TOPICAL REVIEW

Ageing, metabolism and cardiovascular disease

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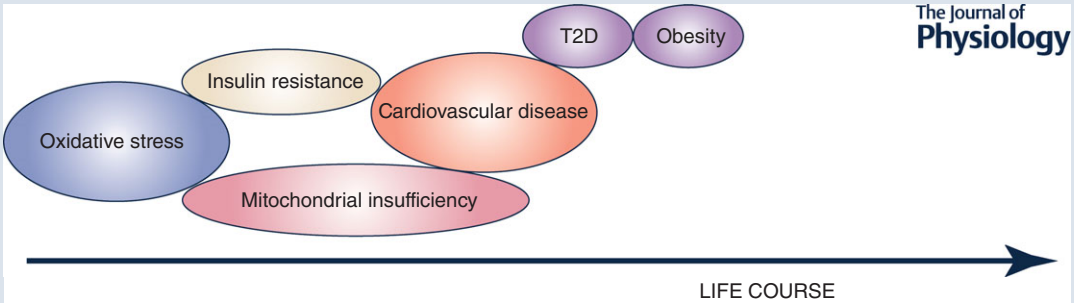
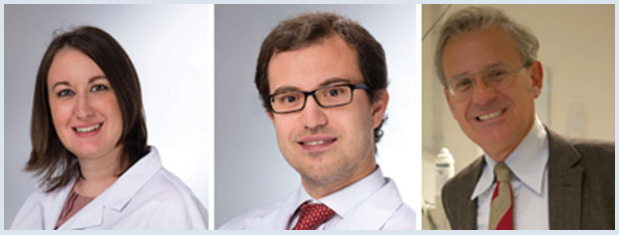


Abstract Age is one of the major risk factors associated with cardiovascular disease (CVD). About one-fifth of the world population will be aged 65 or older by 2030, with an exponential increase in CVD prevalence. It is well established that environmental factors (overnutrition, smoking, pollution, sedentary lifestyles) may lead to premature defects in mitochondrial functionality, insulin signalling, endothelial homeostasis and redox balance, fostering early senescent features. Over the last few years, molecular investigations have unveiled common signalling networks which may link the ageing process with deterioration of cardiovascular homeostasis and metabolic disturbances, namely insulin resistance. These different processes seem to be highly interconnected and their interplay may favour adverse vascular and cardiac phenotypes responsible for myocardial infarction, stroke and heart failure. In the present review, we carefully describe novel molecular cues underpinning ageing, metabolism and CVD. In particular, we describe a dynamic interplay



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between emerging pathways such as FOXOs, AMPK, SIRT1, $p66^{Shc}$, JunD and NF-kB. This overview will provide the background for attractive molecular targets to prevent age-driven pathology in the vasculature and the heart.

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Abstract figure legend Diagram showing the interconnection between ageing, metabolic impairment and cardiovascular disease. An array of molecular events affects cellular homeostasis during the life course, thus fostering accumulation free radicals, mitochondrial damage as well as maladaptive insulin signalling. These processes, in turn, favour the development of cardiometabolic states such as obesity, type 2 diabetes and adverse cardiovascular phenotypes.

Abbreviations ADL, activity of daily living; AGE, advanced glycation end products; AICAR, aminoimidazole carboxamide ribonucleotide; ALDH-2, aldehyde dehydrogenase-2; AMPK, AMP-activated protein kinase; AP-1, activator protein-1; CVD, cardiovascular disease; ECM, extracellular matrix; eNOS, endothelial nitric oxide synthase; Erk, extracellular signal-regulated kinase; FFA, free fatty acids; FOXO, Forkhead transcription factor; HUVECs, human-umbilical-vein-endothelial-cells; ILVM, inappropriate left ventricular mass; LV, left ventricular; MnSOD, manganese superoxide dismutase; NAD, nicotinamide adenine dinucleotide; Nampt, nicotinamide phosphoribosyltransferase; NF-kB, nuclear factor-kappa-B; PGI2, prostaglandin I2; PKCβII, protein kinase C βII; ROS, reactive oxygen species; SA-β-gal, senescence-associated β-galactosidase; TOR, target of rapamycin; VEGF-A, vascular endothelial growth factor-A; VSMCs, vascular smooth muscle cells; miR, microRNA; WT, wild type.

Ageing and cardiovascular disease

Ageing is perhaps the most important risk factor affecting cardiovascular homeostasis (Kovacic *et al.* 2011). Advances in the treatment of cardiovascular disease (CVD) and particularly acute myocardial infarction have prolonged median life-expectancy, with an estimated fourfold increase of people aged more than 75 years (Heidenreich *et al.* 2011; Nichols *et al.* 2014). Accordingly, about one-fifth of the world population will be aged 65 or older by 2030, with an exponential increase in the prevalence of CVD due to the fact that additional 27 million people will have hypertension, 8 million coronary heart disease, 4 million stroke and 3 million heart failure (Heidenreich *et al.* 2011). Moreover, the prevalence of metabolic disturbances, namely metabolic syndrome and diabetes, is significantly increased in the elderly population and further contributes to CV morbidity and mortality (Fadini *et al.* 2011). These numbers may explain why approximately 40% of the deaths in people aged >65 are caused by atherosclerotic disease and its complications (Heidenreich *et al.* 2011). Furthermore, the cost to treat CVD will triple over the next few years. By 2018, CVD costs among those aged 65–79 years are expected to exceed CVD costs among those aged 45–64 years (Heidenreich *et al.* 2011; Tarride *et al.* 2009; Fig. 1). This scenario strengthens the importance of understanding the molecular cues underlying the aetiology of the ageing process and its link with cardiovascular disease phenotypes. So far, the fields of cardiovascular disease and ageing have remained largely separated. More recently, it has been postulated that ageing and CVD are highly interconnected and may share common pathways (Fadini *et al.* 2011). Consistently, researchers have found that many of the factors underlying age-related changes in the arteries are also implicated in the development of CVD (Kovacic *et al.* 2011). In the present article, we provide an overview of emerging molecular pathways implicated in the interplay between senescence, atherosclerotic disease and metabolic disturbances. These novel targets might be considered for the design and development of mechanism-based therapeutic strategies to combat CVD burden over the next decades.

