Pathways for salvage and protection of the heart under stress: novel routes for cardiac rejuvenation

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The world population is aging, and by 2050, there will be more people over the age of 65 than under age 5, and by 2080, two billion of the estimated nine billion people on Earth will be older than 60. Aging itself is a major cardiovascular risk factor, affecting morbidity and mortality of the aging population. At the same time, aging increases the likelihood of the presence of other risk factors. The aged myocardium is characterized by several structural and functional progressive changes that impair its ability to respond appropriately to stressful conditions. Although some progress to understand the complex mechanisms that underlie these phenotypic changes, the molecular pathways that determine the balance between aging and rejuvenation in the aged myocardium remain elusive. In this article, we review molecular mechanisms responsible for the phenotypic changes observed with aging in the heart, providing insight into molecular pathways and pharmacological interventions that may rejuvenate the aged myocardium. A better understanding of these pathways is essential for determining their therapeutic potential in humans, improving the possibility that the increase in life expectancy that we are observing will be accompanied by a parallel increase in healthspan.

Keywords Cardiac aging • Cardiac rejuvenation • Caloric restriction • Aging hormones • Cardiac hypertrophy

1. Introduction

The age profile of the European Union is expected to change dramatically in the coming decades, and nearly one-third of the population will be aged 65 or over by 2060 (http://ec.europa.eu/Ageingreport2012).

Despite some progress in recent years, aging, an evolutionary highly conserved process, still remains poorly understood.1 Aging inevitably leads to a progressive decline in cell and organ function2; however, broad differences in the rate of this process can be observed among individuals. The constant gait of time that leads to progressive functional decline is defined as chronological aging, while the actual status of biological systems and their potential regenerative and rejuvenative capacity are referred to as biological age.3 Usually, chronological age reflects the biological age, whereas, sometimes, biological age appears to be interestingly lower.3 This issue is pivotal in understanding the role of rejuvenation pathways in healthy aging.

Similar to what happens with chronological aging, an increase in the median age of a population may not reflect an increased quality of life. This is essentially due to differences in aging itself and to the increased occurrence of age-related pathologies, which may affect quality of life.4 Therefore, it is crucial to consider that an increase in lifespan, defined as the life expectancy of an individual, does not necessarily mean an increase in healthspan, the expectancy of a good quality of life.

Aging is associated with a higher prevalence of cardiovascular disease (CVD), cancer, degenerative disorders, and immune-mediated diseases, indicating a causative role of this poorly understood process in determining these conditions.5

The cardiovascular system itself is highly affected by the aging process. As previously reported in the Framingham study, age is the primary risk factor for CVD, with 22% increase in the risk of CVD each 5-year increment over 65 years.6 Cardiovascular pathologies such as heart failure (HF), cardiomyopathies, ischaemic heart disease (IHD), cardiac valve abnormalities, and hypertension all appear to increase their burden with age.7

Although IHD still represents the leading cause of mortality in the western world, the development of novel techniques and the improvement of therapies have dramatically overthrown the age-standardized mortality rates for this disease,8 while, as the population ages, chronic heart conditions have become more prevalent,9 in particular HF.

Although HF is traditionally characterized by progressive dilation of the left ventricle accompanied by depressed ejection fraction, namely heart failure with reduced ejection fraction (HFrEF), in part because...
of the increasing aging population, a growing epidemic of HF with normal systolic function, the so-called heart failure with preserved ejection fraction (HFpEF), has been observed, now representing ~50% of patients hospitalized for HF. The different profile of patients with HFpEF that more often are old, obese, diabetic, hypertensive, and female, compared with patients with HFrEF, suggests different physiopathologic determinants. Specific treatment strategies for diastolic dysfunction are very limited, with large clinical trials revealing disappointingly outcomes with drugs like angiotensin-converting enzyme inhibitors or beta adrenergic blockers, which have been applied successfully for systolic HF.

Thus, considering the increase in life expectancy, understanding the molecular mechanisms that regulate aging is crucial to improve the healthspan of the aging populations and reduce the socioeconomic impact of chronic diseases.

In this review, our main focus is to dissect the most important pathways of myocardial aging to understand and underline the main clinically relevant mechanism of the emerging age-related disease and to highlight the possible rejuvenation pathways in cardiovascular biology.

