REVIEW ARTICLE

Molecular Mechanisms of Cardiovascular Aging

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Abstract

BACKGROUND: 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.

CONTENT: We provide an overview of some of the molecular mechanisms involved in regulating lifespan and health, including mitochondria, telomeres, stem cells, sirtuins, Adenosine Monophosphate-activated Protein Kinase, Mammalian Target of Rapamycin and Insulin-like Growth Factor 1. We also provide future perspectives of lifespan and health, which are intimately linked fields.

SUMMARY: Aging remains the biggest non-modifiable risk factor for cardiovascular disease. The biological, structural and mechanical changes in senescent cardiovascular system are thought to contribute in increasing incidence of cardiovascular disease in aging. Understanding the mechanisms contributing to such changes is therefore crucial for both prevention and development of treatment for cardiovascular diseases.

KEYWORDS: cardiovascular aging, mitochondria, telomeres, Sirtuin, stem cells

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Abstrak

LATAR BELAKANG: Rata-rata usia harapan hidup manusia terus meningkat, hingga 20 tahun ke depan jumlah persentase manusia yang berusia lebih dari 65 tahun akan terus bertambah. Pada kelompok usia ini, penyakit kardiovaskular merupakan penyebab kematian yang utama, dan biaya pengobatannya juga akan terus meningkat. Penuaan merupakan bagian yang tidak dapat dihindari dalam hidup dan sayangnya merupakan faktor risiko terbesar untuk penyakit kardiovaskular.

ISI: Kami akan membahas beberapa mekanisme molekular yang terlibat dalam pengaturan proses penuaan dan kesehatan, meliputi mitokondria, telomer, sel punca, sirtuin, *Adenosine Monophosphate-activated Protein Kinase*, *Mammalian Target of Rapamycin*, dan *Insulin-like Growth Factor 1*. Kami juga membahas pandangan ke depan mengenai proses penuaan dan kesehatan yang keduanya saling terkait.

RINGKASAN: Proses penuaan merupakan faktor risiko utama yang tidak dapat dimodifikasi untuk penyakit kardiovaskular. Perubahan biologis, struktural, dan mekanis pada proses penuaan sistem kardiovaskular memiliki peran pada peningkatan kejadian penyakit kardiovaskular pada usia lanjut. Memahami mekanisme molekular yang berperan pada perubahan tersebut sangatlah penting, baik untuk pencegahan maupun pengembangan terapi penyakit kardiovaskular.

KATA KUNCI: penuaan kardiovaskular, mitokondria, telomer, Sirtuin, sel punca

Introduction

Aging is inevitable. Yet for centuries people have tried to slow or stop it, from bathing in the blood of virgin girls to concocting an elixir of life. These days, anti-aging research is on a more scientific footing. And while we are no closer to finding the fountain of youth, humans for a variety of reasons are living longer than ever before.(1)

The World Health Organization estimated that there were 650 million senior citizens in 2007 and the figure is predicted to triple in the next 50 years with about 80% of the elderly population living in developing countries. (2) Both aging and disease result in the same outcome: the impairment of normal biological function. It would not, therefore, be a surprise if tissue dysfunction resulting from an aging mechanism eventually manifested itself as a disease. Therefore, understanding mechanisms of aging would help understand the processes which govern the development and progression of some diseases. This in turn would lead to the development of new therapeutic methods for disease treatment and, more importantly, prevention.

Cellular senescence is the irreversible growth arrest of individual mitotic cells, which as a consequence display a radically altered phenotype that is thought to impair tissue function and predispose tissues to disease development and/or progression as they gradually accumulate. When discussing the impact of senescent cells may have on aging and age-related disease, it is important to take into consideration factors which may result in the removal and replacement of senescent cells. These include apoptosis and the availability of stem cell reserves.(3)

It is not clear to what extent both stem cells and somatic cells play in tissue regeneration, but the functional ability of stem cells appears to become impaired with age.(4) Stem cells express telomerase and are unlikely to become senescent in response to telomere shortening. Some of the changes observed during cellular senescence are likely to be cell-type specific. For example, in senescent vascular endothelial cells, endothelial Nitric Oxide Synthase (eNOS) activity has been found to be decreased.(5,6) Since Nitric Oxide (NO) is important in regulating vascular function, a decline in its production may have detrimental consequences. A reduction in NO production by eNOS, for example, has been suggested to be a significant risk factor for cardiovascular disease (CVD).(7) 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.(8)

