inducing expression of the Nfr2 stress response transcription factor, which induces expression of NADPH:quinone oxidoreductase 1, heme oxygenase I, and glutathione S transferase.[35–37] CR also reduces inflammation by suppressing the activity of vascular adhesion molecules, prostanoids, and inflammatory cytokines in both rodents[38] and humans.[39] Endothelial function is enhanced and both atherosclerosis and arterial stiffness are reduced by CR in rodents.[40,41] With regard to cardiac function, CR delays the age-related decline in diastolic filling accompanied by reductions in inflammation, cardiomyopathy, cardiac fibrosis, and myocardial degeneration.[42] Given all these data, there is considerable interest in understanding how CR works and in finding ways to mimic these effects with pharmacological agents.

Considerable progress has been made in the past decade toward understanding not only how but also why CR works. The original idea that CR works passively by suppressing metabolic rate or reducing damage caused by ROS is being replaced by a fundamentally different model in which CR triggers an active defense response that evolved to promote survival during harsh conditions (Figure 2). At the center of this response are so-called "longevity regulatory" pathways, which include insulin/insulin-like growth factor 1 (IGF-1), the mammalian target of rapamycin (mTOR), AMP-activated kinase (AMPK), and nicotinamide adenine dinucleotide $(NAD)^+$-dependent deacetylases (sirtuins). Although researchers still argue about which longevity gene is most important for the health benefits of CR, the emerging picture is that these genes form a network of redundant pathways and both positive and negative feedbacks (Figures 2 and 3). How these pathways improve health and control one another is the most intense area of research at present in the aging field.

There is increasing evidence that longevity pathways are not only important for understanding how CR works, they may also underlie the health benefits of weight loss and exercise. If this is true, then an individual who reduces caloric intake and starts exercising is improving their health because they are triggering a billion-year-old defense response that evolved to keep organisms alive during adversity. Conversely, when an individual gains adiposity and leads a sedentary lifestyle, they are negatively affecting their cardiovascular system by adding stress to the system and are also inhibiting these natural defense pathways.

The logical extension of this idea is that it should be possible to mimic the beneficial effects of dieting and exercise by tweaking the right pathways, using small molecules. Studies with "CR mimetics" such as resveratrol and metformin (which activate the SIRT1-AMPK system) or rapamycin (which inhibits mTOR), show that it is possible for a rodent to be obese and sedentary while maintaining the physiology of a lean animal.[43–47] Recent work has also identified a secreting hormone termed irisin, which, when increased, induces energy expenditure in the absence of exercise, positively influencing obesity and glucose homeostasis.[48] However, the overall effect of irisin on cardiovascular disease remains largely unexplored.

Longevity Genes and CVD

A number of longevity genes have been identified in model organisms, including the yeast *Saccharomyces cerevisiae*, the nemotode *Caenorhabditis elegans*, and the fly *Drosophila malanogaster*. Many of these genes and their related pathways have been subsequently assessed for their role in regulating longevity in mice, as well as to begin assessing their roles in CVD.

SIR2

Sirtuins are evolutionary conserved enzymes that function as NAD^+-dependent deacetylases and ribosyltransferases. Mammals have 7 sirtuin members, which have diverse localizations in the cell and regulate a variety of cellular functions including DNA damage repair, cell cycle, metabolic response to nutrient availability, and protection from neurological degeneration.[49] Sirtuins have been observed to modulate aging in yeast to mammals.[50–55] However, a direct role for sirtuins in regulating lifespan in worms and flies has been challenged.[56] Yet, numerous studies have maintained sirtuins as a key component of the longevity network (Figure 3). SIRT1, which is localized primarily in the nucleus, has been observed to regulate the AMPK pathway through deacetylation of the liver kinase B1.[57,58] In addition, SIRT1 regulates the Insulin/IGF-1 pathway through modulation of UCP2 expression and direct regulation of the IGF-1 signaling pathway.[55,59,60] SIRT1 also interacts with TSC1/2, inhibiting mTOR activity.[61,62] In relation to cardiovascular development and disease, SIRT1 knockout mice have greater injury in response to ischemia reperfusion

