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Changes of the coronary arteries and cardiac microvasculature with aging: Implications for translational research and clinical practice

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ABSTRACT

Aging results in functional and structural changes in the cardiovascular system, translating into a progressive increase of mechanical vessel stiffness, due to a combination of changes in micro-RNA expression patterns, autophagy, arterial calcification, smooth muscle cell migration and proliferation. The two pivotal mechanisms of aging-related endothelial dysfunction are oxidative stress and inflammation, even in the absence of clinical disease. A comprehensive understanding of the aging process is emerging as a primary concern in literature, as vascular aging has recently become a target for prevention and treatment of cardiovascular disease. Change of life-style, diet, antioxidant regimens, anti-inflammatory treatments, senolytic drugs counteract the pro-aging pathways or target senescent cells modulating their detrimental effects. Such therapies aim to reduce the ineluctable burden of age and contrast aging-associated cardiovascular dysfunction. This narrative review intends to summarize the macrovascular and microvascular changes related with aging, as a better understanding of the pathways leading to arterial aging may contribute to design new mechanism-based therapeutic approaches to attenuate the features of vascular senescence and its clinical impact on the cardiovascular system.

Age represents the strongest independent correlate of endothelial dysfunction, as well as being an important non-modifiable risk factor for cardiovascular disease (CVD) (Grundy, 1999; Camici et al., 2009). Since the beginning of the 20th century life expectancy in humans has doubled to more than 80 years and the global population is expected to continue to increase over the next decades, due to a raised longevity

Abbreviations: ACE, Angiotensin-converting enzyme; ADAMTS, A Disintegrin and Metalloproteinase with a Thrombospondin type 1 motif; AGEs, Advanced glycation end-products; AT1R, Angiotensin receptor 1; BAX, BCL2-Associated X Protein; BCL-2, B-cell lymphoma 2; BH4, Tetrahydrobiopterin; BK, Bradykinin; BK-2R, Bradykinin receptor – type 2; CAT, Catalase; cGMP, Cyclic Guanosine Monophosphate; COX-1, Cyclooxygenase – 1; COX-2, Cyclooxygenase – 2; CFR, Coronary Flow Reserve; CV, Cardiovascular; CVD, Cardiovascular disease; ECM, Extracellular Matrix; EDH, Endothelium – dependent hyperpolarization; eNOS/NO, Endothelial Nitric oxide Synthases / Nitric Oxide; EPCs, Endothelial progenitor cells; ET, Endothelin; ETA, Endothelin receptor type A; ETB, Endothelin receptor type B; FATp300, Fatty-Acid Transport Protein 300; FCS, Fetal Calf Serum; G6PD, Gucose-6-phosphate Dehydrogenase; GPx, 1 Glutathione Peroxidase – 1; GPx, 2 Glutathione Peroxidase – 2; H_2O_2, Hydrogen peroxide; HDAC, Histone Deacetylase; HDACi, Histone Deacetylase inhibitors; HIF-1, Hypoxia-inducible Factor 1; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase; ICAM1, Intercellular Adhesion Molecule 1; IGF-1, Insulin-Like Growth Factor 1; IL-6, Interleukin-6; IT, Intimal Thickening; LDL, Low Density Lipoprotein; LV, Left Ventricle; LVH, Left Ventricle Hypertrophy; miRNA, micro-RNA; MCP-1, Monocyte Chemoattractant Protein 1; MMPs, Matrix Metalloproteinases; NADPH, Nicotinamide-adenine dinucleotide phosphate; NCX1, Na + -Ca2 + exchanger 1; NRF2, Nuclear erythroid-2-p45-related factor-2; NO, Nitric Oxide; O_2^-, Superoxide; ONOO, Peroxynitrite; p, 21 Protein 21; PDE-5, Phosphodiesterase type 5; PDGF, Platel-derived Growth Factor; PGI, Prostaglandin I; PGH2, Prostaglandin H2; PPAR, – α Peroxisome proliferator-activated receptor alpha; RAGE, Receptor for Advanced Glycation End Products; RNA, Ribo Nucleic Acid; RNS, Reactive Nitrogen Species; ROS, Reactive Oxygen Species; SIRT, 1 Sirtuin 1; SMCs, Smooth Muscle Cells; SN-6, Na + -Ca2 + exchanger 1 inhibitor; SOD, Superoxide dismutase; TNF-α, Tumor necrosis factor alpha; TxA, Thromboxane A; VEGF, Vascular endothelial Growth Factor; vWF, von Willebrand Factor

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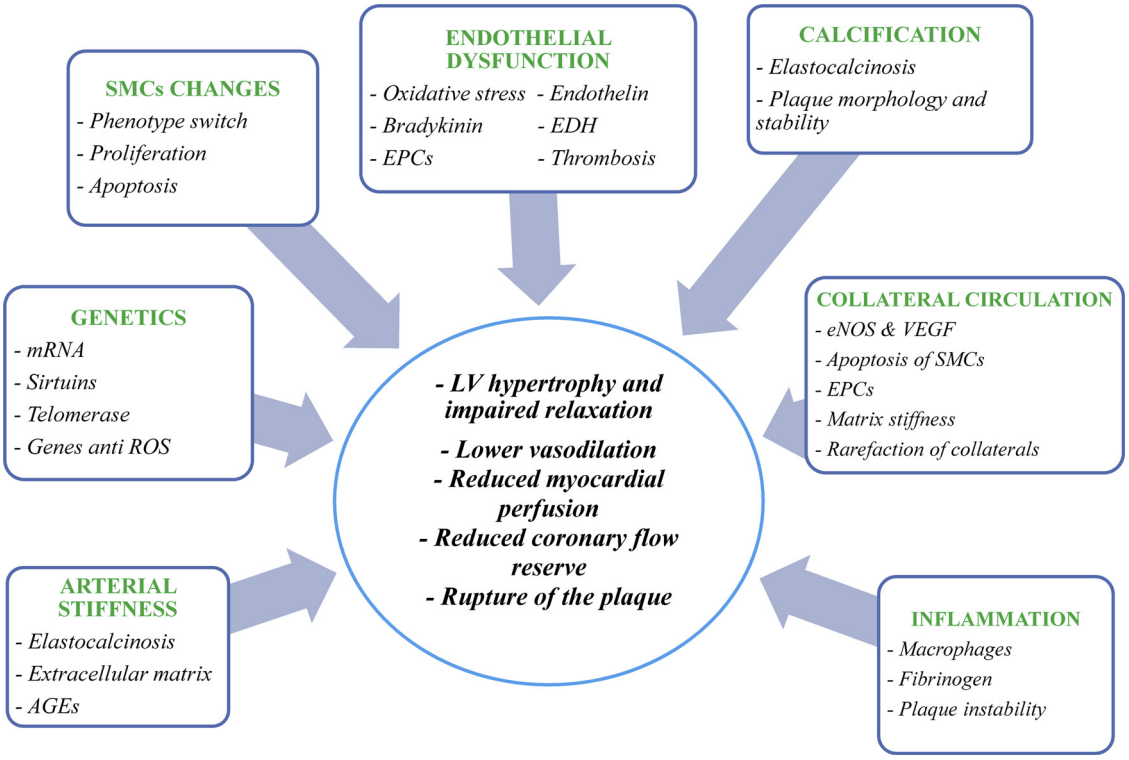

Fig. 1. Aging of the coronary system: from microvascular to macrovascular changes.

and changes in birth rate (Gerland et al., 2014). In 2030, the world population is expected to grow by 23% compared to 2015, whereas the population over 65 years is projected to raise by 57% (Temple and McDonald, 2017). According to World Health Organization, between 2015 and 2050, the proportion of the world's population over 60 years will double from 12% (900 million) to 22% (2 billion), with 80% of older people living in low- and middle-income countries. The financial implications of the aging process are extremely relevant, as individuals aged 65 and over consume most of the health care resources; also, care for patients aged 85 or more costing, on average, twice as much as health care for the younger old (Rich and Adams, 2015; Zolotor and Tippett, 2018). A national economic analysis showed that in 2012 the cost of health care for state residents aged 65 and over was $25 billion, and projections for 2037 show a total cost of care of $69 billion, with an increase of 180% (Zolotor and Tippett, 2018).

Therefore, despite being ineluctable, aging should be considered a process or a problem? A detailed understanding of the aging process is emerging as a primary concern in literature, and vascular aging has become a target for preventive strategy and treatment of CVD therapy (Liu and Chen, 2012). New approaches and the identification of therapeutic targets are needed to reduce the burden of age and contrast aging-associated cardiovascular dysfunction. This narrative review aims to summarize age-associated changes of the coronary arteries and cardiac circulation and the implications of new therapies to prevent organ damage related to vascular dysfunction, trying to enlighten new avenues for future researches.

1. From microvascular to macrovascular changes

Aging is a natural process which develops during our whole life, influenced and modified by both environmental and genetic factors. Aging is linked to significant changes in the morphological structure of the coronary vascular wall, that can be seen at the macroscopic and microscopic level, even in individuals without cardiovascular risk factors (Wang and Bennett, 2012; Kane and Howlett, 2018). Aging affects primarily vascular smooth muscle cells (SMCs) and the endothelial cells, resulting in arterial stiffness and calcifications, that impairs blood flow autoregulation and collateral circulation, ultimately leading to a proatherogenic and ischemic environment. Age-related changes in homeostasis could make human vessels also more susceptible to pathological conditions such as hypertension, diabetes and atherosclerotic disease. Aging causes functional and structural changes in CV system, resulting in a progressive increase of mechanical vessel rigidity and stiffness, due to changes in micro-RNA expression patterns, autophagy, arterial calcification, smooth muscle cell migration and proliferation (Tesauro et al., 2017). These modifications reduce the ability of arteries to dilate and tolerate blood pressure fluctuations (Kotsis et al., 2011), this results in decreased cardiac output and increased pulmonary artery pressure during exercise, which developed as the consequence of both increased pulmonary vasculature resistance and higher left ventricular filling pressures (van Empel et al., 2014). Therefore, forces acting on the arterial wall determine an adaptive response consisting in vascular smooth muscle cells phenotype modification, ultimately resulting in intimal thickening. A condition of intimal thickening is associated with enhanced endothelial permeability, and could represent the substrate on which low-level atherosclerotic stimuli could encourage the progress of atherosclerotic lesions (Tesauro et al., 2017; Roberts and Berry, 2017; Roberts, 2018). It has been noticed an increase of endothelial shear stress with aging (Cuhlmann et al., 2011; Xiao et al., 2013), that leads to endothelial cell death, increased platelet and macrophage adhesion to the arterial wall, thereby resulting in a chronic low-grade inflammation (Franceschi et al., 2000). Arterial stiffening caused by excessive fibrosis, reduced elasticity, increased elastin fiber degeneration with associated increased collagen deposition, laminar medial necrosis, growth of the interstitial space, results in increased vasomotor tone and altered tissue perfusion (Harvey et al., 2016), and all those factors eventually lead to tissue and organ damage (Fig. 1). Markers of coronary aging are summarized in Table 1.

1.1. SMCs changes

From the second decade of life, “intimal thickening” (IT) occurs on

Table 1
Markers of coronary aging.