Pathological features of cardiovascular ageing

Ageing is accompanied by changes in vascular structure and function, especially in the large arteries (Safar *et al.* 2010; Kovacic *et al.* 2011). Age-related impairment of vascular function is the result of phenotypic alterations of different cell types, such as endothelial cells, smooth muscle cells and pericytes (Sawabe *et al.* 2010). Morphological changes are in most cases organ-specific and include vascular wall thickening, collagen deposition, perivascular fibrosis and vessel dilatation (Fig. 2). Progressive myointimal thickening is generally due to enhanced elastin degradation and collagen deposition in the vascular media as well as intimal hyperplasia (Scioli *et al.* 2014). Autopsy studies of perfusion-fixed human arteries have shown that thickening is mostly confined to the non-load-bearing intima, rather than the load-bearing-media. Other studies show that both intima and media are involved (O'Rourke *et al.* 2007). Thickening is a key hallmark of the aged vasculature promoting arterial stiffness (Lee *et al.* 2010). Changes in arterial stiffness are also due to an altered vascular

tone, which results from the imbalance between vasoconstriction and vasorelaxation (Zieman *et al.* 2005). Arterial stiffness is a major determinant of vascular impedance, which affects the pulsatile ejection of blood from the heart. The decrease in aortic distensibility that is associated with ageing creates a mismatch between ventricular ejection and aortic flow energies, which results in increased aortic systolic pressure, changes in aortic pressure contour, pulse wave reflection, and characteristic aortic impedance. Experimental work examining arterial mechanical changes during ageing showed that both incremental passive and active circumferential stiffness are significantly enhanced in 23-month-old compared with 6-month-old rats (Gaballa *et al.* 1998). Moreover, ageing was found to increase media thickness, collagen content and the collagen/elastin ratio by 12%, 21% and 38%, respectively (Gaballa *et al.* 1998). By contrast, elastin density and the number of smooth muscle cell nuclei were decreased by 20% and 31%, respectively, with ageing (Gaballa *et al.* 1998). Another established notion is that proximal arteries such as the central aorta and carotid artery dilate with age, resulting in increased lumen diameter. Importantly, adverse vascular remodelling is accompanied by key alterations of endothelial homeostasis. Occurrence of endothelial dysfunction is a major cause of morbidity and mortality (Flammer *et al.* 2012). Endothelium-dependent vasorelaxation is impaired in aged vessels and this phenomenon is associated with increased vascular permeability and inflammation as well as impaired angiogenesis (Kung *et al.* 1995; Kovacic *et al.* 2011). Moreover, endothelial cells become more heterogeneous in size, shape and axial orientation, so that intraluminal blood flow may be less laminar and the number of sites for lipid deposition may increase, thus fostering the formation of atherosclerotic plaques (Chiu *et al.* 2011).

Besides its effect on the vascular system, from Heidenreich *et al.* 2011 ageing has a remarkable effect on the heart (Stern *et al.* 2003). Heart weight increases with age whereas the number of cardiac myocytes is progressively reduced. This continued loss of functional cardiac cells is paralleled by a decline in regenerative activity from 1% per year at age 20 to 0.4% at 75 years (Dai *et al.* 2012). Myocardial fibrosis in the aged myocardium is an important determinant of impaired diastolic and systolic function (Biernacka *et al.* 2011). In this setting, a reduction in cardiac output due to decline in function stimulates the myocardium to compensate by increasing muscle mass (Cheitlin *et al.* 2003). Increased myocardial hypertrophy is an efficient short-term mechanism, but as a compensatory mechanism it is detrimental over the long term since it directly impairs cardiac performance