studies, and injury is attenuated in SIRT1 transgenic mice.[63] Cardiac-specific SIRT1-overexpressing mice show delayed age-dependent cardiomyopathies and reduction of stress-induced apoptosis. However, extensive SIRT1 overexpression (≈ 20 fold) resulted in oxidative stress, apoptosis, and cardiomyopathy.[64,65] Interestingly, SIRT1 is involved in both pressure overload-induced, and Angiotensin II, cardiac hypertrophy in addition to hypertrophy of vascular smooth muscle.[66,67] SIRT1 has been observed to regulate blood vessel growth both in zebrafish and in mice by regulating notch signaling.[68,69] Arterial stiffness is also regulated by SIRT1 through preventing hyperphosphatemia-induced arterial calcification.[70] SIRT3 is localized in the mitochondria, and knockout mice exhibit both age-dependent and exercise/pressure overload–induced cardiac hypertrophy.[71,72] SIRT7 knockout mice develop cardiac hypertrophy and inflammatory cardiomyopathy and are also characterized by an increase in fibrosis.[54] Cardiomyocytes from SIRT7 knockout mice have decreased resistance to oxidative stress and an increase in apoptosis. Although a number of effects of sirtuins on cardiovascular health have been described, the molecular mechanisms by which sirtuins regulate the heart and vasculature are only beginning to be understood. Furthermore, sirtuins have been shown to be involved in some of the beneficial effects of CR and resveratrol, suggesting that positive influence of CR and resveratrol in CVD may be modulated in part through sirtuin proteins.

IGF-1/Growth Hormone

IGF-1 was one of the initial genes to be identified as a longevity gene, where loss of IGF-1 was shown to extend lifespan of *C elegans*.[73] Subsequently, genes involved in the IGF-1 pathway were characterized to modulate lifespan as well, confirming the role of this pathway in lifespan regulation. IGF-1 signaling modulates other factors in the longevity network by inducing mTOR activity through regulation of Akt activity[74] (Figure 3). Mice deficient in IGF-1 die shortly after birth, whereas some live to adulthood but are deficient in growth and are dwarfed. However, IGF-1 receptor–deficient mice die postnatally due to respiratory failure.[75] Overexpression of IGF-1 in the heart prevented myocardial cell death after infarction and reduced ventricular dilation, hypertrophy, and diabetic cardiomyopathy.[76–79] However, in a second overexpression study, IGF-1 led to cardiac hypertrophy and failure[80] as well as to diminished recovery of heart function after acute ischemic challenge.[81] Interestingly, liver-specific IGF-1 knockout antagonized oxidative stress and cell death in cardiomyocytes induced under potent oxidant treatment with paraquat.[82] These differences probably arise from the duality of autocrine and paracrine effects of IGF-1. In *Drosophila*, which have a primitive cardiovascular system, deletion of the IGF-1 homologue (InR) delayed the effects of aging on the fly cardiovascular system.[83] Although a significant role for the IGF-1 pathway in regulating cardiovascular health has been defined, further studies are needed to elucidate the mechanism and clarify the discrepancies that have been observed.

Forkhead Transcription Factors

Forkhead transcription factors (FOXOs) play a role in regulating expression of genes involved in cell growth, proliferation, differentiation, and longevity.[84] FOXOs are also downstream effectors of the IGF-1 signaling cascade and are regulated by sirtuin-mediated deacetylation (Figure 3). Deletion of FOXO1 in mice leads to embryonic lethality, exhibiting deficient vascular and cardiac growth, including underdeveloped dorsal aorta and impaired cardiac looping.[85,86] However, a balance of FOXO1 is critical, as overexpression of FOXO1 in a cardiac-specific manner leads to embryonic lethality, with impaired cardiomyocyte proliferation, reduced heart size, myocardium thickness, and subsequent heart failure.[87] Expression of FOXO3a in mouse hearts resulted in reduction of cardiomyocyte size, suggesting that this FOXO factor functions to reduce hypertrophy.[88] FOXO3a deficiency has been shown to increase eNOS expression and enhances postnatal

vessel formation and maturation.[89] Although recent studies suggest the disparity between the expression of various members of the FOXO family, more research is necessary to understand the role of these transcription factors in regulating cardiovascular development, function, and disease as well as to elucidate how FOXO factors interact with the longevity network in cardiovascular tissues.

Clock 1

Clock 1 (CLK-1)/mammalian CLK-1 (MCLK1) is a hydroxylase localized to the mitochondria that is necessary for the biosynthesis of ubiquinone (coenzyme Q), the essential electron transporter of the mitochondrial respiratory chain.[90] Inactivation of *clk-1* in *C elegans* and partial inactivation in mice extends lifespan of both organisms. MCLK-1 heterozygous mice have yet to be assessed for effects on cardiovascular system function and disease progression. However, these mice are known to exhibit protection from cerebral ischemia and reperfusion, suggesting a potential role in modulation of vascular response to ischemic conditions.