Aging, although an unavoidable cardiovascular risk factor, may overcome all 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.(9)

Aging and CVD

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, CVD will result in 40% of all deaths and rank as the leading cause. Furthermore, the cost to treat CVD will triple in that time. (10,11) Hence, it remains vital that we understand why age is such a critical component of CVD etiology. However, until recently, the fields of CVD and molecular biology of aging have remained largely separate. 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.(12)

A common feature of aging tissues is low-level chronic inflammation, termed sterile inflammation (indicating an absence of detectable pathogens) or inflammaging.(13-16) Chronic inflammation can drive pathology by at least two mechanisms. First, infiltrating immune cells can degrade tissues because they release reactive or toxic moieties. Second, inflammatory cytokines can provoke phenotypic changes that are independent of the immune system in nearby cells. For example, Interleukin-6 (IL-6) and IL-8 can stimulate angiogenesis, disrupt cell-cell communication, impede macrophage function, induce innate immune responses, and promote epithelial and endothelial cell migration and invasion.(17-23) This chronic inflammation may derive partly from an age-related metabolic dysfunction.

Fat tissue, frequently the largest organ in humans, is at the nexus of mechanisms involved in longevity and age-related metabolic dysfunction. Fat distribution and function change dramatically throughout life.(24) Cellular stress and preadipocyte overutilization with aging induce cellular senescence, leading to impaired adipogenesis, failure to sequester lipotoxic fatty acids, inflammatory cytokine and chemokine generation, and innate and adaptive immune response activation. These pro-inflammatory processes may

amplify each other and have systemic consequences.(24)

Chronic inflammation may also derive in part from senescent cells: senescent cells secrete pro-inflammatory cytokines, chemokines, and proteases, termed the Senescence-associated Secretory Phenotype (SASP). (25,26) SASP is primarily a DNA Damage Response (DDR).(27) The SASP, through the inflammatory, growth-promoting, and remodeling factors that it produces, can potentially explain how senescent cells alter tissue microenvironments, attract immune cells, and, ironically, induce malignant phenotypes in nearby cells. Proteins that are associated with the SASP, such as Tumor Necrosis Factor alpha (TNF-α), IL-6, Matrix Metalloproteinases (MMPs), Monocyte Chemoattractant Protein-1 (MCP-1), and Insulin-like Growth Factor Binding Proteins (IGFBPs), increase in multiple tissues with chronological aging(28), and occur in conjunction with sterile inflammation. This finding suggests that SASP is the main driver of age-related inflammation, at least in fat tissue under certain conditions. Thus, selective elimination of senescent cells or their effects might be a means to reduce age-related sterile chronic inflammation, enhance health span, and interrupt the link between aging and chronic disease.(29) 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.(9)

Mitochondria and Cardiovascular Aging

The prevalence of CVD increases dramatically with advancing age. More than 80% of cases of coronary artery disease and ~75% of cases of congestive heart failure are observed in geriatric patients.(30)

Mitochondria plays important roles in a myriad of cellular processes including Adenosine Triphosphate (ATP) production via oxidative phosphorylation, biosynthetic pathways, cellular redox homeostasis, ion homeostasis, oxygen sensing, signaling, and regulation of programmed cell death. Mitochondrial dysfunction is central to theories of aging, because age-related changes of mitochondria are likely to impair a host of cellular physiological functions in parallel and contribute to the development of all common age-related diseases.(31) The evidence supporting the role of mitochondrial oxidative stress, mitochondrial damage and biogenesis as well as the crosstalk between mitochondria and cellular signaling in cardiac and vascular aging.

Aging is known to be associated with mutations in genes of mitochondrial genome, which encodes key proteins of respiratory complex, including components of electron transport chain and ATP synthase complexes.(32) Considerable evidence has been published that with advanced age mitochondrial production of Reactive Oxygen Species (ROS) significantly increases both in the heart(33) and the vasculature(34). Age-dependent mitochondrial dysfunction is closely correlated with abnormal mitochondrial ROS production and detoxification.(35-37) Mitochondria-derived ROS are likely to contribute to the development of chronic low-grade vascular inflammation in aging(34) by activating redox signaling pathways. Furthermore, recent studies suggest that mitochondria-derived ROS contribute to accelerated development of the senescent phenotype in endothelial cells (*i.e.*, by activating Akt.(28) Endothelial cell senescence may impair regenerative and angiogenic capacity of endothelium, its reactivity and promote progression of atherosclerosis by altering secretion of cytokines, growth factors, and proteases in vascular wall. Another potentially important link between mitochondrial oxidative stress and vascular aging is induction of apoptosis. (38,39) Oxidative stress in aging is associated with an increased rate of endothelial apoptosis(40,41), which may contribute to microvascular rarefaction impairing the blood supply of heart(42) and brain(43).