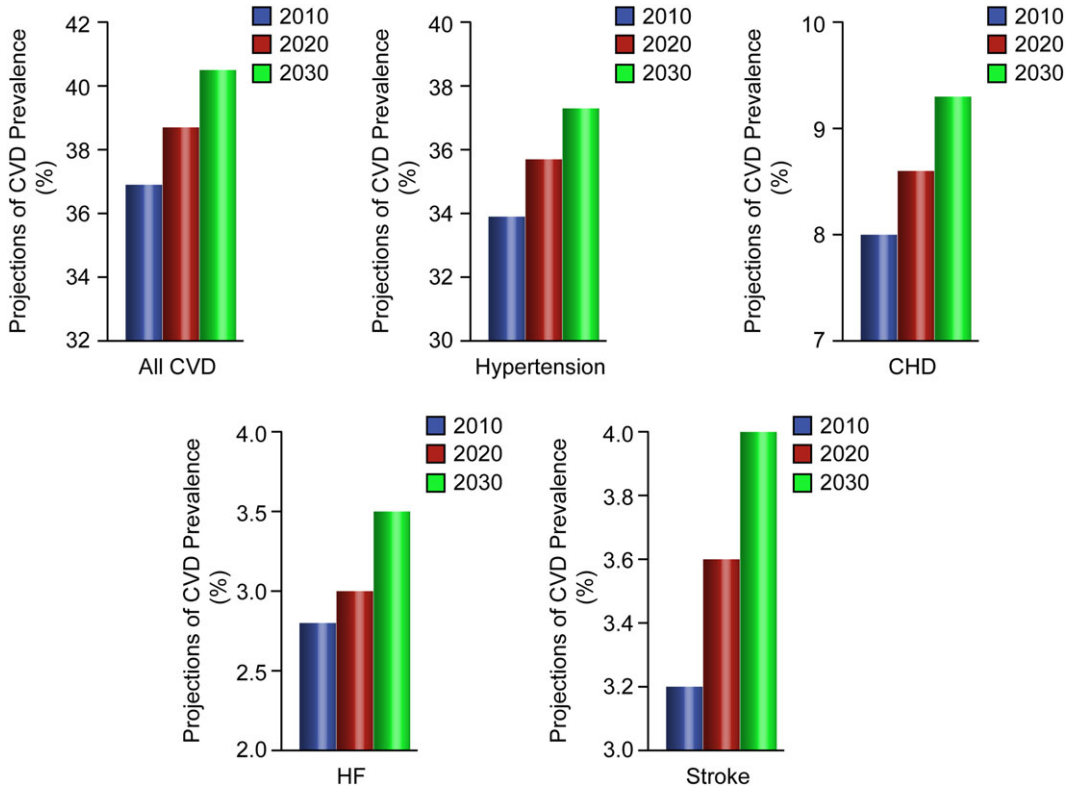

Figure 1. Projections of crude cardiovascular disease prevalence (%), 2010 –2030 in the United States
Modified.

(Isoyama *et al.* 1987). In elderly subjects, the increase in left ventricular (LV) hypertrophy significantly exceeds the predicted values which are based on cardiac workload. In other words, a large part of left ventricular mass is not generated to offset an increase in afterload but rather reflects pathological alterations of cardiac structure likely to be dictated by myocardial fibrosis and inflammation (de Simone *et al.* 2002). This has led to the definition of inappropriate left ventricular mass (ILVM), to indicate an adverse phenotype of LV remodelling. Interestingly, ILVM strongly correlates with systo-diastolic dysfunction as well as cardiovascular mortality (de Simone *et al.* 2002). In the aged heart, fibrosis serves as a pathological substrate for bradi- and tachyarrhythmias (de Jong *et al.* 2011). For example, 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 (Csepe *et al.* 2015). A recent work has clearly demonstrated that age-dependent mitochondrial DNA damage is an important substrate underpinning the pathophysiology of cardiac arrhythmias (Baris *et al.* 2015). In this study, genetically modified mice with accelerated accumulation of mtDNA deletions in the myocardium accumulated few randomly distributed cardiomyocytes with compromised mitochondrial function, which led to spontaneous ventricular premature contractions and atrio-ventricular blocks at the age of 18 months. These symptoms were not caused by a general mitochondrial dysfunction in the entire myocardium, and were not observed in mice at 12 months with significantly lower numbers of dysfunctional cells. These results suggest that the disposition to arrhythmia typically found in the aged human heart might be due to the random accumulation of mtDNA deletions and the subsequent mosaic respiratory chain deficiency (Baris *et al.* 2015). Another important pathological feature associated with ageing is the calcification of aortic and mitral valves which triggers stenosis/insufficiency resulting in cardiac pressure/volume overload (Freeman *et al.* 2005).

Molecular pathways linking senescence, metabolic alterations and cardiovascular phenotypes

Growing evidence is supporting the concept that ageing is able to derail pathways leading to adverse metabolic profile, high blood pressure and altered lipid metabolism. On the other hand, metabolic conditions, namely obesity, diabetes and insulin resistance, are associated with premature features of vascular and cardiac senescence. These aspects highlight a dynamic interplay between ageing, metabolism and cardiovascular disease (Fig. 3).

Mitochondrial adaptor p66^{Shc}

Mechanistic studies over the last decade have demonstrated that the mitochondrial adaptor p66^{Shc} is an important molecular effector which may explain how ageing is connected with metabolic and cardiovascular disease. This enzyme plays a major role in the generation of reactive oxygen species (ROS) (Paneni *et al.* 2012). Several stimuli activate protein kinase C βII (PKCβII)

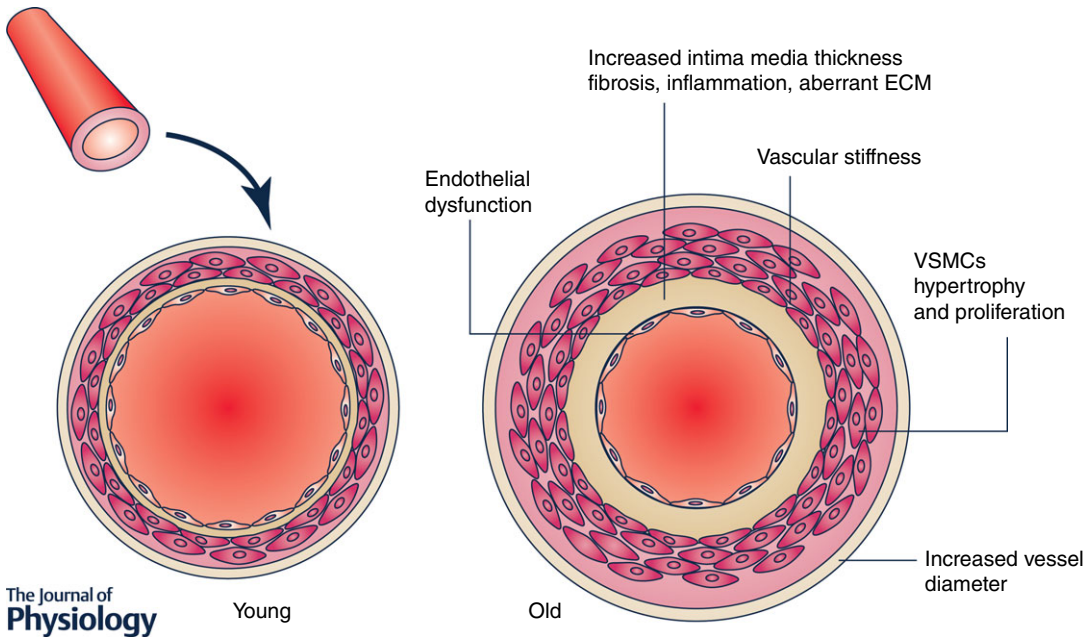

Figure 2. Structural changes of the aged vasculature
ECM, extracellular matrix; VSMCs, vascular smooth muscle cells.