Marker	Relevance score	Effect
Smooth muscle cells and intima		Lipid accumulation, altered ECM production, calcification of arterial wall, rupture of fibrous cap of atherosclerotic disease
- intimal thickening	***	
- phenotype switch from contractile to secretory	***	
- apoptosis (dysfunctional eNOS/NO signaling)	*	
Endothelial dysfunction		Protein inactivation, severe vasomotor dysfunction, decreased function of EPCs promoting endothelial dysfunction
- ROS enzymatic imbalance (activation of cyclooxygenase, NADPH oxidase and mitochondrial electron transport; inactivation of GPx, SOD and catalase) with elevated oxidative stress	***	
- reduced eNOS expression and activity (PI3K/Ras/Akt/MAPK pathway)	**	
- impaired NO activity (by radicals)	**	
- impaired NO activity (by PDE5)	**	
- formation of peroxynitrite	**	
Endothelin		Impaired vasodilation, platelet activation
- loss of type B receptor on SMCs	*	
- increased effects of type A receptor	*	
Bradykinin		Accelerated endothelial senescence, reduction of bradykinin-mediated vasodilation
- reduced response to circulating levels	*	
- downregulation of type 2 receptor (BK-2R) (signal cascade for prostacyclin and NO)	**	
Endothelium dependent hyperpolarization		Impaired vasodilation in response to neurohumoral molecules and blood flow
- inhibition, associated with reduced NO production	**	
Endothelial progenitor cells		Progression of endothelial dysfunction
- reduction, with impaired turnover of endothelial cells	**	
Predisposition to thrombosis		Increased thrombosis and platelet aggregation
- raised COX-1 and COX-2 levels	*	
- increased PGH2-TxA interaction	**	
- increased production of TxA by endothelial cells	*	
- reduction of PGI2	**	
- increased level of vWF and factor VIII	*	
- decreased function of ADAMTS13	**	
Inflammation		Enhanced inflammation, pro-atherogenic environment with production of growth factors and cytokines favoring SMCs changes
- accumulation of macrophages in the intima	***	
- accumulation of fibrinogen and fibrin in vascular wall	***	
- increased plasma concentration of inflammatory molecules (IL-6, TNFα, MCP-1	*	
Elastocalcinosis		Arterial stiffness, increased circumferential stress on fibrous cap, changes in vascular ECM
- osteoblastic differentiation of SMCs by elastin-derived peptides	***	
- calcium inclusions	***	
- impaired MMP activity	**	
- deposition of advanced glycation end-products	*	
Collateral circulation		Reduced collateral-dependent flow recovery, impaired collateral remodeling, collateral rarefaction
- reduced VEGF expression	*	
- reduced HIF1 expression	*	
- indirect effect on SMCs (favoring apoptosis)	**	
- reduced homing of progenitor cells	*	
- reduced clearance of waste products	**	
Micro RNAs		Apoptosis, inhibition of endothelial cells' reproduction
- increased mRNA-29	**	
- increased mRNA-34	**	
Genetic changes		Impaired antioxidant function, raised NADPH oxidase activity, enhanced stress-induced apoptosis, reduced NO levels, apoptosis, autophagy
- reduced sirtuins activity: SIRT1 (regulates JNK phosphorylation, p53 acetylation, eNOS activity and expression, MMP expression)	**	
- telomeres dysfunction (shortening and uncapping)	**	
- reduced Nrf2 levels (regulatory for antioxidant genes as GPx-1, GPx-2, G6PD and CAT)	*	
- increased NCX1 activity	*	

See text for abbreviations. Relevance score is a semiquantitative evaluation, considering the evidences of the literature, and ranges from 1(*) (indicating a weaker effect) to 3 (***) (indicating a stronger effect).

the arterial wall as a physiological response to forces acting on the wall. This thickening contains various layers of SMCs (intimal SMCs and medial layer SMCs, with different phenotype and function) divided from collagen and elastic fibers; in the coronaries IT has an eccentric development, while a concentric one in the aorta (Movat et al., 1958; Stary, 1987; Virmani et al., 2000). IT is characterized by an increased permeability, demonstrated by the presence of phospholipids and cholesterol in the subendothelial space starting from the third decade of life (Virmani et al., 2000). In this situation of increased permeability, low-level atherogenic factors (such as mild hypertension or diabetes) could favor the growth of an advanced atherosclerotic plaque. Accordingly, Spagnoli and al. proved a raised susceptibility of the aged arterial wall to hypercholesterolemia: older rats, when fed with hyperlipemic diet, presented more extensive and severe atherosclerotic

lesions compared with younger rats, with no differences in serum parameters (Spagnoli et al., 1992, 1991). Moreover, in the advanced age, vascular SMCs modify their phenotype, from "contractile" to "secretory" (Bennett et al., 2016; Iyemere et al., 2006); in normal condition, they exhibit a "contractile phenotype", characterized by high expression of genes which encode proteins involved in holding myofilament structure and function (including Smooth Muscle Protein 22-Alpha / Transgelin variant 1 (SM22α /tagln), Smooth muscle cell myosin heavy chain (MYH11), Smooth muscle cell action (ACTA2), and other) (Shanahan and Weissberg, 1999). On the other hand, intimal SMCs exhibit lower levels of contractile proteins and contain fewer myofilaments compared with SMCs of medial layer; moreover, intimal SMCs are similar to immature and dedifferentiated SMCs (Shanahan et al., 1994; Shanahan and Weissberg, 1998; Shanahan et al., 1993). Campbell et al. demonstrated that adult contractile SMCs, isolated from vessels and cultured in vitro, spontaneously develop a phenotypic change and assume several features of intimal SMCs. Thus, the authors established that SMCs of medial layer may dedifferentiate from a contractile phenotype into a synthetic one when they migrate into the intima (after an injury, or in vitro settings, or in atherosclerosis), whereas the opposite transition (from a "secretory" phenotype into a "contractile" phenotype) occurs during vascular development (Chamley-Campbell et al., 1981, 1979). Furthermore, in vitro settings, SMCs acquired macrophage properties and markers and showed a raised capacity for migration, proliferation and secretion of several cytokines and extracellular matrix proteins (Alexander and Owens, 2012). Several studies on human vascular SMCs showed that phenotypic change is also characterized by the acquisition of features of different mesenchymal lineages, including chondrocytic, osteoblastic and adipocytic (Shanahan et al., 1999; Tyson et al., 2003; Davies et al., 2005; Tintut et al., 2003).

These phenotype changes due to vascular aging may contribute to lipid accumulation, altered matrix production and calcification of arterial wall (Roberts, 1986). Significantly, the resultant of some of these phenotypes is SMCs senescence or death, which may lead to a failure of repair causing weakening and potential rupture of the fibrous cap present in stable atherosclerotic lesions (Shanahan and Weissberg, 1999).

Finally, old SMCs exhibit raised susceptibility to apoptosis, an effect mediated by an aging-related dysfunctional eNOS/NO signaling. Interestingly, levels of phosphodiesterase 5 (PDE5) were increased in cells derived from old mice; furthermore, the effect is attenuated pre-incubating the cells with tadalafil (a PDE-5 inhibitor) (Wang et al., 2011).

1.2. Endothelial dysfunction

Several evidences have shown that aging is related to a progressive reduction in endothelial function both in rats as in humans (Donato et al., 2007; Egashira et al., 1993), even in the absence of cardiovascular risk factors. Furthermore, this endothelial dysfunction appears to occur a decade earlier in men compared to women. In women a fast decline begins at around the time of the menopause: this proves a protective effect of estrogens on the arterial wall (Celermajer et al., 1994).

1.2.1. Reactive oxygen species (ROS) and reactive nitrogen species (RNS)

Several studies have proved that age-related decline in endothelial vasomotor reactivity is linked to altered reactive-oxygen species (ROS) endothelial signaling mechanisms: particularly, impaired nitric oxide (NO) activity and production (Csiszar et al., 2002; LeBlanc et al., 2008) and elevated oxidative stress (Donato et al., 2007; Egashira et al., 1993; Celermajer et al., 1994; LeBlanc et al., 2008; Cooke and Davidge, 2003) - first of all, caused by an ineffective buffering of superoxide ($O2^-$) (Csiszar et al., 2002; Kang et al., 2009). NO is a very important factor which ensures vasodilation, and which shows antioxidant and anti-apoptotic effects. Its synthesis begins from arginine, converted in citrulline and NO through endothelial nitric oxide synthase (eNOS) enzyme on the endothelium. In this setting, tetrahydrobiopterin (BH4) is a useful cofactor, which is needed for the right synthesis of the dimeric form of eNOS. Of note, one of the first hits of impaired endothelial function is exactly the oxidation of BH4 due to oxidative stress; in this way, the function of the monomeric form of eNOS is compromised, encouraging the formation of free radicals of oxygen. Moreover, aging-related changes cause a raised ROS production and decreased ROS degradation through different mechanisms. Aging activates cyclooxygenase, xanthine oxidase nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase and mitochondrial electron transport; on the other hand, it inactivates the antioxidant system, including glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase. ROS inactivate NO and activate the PI3K/Ras/Akt/MAPK pathway, leading to the inhibition of eNOS expression and activity (Higashi et al., 2012).

Equally interesting is the role of PDE5: this enzyme deletes NO from circulation and its expression is very high in the endothelial cells of elderly subjects (Wang et al., 2011). Raised PDE5 activity may result in lower levels of cGMP, an important product of NO signaling that prevents apoptosis, promoted vasodilation and decreased oxidative stress. Thus, high levels of PDE5 could lead to increased oxidative stress and higher sensitivity to apoptosis. PDE5 inhibitors, actually used for erectile dysfunction and pulmonary hypertension, may also have a role in delaying eNOS impairment and guarantee vessel's health (Epstein et al., 2012). Furthermore, as Csiszar and al. showed, the reduction of NO-mediated vasodilation and the augmentation in ROS production leads to a raised formation of superoxide ($O2^-$) (Csiszar et al., 2002). In the presence of the $O2^-$ scavenger superoxide dismutase (SOD), $O2^-$ is converted to hydrogen peroxide (H2O2). Miura et al. proved that endothelial H2O2 causes a flow-induced vasodilation of coronary arterioles, that is abolished by H2O2 scavenger catalase and age-related changes in endogenous scavenging processes, breaking the O2/H2O2 system, cause an impaired coronary reaction to flow-induced vasodilation (Miura et al., 2003). Furthermore, the overproduction of O2- soon inactivates NO, forming the highly reactive and toxic peroxynitrite (ONOO), which soon irreversibly inactivates protein and other molecules in endothelial cells (Quijano et al., 2007). ONOO formation rises with aging, leading to vasomotor dysfunction (Sun et al., 2004).

1.2.2. Endothelin (ET)

ET plays its role through two different receptors: ETA on SMCs (vasoconstriction) and ETB on the endothelium and SMCs (vasodilation and vasoconstriction respectively). In young people, endothelin guides normal blood flow in coronary arteries and regulates basal tone of vessels; in elderly ones, there is a loss of ETB receptors on SMCs (already reduced in number) and consequently a lower vasodilation (Van Guilder et al., 2007).

1.2.3. Bradykinin

The vasodilation conducted by bradykinin works through signaling molecules such as NO and prostanoids. Studies have demonstrated a progressively reduced response to bradykinin in elderly patients (over 65 years old), without relevant changes in local reaction. Moreover, NO inhibition reduces critically bradykinin-mediated vasodilation (Feher et al., 2014). Several studies have demonstrated cardioprotective effects of bradykinin type 2 receptor (BK-2R) (Li et al., 2007; Venema, 2002): BK-2R and its second messengers (prostacyclin and NO) support heart function while loss of BK-2R is linked to different CV disorders (Kakoki et al., 2006); (Maestri et al., 2003); (Emanueli et al., 1999). Nurmi and al. (Nurmi et al., 2012) showed that aging is associated with down-regulation of BK-2R expression in endothelial cells, leading to accelerated endothelial senescence.