2. Cardiac aging

Aging is a major cardiovascular risk factor, affecting morbidity and mortality of the aging population. At the same time, aging itself increases the likelihood of the presence of other risk factors, i.e. hypertension, dyslipidemia, and diabetes. Cardiovascular aging is characterized by several structural and functional changes that eventually impair the capability of the myocardium to properly accommodate to stressful conditions.

Aging in humans is accompanied by a progressive deterioration of cardiac function, affecting in particular the diastolic filling properties, and this decline is observed at as early as the age of 20 years in humans. By 80 years of age, the reduction in early diastolic filling is as profound as 50%.

Cardiomyocyte hypertrophy, increased apoptosis, decreased myocyte number, and remodelling of the extracellular matrix (ECM), hallmarks of cardiac aging, contribute to reduced left ventricular (LV) compliance and to LV stiffening, pathognomonic of HFpEF. Increased LV mass has been found both in humans and in animals of older age. Several studies highlighted the role of cardiomyocyte hypertrophy as a translational hallmark of cardiac aging, being frequently observed in either humans, mice, or rats. Myocyte hypertrophy, either primary or secondary to hypertension, increases passive stiffness of the LV, which in turn may contribute to diastolic dysfunction.

Similar changes have been observed in several animal models from invertebrates to non-human primates, recapitulating numerous age-dependent cardiac remodelling and functional changes observed in humans. In particular, rodents present age-dependent cardiomyocyte hypertrophy associated with myocardial fibrosis and mitochondrial dysfunction, all concurring to impair LV filling properties. Mouse LV mass and left atrial dimension increase with a linear age-dependent trend, and functional impairment is caused by a decrease in fractional shortening (FS) and diastolic function associated with a reduction in myocardial performance index (MPI). Recent findings have highlighted the importance of the short-lived African Killifish as an emerging model of aging, with a potential to provide insight into cardiac aging-related diseases. The short life of this fish may, however, represent a limitation to study changes that naturally occur late in life. In the aging myocardium, the increased deposition of collagen, the augmented cross-linking, and the increased ratio of type I to type III collagen coupled with decreased elastin content and increased fibronectin are the fundamental changes occurring with ECM remodelling inducing diastolic dysfunction. All these changes observed with aging may contribute to reduced exercise tolerance and to augmented susceptibility to signs and symptoms of HF and are responsible for the phenotypical manifestation of HFpEF typical of the elderly. With age, increased serum levels of angiotensin II (Ang-II), one of the most important vascular modifiers involved with remodelling, fibrosis, and reactive oxygen species (ROS) production, are observed both in rats and in mice. Several studies have shown in mice that reducing presynaptic choline transporter promotes age-dependent fibrosis and hypertrophy, and the type 5 adenyl cyclase knock-out mice appears to be protected from cardiac fibrosis and hypertrophy related to aging, confirming the detrimental role of excessive adrenergic stimulation in promoting cardiac aging.

In humans, similar cardiovascular changes are observed in genetically determined accelerated aging pathologies like Down’s syndrome and the Hutchinson–Gilford progeria syndrome. In Down’s syndrome, the incidence of HF is more than doubled. In progeria, the association with myocardial fibrosis, lipofuscin deposition, and myocardial hypertrophy is more frequently observed.

3. Anatomical, histological, and molecular correlates of myocardial aging

3.1. Extracellular matrix

Aging results in ECM remodelling due to reduced collagen turnover rates, increased levels of fibrillar collagen, elevated levels of collagen mRNA, and post-translational modifications of collagen, both in humans and in animals. These changes increase the stiffness of the myocardium and mediate diastolic dysfunction. The impact of aging on collagen type I underlines the complex interplay of bidimensional deposition of collagen and more specifically its tridimensional (3D) structure in a 3D model of cardiac ECM.

The balance between the matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) tightly regulates ECM composition, and the activity of transforming growth factor-β (TGF-β) plays a major role in determining a proteolytic balance that favours accumulation of ECM and transformation of fibroblast into their more active phenotype, the myofibroblasts.

Aging is characterized by a shift favouring ECM accumulation and myofibroblast transformation associated with concentric remodelling and decreased LV diastolic function.

Interestingly, in a mouse model of cardiac aging, selective deletion of MMP-9 has been shown to improve angiogenesis, attenuate inflammation, and ameliorate ECM remodelling, underlining the interconnection between endothelial function and ECM composition.