AMPK

The AMP-activated protein kinase is involved in glucose and lipid metabolism, cell growth and autophagy, cellular polarity, and gene expression.[91] AMPK modulates mitochondrial function by triggering the destruction of defective mitochondria through a process of mitochondrial-specific autophagy termed mitophagy and induction of new mitochondria through activation of mitochondrial biogenesis.[91] Composed of 3 subunits, which are regulated by binding AMP, AMPK acts as a metabolic sensor, measuring the relative AMP:ATP ratio.[91] Within the longevity network (Figure 3), AMPK regulates mTOR through direct phosphorylation of the TSC1/2 complex,[92] modulates the IGF-1 pathway through the extracellular signal-regulated kinase (Erk) cascade,[93] and controls sirtuin activity by regulating the abundance of NAD and nicotinamide phosphoribosyltransferase (Nampt).[94,95] AMPK is cardioprotective during ischemia and reperfusion. Mouse models using dominant-negative AMPK have exacerbated myocardial infarction.[96,97] Activation of AMPK by metformin reduces pressure overload–induced cardiac hypertrophy.[98,99] Mutations in the regulatory $\gamma 2$ subunit of AMPK lead to an inherited syndrome of hypertrophic cardiomyopathy and ventricular preexcitation.[100–102] Being involved in energy sensing, AMPK may be a mediator of the positive effects of CR and resveratrol on both longevity and CVD. Consistent with this notion, many of the effects of resveratrol are lost in AMPK knockout mice.[103] Further studies are necessary to determine the interaction of these dietary regimens, AMPK, and cardiovascular heath.

p66shc

The mammalian p66shc is a splice variant of the Shc locus, encoding proteins carrying a Src-homology 2 domain, a collagen-homology region, and a phosphotyrosine-binding domain. The splice variant p66sch contains a unique N-terminal region, which functions as a redox enzyme modulating mitochondrial ROS.[104] Loss of p66shc leads a decrease in ROS and lifespan extension.[105] In the cardiovascular system, loss of p66shc blocks the decline in cardiac progenitor cell senescence, decreases DNA damage, necrosis, and apoptosis, and preserves LV volume and function, thus reducing heart failure.[106] Loss of p66shc also prevented Angiotensin II–induced hypertrophy and apoptotic cell death.[107] Furthermore, consistent with a central role of ROS in the pathogenesis of atherosclerosis, loss of p66sch protected mice from aortic lesions when placed on a high-fat diet.[108]

Catalase

Catalase converts one of the major ROS, hydrogen peroxide, into water and oxygen. Targeting peroxisomal catalase to the mitochondria, leads to ≈ 20% lifespan extension, lending support to the oxidative theory of aging.[109] These mice also demonstrated reduction in age-dependent LV hypertrophy and diastolic dysfunction.[110] In addition, these mice were also protected from Angiotensin II–induced cardiac hypertrophy as well as pressure overload–induced and G-α-q overexpression–induced heart failure.[111] Similar to p66shc knockouts, mitochondrial-targeted catalase mice emphasize the role of ROS in CVD.

Pituitary Transcription Factor 1 and Prophet of Pituitary Transcription Factor 1

The pituitary transcription factor 1 (Pit-1) is involved in the transcriptional program for normal development and function of the pituitary gland. Patients with mutants in Pit-1 have growth hormone deficiencies.[112] Prop-1, or Prophet of Pit-1, is also a pituitary transcription factor. Prop-1 mutations in patients exhibit secondary hypogonadism in addition to the deficiencies of growth hormone, prolactin, and thyroid-stimulating hormone also seen in patients with Pit-1 mutations.[113] Mutations in Prop-1 are the genetic basis for the phenotypes observed in the long-lived Ames dwarf mice. Little is known about the role of these gene alterations in cardiovascular function. Cardiomyocyte size, however, was reduced in young and old Ames dwarf mice compared with wild-type. Collagen content was reduced only in the young mice, suggesting that Ames dwarf mice may receive some longevity benefit from the reduced cardiomyocyte cell size and a period of reduced collagen content in the heart during adulthood.[114]

Klotho

Klotho was discovered as a gene that is mutated in a mouse strain presenting multiple premature aging phenotypes and a shortened lifespan.[115] In addition, overexpression of Klotho increases lifespan.[116] The Klotho gene encodes a single-pass transmembrane protein that is expressed primarily in the renal tubular cells and functions in phosphate reabsorption and metabolism.[117] Klotho is also observed as a circulating hormone-like factor, due to proteolytic cleavage by membrane-anchored proteases.[116] In the heart, Klotho is expressed in the sinoatrial node region and is required for the sinoatrial node to function as a pacemaker under stress.[25]