1.2.4. Endothelium-dependent hyperpolarization

The vascular tone is regulated by the endothelium not only through

several vasoactive molecules but also through the so called "endothelium-dependent hyperpolarization" (EDH), a pathway related to the hyperpolarization of both endothelial and vascular SMCs (Feletou and Vanhoutte, 1988; Busse et al., 2002). From a biochemical point of view, this pathway involves a raised intracellular Ca 2+ concentration obtained through the activation of transient receptor potential channels (Filosa et al., 2013; Burnham et al., 2002). EDH is especially observed in resistance arteries and helps the regulation of vascular tone, contributing to maintain CV homeostasis and normal blood pressure; indeed, this pathway is activated in response to shear stress by neurohumoral molecules and flowing blood (Feletou, 2016). Alterations in this pathway could lead to the endothelial dysfunction observed in several CV diseases or reversely compensate for the loss in NO bioavailability (Feletou, 2016). In aged rats, several studies observed a reduced EDH together with a progressive decreased release of NO and an increased production of contracting factors (Fujii et al., 1993; Mantelli et al., 1995; Nakashima and Vanhoutte, 1993). Furthermore, aging causes also an important inhibition of EDH in coronary and peripheral arteries (Urakami-Harasawa et al., 1997) and alters conducted dilation in coronary arterioles (Feher et al., 2014).

1.2.5. Endothelial progenitor cells

Endothelial progenitor cells (EPCs) have a significant role in endothelial function. In the early phase of endothelial dysfunction, damaged endothelial cells should be replaced by bone-marrow-derived EPCs; thus, a reduction of EPCs in bone marrow or their decreased mobilization could lead to the progression of endothelial dysfunction (Higashi et al., 2012). A relationship between EPCs and endothelial function exists both in normal subjects and in patients with CVD (Hill et al., 2003; Umemura et al., 2008), suggesting that the number of circulating EPCs could be considered as a predictor of CV events (Werner et al., 2005). The increased oxidative stress seen in aging leads to a reduced NO bioavailability and a decreased function of EPCs: this could produce a vicious circle that promotes endothelial dysfunction in aging (Murasawa et al., 2002; Ballard and Edelberg, 2007).

1.2.6. Predisposition to thrombosis

Aging impairs several mechanisms related to hemostasis, such as coagulation, platelet function and fibrinolysis, favoring thrombosis development. Aging is associated to raised COX-1 and COX-2 levels (Tang and Vanhoutte, 2008; Wong et al., 2009), thus increasing untransformed PGH2, which interacts with thromboxane A (TxA) and induces platelet aggregation. Moreover, with aging, TxA, mainly produced by platelets, is also secreted by endothelial cells (Tang and Vanhoutte, 2008). On the other hand, aging causes a reduction of plasmatic levels of PGI2 (Gotoh et al., 1983, 1982), one of the main molecules by which the endothelium negatively modulates platelets. The final effect is an imbalance between PGI and TxA pathway that promotes thrombosis in the elderly. Furthermore, plasmatic levels of vWF and factor VIII increase with aging, inducing an hypercoagulative state (Conlan et al., 1993; Favaloro et al., 2005). The function of ADAMTS13, the enzyme necessary for cleaving vWF, is also reduced in elderly, leading to an accumulation of high molecular weight multimers of VWF, that are pro-thrombotic and able to link higher amount of platelets (Coppola et al., 2003; Shim et al., 2008). Endothelial NO inhibits platelets aggregation and their adhesion to endothelium (Radomski et al., 1987). As seen in previous sections, aging is characterized by an impaired NO activity and production, thus predisposing to platelet activation and thrombosis. Endothelin 1 (ET-1) is a molecule with pleiotropic effects, including implications in the regulation of hemostasis. In particular, ET-1 activates platelets when linked to endothelin receptor A (ETA) while inhibits them interacting with endothelin receptors B (ETB) (Dockrell et al., 1996; Labonte et al., 2001). In aging, as above mentioned, ETB activity is reduced while ETA-related effects are increased (Van Guilder et al., 2007).

1.2.7. Cardiac effects of endothelial dysfunction

The macroscopic counterpart of endothelial dysfunction is the reduced coronary flow reserve (CFR) (LeBlanc and Hoying, 2016). The myocardium is characterized by a very limited anaerobic tolerance and it needs a continuous supply of oxygen from the coronaries. In normal condition, an immediate increase in coronary flow is possible due to local metabolic feedback, especially releasing or constricting or dilating factors. Endothelial products (such as prostacyclin, NO and endothelin) promote dilation or constriction of SMCs. As previously described, aging-related changes produce endothelial dysfunction, that leads to reduced flow reserve while maintaining baseline flow capacity analogous to young levels (Hachamovitch et al., 1989). Differently, after a vascular insult, such as an ischemic event, both baseline and maximum coronary flow decrease, resulting in at least 50% decline in coronary reserve (Karam et al., 1990; Leblanc et al., 2012). CFR is related to LV systolic, diastolic function (Logstrup et al., 2010) and myocardial viability (Ragosta et al., 2001). Indeed, Czemin and al demonstrated that, in old humans, the impairment in CFR increases the risk for heart failure (Czernin et al., 1993).

1.3. Inflammation

Aging increases inflammation within the vascular wall (Wang and Bennett, 2012). Macrophages are the most important cell type in this process; indeed, inflammation in aged arteries is characterized by an exorbitant accumulation of macrophages in the intima of the arterial wall, and this happens both in the presence as in absence of lipid-rich lesions (Orlandi et al., 2006; Lakatta, 2003). Rodriguez-Menocal et al (Rodriguez-Menocal et al., 2014) showed that, after an injury, arteries of old rats contain more macrophages than those of young animals. They demonstrated raised fibrinogen levels in the arterial wall of aged rats in response to injury; this may reflect an excessive deposition of plasma fibrinogen and an increased local secretion of fibrinogen by platelets. Increased levels of fibrinogen/fibrin could promote rolling, adhesion and infiltration of leukocytes into the vascular wall (Kamath and Lip, 2003), which, in turn, produce growth factors and proinflammatory cytokines favoring an increased proliferation and migration of vascular SMCs (Spinetti et al., 2004). Vascular alterations caused by advanced age create a proatherogenic environment negatively influencing destabilization and progression of atherosclerotic stable plaques. Therefore, histological composition of the plaque and local inflammatory response are influenced by aging (Franceschi et al., 2000; Csiszar et al., 1985); age-related oxidative stress promotes local inflammation, resulting in an increased plasma concentration of inflammatory proteins (including IL-6, TNFα and monocyte chemoattractant protein-1 MCP-1), and thus in the modification of the atherosclerotic plaque (Csiszar et al., 1985; El Assar et al., 2013; Ungvari et al., 2010).

1.4. Elastocalcinosis and arterial stiffness

One of the main feature of vascular aging is a raised arterial stiffness, described as an increased resistance of the vascular wall to dilation (Gavish and Izzo, 2016). Different structural changes are implicated in the age-related stiffness (Cecelja and Chowienczyk, 2016; Hanna et al., 1985); all those mechanisms play towards an increase in wall-to-lumen ratio, resulting in vascular enlargement and consequently worsening blood distribution (Thijssen et al., 2016). First of all, in older humans, calcification occurs in atherosclerotic plaque in the intima and within the tunica media of arterial wall, around elastin fibers (the so called "elastocalcinosis"), leading to a reduced elasticity of the arterial wall (Demer and Tintut, 2008; Niederhoffer et al., 1997). This is an active process in which SMCs go through osteoblastic differentiation, expressing several proteins usually related to bone formation (Demer, 2002) and delivering vesicles into the extracellular matrix used for the storage of hydroxyapatite crystals (Kim, 1976;

Reynolds et al., 2004). Calcific content of an atherosclerotic plaque plays a main role in its destabilization, changing mechanical characteristics of the plaque and promoting its rupture (Alexopoulos and Raggi, 2009). The theoretical model proposed by Vengrenyuk and al (Vengrenyuk et al., 2006) predicts that plaque calcium inclusions intensify the circumferential stress of the fibrous cap and promote his rupture. Several studies confirmed this hypothesis, showing that microcalcifications act as intensifier of the background stress in the cap depending on number, location, alignment and spacing (Maldonado et al., 2012; Cardoso et al., 2014; Kelly-Arnold et al., 2013; Rambhia et al., 2012). Furthermore, as Mauriello and al. showed (Mauriello et al., 2013), coronary calcification can be considered an indirect plaque vulnerability factor, independently of grading of coronary stenosis. Matrix metalloproteinases (MMPs) degrade elements of extracellular matrix, including elastin; then, elastin derived peptides (elastokines or matrikines) activate SMCs osteogenic differentiation, promoting calcification and arterial stiffness (Duca et al., 2004; Yasmin et al., 2006, 2005). Moreover, different studies suggested that age-related formation of advanced glycation end-products (AGEs) in the arterial wall may also lead to increased vascular stiffness (Schwartz and Reidy, 1987; Zieman and Kass, 2004). AGEs can link to collagen, producing changes in the mechanical characteristics of the arterial wall (Nenna et al., 2015). The non-enzymatic glycosylation of some proteins in the vasculature and the inversion of the elastin/collagen ratio (with an increased collagen and a decreased elastin production), enhance the mechanical rigidity of the arterial wall (Sun et al., 2004; Aronson, 2003; Mauriello et al., 1992; Nilsson, 2014; Sell and Monnier, 2012).

The combined decrease in distensibility and elasticity of the arteries induces their reduced compliance causing an increase in systolic and a decrease in diastolic blood pressure. The increased systolic afterload leads to left ventricle hypertrophy (LVH) and consequently to raised LV oxygen requests. Furthermore, LVH enhances the systole's duration and decreases the diastole's length. Thus, oxygen requirements start to be not satisfied due to decreased diastolic pressure and reduced length of diastole (Gavish and Izzo, 2016); myocardial ischemia occurs from higher request and decreased coronary flow, independently of coronary stenosis (Ferro et al., 1995).

1.5. Impairment in collateral circulation

Aging reduces collateral-dependent flow recovery after an acute vascular occlusion, which results in more severe ischemic tissue injury (Moriya and Minamino, 2017; Rivard et al., 1999). Aging causes endothelial dysfunction (Bosch-Marce et al., 2007), damaging eNOS pathways in collaterals (Dai and Faber, 2010), increasing oxidative stress in arteries (Csiszar et al., 2002) and reducing the expression of VEGF (vascular endothelial growth factor) and HIF-1 (hypoxia-inducible factor-1) (Bosch-Marce et al., 2007). Also, aging increases endothelial cells and SMCs susceptibility to apoptosis (Wang et al., 2011; Hoffmann et al., 2001) contributing to impaired collateral remodeling and collateral rarefaction. Furthermore, age-related changes compromise mobilization and homing of mononuclear and progenitor cells from bone marrow to the perivascular region of collaterals (Wang et al., 2011; Xin et al., 2010); in this way, collateral are exposed to reduced secreted growth factors and cytokines that normally contribute to their remodeling. Finally, also the raised arterial stiffness, mediated by changes in extracellular matrix and raised collagen deposition, could impair collateral remodeling (Gavish and Izzo, 2016; Cecelja and Chowienczyk, 2016). Faber and al. demonstrated that aging causes collateral rarefaction and reduced collateral diameter, leading to insufficiency and rarefaction of the collateral circulation (Faber et al., 2011). These effects were related to a drop in collateral diameter, number and remodeling. Moreover, also angiogenesis was impaired by aging in this mice-model and rarefaction was related to altered eNOS/NO signaling in the endothelium. Thus, these findings suggest that aging may contribute significantly to an increased severity of ischemic tissue injury (Faber et al., 2011).

2. Genetic bases of aging

MicroRNAs (mRNAs) are considered key elements in the pathogenesis of several cardiovascular diseases and two of them, mRNA-29 and mRNA-34, are linked to cellular senescence and endothelial functioning. The overexpression of mRNA-29 leads to cellular apoptosis; similarly, mRNA-34 inhibits the endothelial cells' reproduction, favoring cellular death (Ghebre et al., 2016). Moreover, a recent clinical study assessed a lower rate of coronary and Alzheimer's diseases in an 80-year-old population. These patients did not suffer from any major disease (cancer, myocardial infarction, diabetes, dementia) and they were not taking any drug at the time of the study; however, their genetic analysis showed an overexpression of FOXP3 gene and an underexpression of ApoE-ε4 gene (Erikson et al., 2016).