isoform to induce Ser-36 phosphorylation of p66Shc, allowing transfer of the protein from the cytosol to the mitochondrion where it fosters ROS accumulation by oxidizing cytochrome c (Giorgio *et al.* 2005). This latter event leads to mitochondrial disruption, release of solutes and water, and subsequent apoptotic programmes. Intracellular free radicals are reduced in cells lacking the *p66*^{Shc} gene (*p66*$^{Shc-/-}$ cells), and both systemic and intracellular free radicals are diminished in *p66*$^{Shcc-/-}$ mouse models exposed to high oxidative stress (Giorgio *et al.* 2005; Camici *et al.* 2008). An interesting observation from our lab was that endothelium-dependent relaxation in response to acetylcholine is age-dependently impaired in wild-type but not in *p66*$^{Shcc-/-}$ mice (Francia *et al.* 2004). This phenomenon was largely explained by preservation of nitric oxide availability in mice lacking the *p66*^{Shc} gene (Francia *et al.* 2004). Further work has demonstrated that p66^{Shc} activation is critically involved in different processes including adipogenesis, insulin resistance and diabetes-related cardiovascular complications (Camici *et al.* 2007; Berniakovich *et al.* 2008; Paneni *et al.* 2014). More recently, we found that vascular p66Shc levels are increased in genetically obese mice and participate in endothelial insulin resistance (Paneni *et al.* 2014). In this study, endothelium-dependent relaxations to insulin were blunted in obese as compared to WT mice. Interestingly, *in vivo* gene silencing of p66^{Shc} restored insulin response via the IRS-1–Akt–eNOS pathway (Paneni *et al.* 2014). Furthermore, p66^{Shc} knockdown in endothelial cells isolated from obese mice attenuated ROS production, free fatty acids (FFA) oxidation and prevented dysregulation of redox-sensitive pathways such as nuclear factor-kappa-B (NF-kB), advanced glycation end products (AGE) precursor methylglyoxal and prostaglandin I2 (PGI2) synthase (Paneni *et al.* 2014). Activation of p66^{Shc} also interferes with the acquisition of the heart senescent phenotype and the development of heart failure in diabetic mice. Indeed, diabetic *p66*$^{Shcc-/-}$ mice were protected against myocardial oxidative stress, apoptosis and telomere shortening. Moreover, ablation of the *p66*^{Shc} gene in cardiac stem cells preserved the growth reserve of the heart (Rota *et al.* 2006). The clinical relevance of p66^{Shc} is supported by the notion that *p66*^{Shc} gene expression is increased in mononuclear cells obtained from patients