The molecular mechanisms underlying age-related increases in mitochondrial oxidative stress in the cardiovascular system are multifaceted and likely involve cell-autonomous effects, including a significant decline in reduced glutathione content (44), dysregulation of antioxidant defense mechanisms (*e.g.*, peroxynitrite-mediated nitration and inhibition of Manganese Superoxide Dismutase (MnSOD))(39), and a dysfunctional electron transport chain(45,46). Recent studies suggest that age-related changes in endocrine/paracrine regulatory mechanisms-including activation of the renin-angiotensin-aldosterone system, adrenergic signaling, and an age-related dysfunction of growth hormone/Insulin-like Growth Factor-1 (IGF-1) signaling also have an important role in promoting mitochondrial oxidative stress in the aged cardiovascular system.(31)

In heart and the vasculature of young animals in response to increased production of mitochondria-derived ROS, an adaptive Nuclear Factor (NF)-E2-related factor 2 (Nrf2)-driven antioxidant defense mechanism manifests, which upregulates Antioxidant Response Element (ARE)-driven expression of detoxifying and antioxidant enzymes and the cystine/glutamate transporter involved in glutathione biosynthesis.(47,48) Recent findings demonstrate that in aging vessels increased production of ROS by mitochondria and other sources fails to activate Nrf2 resulting in increased cellular sensitivity to the deleterious effects of oxidative stressors.(47-49)

Mitochondria are highly dynamic organelles, and dysregulation of mitochondrial turnover is likely one of the intrinsic causes of mitochondrial dysfunction, which contributes to dysregulation of cell metabolism, oxidative stress, and altered signal transduction during the aging process.(50) The removal of dysfunctional mitochondria through autophagy is crucial for the maintenance of cell viability.(51) The efficiency of this process declines with advancing age, which may be critically involved in heart senescence and in age-related CVD.(52,53) Regardless of the mechanism(s) primarily responsible for mitochondrial decay during aging, mitochondrial quality control is essential for the preservation of cardiomyocyte homeostasis. This task is accomplished through the complex coordination of several processes.(54)

Mitophagy is a highly selective process that can promote the elimination of dysfunctional or unnecessary mitochondria. The loss of mitochondrial membrane potential ($\Delta\psi m$) represents a major trigger of mitophagy.(55) Although the molecular regulation of mitophagy has not yet been completely elucidated, the Mammalian Target of Rapamycin (mTOR)/Adenosine Monophosphate (AMP)-activated Protein Kinase (AMPK) pathway is proposed to be a major checkpoint.(56) AMPK, in addition to stimulating mitochondrial removal through autophagy, enhances activity of Sirtuin-1 (SIRT1) and its downstream target Peroxisome Proliferator-activated Receptor gamma Coactivator-1 alpha (PGC-1α), resulting in stimulation of mitochondrial biogenesis.(57) Hence, through the activity of AMPK, mitophagy and mitochondrial biogenesis are coordinately regulated, maintaining a healthy and functional pool of mitochondria in the cell.(58)

Aging is associated with impaired mitochondrial biogenesis and reduced mitochondrial mass in the vascular endothelial and smooth muscle cells.(46,59,60) Available evidence suggests that in the aged vasculature, because of an increased production of ROS and downregulation and uncoupling of eNOS, the bioavailability of NO is significantly decreased(61), which results in a downregulation of PGC-1α and consequential dysregulation of constituents of the electron transport chain and other mitochondrial proteins(46). It is likely that decreased NO bioavailability is causally linked to dysfunction of mitochondrial biogenesis in other organs as well during aging.(60,62) Aging-associated phenotypes have been linked not only to mitochondrial dysfunction but also to aberrant mitochondrial biogenesis caused by impaired retrograde signaling regulated by nuclear genes and factors dependent on mitochondrial metabolism (*e.g.*, ATP, Ca^{2+}, ROS, NO, Nicotinamide Adenine Dinucleotide $(NAD)^{+}$/NADH).(63) Pathways that improve mitochondrial function, attenuate mitochondrial oxidative stress, and regulate mitochondrial biogenesis have recently emerged as potential therapeutic targets for prevention of the development of age-related CVD.