Moreover, sirtuins, NAD + related histone-deacethylase enzymes, have been recently defined as "the fountain of youth", controlling and preventing aging-related diseases. Sirtuins are involved in different metabolic processes, such as fatty acids oxidation, urea cycle, gluconeogenesis and oxidative phosphorylation. Indeed, sirtuins play an important role in adaptation to stress, energy management and cell plasticity (Cencioni et al., 2015). In human species, seven sirtuins families have been described, distributed in mitochondria, endothelial cells and nucleus. SIRT1 is a regulator gene which provides an antioxidant function and reduces stress-induced apoptosis. Specifically, it regulates Jun NH2-terminal kinase (JNK) phosphorylation, p53 acetylation and eNOS activity and expression, attenuating endothelial senescence and alterations (Ota et al., 2007). Indeed, acetylation of p53 favors DNA's stabilization and leads to transactivation of several genes (p21, BAX and ICAM1), inducing cell growth arrest (Tang et al., 2008). Moreover, the reduction in SIRT1 activity during aging promotes an increased acetylation of eNOS and a raised NADPH oxidase activity, resulting reduced levels of NO that leads to endothelial dysfunction (Donato et al., 2011; Zarzuelo et al., 2013). Furthermore, SIRT1 depletion may lead to a down-regulation of MMP-14, favoring a pro-fibrosing phenotype and vascular rarefaction (Vasko et al., 2014).

Telomeres' dysfunction is another crucial factor involved in age-related vascular dysfunction and cellular senescence. When telomeric DNA becomes too short, the cell activates several pathways that lead to apoptosis (Moyzis et al., 1988). Another crucial function of telomeres is the formation of the t-loop at the chromosome's end, creating a so-called "capped" telomere; the T-loop structure preserves DNA from damages by repair machinery. A breakdown of this structure, defined as "uncapping", could lead to a rapid DNA damage and consequent activation of apoptosis' pathway (d'Adda di Fagagna et al., 2003). The telomere uncapping seems to be more relevant than telomere shortening, from a biological point of view. In particular, markers of cellular senescence (such as p-21 induced pathways) are linked to telomere uncapping and not to telomere shortening (Morgan et al., 2018). Interestingly, telomere uncapping is greater in women compared with men, suggesting a possible relation between aging and menopause (Walker et al., 2016). Also, shortened leukocyte telomere length has been associated with subclinical coronary artery atherosclerosis, as expressed by coronary artery calcium (Hunt et al., 2015), and this association might be reverted by a regular physical lifestyle (Diaz et al., 2010); however, those new findings warrant further investigations.

As previously described, aging-related decline in endothelial vasomotor activity is linked to the accumulation of ROS and, consequently, an increased oxidative stress. Cellular defense to reduce and balance this increased oxidative stress involves several transcription factors and signaling proteins which regulate the transcription on different genes encoding anti-oxidants enzymes and proteins. Among these, a transcription factor named nuclear erythroid-2-p45-related factor-2 (Nrf2) regulates different antioxidant genes (such as GPx-1, GPx-2, G6PD and CAT) (Osburn and Kensler, 2008). In normal oxidant condition, Nrf2

binds to specific DNA sequences and promotes the transcription of genes encoding anti-oxidant agents (Pickering et al., 2012), attenuating oxidative stress and the related pathological processes. Zhou and al. (Zhou et al., 2014) showed that the age-related decline in Nrf2 levels could make the cardiovascular system more vulnerable to dysfunction and to heart failure development.

NCX1 is a plasma membrane channel protein highly expressed in heart, kidney and brain. Particularly, it could extrude Ca^{++} from the cytosol, exchanging for Na^{+} as a forward, or vice-versa (Na^{+} out and Ca^{++} in) as a reverse mode (Lytton, 2007). Several studies have shown NCX1 works in a reverse mode in different pathological conditions, including myocardial necrosis, cancer, diabetes and arrhythmias (Antoons et al., 2012; Svensson et al., 2011). Osanai et al. Osanai et al. (2018) demonstrated that NCX1 plays an important role in aging: treatment with SN-6, a NCX1 inhibitor, leads to the reduction of the expression of aging hallmarks (chromatin remodeling, histone modification, impairing autophagy). Furthermore, they discovered a new gene named NM-026333 that bounds NCX1 suppressing its reverse mode, suggesting it as a novel anti-aging gene Osanai et al. (2018).

3. From vascular to structural changes in the aging heart

Changes in coronary microvasculature are linked to both structural and functional changes in the aging heart, that are intrinsically associated with heart failure and arrhythmias (Kannel et al., 1987; Strait and Lakatta, 2012). Structurally, the heart thickens and stiffens with age resulting in the increased imposition of a number of functional demands; functionally, a number of changes which assist the resting heart to deal with the effects of aging cause significant functional deficits with exercise or stress, thereby lowering the cardiac reserve; additionally, the aging heart has a reduced number of cardioprotective mechanisms and repair process (Strait and Lakatta, 2012).

Cardiac fibroblasts and connectival ECM are considered crucial mediators of fundamental biological processes occurring in the aging heart. However, it is extremely difficult to discern whether those changes reflect a direct effect of aging on cardiac structures (e.g. ECM) or are the consequence of the aged vasculature supplying those tissues. The dynamic profile and polyhedral properties of cardiac fibroblasts account for their role in ECM remodeling, modulation of post-injury tissue homeostasis, regulation of cardiomyocyte function and influence in the normal activity of the conduction system (Nguyen et al., 2014). While cardiac fibroblasts are responsible of deposition of altered ECM components during pathophysiological states (Cieslik et al., 2014), it is increasingly evident an inverse modulating action exerted by the ECM itself on the fibroblasts, regulating their secretory activities or phenotype switches and eventually realizing a amphidromous circle (Dobaczewski et al., 2012). Therefore, alterations of ECM function in the elderly might exert a detrimental effect on the overall ventricular function and cardiac performance with enormous reflexes on the clinical scenario. The clinical picture of the aging ventricle is mainly characterized by progressive increase in LV wall thickness with a typical pattern favoring the interventricular septum more than the free wall (Kovacs et al., 2009). The result is a progressive shift in the overall LV shape which abandons the typical elongated ellipsoid geometry to a more spherical profile with significant implication on contractility efficiency (Hees et al., 2002). The spherical shape leads to an increased wall stress: rather than systolic LV function, aging seems to predominantly affect the diastolic function. Echocardiographic and radionuclide studies demonstrated that the typical early diastolic filling is progressively retarded in aged population (Downes et al., 1989) and that myocardial diastolic stiffness is augmented with prolonged isovolumic relaxation, also suggesting an age-related impairment of calcium accumulation by the sarcoplasmic reticulum (Strait and Lakatta, 2012; Fleg and Strait, 2012). Increased wall thickness and augmented ventricular stiffening also predispose to subendocardial ischemia, because of an increased distance between epicardial coronary arteries and subendocardial myocardial cells, in association to augmented LV fibers afterload and oxygen demand. To this extent, a mismatch between oxygen demand and capillary growth has been demonstrated in older hearts (Hachamovitch et al., 1989). In addition, the well-known increased peripheral pressures, blunting the intramyocardial perfusion pressure gradient. These age-related structural and functional changes might overlay to the prolonged exposition of the elderly to cardiovascular risk factors, hypertension, and susceptibility to atherosclerosis, so that coronary events and heart failure become more common in this subpopulation (Fleg and Strait, 2012). Occurrence of ischemic events over this aged substrate is even more daunting in the clinical and surgical scenario carrying increased rate of complications and mortality. Dilated cardiomyopathy resulting from aging process and superimposed ischemic disease are often requiring medical or surgical therapy and a comprehensive knowledge on the biological alterations occurring in these circumstances is fundamental to develop targeted and efficient therapeutic strategies (Strait and Lakatta, 2012; Chantler and Lakatta, 2012).

Considering the electrical side of the heart, it is known that aging is associated with a generalized increase in elastic and collagenous tissue which result in fat accumulation around the sinoatrial node (Strait and Lakatta, 2012). Also, the increased LV wall thickness and left atrial size alter ECM deposition and increase left atrial volume, and this is associated with increased incidence of arrhythmias (Spadaccio et al., 2015). In addition, aging is associated with enhanced sodium/calcium (Na/Ca) exchanger function that results in cytosolic Ca2+ overload (Hakim and Shen, 2014). Atrial fibrillation (AF) is found in approximately 5% of subjects over age 60 years, a rate 10-fold higher than the general adult population, and its incidence is known to increase with age (Strait and Lakatta, 2012; Lin et al., 2018); (Andrade et al., 2014); (Rahman et al., 2014). Approximately 70% of patients with AF are between 65 and 85 years old, and it is projected that by 2050 most of patients with AF will be aged 80 or more (Hakim and Shen, 2014; da Silva, 2015). Improved recognition of the underlying mechanisms leading to AF appears to be crucial to develop improved methods for AF prevention and management in the elderly (Andrade et al., 2014; Morin et al., 2016), as many clinically relevant issues are known to complicate AF treatment in such fragile patients (comorbidities, organ dysfunction, drug interactions, side effects, poor compliance to medications) (Hakim and Shen, 2014).

4. Implications for clinical practice and translational research

For the above considerations, it appears how important is to ensure a healthy advanced age among other social and economic issues. Molecular mechanisms underlying aging may be candidate for targeting by interventions to prevent and delay the arteries' aging (Tesauro et al., 2017). Therapeutic strategies focused on multi-target interventions (Fig. 2) rather than single target therapy could provide greater potential to reduce atherosclerosis and vascular aging (Liu and Chen, 2012). An appropriate control of aging process represents the main target and may explain the efficacy of many approaches (both pharmacological and nutritional) currently used in clinical practice (Scioli et al., 2014). Therapeutic strategies to delay coronary aging are summarized in Table 2.

4.1. Aerobic exercise

The term "modifiable molecular mediators" (MMM) (Barton, 2014) identifies biological factors, like ET-1 or Angiotensin II, that could be targeted either by drugs or also by other interventions, in order to slow down or even reverse the pathological process. Regular physical exercise is one of the most powerful means to increase the bioactivity of nitric oxide (NO), which is an important inhibitor of ET-1 production and activity; additionally, exercise training normalizes the age-associated shift in redox balance (Camici et al., 2015). Results from several randomized trials indicate that regular aerobic exercise training