Target of Rapamycin/Rapamycin

Target of rapamycin (TOR), is a serine-threonine protein kinase that is inhibited by the bacterial product rapamycin. Conserved from yeast to humans, TOR integrates signaling from insulin and growth factors as well as sensing intracellular amino acid levels to regulate cell size and growth, proliferation, survival, motility, protein synthesis, and transcription.[118] Deletion of TOR or treatment with rapamycin has been observed to extend lifespan in yeast, worms, flies, and mammals.[45,119–121] Within the longevity network, mTOR tends to play an effector role of upstream sirtuin/AMPK/IGF-1 activity (Figure 3). However, in yeast, one proposed mechanism by which TOR inhibition regulates lifespan is by enhancing multicopy suppressor of SNF1 protein 2/4 transcriptional induction of the NAD synthesis enzyme Pnc1 (yeast homolog of Nampt), thus activating sirtuin proteins.[122] Conservation of this pathway in higher organisms has yet to be described. Inhibition of mTOR signaling in the heart represses cardiac hypertrophy mediated by pressure overload, potentially through blocking of mTOR control of protein translation and cell size.[123,124] Furthermore, mTOR, through its involvement in the PI3K/AKT signaling pathway, plays a role in mediating hypoxia-induced angiogenesis in tumors, through regulation of hypoxia-inducible factor-1α stabilization and vascular endothelial growth factor (VEGF) expression. [125] Inhibition of PI3K/AKT/mTOR pathway through such agents as PI3K/AKT inhibitors LY294002 and wortmannin and the

mTOR inhibitor rapamycin leads to decreases in VEGF secretion and angiogenesis.[126] mTOR negatively regulates autophagy, and inhibition of mTOR, either through nutrient-poor conditions or rapamycin treatment, activates autophagy, allowing for the destruction of defective molecules and organelles and promoting health of cardiovascular tissues.[127] The PI3K/AKT/mTOR pathway lies at the intersections of numerous signaling pathways, thus positioning this pathway as a vital mediator of aging and the cardiovascular system.

BubR1

BubR1 is a serine/threonine protein kinase and an inhibitor of the anaphase-promoting complex, regulating the mitotic spindle assembly checkpoint.[128] Heterozygous mice develop normally but are tumor-prone, and mice with near-deficient levels (homozygous BubR1 hypomorphic mice) develop premature aging phenotypes and have a shortened lifespan.[129,130] BubR1 hypomorphic mice exhibit arrhythmias, thought to be the primary mechanism leading to their death.[19] In addition, arterial wall thickness and inner diameter were reduced in loss-of-BubR1 mice, with increased fibrosis and reduced elastic properties.[131] Future studies should illuminate the role that this mitotic regulator plays in the heart, which is largely postmitotic.

A Cardiovascular Aging Review Series

The work discussed in the following articles in the series highlights the recent progress in understanding the mechanisms by which aging and obesity reduce cardiovascular function and how we may use this knowledge to combat cardiovascular disease. In the first part of the series, Leeuwenburgh, Marzetti, and colleagues examine involvement of mitochondrial dysfunction and deregulated autophagy on cardiovascular aging. Extensive evidence has been presented that mitochondrial function declines with age in a majority of tissues. The triggering cause and molecular mechanism of age-associated mitochondrial decline is unclear, but the functional consequences at the cellular and tissue levels have been well documented. Mitochondria generate energy through oxidative phosphorylation, of which a byproduct is an increase in ROS production, leading to free radical– imposed damage to macromolecules and cellular components. Control of mitochondria quality can occur at 3 basic stages: repair of damaged mitochondria, removal of dysfunctional mitochondria through autophagy, and generation of new mitochondria through biogenesis.[132] The current knowledge of the mechanisms responsible for cardiac mitochondrial dysfunction and abnormal ROS production in advanced age will be reviewed; the role that autophagy plays in controlling mitochondria quality will be discussed in the context of cardiac hypertrophy, ischemia, heart failure, and diabetic cardiomyopathy.