Recently, Meyer et al. investigated the role of premature senescence in myocardial fibrosis in animal models of transverse aortic constriction and b1-adrenoeceptor transgenic mice. Intriguingly, the authors concluded that premature senescence of myofibroblast could reduce the fibrotic burden and ameliorate the cardiac function marshalling the role of ‘prosenescence’ cardiotropic myofibroblast induction as a potential therapeutic target for cardiac aging.

Clear understanding of the specific mechanism underlying the role of myofibroblast and ECM remodelling in cardiac aging needs further
3.2. Titin
Titin, the largest molecule in myocytes, modulates myocyte stiffness through phosphorylation of its spring-like domain. Genetic mutations of titin are highly related to the onset of several cardiomyopathies. Aging is associated with post-translational modifications of the spring-like domain of titin that contribute to decreased cardiomyocyte compliance and promote diastolic dysfunction. Recently, Zile et al. reported that patients with hypertension and HfPEF had increased collagen-dependent and titin-dependent stiffness, confirming the interaction between these two factors in the development of diastolic dysfunction in elderly. In old dogs, treatment with sildenafil alone and sildenafil plus brain natriuretic peptide (BNP) increased phosphorylation level of decreased titin-based passive stiffness, ameliorating diastolic function. These findings strongly suggest that titin regulates myocyte stiffness and the complex interplay between cardiomyocytes and ECM is a possible therapeutic target to ameliorate cardiac aging.

3.3. Calcium homeostasis
Calcium (Ca$^{2+}$) has complex and wide pleiotropic effects in cardiomyocyte biology. Increased beta adrenergic activity, observed with aging, alters Ca$^{2+}$ cycling through phosphorylation of different sites, namely phospholamban, Ca$^{2+}$/calmodulin-dependent protein kinase (CaMK), L-type Ca$^{2+}$ channels, and Na$^+$/Ca$^{2+}$ exchanger (NCX), and also through decreased expression of sarcoplasmic/endoplasmic reticulum Ca$^{2+}$-ATPase (SERCA) and alteration in ryanodine receptors (RyRs). Howlett et al. demonstrated that, in preparation of isolated ventricular myocytes from old mice, when compared with cells from young mice, Ca$^{2+}$ transient amplitudes are reduced, while occurrence of Ca$^{2+}$ sparks increases. Activation of calcium–calmodulin (CaM)-dependent pathways leads to hypertrophic response in human cardiomyocytes. This pathway is activated by excessive Ca$^{2+}$ load within the cytoplasm, partially due to increased calcium entry through non-selective cation channels, the so-called transient receptor potential channels (TRPCs), which modulate cardiac hypertrophy and fibroblast proliferation both in physiological condition and in aging. Inositol-1,4,5-triphosphate (IP$_3$) receptors, responsible for the release of Ca$^{2+}$ within the cytoplasm, are also increased in the myocardium of 26-month-old rats compared with 5- and 15-month-old rats. These receptors appear to be upregulated in failing human hearts, probably mediating cell fate in this setting. Thus, the complex interplay between calcium-induced calcium release, store-operated calcium entry (SOCE), and TRPCs may participate in regional regulatory events promoting cardiac hypertrophy and affecting lusitropism in aged mice.

Del Monte et al. have shown that adenoviral transfection of SERCA2a restored contractile function in failing human cardiomyocytes. These data were subsequently confirmed by Schmidt et al., showing that transfection of SERCA2a is able to restore normal Ca$^{2+}$ cycling and ameliorate diastolic function in the senescent rat myocardium. In addition, activation of insulin-like growth factor 1 (IGF-1) in old animals ameliorated cardiomyocyte contractile dysfunction and increased the SERCA activity. Though promising, results in humans appear controversial. A Phase 1/Phase 2 clinical trial using an adeno-associated virus serotype 1 (AAV1) vector carrying SERCA2a in patients with end-stage HF ameliorated LV geometry and reduced hospitalization times. These results inspired a Phase 2b trial; however, intracoronary administration of AAV1/SERCA2a did not improve clinical outcomes in patients with HFrEF. A clinical trial using the same strategy has been recently confirmed in patients with end-stage HF with left ventricular assist device (LVAD) (https://clinicaltrials.gov/ct2/show/NCT00534703); however, the results are not available yet. In contrast, recent findings indicate a potential beneficial role of airway-based delivery of AAV1/SERCA2a to induce vascular SERCA2a overexpression in pulmonary arteries resulting in amelioration of pulmonary haemodynamics and RV performance, potentially reopening the question regarding feasibility and efficiency of this type of therapy in the setting of HF. Indeed, many aspects related to the delivery methods, including the titre and amount of empty viral capsids as decoys for preformed AAV antibodies, may have influenced the results of the different trials. These results encourage further investigations to clarify the role of enhanced calcium handling in HF and at the same time provide evidence for a clear role of gene therapy in treating CVDs.