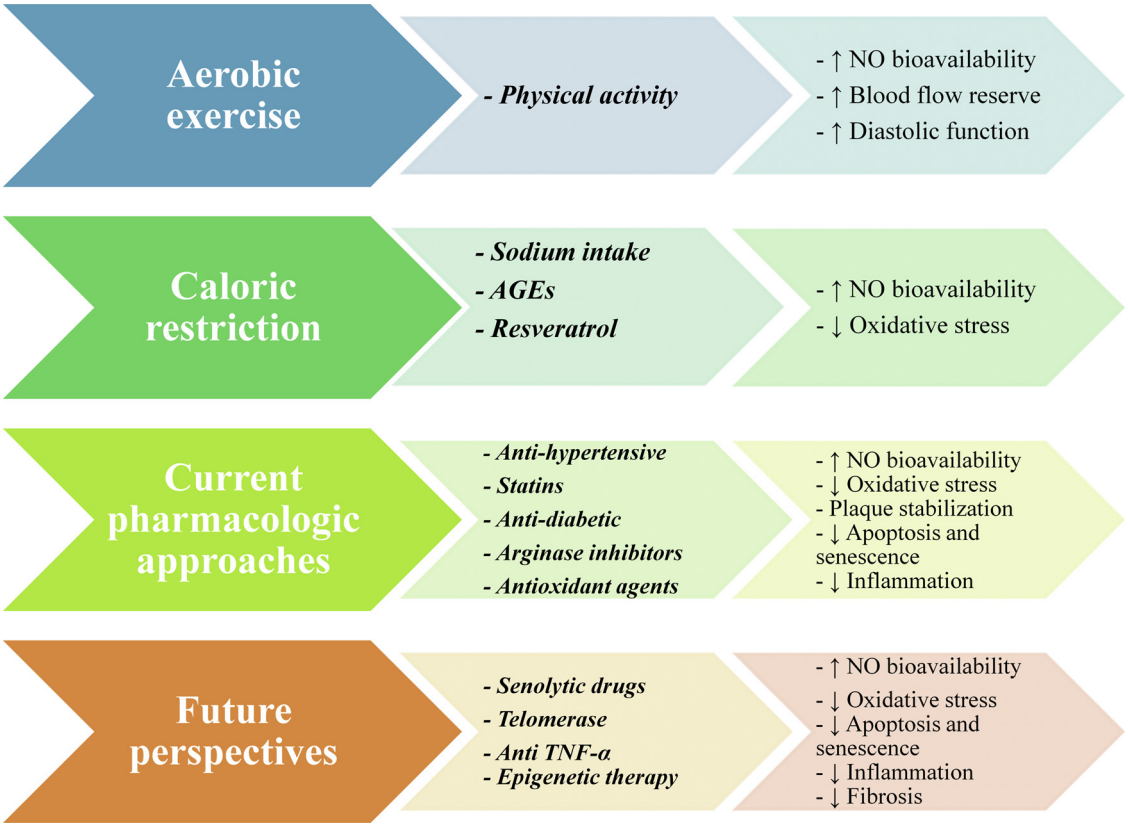

Fig. 2. Clinical practice: multi-target interventions and their effects.

improve both the function and structural health of the arterial walls (Fontana, 2018; Nowak et al., 2018). Exercise actualizes its beneficial effects on endothelium through different mechanisms: provisional increases in shear stress, raised systemic metabolism, anti-inflammatory actions (such as suppression of TNFα), protection against TNFα-induced vascular impairment (Ungvari et al., 2008). Interestingly, physical training increases the activity of SIRT-1 by enhancing nicotinamide phospho-ribosyl-transferase and thereby the production of sirtuin-fueling NAD+ (Camici et al., 2015). Physical activity, regularly practiced at any age, may restore both mechanical and functional capacity of the vascular SMCs of coronary arteries, promoting a better distribution of coronary blood flow and oxygen supply in the endocardial and epicardial regions (Hanna et al., 1985). Moreover, prolonged aerobic exercise has been established to enhance coronary blood flow reserve to adenosine infusion and coronary blood flow responses to intracoronary administration of acetylcholine, with larger benefits in populations with cardiometabolic disorders (Ashor et al., 2015; Laughlin et al., 2012). The results of a clinical study (Riordan et al., 2008) enrolling nonobese, middle-aged people of both sexes without clinical evidence of cardiovascular disease have showed that endurance-exercise-induced and caloric restriction-induced weight loss improve diastolic function, as suggested by the decrease in isovolumic relaxation time. Therefore, the improvements in diastolic function that occurred in response to exercise and caloric restriction weight loss, may be considered as the restoration of a more youthful cardiac phenotype (Fontana, 2018; Leibowitz et al., 2016; Abou et al., 2017).

No male/female differences have been found after a 3-months walking aerobic exercise training (Moreau et al., 2003; Tanaka et al., 2000), and the articles that specifically examined the gender effect in response to more intense aerobic exercise confirmed no significant differences (Nowak et al., 2018; Whipple et al., 2018). Individual habits and functional capacities are emerging as new determinants of exercise program, as a high interindividual variability in response of maximal oxygen consumption to aerobic exercise was prevalent among older adults, ranging from 2% to more than 60% (Whipple et al., 2018). Age, sex, race, and body mass index are unrelated with non-response to aerobic exercise (Whipple et al., 2018). Therefore, future aerobic programs should be tailored to assist older adults in achieving optimal benefit from exercise programs through a personalization of the physical activities taking into account individual capacity (Fontana, 2018).

4.2. Caloric restriction

Data indicate that intermittent fasting and adjusted diurnal rhythm of feeding, lowered intake of protein and specific amino acids, and nutritional modulation of the microbiome can also be important to prevent senescence (Fontana and Partridge, 2015). The anti-aging action of caloric restriction seems to be linked to the attenuation of the age-associated increase in oxidative stress (Bevilacqua et al., 2005; Sohal and Weindruch, 1996) and to enhanced NO bioavailability (Jablonski et al., 2013). The specific mechanisms by which caloric restriction promotes a youthful endothelial phenotype involve a plethora of metabolic pathways (Ungvari et al., 2010); first of all, it improves upregulation and activation of eNOS, with subsequent increased bioavailability of NO. In addition, caloric restriction increases insulin sensitivity, decreasing the activation of receptor for advanced glycation end products (Fontana, 2018; Weiss et al., 2006), reduces oxidative stress-induced DNA damage (Fontana, 2018; Hofer et al., 2008) and enhances adipokine and cytokine production, acting on dyslipidemia and chronic inflammation (Fontana et al., 2004; Meydani et al., 2016). Interestingly, dietary restriction of AGEs was evaluated as an effective and easy-to-use method to reduce the overall amount of AGEs (Nenna et al., 2015), with potential benefits in cardiovascular complications by reducing glycation and oxidation of LDL (Cai et al., 2004).

Dietary sodium restriction represents an important public health strategy to maintain or restore healthy vascular aging. Also, dietary

Table 2
Therapeutic strategies to delay coronary aging.

Strategy	Microvascular effect	Macrovascular effect
Aerobic exercise	increased NO bioactivity optimized redox balance optimized endothelial function (suppression of TNFα, expression of SIRT-1)	increased blood flow reserve improved diastolic function
Caloric restriction	attenuated oxidative stress enhanced upregulation and activation of eNOS increased NO bioavailability increased insulin-sensitivity reduced activation of receptor for AGEs reduced oxidative stress-induced DNA damage	improved diastolic function improved heart rate variability reduced arterial stiffness optimization of blood pressure
Anti-hypertensive drugs (ACEi or ARB)	improved endothelial function reduced structural adverse remodeling reduced arterial wall fibrosis reduced accumulation and activation of inflammatory cells	reduced mean arterial pressure improved vascular reactivity decreased mortality from myocardial infarction
Statins	reduced LDL plasmatic level improved endothelial function improved NO bioavailability reduced vascular inflammation reduced progression of intimal thickening stabilization of atherosclerotic plaque reduced endothelin-1, increased prostacyclin inhibited AGEs-induced RAGE expression inhibition of ROS and ROS-mediated telomere shortening inhibition of SMCs apoptosis protection against DNA damage (via inhibition of prenylation)	improved renal and cardiovascular function
Anti-diabetic drugs (metformin)	decreased insulin levels decreased IGF-1 signaling reduced ROS production and DNA damage	promotes weight loss
Arginase inhibitors	increased NO bioavailability	needs further validation for specific effect
Antioxidant agents (resveratrol)	activation of SIRT-1 inhibition of NF-kB upregulation of eNOS and antioxidant enzymes prevention of ROS-induced apoptosis improved insulin sensitivity	needs further validation for specific effect
Senolytic drugs (e.g. dasatinib)	increased NO bioavailability (?) senescence delaying activity (?)	needs further validation for specific effect
Telomerase	senescence delaying activity (?)	needs further validation for specific effect
Anti TNF-α	reduced apoptosis (?) downregulation of NADPH oxidase activity (?)	needs further validation for specific effect
Epigenetic therapy (e.g. FATp300 inhibitors, HDACi, specific miRNA inhibitors)	reduced fibrosis (?)	needs further validation for specific effect

See text for abbreviations.

sodium restriction rapidly improves carotid artery compliance, an important index of arterial stiffness, in middle aged and older adults with moderate hypertension (Nowak et al., 2018). Long-term caloric restriction results in profound and sustained beneficial effects on the major risk factors for cardiovascular disease, and was associated with improved diastolic function and improved heart rate variability, indicating that caloric restriction might retard the age-associated changes in cardiovascular function (Fontana, 2018). The traditional Mediterranean diet, which is characterized by a low consumption of animal products and high intake of vegetables, minimally processed grains, fruits, extra-virgin olive oil, and a small intake of red wine, is very high in antioxidant and anti-inflammatory compounds. In the new analysis of the PREDIMED trial, patients with Mediterranean diet supplemented with olive oil, compared with a control diet, had a significant reduction in circulating oxidized-LDL and inflammatory markers, notably associated with a 30% reduction in the incidence of major cardiovascular events over a median follow-up of 5 years (Estruch et al., 2018). Based on a theoretic ideal intake of 1800–2000 for women and 2000–2200 kcal for men, some dietary recommendations were made (Estruch et al., 2018; Katz and Meller, 2014): fresh fruits and vegetables should for 2 or more servings per day, fish and legumes for 3 or more servings per week, white meat instead of red meat which should be limited to 1 serving each other day. Soda drinks, spread fats and commercial bakery goods or sweets should be limited.

4.3. Current pharmacologic approaches

4.3.1. Anti-hypertensive drugs

The evidence suggests that antihypertensive drugs improve endothelial function, mean arterial blood pressure, vessel tone, and reduce structural remodeling. Angiotensin-converting enzyme (ACE)-inhibitors improve smooth muscle hypertrophy (Jani and Rajkumar, 2006), reduce arterial wall fibrosis, decrease accumulation and activation of inflammatory cells. ACE-inhibitors improve endothelial function in epicardial, brachial, renal and subcutaneous circulation (Virdis et al., 2011). Angiotensin II type I receptor (AT1R), expressed in the vascular cells, is a mediator of vascular aging and its vascular effects may be reduced by chronic AT1R blockage (ARB) (Papakatsika et al., 2016). Inhibition of endothelial ACE is able to reverse complex phenotype of activated pro-thrombotic, pro-inflammatory endothelium towards healthy quiescent phenotype, and this contributes significantly to their clinical benefits, such as decrease in mortality from myocardial infarction and stroke (Kampoli et al., 2012). Several studies showed beneficial effects of Ace inhibitors on endothelial dysfunction and, in particular, ACEi with a sulfhydryl (SH) group allow an improved endothelial function (Buikema et al., 2000; Pasini et al., 2007); indeed, the SH-groups are released in the vascular wall, stabilizing NO and preventing its breakdown by circulating free radicals.

4.3.2. Statins

Statins are HMG-CoA reductase competitive inhibitors, able to induce a reduction of HMG- CoA conversion into mevalonate and subsequently into cholesterol (Istvan and Deisenhofer, 2001), resulting in a significant reduction of hepatic cholesterol synthesis and enhanced local expression of LDL receptors. Thus, statins improve removal of circulating LDL lowering their plasmatic levels (Biasucci et al., 2010). Statins also improve endothelial function, reduce vascular inflammation, reduce progression of intimal thickness in coronary arteries and stabilize atherosclerotic plaque (Jani and Rajkumar, 2006). Statins reduce the levels of endothelin-1 by decreasing the mRNA of its precursor pre-proendothelin-1, increase the production of the vasodilator prostacyclin, reduce LDL oxidation and reduce inflammatory mediators, such as C-Reactive Protein (Ghaffari et al., 2011; Mason et al., 2004). They represent the pleiotropic drugs for excellence (Nenna et al., 2015), improving renal and cardiovascular function also in diabetic patients (Akira et al., 2006). Moreover, statins dose-dependently inhibit AGEs-induced RAGE expression, ROS and apoptosis via PPAR-alfa independent mechanisms (Ishibashi et al., 2012). Other potentially implicated pathways targeted by statins are: modulation of smooth muscle tone by increasing NO bioavailability (Wang et al., 2012); reduction of intracellular reactive oxygen species (ROS) generation (Hong et al., 2006; Manfredini et al., 2010); immuno-modulatory actions (Hakamada-Taguchi et al., 2003; Kwak et al., 2000); stimulation of the angiogenic process (Shen et al., 2011). Recently, statins have been also demonstrated to reduce cholesterol levels in erythrocyte membranes, considered a marker of clinical instability in patients with coronary artery disease (Tziakas et al., 2009). They improve mobilization, differentiation and prevention of cellular senescence, reducing of apoptosis of different vascular cell types (Assmus et al., 2003; Satoh et al., 2009). Furthermore, statins may protect against DNA damage (Olivieri et al., 2012), by inhibition of protein prenylation involved in inducing DNA damage, inhibition of downstream signaling from damaged DNA and acceleration of repairing damaged DNA (Mahmoudi et al., 2008; Wassmann et al., 2002). Statin treatment contributes to minimizing telomere shortening by inhibition of ROS production (Chen et al., 2010; Puccetti et al., 2011).