In the second part of the series, Fontana, Vinciguerra, and Longo take a look at the role of growth factors on cardiovascular aging. Growth factors were at the center of initial studies pertaining to the molecular genetics of lifespan extension. Kenyon and colleagues found that loss of insulin-like growth factor 1 (IGF-1) signaling led to lifespan extension in *C elegans*.[73] IGF-1 pathways regulate downstream phosphorylation cascades including the MAPK and mTOR pathways, leading to effects on cell growth, proliferation, and survival, in addition to lipid and glucose metabolism. The role of IGF-1 in cardiac function and aging is largely unknown, and the recent data present a complex picture of positive and negative influences of growth factors on cardiovascular health. Furthermore, clinical studies rely heavily on associations, and the limited direct mouse studies have given conflicting results. The pathways regulated by growth factors in cardiomyocytes and the effect of these pathways on cardiac aging will be discussed, including effects of IGF-1 on cardiac stem cell and myocardial regeneration. In addition, the role of adiposity of cardiac tissue is touched on.

In the third part of the series, Dai, Rabinovitch, and Ungvari revisit the role of mitochondria and cardiovascular aging. Mitochondria are the cellular sources for energy generation and are critically important for high energy–demanding tissues such as the heart. However, due to their production of ROS, they are central to the rate of aging of these tissues. The role of mitochondria dysfunction and oxidative stress in cardiovascular aging is exemplified by murine models that reduce mitochondria ROS, which have reduced age-associated changes in the heart.[133] This review discusses the role and molecular mechanisms regulating oxidative stress in the aging cardiovascular system. Furthermore, discussions touch on the role of retrograde mitochondrial signaling (communication from the mitochondria to the nucleus) and mitochondrial biogenesis in cardiovascular health, including therapeutic targets to regulating mitochondria to diminish cardiovascular aging.

In the fourth part of the series, DePinho, Moslehi, and Sahin consider the implications of telomere attrition and mitochondria in the aging heart. Loss of telomeric DNA, during each successive cell division has been a central instigator in cellular aging since the identification of cellular senescence by Hayflick.[134] Subsequently, extensive studies attribute telomere attrition to degeneration of stem and progenitor cell compartments and other highly dividing tissues. However, a number of recent studies have found that loss of telomeres has deleterious effects in quiescent tissues and further characterize signaling pathways that are activated in response to loss of telomeric DNA.[135] Specifically, the role of telomere attrition in augmenting age-related cardiac decline are discussed, including details of a p53/ mitochondrial signaling pathway.

In the fifth part of the series, Oellerich and Potente provide a current view of the role of FOXOs/sirtuins in vascular aging. FOXOs have been associated with longevity due to their role as downstream effectors of IGF-1 signaling.[136] Similarly, sirtuins have been associated with lifespan and age-related diseases.[49] Both FOXO factors and sirtuins have been associated with vascular development and cardiac function during aging. The upstream regulators of FOXO/sirtuin factors and downstream effector pathway involving these factors are discussed, with particular emphasis on vascular flow and hypoxic responses leading to modulators of these factors.

In the sixth part of the series, Lahteenvuo and Rosenzweig evaluate the role of angiogenesis impairment and endothelial dysfunction in cardiovascular aging. Age-related impairment of angiogenesis contributes to increased end-organ damage seen in the elderly and directly links cardiovascular health with systemic tissue homeostasis.[137] Angiogenesis is critical in the elderly as a mechanism for repairing tissues after damage caused by events such as ischemic stroke, myocardial infarction and ischemia, and lower-limb ischemia. Processes and pathways contributing to impairment of angiogenesis, including cellular senescence, telomere attrition, oxidative damage, NO, hypoxia, and vascular growth factors are discussed, in addition to therapeutic approaches for improving angiogenesis in the elderly.

In the final part of the series, Tartar and Bodmer discuss nonmammalian models of cardiovascular aging. Various model organisms have rudimentary heart and circulatory systems, such as those observed in zebrafish and *Drosophila*. Even with a rudimentary cardiovascular system, the powerful use of genetics in these systems has, and will continue to, allow for these models to be useful in discovering novel pathways regulating cardiovascular biology. Although zebrafish are rarely used for aging research, *Drosophila* has been used in discovery and confirmation of various aging pathways. This review discusses the history and discoveries made in various nonmammalian models both in terms of longevity and cardiovascular aging.

Aging, although an unavoidable cardiovascular risk factor, may overcome all the other risk factors collectively. Therefore, understanding fundamental mechanisms that dictate the pace of aging could lead to significant advancements into both preventative and therapeutic treatments of CVD. The articles presented in this review series shed light the interconnected roles of aging and cardiovascular research and provide a perspective on new and exciting areas to investigate by integrating these two tightly linked research areas.