Interestingly, calcium homeostasis appears to be regulated by sexual hormones, specifically by testosterone. As recently highlighted, testosterone concentration decreases with aging in men and is associated with poorer quality of life. Testosterone treatment has already shown to inhibit coronary plaque progression in IHD. In the setting of cardiac aging, treatment of older men with testosterone may ameliorate cardiac contraction and calcium homeostasis, although the mechanism underlying these effects has still to be clarified.

3.4. Excitation–contraction coupling and late Na current
In the elderly, different adaptation mechanisms contribute to the genesis of arrhythmias and, in particular of atrial fibrillation (AF), a common hallmark of cardiac aging.

Isolated atrial cardiomyocytes from old humans and old dogs present reduced activity of Ca$^{2+}$-handling proteins and altered atrial Ca$^{2+}$ homeostasis when compared with young individuals, contributing to the genesis of AF. Modulation of the action potential (AP) duration results in increased Ca$^{2+}$ entry through L-Type Ca$^{2+}$ channels providing inotropic support to the aged myocardium. Signore et al. demonstrated that aging is associated with an increase in the late Na$^+$ current (INaL) in cardiomyocytes, which in turn prolongs the AP and alters Ca$^{2+}$ cycling and myocyte contractility. In vitro inhibition of INaL in old myocytes shortened the AP, corrected the kinetics of Ca$^{2+}$ transients, and ameliorated cell contraction and relaxation, while improving diastolic function both in mice and in dogs.

Ranolazine, an INaL blocker, was demonstrated to prevent recurrences of angina and improve exercise tolerance in patients with chronic IHD.

Finally, in a model of hypertensive rats, blocking the INaL, through either tetrodotoxin or ranolazine, ameliorated diastolic dysfunction reducing the end-diastolic pressure–volume relationship slope and enhancing cardiomyocyte relaxation. Moreover, blockade of INaL slowed the progression of hypertensive HF through an improvement of ultrastructural and physiological defects. Taken together, all these observations highlight that defects in the electromechanical properties of cardiomyocytes are crucial determinants altering cardiac performance in the elderly.
3.5. Neurohormonal activation

The role of the renin–angiotensin–aldosterone system (RAAS) in cardiac pathophysiology is well established, and the modulation of this axis is pivotal in the management of patients affected by HF. Increased activity of RAAS leads to phenotypical manifestation of cardiac aging in animal models, and treatment with angiotensin-converting enzyme inhibitors (ACE-I) or angiotensin receptor blockers (ARBs) is associated with prolonged lifespan of the treated animals. High levels of Ang-II in fibrotic human hearts are associated with cardiac aging phenotype, and inhibition of Ang-II signalling in aged animals significantly reduces cardiac hypertrophy and myocardial fibrosis, improving survival.

Ang-II increases endothelin-1 (ET-1) levels, and treatment with ACE-I and ARBs is associated with decreased levels of ET-1, protecting against the effect of aging in the vascular system. Aging itself is also associated with increased levels of ET-1. Local levels of ET-1 correlate with vasoconstrictor tone in peripheral arteries, which explain one of the mechanisms of the increased incidence of hypertension with aging. This factor mediates TGF-β expression, leading to increased collagen deposition both in old humans and in old rats. At the same time, ET-1 is also crucial to maintain normal cardiac function and exerts its effect through reduction of superoxide and MMP-9 levels.

Interestingly, increased expression of Klotho gene, which has been shown to increase lifespan in mice, appears to prevent up-regulation of ET-1 and peripheral vascular disease in hypertensive rats.

Excessive levels of Ang-I and Ang-II stimulate the production of Ang 1–9 and Ang 1–7 via endo- or carboxy-peptidases through ACE-2, which is up-regulated by ACE-I, drugs widely used in the elderly. ACE-2-mediated catabolism of Ang-II is likely to play a major role in cardiovascular protection. Protective effects of Ang 1–7 go beyond the blood pressure effects, are mediated through Mas receptors, and are also associated with reduced cardiomyocyte autophagy and oxidative stress. Moreover, the other catabolite of ACE-2, Ang 1–9, appears to reduce Ang-II levels, competing with Ang-I at the ACE active site, and increases circulating levels of Ang 1–7. The administration of Ang 1–9 reduces cardiac hypertrophy and ameliorates cardiac function in rats after coronary artery ligation, reducing cardiac fibrosis and improving endothelial function in hypertensive rats, effects that appear independent of Mas receptor activation. This important pathway intercedes the most widely used therapeutic intervention in patients with cardiovascular risk factors, ACE-I or ARBs. Determining which compounds are capable to promote the production of these metabolites may provide an important therapeutic tool to halt the aging process.