4.4. Anti-diabetic drugs

Untreated diabetic patients have anticipated manifestations of aging, such as vascular damage (Anisimov, 2013). In this settings, metformin has been demonstrated to have relevant pleiotropic effects, although the exact mechanisms of action remains unclear (Barzilai et al., 2016). Metformin leads to decreased insulin levels, decreased IGF-1 signaling (Liu et al., 2011), inhibition of mitochondrial complex 1 in the electron transport chain, reduction in endogenous production of ROS (Batandier et al., 2006); (Bridges et al., 2014); (Zheng et al., 2012) and DNA damage (Algire et al., 2012). Metformin may also modify arterial stiffness and induce lower blood pressure by promoting weight loss (Agarwal et al., 2010). Furthermore, this drug favorably influences other metabolic and cellular processes closely associated with the development of age-related conditions, such as inflammation (Saisho, 2015), autophagy (Song et al., 2015; Xie et al., 2011) and cellular senescence (Jadhav et al., 2013; Moiseeva et al., 2013).

4.4.1. Arginase inhibitors

L-arginine is an aminoacid used as a substrate for endothelial nitric oxide synthase for the synthesis of NO (Tentolouris et al., 2000; Tousoulis et al., 2002). Increased arginase enzyme activity may lead to local L-arginine deficiency in certain intracellular compartments and subsequently decreased NO production (Kampoli et al., 2012). Thus, arginase inhibitors may improve microvascular function and mitigate myocardial injury following ischemia–reperfusion by increasing NO bioavailability (Gronros et al., 2013), but this hypothesis should be validated in clinical settings.

4.4.2. Antioxidant agents

The most important mechanism by which oxidative stress influence endothelial function is the inactivation of nitric oxide by superoxide anions and oxidized LDLs (Antoniades et al., 2004; Ohara et al., 1993); thus, based on these findings, researchers tried to find a relation between several antioxidants and endothelial function (Kampoli et al., 2012). Vitamin E was found to preserve endothelial vasomotor function in patients with coronary atherosclerosis and its supplementation corrects the oxidant/anti-oxidant balance in plasma; however, no direct effect inside the atherosclerotic plaques was observed with the administration of this molecule (Heart Protection Study Collaborative G, 2002). Acute ascorbic acid administration increases muscle blood flow during dynamic exercise and this is associated with improved endothelium-dependent vasodilation increasing the bioavailability of NO derived from the NOS pathway (Kampoli et al., 2012). Some works focused the attention on antioxidant agents (Armour et al., 1985) and free radical scavengers such as N-acetyl-cysteine and propionyl-L-carnitine (Radomski et al., 1987; Brass et al., 2013), to prevent or reduce the age progression of organ microvascular dysfunction and myocardial remodeling. Resveratrol, a polyphenolic SIRT1-activating compound found in grapes, wine, nuts and other plants source, has been demonstrated to exert several beneficial effects on cardiovascular and cerebrovascular diseases in animal and in vitro models performed on endothelial cells (Camici et al., 2015). Resveratrol promotes vasoprotection through different molecular mechanisms: direct inhibition of NF-κB, upregulation of eNOS and antioxidant enzymes, induction of mitochondrial biogenesis, prevention of oxidative stress–induced apoptosis (Ungvari et al., 2010; Csiszar et al., 2009); (Csiszar et al., 2008); (Ungvari et al., 2007). Moreover, it also improves insulin sensitivity and induces lower plasma glucose levels (Banks et al., 2008; Carrizzo et al., 2013).

4.5. Future perspectives

4.5.1. Senolytic drugs

Several pathologies derive from senescence of general population. For this reason, it is useful to target therapies or prevention strategies for delay diseases of elderly. A newer class of drugs with this aim is represented by “senolytics”; these molecules, discovered in 2015, may be used to inhibit and slow aging pathways involving in the development of several pathologies (Zhu et al., 2015). Among these molecules, Dasatinib, quercetin and navitoclax are directed toward BLC-2 apoptotic pathway. Indeed, senolytic treatment resulted in significant reduction in senescent cell markers in an animal model of aging (Roos et al., 2016). Moreover, senolytic treatment significantly improved vasomotor function due to an important increase in NO bioavailability in aged mice (Roos et al., 2016). Tocotrienols and quercetin, albeit belonging to different phytochemical classes, display similar and promising effects "in vitro" when tested in normal and cancer cells. Both compounds have been shown to induce senescence and promote apoptosis in a multitude of cancer lines (Malavolta et al., 2016). Conversely, they demonstrate senescence delaying activity in primary cells and rejuvenating effects in senescent cells. Using single or combination of senolytic drugs should prolong lifespan and improve vascular reactivity to acetylcholine and nitrates (Kirkland et al., 2017). Moreover, some studies prove that mice with reduced “senescence burden”, when treated with senolytic drugs, show better cardiac contractility and consequently major ejection fraction. Other advantages of this pharmacological class are reliability with short-cycle administration and low side effects also if two drugs in combination are used (Kirkland and Tchkonia, 2017).

4.5.2. Telomerase

Evidence suggests that cardiovascular repair systems become progressively impaired with age, due to reduced production of proangiogenic factors and a decreased number and function of stem cells

(Minamino and Komuro, 2008). Several studies have shown that somatic cells, including vascular cells, develop progressive telomere shortening due to low telomerase activity. The introduction of telomerase in vitro into human endothelial cells may prevent progression of senescence-related endothelial dysfunction, including the increase in monocyte adhesion to endothelial cells and the decrease in endothelial nitric oxide synthase activity (Matsushita et al., 2001; Minamino et al., 2002), but further studies are warranted.

4.5.3. Anti TNF-α

Vascular aging is associated with deregulation of TNF-α expression (Csiszar et al., 2002; Scioli et al., 2014), whose level increases with aging and correlates with morbidity and mortality in the elderly patients (Bruunsgaard et al., 2000; Harris et al., 1999). Therefore, an anti-TNF-α treatment may perform vasoprotective effects, with a reduction of endothelial cell apoptosis and the downregulation of NAD(P)H oxidases activity (Csiszar et al., 2007). Future studies will elucidate these mechanisms and their clinical implications.

4.5.4. Epigenetic therapy

In the last 25 years, multiple studies have investigated the molecular basis of organ fibrosis and highlighted its multi-factorial genetic, epigenetic, and environmental regulation (Watson et al., 2016); (Zhang et al., 2017); (Ghosh et al., 2017). Epigenetic changes, such as DNA methylation, histone modifications, and miRNAs have been involved in vascular aging (Zhang et al., 2017) and reverting such epigenetic transformations might reduce age-associated myocardial fibrotic degeneration. Demethylating agents, such as 5-aza-2′-deoxycytidine, 5-azacytidine, some selective histone deacetylase inhibitors (including mocetinostat, trichostatin A, and MPT0E01), have been shown to have a direct action on important inducers of cardiac fibrosis (Watson et al., 2016; Grimaldi et al., 2017). Targeting FATp300 might reduce pathologic profibrogenic signaling and organ fibrosis. Though few FATp300 inhibitors have been tested, exhaustive in vivo studies are needed to determine their pharmacokinetics, safety and possible teratogenic effects. Additionally, while excessive levels of FATp300 are detrimental, normal functioning of FATp300 is necessary for tissue homeostasis. Hence, normalization of elevated FATp300 signaling— rather than complete suppression—by safe, soluble, cell permeable inhibitors is an attractive anti-fibrosis strategy. Based on in vitro and in vivo studies, histone deacetylases inhibitors (HDACi) have also generated considerable interest as novel antifibrotic drugs. Although multiple HDAC inhibitors are being tested in clinical trials for cancer treatment, no clinical trial utilizing HDAC inhibitors for cardiac fibrosis has been published. It is important to highlight those HDACs whose alteration is primarily dictated by the initiating stress and altered HDACs affect multi-gene expression. Hence, it is necessary that only the disease-mediating specific classes of HDACs are targeted to avoid any potential adverse effects of pan-HDAC inhibition (Ghosh et al., 2017).

At present extensive studies on the altered expression profiles of cardio-protective and cardio- pathologic microRNAs in hypertension- or MI-induced myocardial fibrosis have been well documented. As individual miRNA can influence multiple pathways simultaneously in a context dependent-manner, normalizing the levels of that altered miRNA either by overexpression (antifibrogenic miRNA) or silencing (profibrogenic miRNA) will normalize multiple pathways associated with profibrogenic responses and prevent cardiac fibrogenesis. Therefore, targeting specific microRNA may be a potential therapeutic approach to halt the progression of cardiac fibrosis and improve cardiac functions, but studies are lacking. Additionally, targeting any specific microRNA to ameliorate cardiac fibrogenesis will be challenging as each microRNA targets numerous mRNAs in a cell and signal specific manner (Zhang et al., 2017; Ghosh et al., 2017).

5. Conclusion

Aging has never been so young and innovative in medical literature, and the renovated interest for new approaches and new therapeutic targets to reduce the burden of aging-associated cardiovascular dysfunction will have profound implications in the near future. Microvascular changes obviously play a pivotal role for end-organ damage related to vascular dysfunction. In an attempt to reduce the distance between the clinical side of the research on aging, mainly limited to descriptive statistics, and its biological side, focused on the molecular mechanisms, the possible points of cross-talking should be investigated in future researches with “from bench to bedside” and “from bedside to bench” approaches.

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Declaration of Competing Interest

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.mad.2019.111161.