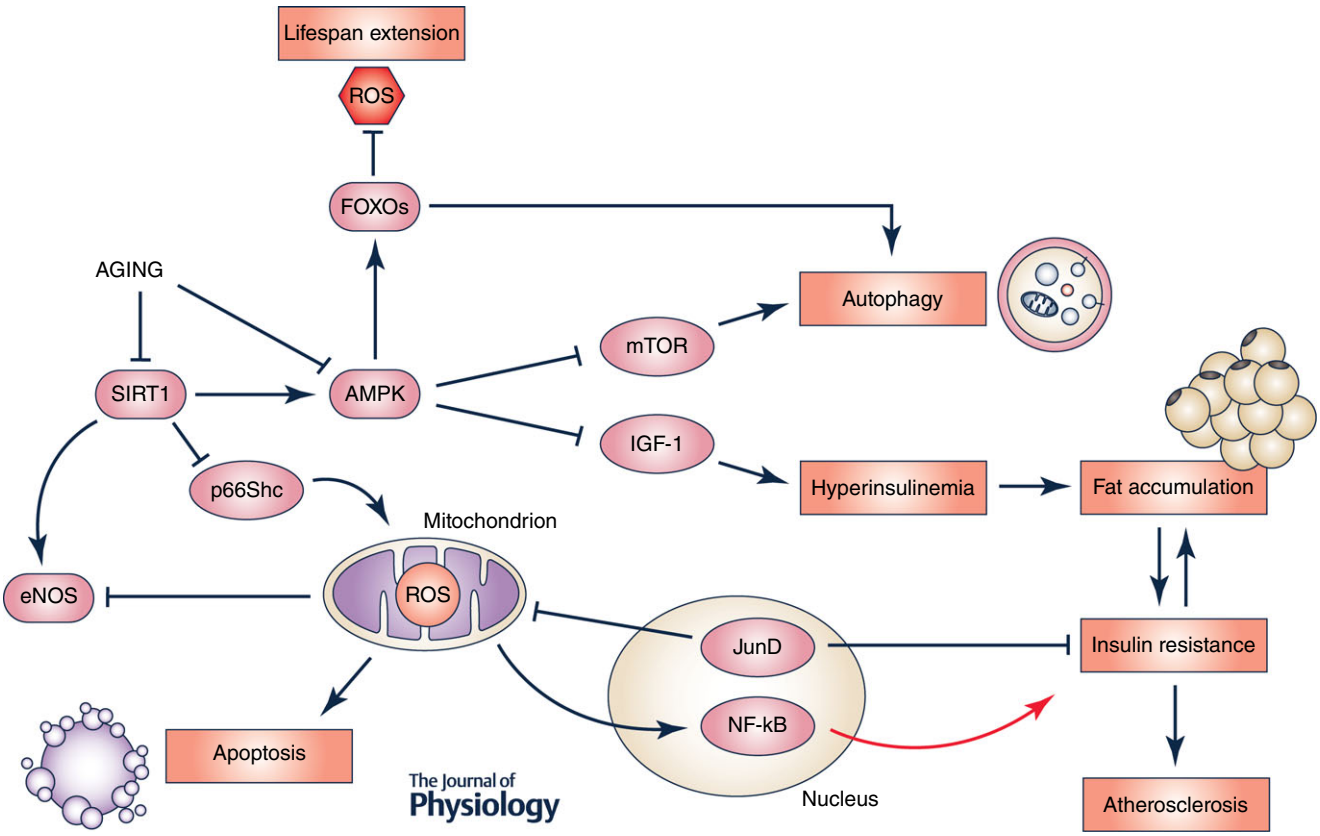

Figure 3. Schematic representation of the molecular pathways linking ageing, metabolism and cardiovascular disease
ROS, reactive oxygen species; eNOS, endothelial nitric oxide synthase; NF-kB, nuclear factor-kappa B; AMPK, AMP-activated protein kinase; FOXOs, Forkhead box O; IGF-1, insulin growth factor-1; mTOR, mammalian target of rapamycin.

with type 2 diabetes and coronary artery disease (Pagnin *et al.* 2005; Franzeck *et al.* 2012). Moreover, a recent study showed that $p66^{Shc}$ expression is higher in fibroblasts isolated from centenarians (Pandolfi *et al.* 2005). This finding probably indicates that $p66^{Shc}$ expression may increase in a time-dependent manner. Taken together, these findings show that increased $p66^{Shc}$ expression during the life-course may foster ROS accumulation with subsequent deregulation of pathways implicated in mitochondrial dysfunction, fat accumulation, insulin resistance and diabetes.

AMP-activated protein kinase

The functional AMP-activated protein kinase (AMPK) is a heterotrimer consisting of a catalytic alpha (α), a regulatory gamma (γ) and a scaffolding beta (β) subunit and is activated by low cellular energy status (Salminen *et al.* 2012). AMPK activation orchestrates many biochemical events including glucose uptake, glycolysis, oxidation of free fatty acids (FFAs) and mitochondrial biogenesis (Towler *et al.* 2007). These processes significantly contribute to raise ATP levels and restore myocardial contractile efficiency and vascular responses. AMPK also activates endothelial nitric oxide synthase (eNOS), and promotes autophagy and mitophagy, thus preventing mitochondrial insufficiency, inflammation and cellular death (Alers *et al.* 2012). Autophagy is a major intracellular degradation process recognized to play a central role in cell survival and longevity (He *et al.* 2009). In recent years, this phenomenon has emerged as a unifying downstream feature of several evolutionarily conserved anti-ageing interventions including both dietary restrictions and reduced target of rapamycin (TOR) signalling (Jung *et al.* 2010). A recent study found that upregulation of AMPK in the adult *Drosophila* nervous system induces autophagy both in the brain and in the intestinal epithelium. Interestingly, induction of autophagy was linked to improved intestinal homeostasis during ageing and increased lifespan by 30% (Ulgherait *et al.* 2014). AMPK is also a master regulator of key molecular effectors involved in metabolic processes, longevity and cardiovascular homeostasis. It modulates mTOR signalling by directly phosphorylating the TSC1/2 complex, regulates the IGF-1 pathway through the extracellular signal-regulated kinase (Erk) cascade, and controls sirtuin activity by regulating the abundance of nicotinamide adenine dinucleotide (NAD) and nicotinamide phosphoribosyltransferase (Nampt) (Salminen *et al.* 2012). Old mice (28–30 months) display reduced arterial AMPK expression compared to 3- to 6-month-old animals. Pharmacological activation of AMPK by aminoimidazole carboxamide ribonucleotide (AICAR) for 2 weeks increased arterial AMPK and reversed ROS-driven endothelial dysfunction (Lesniewski *et al.* 2012). Moreover, AMPK activation has shown to diminish senescence-associated β-galactosidase (SA-β-gal) staining while restoring proliferation of vascular smooth muscle cells (Sung *et al.* 2011). Interestingly, recent evidence indicates that AMPK is amenable to pharmacological intervention and, hence, represents a potentially 'druggable' target to prevent ageing-related features. Experimental work has indeed shown that dietary curcumin administration for 1 month remarkably restored cerebrovascular endothelium-dependent vasorelaxation in aged rats. In cerebral arteries from old animals and cultured endothelial cells, curcumin promoted AMPK phosphorylation and reduced ROS production. Interestingly, the beneficial effects of curcumin were no longer seen following AMPK inhibition (Pu *et al.* 2013). Meformin, a biguanide often used in the treatment of diabetes, is capable of inducing AMPK activation by phosphorylating a key regulatory site (Thr-172) on the catalytic (α) subunit. Administration of metformin before, during or after myocardial ischaemia has been shown to prevent ischaemia–reperfusion injury and adverse remodelling of the left ventricle (El Messaoudi *et al.* 2011). Consistently, metformin has been shown to preserve insulin secretion by promoting the AMPK-dependent autophagic response in pancreatic beta cells (Jiang *et al.* 2014). However, in a recent randomized trial chronic treatment with metformin did not affect the p-AMPK/total AMPK ratio in peripheral blood mononuclear cells isolated from prediabetic subjects (Vigili de Kreutzenberg *et al.* 2015). Moreover, high dose resveratrol stimulated differentiation of vascular smooth muscle cells (VSMCs) through AMPK-mediated inhibition of the mTORC1 pathway, allowing activation of the Akt kinase. These latter findings hint that pharmacological activation of AMPK may play a role in VSMC phenotypic plasticity, thus promoting vessel maintenance, repair and adaptation to vascular changes associated with ageing (Thompson *et al.* 2014).