In elderly patients, elevated levels of natriuretic peptides (NPs) are found even in physiological conditions, and administration of atrial natriuretic peptide (ANP) or chronic inhibition of nephrillin (Nep), the enzyme responsible for NPs degradation, appears to ameliorate the aging phenotype in animal models. Recently, in humans, a new drug composed of valsartan and sacubitril, LCZ-696, which combines the favourable effect of angiotensin type 1 receptor (AT1R) blockers and NPE inhibition, demonstrated its favourable effects in the reduction of death or hospitalization for HF. Administration of this compound reduces myocardial fibrosis and cardiac hypertrophy, reduces levels of circulating high-sensitivity cardiac troponin, a marker of subtle myocardium damage; and have several favourable effects on renal and cerebral functions in the setting of HfPEF. Thus, to further investigate the role of this drug in patients affected by HfPEF, a Phase III clinical trial has been designed (https://clinicaltrials.gov/ct2/show/NCT01920711), results of which are expected by 2020.

3.6. Mitochondria and oxidative imbalance

Increased inflammation with aging is characterized by increased gene expressions of interleukin (IL)-1β, IL-6, tumour necrosis factor (TNF)-α, cyclooxygenase (COX)-2, and inducible nitric oxide synthases (iNOS) resulting in augmented oxidative stress through generation of ROS, ultimately contributing to the onset of cardiac aging. Chronic inflammation and excessive ROS production damage directly mitochondria, and the damage of mitochondria leads to increased ROS production, which results in a vicious circle affecting healthy aging. Aging also results in increased production and decreased detoxification of mitochondrial ROS, directly damaging cardiomyocytes. Indeed, reduction of myocyte oxidative stress through overexpression of mitochondrial catalase (mCAT) has protective effects against cardiac hypertrophy and fibrosis ameliorating the aging phenotype.

Accumulation of damaged mitochondria with aging is also secondary to a reduction of mitophagy, a specific form of macroautophagy responsible for the elimination of dysfunctional mitochondria. Clearance of dysfunctional mitochondria appears to be controlled by AMP-activated protein kinase (AMPK) and mammalian target of rapamycin (mTOR) complex 1 (mTORC1) balance, and indeed, activation of AMPK or use of rapamycin, an inhibitor of the mTOR pathway, promotes beneficial autophagy.

In addition, increased oxidative stress activates ECM remodelling and profibrotic pathways directly through the activation of TGF-β1 pathway by the NADPH oxidase 4 (NOX4)-dependent generation of H₂O₂ and the conversion of cardiac fibroblast to myofibroblast. Ang-II stimulates NOX4 on the mitochondrial membrane favouring the vicious cycle of oxidative stress and mitochondrial damage, thus promoting cardiac aging. Scavenging of ROS mediated by catalase targeted to mitochondrial appears as a feasible therapeutic approach to attenuate cardiac ECM remodelling and ameliorate myocardial fibrosis in aged mice hearts.

With aging, mitochondrial dysfunction produces oxidative imbalance favouring myocyte hypertrophy, ECM remodelling, and stimulating myofibroblast activity. All these aspects are crucial for the development of the aging phenotype, and interventions on these pathways may prevent the progression of cardiac aging.

Some of these aspects appear to be modulated by AMPK–NAD-dependent deacetylase sirtuin (SIRT)-1 axis through NF-kB activity and through FoxO/Daf-16 and Nrf2/SKN-1 activation, enhancing cell survival.

Thus, several potential targets have been proposed to ameliorate mitochondrial dysfunction. Caloric restriction (CR) physiologically represses the mTOR pathway and activates AMPK. Salicylate and metformin, drugs widely used in humans, activate AMPK either by a direct interaction or by increasing ADP:ATP ratio, respectively. Rapamycin mimics CR beneficial effects and extends lifespan, reduces LV hypertrophy, improves diastolic function in mice, and promotes mitochondrial remodelling. An interesting Phase I clinical trial conducted in old adults with IHD treated with three different doses of rapamycin and aimed to evaluate frailty and its effects on senescence-associated secretory phenotype has been recently concluded (https://clinicaltrials.gov/ct2/show/results/NCT01649960); however, results from this study are not yet available. A positive result
of this trial would contribute to enhance the evidence that mimicking the effects of CR is clearly a potential therapy in age-related CVDs in humans.