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Non-standard Abbreviations and Acronyms

Akt	also known as protein kinase B
AMPK	AMP-activated protein kinase
BubR1	budding uninhibited by benzimidazoles related 1
CLK-1	clock 1
CR	calorie restriction
CVD	cardiovascular disease
eNOS	endothelial nitric oxide synthetase
EP	electrophysiology
Erk	extracellular signal-regulated kinase
FOXO	forkhead transcription factors
IGF-1	insulin-like growth factor-1
LV	left ventricle
MCLK1	mammalian clock 1
mTOR	mammalian target of rapamycin
NAD	nicotinamide adenine dinucleotide
NADPH	reduced nicotinamide adenine dinucleotide phosphate
Nampt	nicotinamide phosphoribosyltransferase
PI3K	phosphatidylinositol-3-kinase
Pit-1	pituitary transcription factor 1
PNC1	pyrazinamidase and nicotinamidase 1
Prop-1	prophet of Pit-1
ROS	reactive oxygen species
SIR2	silent information regulator 2
SIRT	sirtuin

TOR	target of rapamycin
TSC1/2	tuberous sclerosis complex 1/2
UCP2	uncoupling protein 2
VEGF	vascular endothelial growth factor

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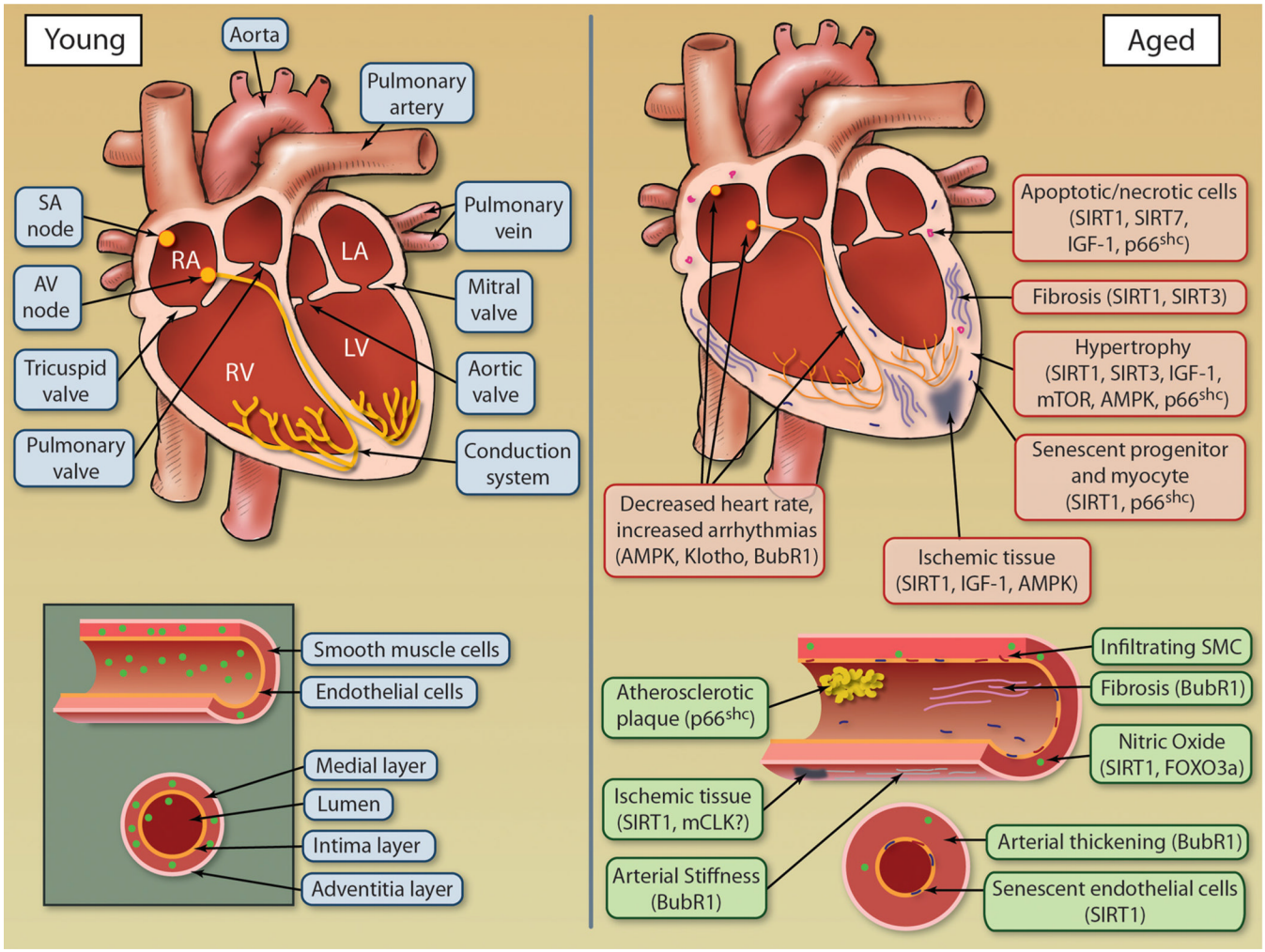