The important role of mitochondrial oxidative stress and mitochondrial dysfunction in age-related cardiovascular pathologies is evident, and we are at the beginning of an exciting phase of research on understanding the genetic and epigenetic mechanisms underlying the mitochondrial alterations that occur with age.(31)

Telomeres and Cardiovascular Aging

Hypothesized molecular mechanisms for aging in modern biology have abounded. These have included stem cell

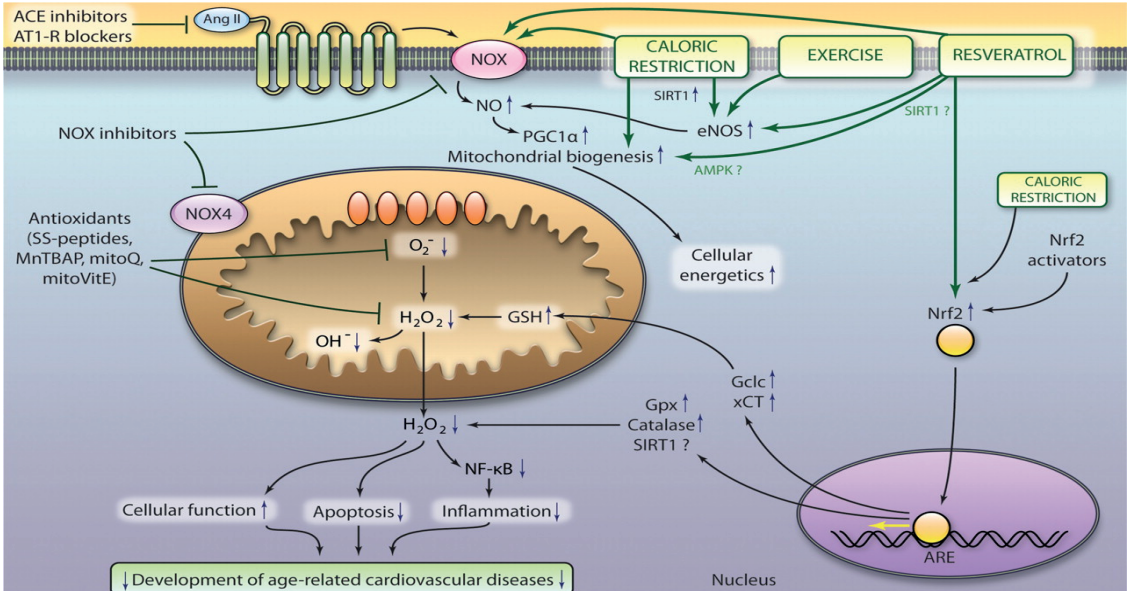

Figure 1. Summary of mitochondrial-targeted interventions and their therapeutic potential in aging.(31) (Adapted with permission from American Heart Association).

failure, mitochondrial dysfunction, genotoxic stress, and epigenetic changes. Recent cumulative evidence points to telomere shortening as sufficient to provoke all these mechanisms. The manifestations of telomere-mediated disease, especially in adults, can be subtle and are often indistinguishable from the slow, gradual functional decline that is a hallmark of aging. The compelling clinical evidence therefore points to telomere shortening itself as being sufficient, or perhaps more broadly representing forms of genotoxic stress that contribute to age-related changes.(64)

Telomeres define the ends of linear chromosomes. They are made up of repetitive DNA sequences that are bound by specialized proteins. The human telomeric DNA sequence is a tandem repeat of TTAGGG that extends several kilobases.(65-67) The telomere-binding complex of proteins, known as shelterin, together with telomere DNA, functions as a dynamic unit that protects chromosome ends from being recognized as broken DNA, thus preventing their degradation and participation in fusion events.(68) Telomeres are therefore essential for the maintenance of genomic integrity. Telomerase is the specialized polymerase that synthesizes new telomere repeats.(69,70) It offsets the shortening that normally occurs with cell division since the replication machinery does not copy fully to the ends. Telomerase has two essential core components, Telomerase Reverse Transcriptase (TERT) and Telomerase RNA (TR), the latter of which provides the template for telomere repeat addition.(71-73) Telomeres have long been linked to processes of cellular aging. Telomere length shortens with age and predicts the onset of replicative senescence. When telomeres become critically short, they become dysfunctional and activate a DNA damage response that resembles double-strand breaks.(74) The resulting signaling cascade provokes apoptosis and/or a permanent cell cycle arrest that, until recently, has been considered the primary functional consequence of senescence.(64)