Coenzyme Q (mitoQ) and plastoquinone (SkQ1) have already demonstrated to exert protective effects on mitochondria in models of ischaemia–reperfusion and on cardiac hypertrophy. Their role in cardiac aging, however, has to be further investigated. Controversies on the potential pro-oxidative effects of mitoQ might halt, however, its broader use in the setting of cardiac aging.

Statins, the most widely used lipid-lowering drugs, also exert anti-aging effects by improving the redox status through inhibition of Rac1 and NADPH oxidase activity and by inhibiting telomere attrition. Moreover, activation of peroxisome proliferator-activated receptors (PPARs), a superfamily of ligand-activated nuclear transcriptional factors, regulates energy homeostasis, reduces inflammation, inhibits oxidative stress and apoptosis, and mitigates the effects of cardiac aging.

Recently, a novel approach to deliver antioxidants to the inner membrane of the mitochondria using the Szeto-Schiller (SS) peptides was shown to restore mitochondrial plasticity. These mitochondrial-targeted peptides ameliorate function of the organelle, improve energetic properties, and reduce oxidative cell death, suggesting a possible role in targeting the effects of cardiac aging.

In summary, oxidative imbalance and mitochondrial dysfunction are key modulators of cardiac aging. The potential role of proper antioxidant compounds and of several widely used drugs, which impact on mitochondrial dysfunction and reduce the oxidative burden, may help promote positive effects to improve the aged phenotype.

### 3.7. Telomere and telomerase

The telomere–telomerase axis has a controversial role in the progression of the functional decline observed with cardiac aging. Telomeric shortening is a biomarker of lifetime stress, and the stress-related telomere attrition is responsible for accelerated aging. Short leukocyte telomere length (LTL) is a hallmark of aging and is associated with CVD. Interestingly, longer LTLs are associated with better pulmonary performance in humans, and, although the molecular mechanism underlying this effect is still unclear, endurance-exercise training may improve the aging phenotype by promoting the expression of telomere-regulating genes. No direct evidences indicate that telomere length correlates with mortality in humans; however, to confirm the putative role of telomere length in aging, patients with autosomal-dominant dyskeratosis congenita have short telomeres due to mutations in the telomerase and show signs of premature aging and a reduced lifespan. Similarly, in C57Bl/6 mice, knockout of either the telomerase reverse transcriptase (TERT) or telomerase RNA component (TERC) of telomerase promotes accelerated aging and reduces lifespan of these animals. Furthermore, short telomeres are associated with reduced exercise-specific signalling mechanisms in mice. Specifically to cardiac aging, telomere attrition may impact on either the replicative capacity of progenitor cells or directly cardiomyocytes. Telomere attrition activates p53 affecting mitochondrial function in cardiomyocytes by repressing peroxisome PGC-1α and PGC-1β, thus promoting cardiac aging. Although the molecular pathway responsible for this interaction and the development of the aged phenotype has not yet been completely characterized, the telomere–p53–PGC–mitochondria axis represents a key modulator in the setting of human aging.

### 4. Novel strategies to rejuvenate the aged myocardium

#### 4.1. Reversing age-related cardiac hypertrophy

The evidence that circulating factors in a young individual can rejuvenate the aging phenotypes has sparked a lot of interest in the scientific community. A number of studies, mainly based on the revival of parabiosis, a surgical technique very popular during the 1970s to study the effect of a shared circulation in two animals, have shown the potential that humoral factors can control the aging process in different tissues. Heterochronic parabiosis, a specific experimental procedure whereby two animals of different ages are joined together, has shown the potential to reverse age-related cardiac hypertrophy in mice. By proteomics studies, growth differentiation factor 11 (GDF11), a member of the activin/TGF-β superfamily, was identified as a factor that carries the potential to reverse cardiac hypertrophy.