References

Grundy, S.M., 1999. Age as a risk factor: you are as old as your arteries. Am. J. Cardiol. 83 (1455-1457), a1457.
Camici, G.G., Sudano, I., Noll, G., Tanner, F.C., Luscher, T.F., 2009. Molecular pathways of aging and hypertension. Curr. Opin. Nephrol. Hypertens. 18, 134–137.
Gerland, P., Raftery, A.E., Sevcikova, H., Li, N., Gu, D., Spoorenberg, T., Alkema, L., Fosdick, B.K., Chunn, J., Lalic, N., Bay, G., Buettner, T., Heilig, G.K., Wilmoth, J., 2014. World population stabilization unlikely this century. Science 346, 234–237.
Temple, J.B., McDonald, P.F., 2017. Population ageing and the labour force: 2000-2015 and 2015-2030. Australas. J. Ageing 36, 264–270.
Rich, P.B., Adams, S.D., 2015. Health care: economic impact of caring for geriatric patients. Surg. Clin. North Am. 95, 11–21.
Zolotor, A.J., Tippett, R., 2018. Running the numbers: aging and cost of health care in North Carolina. N. C. Med. J. 79, 66–69.
Liu, Y., Chen, K.J., 2012. Atherosclerosis, vascular aging and therapeutic strategies. Chin. J. Integr. Med. 18, 83–87.
Wang, J.C., Bennett, M., 2012. Aging and atherosclerosis: mechanisms, functional consequences, and potential therapeutics for cellular senescence. Circ. Res. 111, 245–259.
Kane, A.E., Howlett, S.E., 2018. Differences in cardiovascular aging in men and women. Adv. Exp. Med. Biol. 1065, 389–411.
Tesauro, M., Mauriello, A., Rovella, V., Annicchiarico-Petruzzelli, M., Cardillo, C., Melino, G., Di Daniele, N., 2017. Arterial ageing: from endothelial dysfunction to vascular calcification. J. Intern. Med. 281, 471–482.
Kotsis, V., Stabouli, S., Karafillis, I., Nilsson, P., 2011. Early vascular aging and the role of central blood pressure. J. Hypertens. 29, 1847–1853.
van Empel, V.P., Kaye, D.M., Borlaug, B.A., 2014. Effects of healthy aging on the cardiopulmonary hemodynamic response to exercise. Am. J. Cardiol. 114, 131–135.
Roberts, W.C., Berry, A.E., 2017. Frequency of coronary endarterectomy in patients undergoing coronary artery bypass grafting at a single tertiary Texas hospital 2010 to 2016 with morphologic studies of the operatively excised specimens. Am. J. Cardiol. 120, 2164–2169.
Roberts, W.C., 2018. Quantitative extent of atherosclerotic plaque in the major epicardial coronary arteries in patients with fatal coronary heart disease, in coronary endarterectomy specimens, in aorta-coronary saphenous venous conduits, and means to prevent the plaques: a review after studying the coronary arteries for 50 years. Am. J. Cardiol. 121, 1413–1435.
Cuhlmann, S., Van der Heiden, K., Saliba, D., Tremoleda, J.L., Khalil, M., Zakkar, M., Chaudhury, H., Luong le, A., Mason, J.C., Udalova, I., Gsell, W., Jones, H., Haskard, D.O., Krams, R., Evans, P.C., 2011. Disturbed blood flow induces RelA expression via c-Jun N-terminal kinase 1: a novel mode of NF-kappaB regulation that promotes arterial inflammation. Circ. Res. 108, 950–959.
Xiao, H., Lu, M., Lin, T.Y., Chen, Z., Chen, G., Wang, W.C., Marin, T., Shentu, T.P., Wen,

L., Gongol, B., Sun, W., Liang, X., Chen, J., Huang, H.D., Pedra, J.H., Johnson, D.A., Shyy, J.Y., 2013. Sterol regulatory element binding protein 2 activation of NLRP3 inflammasome in endothelium mediates hemodynamic-induced atherosclerosis susceptibility. Circulation 128, 632–642.
Franceschi, C., Bonafe, M., Valensin, S., Olivieri, F., De Luca, M., Ottaviani, E., De Benedictis, G., 2000. Inflamm-aging. An evolutionary perspective on immunosenescence. Ann. N. Y. Acad. Sci. 908, 244–254.
Harvey, A., Montezano, A.C., Lopes, R.A., Rios, F., Touyz, R.M., 2016. Vascular fibrosis in aging and hypertension: molecular mechanisms and clinical implications. Can. J. Cardiol. 32, 659–668.
Movat, H.Z., More, R.H., Haust, M.D., 1958. The diffuse intimal thickening of the human aorta with aging. Am. J. Pathol. 34, 1023–1031.
Stary, H.C., 1987. Macrophages, macrophage foam cells, and eccentric intimal thickening in the coronary arteries of young children. Atherosclerosis 64, 91–108.
Virmani, R., Kolodgie, F.D., Burke, A.P., Farb, A., Schwartz, S.M., 2000. Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler. Thromb. Vasc. Biol. 20, 1262–1275.
Spagnoli, L.G., Orlandi, A., Mauriello, A., De Angelis, C., Ramacci, M.T., 1992. Age-dependent increase of rabbit aortic atherosclerosis. A morphometric approach. Pathol. Res. Pract. 188, 637–642.
Spagnoli, L.G., Orlandi, A., Mauriello, A., Santeusanio, G., de Angelis, C., Lucreziotti, R., Ramacci, M.T., 1991. Aging and atherosclerosis in the rabbit. 1. Distribution, prevalence and morphology of atherosclerotic lesions. Atherosclerosis 89, 11–24.
Bennett, M.R., Sinha, S., Owens, G.K., 2016. Vascular smooth muscle cells in atherosclerosis. Circ. Res. 118, 692–702.
Iyemere, V.P., Proudfoot, D., Weissberg, P.L., Shanahan, C.M., 2006. Vascular smooth muscle cell phenotypic plasticity and the regulation of vascular calcification. J. Intern. Med. 260, 192–210.
Shanahan, C.M., Weissberg, P.L., 1999. Smooth muscle cell phenotypes in atherosclerotic lesions. Curr. Opin. Lipidol. 10, 507–513.
Shanahan, C.M., Cary, N.R., Metcalfe, J.C., Weissberg, P.L., 1994. High expression of genes for calcification-regulating proteins in human atherosclerotic plaques. J. Clin. Invest. 93, 2393–2402.
Shanahan, C.M., Weissberg, P.L., 1998. Smooth muscle cell heterogeneity: patterns of gene expression in vascular smooth muscle cells in vitro and in vivo. Arterioscler. Thromb. Vasc. Biol. 18, 333–338.
Shanahan, C.M., Weissberg, P.L., Metcalfe, J.C., 1993. Isolation of gene markers of differentiated and proliferating vascular smooth muscle cells. Circ. Res. 73, 193–204.
Chamley-Campbell, J.H., Campbell, G.R., Ross, R., 1981. Phenotype-dependent response of cultured aortic smooth muscle to serum mitogens. J. Cell Biol. 89, 379–383.
Chamley-Campbell, J., Campbell, G.R., Ross, R., 1979. The smooth muscle cell in culture. Physiol. Rev. 59, 1–61.
Alexander, M.R., Owens, G.K., 2012. Epigenetic control of smooth muscle cell differentiation and phenotypic switching in vascular development and disease. Annu. Rev. Physiol. 74, 13–40.
Shanahan, C.M., Cary, N.R., Salisbury, J.R., Proudfoot, D., Weissberg, P.L., Edmonds, M.E., 1999. Medial localization of mineralization-regulating proteins in association with Monckeberg's sclerosis: evidence for smooth muscle cell-mediated vascular calcification. Circulation 100, 2168–2176.
Tyson, K.L., Reynolds, J.L., McNair, R., Zhang, Q., Weissberg, P.L., Shanahan, C.M., 2003. Osteo/chondrocytic transcription factors and their target genes exhibit distinct patterns of expression in human arterial calcification. Arterioscler. Thromb. Vasc. Biol. 23, 489–494.
Davies, J.D., Carpenter, K.L., Challis, I.R., Figg, N.L., McNair, R., Proudfoot, D., Weissberg, P.L., Shanahan, C.M., 2005. Adipocytic differentiation and liver x receptor pathways regulate the accumulation of triacylglycerols in human vascular smooth muscle cells. J. Biol. Chem. 280, 3911–3919.
Tintut, Y., Alfonso, Z., Saini, T., Radcliff, K., Watson, K., Bostrom, K., Demer, L.L., 2003. Multilineage potential of cells from the artery wall. Circulation 108, 2505–2510.
Roberts, W.C., 1986. The senile cardiac calcification syndrome. Am. J. Cardiol. 58, 572–574.
Wang, J., Peng, X., Lassance-Soares, R.M., Najafi, A.H., Alderman, L.O., Sood, S., Xue, Z., Chan, R., Faber, J.E., Epstein, S.E., Burnett, M.S., 2011. Aging-induced collateral dysfunction: impaired responsiveness of collaterals and susceptibility to apoptosis via dysfunctional eNOS signaling. J. Cardiovasc. Transl. Res. 4, 779–789.
Donato, A.J., Eskurza, I., Silver, A.E., Levy, A.S., Pierce, G.L., Gates, P.E., Seals, D.R., 2007. Direct evidence of endothelial oxidative stress with aging in humans: relation to impaired endothelium-dependent dilation and upregulation of nuclear factor-kappaB. Circ. Res. 100, 1659–1666.
Egashira, K., Inou, T., Hirooka, Y., Kai, H., Sugimachi, M., Suzuki, S., Kuga, T., Urabe, Y., Takeshita, A., 1993. Effects of age on endothelium-dependent vasodilation of resistance coronary artery by acetylcholine in humans. Circulation 88, 77–81.
Celermajer, D.S., Sorensen, K.E., Spiegelhalter, D.J., Georgakopoulos, D., Robinson, J., Deanfield, J.E., 1994. Aging is associated with endothelial dysfunction in healthy men years before the age-related decline in women. J. Am. Coll. Cardiol. 24, 471–476.
Csiszar, A., Ungvari, Z., Edwards, J.G., Kaminski, P., Wolin, M.S., Koller, A., Kaley, G., 2002. Aging-induced phenotypic changes and oxidative stress impair coronary arteriolar function. Circ. Res. 90, 1159–1166.
LeBlanc, A.J., Shipley, R.D., Kang, L.S., Muller-Delp, J.M., 2008. Age impairs Flk-1 signaling and NO-mediated vasodilation in coronary arterioles. Am. J. Physiol. Heart Circ. Physiol. 295, H2280–2288.
Cooke, C.L., Davidge, S.T., 2003. Endothelial-dependent vasodilation is reduced in mesenteric arteries from superoxide dismutase knockout mice. Cardiovasc. Res. 60, 635–642.
Kang, L.S., Reyes, R.A., Muller-Delp, J.M., 2009. Aging impairs flow-induced dilation in coronary arterioles: role of NO and H(2)O(2). Am. J. Physiol. Heart Circ. Physiol. 297, H1087–1095.
Higashi, Y., Kihara, Y., Noma, K., 2012. Endothelial dysfunction and hypertension in aging. Hypertens. Res. 35, 1039–1047.
Epstein, S.E., Lassance-Soares, R.M., Faber, J.E., Burnett, M.S., 2012. Effects of aging on the collateral circulation, and therapeutic implications. Circulation 125, 3211–3219.
Miura, H., Bosnjak, J.J., Ning, G., Saito, T., Miura, M., Gutterman, D.D., 2003. Role for hydrogen peroxide in flow-induced dilation of human coronary arterioles. Circ. Res. 92, e31–40.
Quijano, C., Castro, L., Peluffo, G., Valez, V., Radi, R., 2007. Enhanced mitochondrial superoxide in hyperglycemic endothelial cells: direct measurements and formation of hydrogen peroxide and peroxynitrite. Am. J. Physiol. Heart Circ. Physiol. 293, H3404–3414.
Sun, D., Huang, A., Yan, E.H., Wu, Z., Yan, C., Kaminski, P.M., Oury, T.D., Wolin, M.S., Kaley, G., 2004. Reduced release of nitric oxide to shear stress in mesenteric arteries of aged rats. Am. J. Physiol. Heart Circ. Physiol. 286 H2249-2256.
Van Guilder, G.P., Westby, C.M., Greiner, J.J., Stauffer, B.L., DeSouza, C.A., 2007. Endothelin-1 vasoconstrictor tone increases with age in healthy men but can be reduced by regular aerobic exercise. Hypertension 50, 403–409.
Feher, A., Broskova, Z., Bagi, Z., 2014. Age-related impairment of conducted dilation in human coronary arterioles. Am. J. Physiol. Heart Circ. Physiol. 306, H1595–1601.
Li, H.J., Yin, H., Yao, Y.Y., Shen, B., Bader, M., Chao, L., Chao, J., 2007. Tissue kallikrein protects against pressure overload-induced cardiac hypertrophy through kinin B2 receptor and glycogen synthase kinase-3beta activation. Cardiovasc. Res. 73, 130–142.
Venema, R.C., 2002. Post-translational mechanisms of endothelial nitric oxide synthase regulation by bradykinin. Int. Immunopharmacol. 2, 1755–1762.
Kakoki, M., Kizer, C.M., Yi, X., Takahashi, N., Kim, H.S., Bagnell, C.R., Edgell, C.J., Maeda, N., Jennette, J.C., Smithies, O., 2006. Senescence-associated phenotypes in Akita diabetic mice are enhanced by absence of bradykinin B2 receptors. J. Clin. Invest. 116, 1302–1309.
Maestri, R., Milia, A.F., Salis, M.B., Graiani, G., Lagrasta, C., Monica, M., Corradi, D., Emanueli, C., Madeddu, P., 2003. Cardiac hypertrophy and microvascular deficit in kinin B2 receptor knockout mice. Hypertension 41, 1151–1155.
Emanueli, C., Maestri, R., Corradi, D., Marchione, R., Minasi, A., Tozzi, M.G., Salis, M.B., Straino, S., Capogrossi, M.C., Olivetti, G., Madeddu, P., 1999. Dilated and failing cardiomyopathy in bradykinin B(2) receptor knockout mice. Circulation 100, 2359–2365.
Nurmi, L., Heikkila, H.M., Vapaatalo, H., Kovanen, P.T., Lindstedt, K.A., 2012. Downregulation of Bradykinin type 2 receptor expression in cardiac endothelial cells during senescence. J. Vasc. Res. 49, 13–23.
Feletou, M., Vanhoutte, P.M., 1988. Endothelium-dependent hyperpolarization of canine coronary smooth muscle. Br. J. Pharmacol. 93, 515–524.
Busse, R., Edwards, G., Feletou, M., Fleming, I., Vanhoutte, P.M., Weston, A.H., 2002. EDHF: bringing the concepts together. Trends Pharmacol. Sci. 23, 374–380.
Filosa, J.A., Yao, X., Rath, G., 2013. TRPV4 and the regulation of vascular tone. J. Cardiovasc. Pharmacol. 61, 113–119.
Burnham, M.P., Bychkov, R., Feletou, M., Richards, G.R., Vanhoutte, P.M., Weston, A.H., Edwards, G., 2002. Characterization of an apamin-sensitive small-conductance Ca(2+)-activated K(+) channel in porcine coronary artery endothelium: relevance to EDHF. Br. J. Pharmacol. 135, 1133–1143.
Feletou, M., 2016. Endothelium-Dependent Hyperpolarization and Endothelial Dysfunction. J. Cardiovasc. Pharmacol. 67, 373–387.
Fujii, K., Ohmori, S., Tominaga, M., Abe, I., Takata, Y., Ohya, Y., Kobayashi, K., Fujishima, M., 1993. Age-related changes in endothelium-dependent hyperpolarization in the rat mesenteric artery. Am. J. Physiol. 265, H509–516.
Mantelli, L., Amerini, S., Ledda, F., 1995. Roles of nitric oxide and endothelium-derived hyperpolarizing factor in vasorelaxant effect of acetylcholine as influenced by aging and hypertension. J. Cardiovasc. Pharmacol. 25, 595–602.
Nakashima, M., Vanhoutte, P.M., 1993. Age-dependent decrease in endothelium-dependent hyperpolarizations to endothelin-3 in the rat mesenteric artery. J. Cardiovasc. Pharmacol. 22 (Suppl 8), S352–354.
Urakami-Harasawa, L., Shimokawa, H., Nakashima, M., Egashira, K., Takeshita, A., 1997. Importance of endothelium-derived hyperpolarizing factor in human arteries. J. Clin. Invest. 100, 2793–2799.
Hill, J.M., Zalos, G., Halcox, J.P., Schenke, W.H., Waclawiw, M.A., Quyyumi, A.A., Finkel, T., 2003. Circulating endothelial progenitor cells, vascular function, and cardiovascular risk. N. Engl. J. Med. 348, 593–600.
Umemura, T., Soga, J., Hidaka, T., Takemoto, H., Nakamura, S., Jitsuiki, D., Nishioka, K., Goto, C., Teragawa, H., Yoshizumi, M., Chayama, K., Higashi, Y., 2008. Aging and hypertension are independent risk factors for reduced number of circulating endothelial progenitor cells. Am. J. Hypertens. 21, 1203–1209.
Werner, N., Kosiol, S., Schiegl, T., Ahlers, P., Walenta, K., Link, A., Bohm, M., Nickenig, G., 2005. Circulating endothelial progenitor cells and cardiovascular outcomes. N. Engl. J. Med. 353, 999–1007.
Murasawa, S., Llevadot, J., Silver, M., Isner, J.M., Losordo, D.W., Asahara, T., 2002. Constitutive human telomerase reverse transcriptase expression enhances regenerative properties of endothelial progenitor cells. Circulation 106, 1133–1139.
Ballard, V.L., Edelberg, J.M., 2007. Stem cells and the regeneration of the aging cardiovascular system. Circ. Res. 100, 1116–1127.
Tang, E.H., Vanhoutte, P.M., 2008. Gene expression changes of prostanoid synthases in endothelial cells and prostanoid receptors in vascular smooth muscle cells caused by aging and hypertension. Physiol. Genomics 32, 409–418.
Wong, S.L., Leung, F.P., Lau, C.W., Au, C.L., Yung, L.M., Yao, X., Chen, Z.Y., Vanhoutte, P.M., Gollasch, M., Huang, Y., 2009. Cyclooxygenase-2-derived prostaglandin F2alpha mediates endothelium-dependent contractions in the aortae of hamsters with