Figure 1. Age-dependent changes to cardiovascular tissues

Both the heart and vasculature undergo numerous alterations during aging as a result of deregulation of molecular longevity pathways, leading to compromised function. Illustration credit: Cosmocyte/Ben Smith.

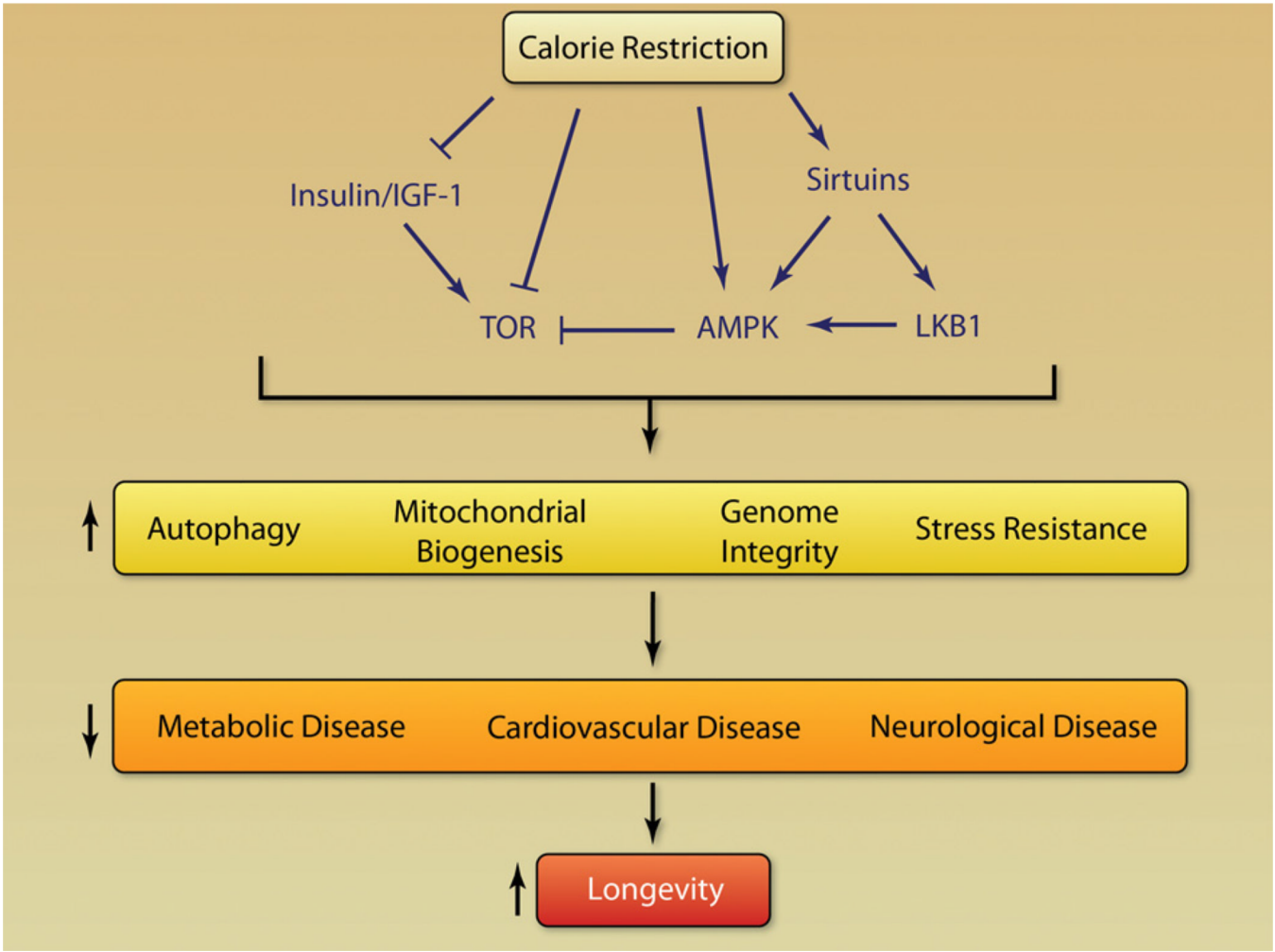

Figure 2. Pathway leading from calorie restriction to longevity

Calorie restriction modulates factors in the longevity network, leading to alterations in cellular responses to stress, affecting common diseases of aging and influencing longevity.