SIRT1

The *SIRT1* gene belongs to the family of nicotinamide adenine dinucleotide (NAD)-dependent proteins and is considered a major gatekeeper against oxidative stress, inflammation and cardiovascular ageing (Pillarisetti *et al.* 2008). SIRT1 protects the heart from senescent features, ischaemia/reperfusion injury, hypertrophy and cardiomyocyte apoptosis (Alcendor *et al.* 2007). SIRT1 overexpression leads to reduced myocardial hypertrophy, interstitial fibrosis, oxidative stress and senescent markers such as p15INK4b and p19ARF (Sundaresan *et al.* 2011). Moreover, pharmacological activation of SIRT1 by resveratrol attenuates ageing-induced elevations of fibrotic

collagen deposition and markers of oxidative damage including 4HNE and nitrotyrosine (Sin *et al.* 2014). These changes were associated with a significant improvement in cardiac function, as assessed by ejection fraction and fractional shortening. Moreover, SIRT1 ameliorates endothelial function, prevents macrophage foam cell formation and calcification of vascular smooth muscle cells (Stein *et al.* 2011). Age-dependent downregulation of SIRT1 favours acetylation of nuclear factor (NF)-kB p65, leading to its nuclear translocation and transcription of inflammatory genes (Yeung *et al.* 2004). Recent work has shown that sirtuin expression can be modulated by epigenetic changes, namely increased DNA methylation and non-coding RNAs such as miR-200a and miR-34a (Eades *et al.* 2011; Sahin *et al.* 2014; Yu *et al.* 2015). The maintenance of SIRT1 homeostasis is fundamental to repression of detrimental pathways of arterial ageing such as the Forkhead transcription factor (FOXO) pathway (Brunet *et al.* 2004). Specifically, SIRT1 has the ability to deacetylate FOXO 1, 3 and 4 in the nucleus thus preventing DNA damage, cell cycle arrest and oxidative stress (Brunet *et al.* 2004; van der Horst *et al.* 2004; Kobayashi *et al.* 2005). Moreover, SIRT1 deacetylates LKB1 leading to activation of the final effector enzyme AMPK, a central energy regulator involved in glucose homeostasis, maintenance of cellular ATP levels and endothelial integrity via regulation of eNOS activity and autophagy (Mattagajasingh *et al.* 2007). Perturbation of the SIRT1–LKB1–AMPK pathway leads to energy imbalance, cellular stress and activation of the apoptotic machinery, thus contributing to vascular ageing (Pillarisetti *et al.* 2008). A randomized, placebo-controlled trial including 38 prediabetic subjects, showed that treatment with metformin (1500 mg day^{-1}) significantly restored the SIRT1–mTOR–AMPK axis while suppressing senescent markers such as p53 and p21 in peripheral blood mononuclear cells (Vigili de Kreutzenberg *et al.* 2015). SIRT1 inhibition also affected telomere length in these patients (Vigili de Kreutzenberg *et al.* 2015). Another important mechanism whereby SIRT1 prevents cardiovascular ageing is the activation of endothelial nitric oxide synthase (eNOS) (Stein *et al.* 2011). In human umbilical vein endothelial cells (HUVECs), SIRT1 activation protects against ROS-induced premature senescence by increasing eNOS protein expression and activity (Ota *et al.* 2007). These data were further strengthened by the observation that microRNA (miR)-217, an endogenous inhibitor of SIRT1, triggers endothelial senescence by suppressing SIRT1-dependent eNOS functionality (Menghini *et al.* 2009). Taken together, these results indicate that the SIRT1–eNOS axis could be a potential target against vascular ageing and age-related vascular diseases. Pharmacological approaches to inhibition of SIRT1 have been shown to prevent maladaptive pathways promoting ageing, and metabolic and cardiovascular disease (Winnik *et al.* 2015). Among different compounds, resveratrol is of particular importance. This is a polyphenol naturally present in several plants that is identified as a small-molecule activator of SIRT1. Modulation of SIRT1 activity by resveratrol suppresses oxidant and inflammatory genes by altering the epigenetic status of their promoter (Fernandez *et al.* 2011). Indeed, SIRT1-induced deacetylation of histone tails reduce chromatin accessibility to transcription factors, thereby reducing the expression of detrimental genes such as the adaptor $p66^{Shc}$ (Paneni *et al.* 2013*b*; Costantino *et al.* 2015). Beside epigenetic regulation, resveratrol also has effects on mitochondrial metabolism and insulin sensitivity by activating the AMPK–SIRT1–PGC-1α axis (Lagouge *et al.* 2006). Chronic treatment with resveratrol improves endothelial function and prevents metabolic disturbances in obese individuals (Beaudeux *et al.* 2010). Furthermore, resveratrol improved diastolic function in patients with coronary artery disease (Magyar *et al.* 2012). Many experimental studies have shown that resveratrol prolongs lifespan in different animal models. In mammals, controversial results have also been found and they seem to depend on the composition of the diet supplied with the resveratrol supplementation (Fernandez *et al.* 2011). However, it has been claimed that resveratrol may have other targets than sirtuins and the direct activation of sirtuins by resveratrol is currently a matter of debate (Costantino *et al.* 2015). Further research is needed to fully understand the potential and clinical application of resveratrol as a SIRT1-activating approach.