Recent publications, however, have raised questions about the age-dependent reduction of GDF11 and its antihypertrophic effect. The results of these non-confirmatory studies, however, have been challenged by Poggioli et al. in a recent publication. These apparently conflicting results can be explained by the lack of a specific detection reagent that can reliably discriminate GDF11 from its homologous myostatin (GDF8 or MSTN) and by differences in source and doses of the recombinant proteins used. While the effect of GDF11 initially seemed to be restricted to age-induced cardiac hypertrophy, Poggioli et al. showed that this factor also exerts a dose-dependent effect in reducing cardiac hypertrophy in young mice, extending its therapeutic potential to different forms of cardiac hypertrophy. These findings in mice are also supported by data from a prospective cohort study showing how GDF11 and MSTN might have similar cardioprotective properties in humans. Over 900 plasma samples in subjects with stable coronary heart disease (CHD) from the Heart and Soul prospective observational cohort were analysed using aptamers to measure the levels of circulating factors, because this assay does not discriminate GDF11 from GDF8, the authors indicate the circulating factor as GDF11/8. Results from this study have shown that older individuals have significantly lower levels of GDF11/8 and that a strong correlation is present between low plasma GDF11/8 concentrations and cardiovascular and mortality outcomes, with significantly more cardiac hypertrophy detected by echocardiography in individuals with low levels of the factor.

While it is attractive to imagine that systemic administration of an age-regulating factor could reverse the aging phenotype in humans, it is critical first to consider that the effects of sustained supernormal levels of those factors are unpredictable, especially when patients are young. Thus, a more specific approach to target specifically cardiomyocytes without interfering with circulating levels of aging hormones is necessary.

#### 4.2. MicroRNAs

MicroRNAs (miRNAs) play a key role in regulating cardiovascular regeneration, aging, and remodelling.

In Caenorhabditis elegans, lin-4 miRNA, which controls the developmental progression, appears to modulate lifespan and accelerate tissue aging. Indeed, loss of function of this miRNA shortens lifespan, while knocking down the lin-4 target expands it. Moreover, several other miRNAs, such as mir-71, mir-238, mir-239, and mir-246, have been...
associated with altered lifespan, and overexpression of mir-71 and mir-246 interestingly increases the lifespan of C. elegans.\textsuperscript{155,156}

In mammals, several miRNAs have been associated with aging. Hooten et al. have shown that miR-151a-5p, miR-181a-5p, and miR-1248 were significantly decreased in the serum of old humans compared with their younger counterparts.\textsuperscript{157} These data were confirmed also in non-human primates; however, their role in cardiovascular biology needs to be further investigated.

Apoptosis and cellular senescence appear to be mediated by miR-34a, a miRNA upregulated in cardiac aging,\textsuperscript{158} which inhibits protein phosphatase-1 regulatory subunit-10 (PNUTS) exacerbating telomere attrition, DNA damage, and fibrosis.\textsuperscript{159} Cardiac fibroblast and fibrosis, instead, are modulated by two different miRNAs. miR-22 activates cardiac fibroblast increasing their migration and senescence,\textsuperscript{160} whereas the miR-17–92 cluster activates TGF-β/connective tissue growth factor (CTGF) and thrombospondin-1 pathways, regulating ECM remodelling.\textsuperscript{161} Efficacy of cardiac repair and angiogenesis is affected by loss of miR-126 and miR-130.\textsuperscript{162} Cardiac repair and angiogenesis are also impaired by the increased senescence of endothelial stem cell due to the age-dependent increase of miR-10a and miR-21a.\textsuperscript{163}

Taken together, these findings support the potential of miRNAs to treat cardiac aging. A major limitation could be represented by the multiplicity of targets of single miRNAs that may elicit unwanted effects. However, understanding their role in cardiac aging may help to identify specific targets to delay the onset of a dysfunctional aging myocardium.

### 4.3. Metabolism and antioxidants

Longevity pathways play an important role in regulating mitochondrial biogenesis and function, in yeast and eukaryotes, including mammals,\textsuperscript{164} representing a critical biological aspect to promote healthy cardiac aging. This modulation is controlled mainly by PPAR-γ coactivator 1-α (PGC-1α).\textsuperscript{165} a potential target for correcting the effects of age on mitochondrial dysfunction.