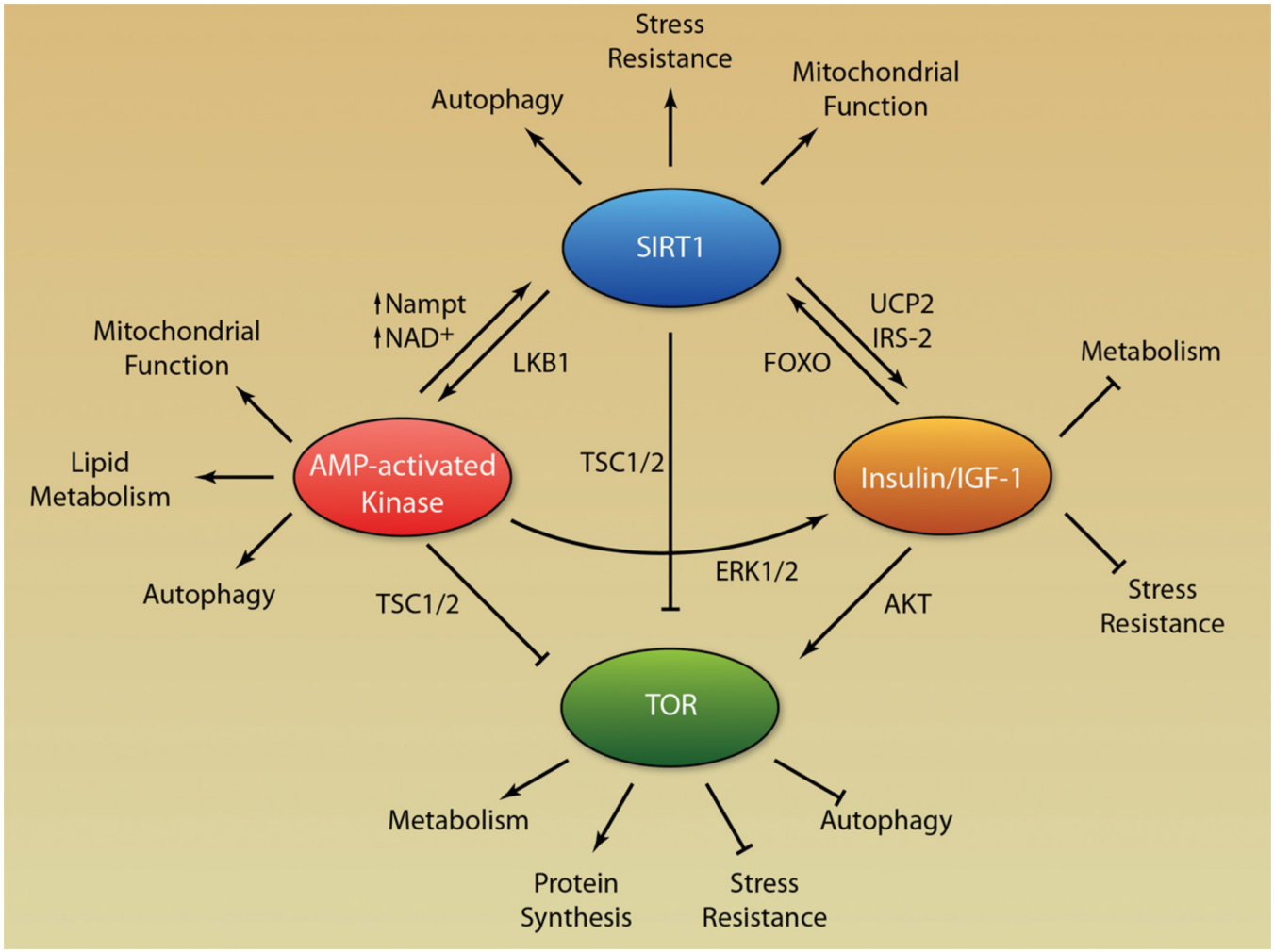

Figure 3. Model for a longevity network

Positive and negative feedback regulation between genes involved in lifespan and age-related diseases including sirtuins, AMPK, IGF-1, and TOR.