Cumulative studies in humans with telomere maintenance disorders and telomerase knock-out mice have demonstrated that short telomeres precipitate functional decline in different tissues, including the cardiovascular system.(75) Mechanistically, telomere dysfunction-driven tissue compromise is thought to be secondary to the activation of DNA damage signaling pathways that converge on p53, a central executor of the DNA damage response pathway.(76) p53 activation induces senescent and apoptosis pathways, particularly in stem cell and progenitor compartments of highly regenerative organs. The elimination of stem and progenitor cells is thought to be the driving force in the development of tissue defects.(77)

Telomere dysfunction-activated p53 directly leads to mitochondrial and metabolic compromise through the repression of the master regulators of mitochondrial biogenesis and function, PGC-1α and PGC-1β.(78) Given that an accelerated rate of telomere shortening may be expected from the increased cellular turnover associated to inflammation occurring in atherosclerosis, and from the action of several cardiovascular risk factors (*e.g.*, oxidative stress, hypertension, diabetes, smoking, psychological stress), telomere exhaustion may be a surrogate marker of CVD.(79)

Sirtuin, Class O of Forkhead Box Transcription Factors (FOXO) and Cardiovascular Aging

Age is one of the major risk factors associated with CVD. Part of this complex phenomenon is the deterioration of tissues that constitute heart and its associated vasculature. Aging results in a progressive functional and structural decline in multiple organs, and in particular, has profound effects on heart and arterial system. Age-related cardiac and vascular changes include impaired endothelial function and intimal proliferation(80), increased arterial stiffness(8,81-85), left ventricular (LV) diastolic dysfunction(12,86,87), LV pathological hypertrophy(88), diminished LV systolic reverse capacity(12,87), decreased heart rate variability(89-91), and a reduction in maximal heart rate(92). Furthermore, as a consequence of aging, the interaction between the heart and arterial system is altered to preserve ventricle–arterial homeostasis.

Sirtuins post-translationally modulate the function of many cellular proteins that undergo reversible acetylation-deacetylation cycles, affecting physiological responses that have implications for treating diseases of aging.(93) Sirtuin proteins bolster stress resistance of mammalian cells by virtue of their abilities to remodel metabolism, alter inflammatory responses, and enhance the ability to cope with oxidative species. Because many of these same pathways are pathologically altered in the aged, activation of sirtuins represents a feasible means for attenuating age-related CVD.(94)

In mammals there are seven members of the sirtuin family, SIRT1-7, of which SIRT1 has become the most well-studied protein. The expression level of SIRT1 increases upon calories restriction (CR) in several rodent and human tissues, such as white adipose, liver, skeletal muscle, brain and kidney.(95-98) SIRT1 activates PGC-1α by deacetylation of lysine residues(99,100), which results in increased mitochondriogenesis(99,101). A decline in mitochondrial function with age is thought to be a contributing factor to insulin resistance and age-related cancers.(102,103) Interestingly, CR elicits similar

improvements in mitochondrial function.(104-107) Therefore, it is possible that a small-molecule activator of SIRT1 may activate some of the same pathways that are modified by CR and could be a therapy for diseases of aging.

Two different enzymatic activities have been reported for the sirtuins: an Adenosine Diphosphate (ADP)-ribosyl transferase(108,109), and/or a deacetylase activity(110-112). The most important and well-studied protein of this family, SIRT1, is an NAD^+-dependent deacetylase.(113) The deacetylation reaction removes an acetyl group from the lysine side chains of a protein substrate while cleaving NAD^+ in the process to generate the deacetylated protein 2′-O-acetyl-ADP-ribose and nicotinamide. Overexpression or activation of SIRT1 has been shown to modulate mitochondrial biogenesis, metabolic rate, insulin sensitivity, glucose and lipid metabolism.(114-119) When there is a limiting supply of fuel substrates to produce the required amount of ATP, concentrations of NAD are elevated. As SIRT1 activity is increased by elevated NAD levels, it may therefore act as a sensor of cellular NAD^+/NADH levels, contributing to the adaptive changes in the activity of transcription factors, co-activators or co-repressors (for example, nuclear receptor interacting protein 1 (NRIP1), peroxisome proliferate-activated receptor-α (PPAR-α) and PGC-1α that have key roles in metabolic adaptations to nutrient availability.