Forkhead transcription factors (FOXOs). Forkhead transcription factors (FOXOs) regulate the expression of genes involved in cell growth, proliferation, differentiation and longevity (Ronnebaum *et al.* 2010). FOXOs are also modulated by SIRT1-mediated deacetylation and IGF-1 signalling (Giannakou *et al.* 2004; Gan *et al.* 2005). Inactivation of sirtuins during ageing favours FOXO acetylation and subsequent transcription of FOXO-dependent genes favouring cellular apoptosis, cell cycle arrest, accumulation of ROS and metabolic derangements. Moreover, FOXOs control the expression of many autophagy-related genes and are important for lifespan extension (van der Horst *et al.* 2007; Webb *et al.* 2014). A role for the FOXO family in autophagy was first described in murine models of muscle atrophy, an age-related condition to which several degradative pathways contribute, especially autophagy (Lapierre *et al.* 2015). In the muscle, FOXO1 and FOXO3 elevate the autophagic flux by increasing the expression of autophagy genes mainly working as part of the core machinery and additionally increase protein degradation via the proteasomal pathway (Sanchez *et al.* 2014). In particular, FOXO3 increases the capacity of the lysosome to degrade

incoming cargo, indicating a role for lysosomal function in muscle atrophy (Zhao *et al.* 2007). Other FOXOs (FOXO1, FOXO4, and FOXO6) also play roles in proteostasis and autophagy (Lapierre *et al.* 2015).

FOXOs are key downstream effectors of the PI3K–Akt pathway (Ronnebaum *et al.* 2010). Following stimulation of PI3K–Akt signalling by growth factors, Akt phosphorylates FOXOs on three conserved residues, which leads to their cytoplasmic sequestration and inactivation (Huang *et al.* 2007). By contrast, Akt is inactive in the aged vasculature, thus promoting FOXOs-dependent transcriptional programmes culminating with endothelial senescent phenotypes (Ronnebaum *et al.* 2010). Expression of FOXO3a in mouse hearts resulted in reduction of cardiomyocyte size, suggesting that this FOXO factor functions to reduce hypertrophy (Ronnebaum *et al.* 2010). Indeed, FOXO1 or FOXO3 in cardiomyocytes attenuate calcineurin phosphatase activity and inhibit agonist-induced hypertrophic growth (Ni *et al.* 2006). FOXO3a deficiency has been shown to increase eNOS expression and enhances postnatal vessel formation and maturation (Salih *et al.* 2008). Loss of insulin signalling is emerging as an important factor underpinning impaired lifespan (Paneni *et al.* 2015). A very recent study found that insulin signalling via phosphorylation of FOXO-1 and consequent eNOS stimulation was selectively impaired in visceral adipose tissue and endothelial cells of obese subjects (Karki *et al.* 2015). Interestingly, pharmacological antagonism of FOXO1 by AS184256 and gene silencing reversed insulin resistance and restored endothelial eNOS functionality in endothelial cells from obese subjects (Karki *et al.* 2015). Further work has demonstrated that FOXO3 is required for lifespan extension in mice undergoing dietary restriction. Indeed, low caloric intake was associated with increased survival in wild-type (WT) mice but not in Foxo3-knockout heterozygous (+/−) and homozygous (−/−) animals (Shimokawa *et al.* 2015). In line with these data, FOXO3A single nucleotide polymorphisms (SNPs) were significantly associated with cognitive function, handgrip strength, activity of daily living (ADL), and self-rated health in a cohort of 1088 oldest-old Danes (age 92–93 years) (Soerensen *et al.* 2015). Understanding the biology of FOXOs may have important implications for the prevention of ageing phenotypes.

Nuclear factor kappa-B

Nuclear factor kappa-B (NF-kB) is an important transcription factor expressed in all mammalian cell types (Brasier *et al.* 2006). It is responsible for regulating gene expression of factors that control cell adhesion, proliferation, inflammation, redox state, and tissue-specific enzymes (Baker *et al.* 2011). Activation of NF-kB mediates vascular and myocardial inflammation in metabolic and age-related diseases. A recent study clearly demonstrated that endothelial suppression of NF-kB prolongs lifespan in mice and ameliorates obesity-induced endothelial insulin resistance. Hasegawa and co-workers have shown that transgenic mice with endothelium-specific overexpression of the inhibitory NF-kB subunit IkBα (E-DNIkB) were protected against insulin resistance in adipose tissue and skeletal muscle (Hasegawa *et al.* 2012). In these mice, obesity-induced macrophage infiltration of adipose tissue and plasma oxidative stress markers were reduced whereas blood flow and muscle mitochondrial content were increased. Of note, capillary recruitment and subsequent insulin delivery were explained by restoration of NO levels in E-DNIkB animals (Hasegawa *et al.* 2012). Impaired insulin signalling is indeed an important hallmark linking metabolic disease with premature ageing of the CV system (Rask-Madsen *et al.* 2011). The importance of inflammation in the pathogenesis of metabolic disturbances is strengthened by the observation that proinflammatory endothelial activation detected by molecular imageing in obese non-human primates coincides with onset of insulin resistance and progressively increases with duration of altered glucose homeostasis (Chadderdon *et al.* 2014). The relevance of these findings is supported by the notion that NF-κB protein is elevated in vascular endothelial cells isolated from obese and aged adults compared with normal-weight and young controls (Seals *et al.* 2011). Moreover, age-dependent NF-κB activation was associated with systemic inflammation and impaired endothelial dependent dilatation (Tabit *et al.* 2013). NF-kB is also a potent mediator of age-induced myocardial inflammation and fibrosis. Accordingly, NF-kB suppression, using direct gene delivery of short hairpin p65 RNA, attenuates remodelling and cardiac hypertrophy (Tas *et al.* 2009). These data validate NF-kB as a therapeutic target to prevent cardiac disease in the elderly.