Nutrient uptake is also crucial in determining cardiac aging, and CR increases lifespan and healthspan across the entire eukariota domain.\textsuperscript{166} CR protects against heart disease, diabetes, cancer, obesity, and neurodegenerative disease\textsuperscript{167} and reduces age-dependent loss of cardiac function, fibrosis, and apoptotic pathways activation, either as a preventive or a therapeutic approach in young and aged animals.\textsuperscript{168}
CR decreases mitochondrial ROS production, reduces the cell metabolic rate, and triggers the activation of longevity pathways that improve organism resistance to hostile conditions.\(^5\) Insulin/IGF-1/mTOR signalling pathway, nicotinamide adenine dinucleotide-dependent deacetylases (sirtuins), and AMPK act as key nutrient sensors involved in cardiac and organism longevity and are able to increase autophagy, stress resistance, genomic stability, and mitochondrial turnover.\(^5\) Sirtuins, important key regulators of the aging process, retard cardiac aging in animal models.\(^9\) Conserved from lower organisms, such as yeast, flies, and worms to humans, the seven members of the sirtuins class of molecules are diversely positioned in the nucleus and cytosol responding to the cellular energy balance through their NAD\(^+\) cofactor.\(^9\) Sirtuins deacetylate PGC-1\(\alpha\) favouring the expression of NAD synthetic enzyme nicotinamide phosphoribosyltransferase and activating the AMPK activator kinase LKB1.\(^{169}\) On the other hand, SIRT1 reduces metabolic turnover rate and represses HIF-1\(\alpha\).\(^{170}\) All these effects reduce ROS formations and promote mitochondrial turnover. SRT1720 and SRT2104 are two synthetic activators of SIRT1, capable of extending mice lifespan and providing cardiovascular protection.\(^{171}\) SRT2104 has also been tested in clinical trials confirming safety and biological activity in elderly humans.\(^{172}\) Noteworthy, the role of SIRT1 activation is controversial.\(^{173}\) Mild overexpression of SIRT1 (up to 7.5-fold) reduces the age-dependent cardiac dysfunction and remodelling induced by oxidative stress.\(^9\) However, 12.5-fold overexpression of SIRT1 increases oxidative stress, promotes cardiomyocyte hypertrophy, and activates apoptosis.\(^9\) Additionally, SIRT1 haploinsufficiency has a protective role against pressure overload-induced hypertrophy and failure.\(^{174}\) Resveratrol administration modulates and restores antioxidant capacity of the cells, improves mitochondrial function, and activates the sirtuin pathways.\(^{175,176}\) Resveratrol treatment ameliorates cardiac function\(^{177}\) activating SIRT1 and reducing the pro-apoptotic signalling mediated by Foxo-1 in old animals.\(^{178}\) The role of resveratrol on cardiac aging in humans is still unclear.\(^{179}\) Physical activity also delays aging and promotes mitochondrial rejuvenation in animal models.\(^{180}\) In particular, endurance-exercise training stimulates insulin sensitivity and energy expenditure; this results in increased median lifespan through activation of sirtuin and amelioration of mitochondrial function.\(^{181}\)

Figure 2. Schematic representation of the putative pathways to rejuvenate the aged myocardium. Therapeutic approaches that interfere with the molecular pathways that are involved in cardiac aging have the potential to delay or reverse the onset of chronic cardiovascular conditions and reverse cardiac dysfunction.
Pharmacological interventions to redirect energy expenditure in favour of cellular maintenance and repair may represent the hinge to halt and reverse cardiac aging. Further studies are needed to investigate the molecular pathways of these compounds; however, suggesting a correct lifestyle to everyone appears to be the cornerstone to improve quality of life.

5. Future perspectives and conclusions

In a world where the aging population is rapidly increasing, the recent progress of our understanding of the molecular processes that regulate cardiovascular aging (Figure 1) has provided a novel and innovative approach to reverse or delay age-related diseases (Figure 2). Although a theory that integrates all of the different intriguing observations in the field of aging is still needed, recent studies have provided a number of evidences indicating that a switch from energy expenditure for reproductive and biosynthetic needs towards maintenance and repair could be the key to extend lifespan and healthspan. Several therapeutic approaches that interfere with the molecular pathways that are involved in cardiac aging have shown the potential to delay or reverse the onset of chronic cardiovascular conditions and reverse cardiac dysfunction. In particular, modulation of cardiac metabolism, organelles and proteins turnover, and gene expression using CR, pharmacological therapy, recombinant proteins, and miRNAs represents a promising approach to reverse or delay age-related diseases (Figure 2).

The possibility to ‘rejuvenate’ the aged myocardium has enormous implications in cardiovascular pathology, and it will favour the chances that the increase in life expectancy observed will be accompanied by a parallel increase in healthspan.

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Novel routes for cardiac rejuvenation