SIRT1 activation can improve cardiac function through effects on multiple pathways including improved vasorelaxation (possibly through K-channel inhibition), anti-inflammatory activity on macrophages and foam-cell formation. This is in addition to increased scavenging of ROS, increased NO synthase activity, reduced platelet aggregation, angiogenesis activity and anti-apoptosis activity.(120-129) As an example, SIRT1 regulates the activity of PGC-1α, a central factor in controlling energy state and contractile function in cardiac muscle.(130)

SIRT1-induced angiogenic activity is probably mediated via FOXO transcription factors that regulate blood vessel endothelial development.(131,132) The interaction between SIRT1 and FOXO proteins also leads to cardioprotection in a p53-dependent manner against a number of different stressors.(133,134) Indeed, SIRT1 expression is elevated during vascular development, whereas loss of its activity leads to limited blood vessel sprouting, defective blood vessel formation and attenuated ischaemia-induced neovascularization.

One of the intermediates in insulin/IGF-1 signaling cascade is activation of serine/threonine kinase Akt/Protein Kinase B (PKB) and Serum/Glucocorticoid-regulated Kinase (SGK).(135) From the standpoint of aging, the most important Akt and SGK substrates appear to be FOXOs. (136,137) Akt and SGK-mediated phosphorylation of FOXOs results in FOXO sequestration in the cytoplasm. Mutations that decrease signaling though the insulin/IGF-1 signalling pathway reduce the phosphorylation of FOXOs, resulting in nuclear translocation.(138,139) In the nucleus, FOXOs modulate the expression of genes that increase lifespan.(140-142) Thus, IGF-1 signaling is likely to promote a chronic aging-promoting effect on cardiomyocytes. These sensitizing effects of growth factor signaling genes in various mammalian cell types may be mediated in part by the inactivation of FOXO forkhead stress resistance transcription factors regulate cellular protection in part by modulating the expression of antioxidant enzymes such as

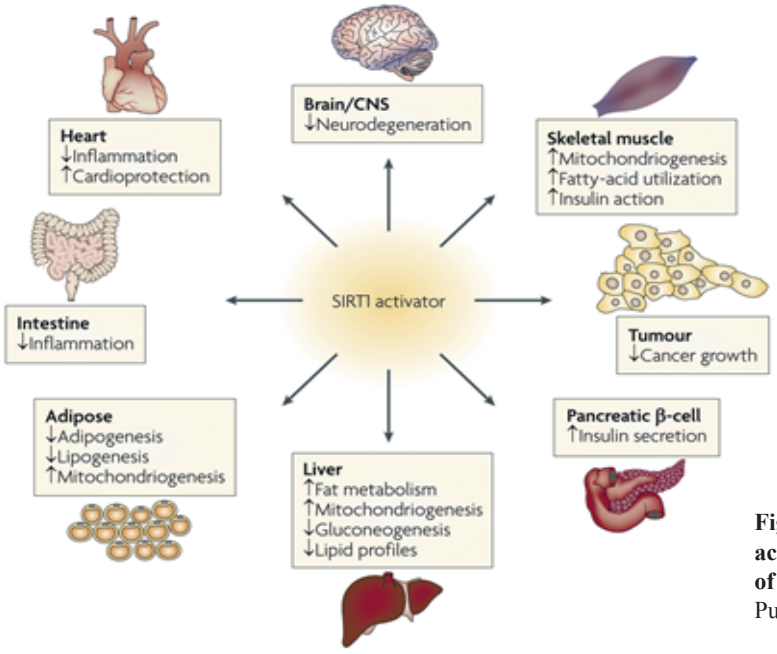

Figure 2. Multiple target organs in which SIRT1 activators can potentially have effects to treat diseases of aging . (93) (Adapted with permission from Nature Publishing Group).