Transcription factor JunD

Growing evidence is supporting the notion that the activator protein-1 (AP-1) transcription factor JunD is a key molecule implicated in age-related diseases, mostly by modulating oxidative stress levels. The transcription factor AP-1 is a heterodimeric complex which is composed of several proteins belonging to the c-Fos, c-Jun, ATF and JDP families (Hirai *et al.* 1989). AP-1 modulates gene expression in response to a variety of stimuli, including bacterial and viral infections, stress, cytokines and growth factors. Moreover, this molecular complex regulates transcriptional programmes implicated in cellular differentiation, proliferation, and apoptosis (Hirai *et al.* 1989). Over the last few years, several investigations have unveiled a critical role for the AP-1 component JunD

in cell growth and survival as well as in the regulation of redox signalling by modulating genes involved in anti-oxidant defence and ROS production (Gerald *et al.* 2004). In this regard, we have recently reported that JunD is highly relevant for cardiovascular integrity during the life course (Paneni *et al.* 2013*a*). An interesting experimental observation from this study is that vascular JunD expression progressively declines with ageing, thus altering the balance between pro-oxidant (NADPH oxidase) and antioxidant enzymes (manganese superoxide dismutase (MnSOD) and aldehyde dehydrogenase-2 (ALDH-2)), with subsequent accumulation of free radicals. Indeed, genetic deletion of JunD in young mice (6 months old) was associated with premature disturbances of redox signalling, mitochondrial disruption and endothelial dysfunction. Moreover, young $JunD^{-/-}$ mice displayed premature features of vascular senescence which were comparable with those observed in aged animals (22 months old) (Paneni *et al.* 2013*a*). We found that age-dependent downregulation of JunD was the result of epigenetic changes occurring on its promoter, namely increased CpG methylation (Paneni *et al.* 2013*a*). This latter finding is in line with the notion that postgenomic mechanisms (i.e. DNA methylation, histone modifications and non-coding RNAs) may significantly alter the expression of genes relevant to senescence, metabolic disorders and cardiovascular damage (Lopez-Otin *et al.* 2013). The relevance of JunD in the context of vascular ageing is supported by the observation that *in vivo* overexpression of the transcription factor was able to rescue endothelial dysfunction in aged mice (Paneni *et al.* 2013*a*). Furthermore, we have translated these findings to human subjects by showing that JunD expression is reduced in peripheral blood monocytes isolated from aged compared to young healthy volunteers. Taken together, the results of our recent work imply that JunD can be regarded as an attractive molecular target to prevent or delay age-driven cardiovascular diseases. In agreement with our observations, genetic disruption of JunD promotes pressure overload-induced apoptosis, hypertrophic growth, and angiogenesis in the heart (Ricci *et al.* 2005). By contrast, adenoviral over-expression of wild-type JunD blunted phenylephrine-mediated cardiomyocyte hypertrophy by negatively regulating AP-1 transcriptional activity (Hilfiker-Kleiner *et al.* 2006). Notably, JunD protein levels are decreased in patients with end-stage heart failure, suggesting that the transcription factor may protect against age-related cardiac dysfunction (Hilfiker-Kleiner *et al.* 2005). Furthermore, reduced JunD levels may affect longevity by controlling pathways relevant to angiogenesis and insulin signalling. Insulin–IGF-1 signalling is constitutively stimulated in mice lacking JunD, leading to inactivation of FOXO1, a positive regulator of longevity (Laurent *et al.* 2008). The importance of insulin homeostasis is outlined by experimental observations in *C. elegans* showing that suppression of insulin–IGF-1 signalling increases lifespan. Likewise, substantial increases in lifespan are associated with mutations that reduce insulin–IGF-1 signalling in the fruit fly *Drosophila melanogaster* (van Heemst *et al.* 2010). $JunD^{-/-}$ mice display hyperinsulinaemia, most probably resulting from enhanced pancreatic islet vascularization due to chronic oxidative stress. Indeed, accumulation of free radicals in beta-cells enhances vascular endothelial growth factor-A (VEGF-A) transcription, which in turn increases pancreatic angiogenesis and insulin secretion (Laurent *et al.* 2008). Interestingly enough, long-term treatment with an antioxidant rescued the metabolic disturbances observed in $JunD^{-/-}$ mice (Laurent *et al.* 2008). Indeed, dietary antioxidant supplementation was protective against pancreatic angiogenesis, hyperinsulinaemia, and subsequent activation of insulin signalling cascades in peripheral tissues. Collectively, these data establish a pivotal role for oxidative stress in systemic regulation of insulin and define a key role for the JunD protein in longevity.

Conclusions

In the present review, we have reported novel molecular mechanisms implicated in the interplay between ageing, metabolism and cardiovascular disease. The importance of such emerging signalling pathways is that they may represent common molecular targets to prevent a spectrum of comorbidities driven by senescence, metabolic defects and impaired cardiovascular homeostasis. Premature deregulation of genes involved in oxidative stress, insulin signalling, autophagy and inflammation may anticipate pathological features of the heart and vessels. Of note, most of these pathways are dysregulated in human ageing and a deep understanding of these networks may foster the development of mechanism-based therapeutic approaches to slow down cardiovascular senescence.

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Additional information

Competing interests

None declared.

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