Superoxide Dismutase-2 (SOD2).(144)

The metabolic effects of FOXO1 vary with the tissue, but they generally antagonize the actions of insulin.(144-149) FOXO1 activity itself is negatively regulated by insulin through Akt phosphorylation, which causes it to translocate to the cytoplasm from the nucleus. Whether altered FOXO1 levels or activity can affect lifespan in mammals has not been reported.(150) The integration of individual FOXO and sirtuin family members into various aspects of vessel growth, maintenance, and function provides new perspectives on disease mechanisms of aging, the most important risk factor for medical maladies of the vascular system.(151)

Nutrients and Cardiovascular Aging

CR, a 20% to 40% reduction in calorie intake, which reduces the levels of IGF-1 and other growth factors, has been consistently shown to increase life span and to prevent the development of age-associated cardiovascular functional and structural changes in several model organisms.(152-159) In particular, CR has been shown to improve arterial flow-mediated vasodilation(160,161) and to delay the development of atherosclerotic lesions in rodents(154). CR significantly ameliorates LV diastolic function of the aging heart and reduces arterial stiffness.(153,156,157,160) Moreover, long-term CR has been shown to improve autonomic function and, in particular, to increase the high-frequency component of the heart rate variability spectra, a marker for parasympathetic activity in rats.(158) Finally, long-term CR has a powerful effect in preventing/delaying the age-related increase in the severity of cardiomyopathy in rodents as well as in monkeys.(159,162,163)

CR, defined as a reduction in food intake without malnutrition, is a robust anti-aging intervention and the most powerful physiological inducer of macroautophagy. (164) The modulation of the autophagic response represents a primary mechanism underlying the lifespan-extending properties of CR.(165-167) There are a number of hypotheses regarding the mechanisms by which CR mediates its beneficial effects on aging in lower organisms that could have relevance to slowing cardiovascular aging in humans. These include a decrease in chronic inflammation, a reduction in the levels of various hormones and growth factors, an increased resistance to oxidative stress, as well as the potentiation of antioxidant defense mechanisms.(168)

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. At the center of this response are so-called "longevity regulatory" pathways, which include IGF-1, mTOR, AMPK and NAD^+-dependent deacetylases (sirtuins).(9)

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 vasculature, in part by inducing expression of the Nfr2 stress response transcription factor, which induces expression of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH): Quinone Oxidoreductase 1, Heme Oxygenase 1, and Glutathione S Transferase. (169-171) CR also reduces inflammation by suppressing the activity of vascular adhesion molecules, prostanoids, and inflammatory cytokines in both rodents(172) and humans(173). Endothelial function is enhanced and both atherosclerosis and arterial stiffness are reduced by CR in rodents.(174,175) 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.(176)

Coenzyme (Co)Q contributes to stabilize plasma membrane, regenerates antioxidants such as ascorbate and α-tocopherol, and regulates the extracellularly induced ceramide-dependent apoptosis pathway.(177,178) NAD(P)H-dependent reductases act at the plasma membrane to regenerate CoQH2, contributing to maintain its antioxidant properties. As a whole, both CoQ and its reductases constitute a transplasma membrane antioxidant redox system responsible of the above described functions.(179-181) The upregulation of plasma membrane redox system that occurs during CR decreases the levels of oxidative stress in aged membranes.(182-185) CR modifies composition of fatty acid in plasma membrane, resulting in decreased oxidative damage including lipid peroxidation.(186,187) More importantly, plasma membrane redox activities and also the content of CoQ, which decline with age, are enhanced by CR, providing protection to phospholipids and preventing lipid peroxidation reaction progression.(182-185)

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.(114,188-191) 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. However, the overall effect of irisin on CVD

remains largely unexplored.(192)

Resveratrol has been shown to recapitulate the transcriptional profile and some of the physiological changes that develop under CR. Indeed, both CR and resveratrol supplementation inhibit gene expression profiles associated with cardiac aging in mice. In addition, resveratrol improved survival and reduced the prevalence of cardiac pathology in mice fed a high-calorie diet.(188,193)

In general, CR may affect vascular health both by improving systemic risk factors for coronary artery disease (*e.g.*, plasma lipid and glucose levels, blood pressure) and by modulating cellular functions and gene expression in endothelial and smooth muscle cells that create a microenvironment in the vascular wall, which does not favor atherogenesis (*e.g.*, attenuation of ROS production, anti-inflammatory effects).(49)

Conclusion

Aging, although an unavoidable cardiovascular risk factor, may overcome all the other risk factors collectively. Therefore, understanding how aging mechanisms cause alterations to tissues and understanding the consequences of those alterations brings us one step closer to developing new therapeutic ways of treating and, more importantly, preventing the appearance of age-related CVD.

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