increased impact during aging. Circ. Res. 104, 228–235.
Gotoh, S., Ogihara, T., Nakamaru, M., Masuo, K., Hata, T., Kumahara, Y., 1983. Effect of aging on 6-keto-PGF1 alpha levels in normotensive and essential hypertensive males. Jpn. Circ. J. 47, 309–312.
Gotoh, S., Ogihara, T., Nakamaru, M., Hata, T., Kumahara, Y., 1982. [Levels of plasma 6-keto-PGF1 alpha in normotensive and essential hypertensive males with and without a family history of hypertension and effects of aging]. Nihon Naibunpi Gakkai Zasshi 58, 1341–1349.
Conlan, M.G., Folsom, A.R., Finch, A., Davis, C.E., Sorlie, P., Marcucci, G., Wu, K.K., 1993. Associations of factor VIII and von Willebrand factor with age, race, sex, and risk factors for atherosclerosis. The Atherosclerosis Risk in Communities (ARIC) Study. Thromb. Haemost. 70, 380–385.
Favaloro, E.J., Soltani, S., McDonald, J., Grezchnik, E., Easton, L., Favaloro, J.W., 2005. Reassessment of ABO blood group, sex, and age on laboratory parameters used to diagnose von Willebrand disorder: potential influence on the diagnosis vs the potential association with risk of thrombosis. Am. J. Clin. Pathol. 124, 910–917.
Coppola, R., Mari, D., Lattuada, A., Franceschi, C., 2003. Von Willebrand factor in Italian centenarians. Haematologica 88, 39–43.
Shim, K., Anderson, P.J., Tuley, E.A., Wiswall, E., Sadler, J.E., 2008. Platelet-VWF complexes are preferred substrates of ADAMTS13 under fluid shear stress. Blood 111, 651–657.
Radomski, M.W., Palmer, R.M., Moncada, S., 1987. Endogenous nitric oxide inhibits human platelet adhesion to vascular endothelium. Lancet 2, 1057–1058.
Dockrell, M.E., Webb, D.J., Williams, B.C., 1996. Activation of the endothelin B receptor causes a dose-dependent accumulation of cyclic GMP in human platelets. Blood Coagul. Fibrinolysis 7, 178–180.
Labonte, J., Brochu, I., Honore, J.C., D'Orleans-Juste, P., 2001. Role of ETB and B2 receptors in the ex vivo platelet inhibitory properties of endothelin and bradykinin in the mouse. Br. J. Pharmacol. 132, 934–940.
LeBlanc, A.J., Hoying, J.B., 2016. Adaptation of the Coronary Microcirculation in Aging. Microcirculation 23, 157–167.
Hachamovitch, R., Wicker, P., Capasso, J.M., Anversa, P., 1989. Alterations of coronary blood flow and reserve with aging in Fischer 344 rats. Am. J. Physiol. 256, H66–73.
Karam, R., Healy, B.P., Wicker, P., 1990. Coronary reserve is depressed in postmyocardial infarction reactive cardiac hypertrophy. Circulation 81, 238–246.
Leblanc, A.J., Touroo, J.S., Hoying, J.B., Williams, S.K., 2012. Adipose stromal vascular fraction cell construct sustains coronary microvascular function after acute myocardial infarction. Am. J. Physiol. Heart Circ. Physiol. 302, H973–982.
Logstrup, B.B., Hofsten, D.E., Christophersen, T.B., Moller, J.E., Botker, H.E., Pellikka, P.A., Egstrup, K., 2010. Association between coronary flow reserve, left ventricular systolic function, and myocardial viability in acute myocardial infarction. Eur. J. Echocardiogr. 11, 665–670.
Ragosta, M., Powers, E.R., Samady, H., Gimple, L.W., Sarembock, I.J., Beller, G.A., 2001. Relationship between extent of residual myocardial viability and coronary flow reserve in patients with recent myocardial infarction. Am. Heart J. 141, 456–462.
Czernin, J., Muller, P., Chan, S., Brunken, R.C., Porenta, G., Krivokapich, J., Chen, K., Chan, A., Phelps, M.E., Schelbert, H.R., 1993. Influence of age and hemodynamics on myocardial blood flow and flow reserve. Circulation 88, 62–69.
Orlandi, A., Bochaton-Piallat, M.L., Gabbiani, G., Spagnoli, L.G., 2006. Aging, smooth muscle cells and vascular pathobiology: implications for atherosclerosis. Atherosclerosis 188, 221–230.
Lakatta, E.G., 2003. Arterial and cardiac aging: major shareholders in cardiovascular disease enterprises: part III: cellular and molecular clues to heart and arterial aging. Circulation 107, 490–497.
Rodriguez-Menocal, L., Faridi, M.H., Martinez, L., Shehadeh, L.A., Duque, J.C., Wei, Y., Mesa, A., Pena, A., Gupta, V., Pham, S.M., Vazquez-Padron, R.I., 2014. Macrophage-derived IL-18 and increased fibrinogen deposition are age-related inflammatory signatures of vascular remodeling. Am. J. Physiol. Heart Circ. Physiol. 306, H641–653.
Kamath, S., Lip, G.Y., 2003. Fibrinogen: biochemistry, epidemiology and determinants. Qjm 96, 711–729.
Spinetti, G., Wang, M., Monticone, R., Zhang, J., Zhao, D., Lakatta, E.G., 2004. Rat aortic MCP-1 and its receptor CCR2 increase with age and alter vascular smooth muscle cell function. Arterioscler. Thromb. Vasc. Biol. 24, 1397–1402.
Csiszar, A., Wang, M., Lakatta, E.G., Ungvari, Z., 1985. Inflammation and endothelial dysfunction during aging: role of NF-kappaB. J. Appl. Physiol. 2008 (105), 1333–1341.
El Assar, M., Angulo, J., Rodriguez-Manas, L., 2013. Oxidative stress and vascular inflammation in aging. Free Radic. Biol. Med. 65, 380–401.
Ungvari, Z., Kaley, G., de Cabo, R., Sonntag, W.E., Csiszar, A., 2010. Mechanisms of vascular aging: new perspectives. J. Gerontol. A Biol. Sci. Med. Sci. 65, 1028–1041.
Gavish, B., Izzo Jr., J.L., 2016. Arterial stiffness: going a step beyond. Am. J. Hypertens. 29, 1223–1233.
Cecelja, M., Chowienczyk, P., 2016. Molecular mechanisms of arterial stiffening. Pulse Basel (Basel) 4, 43–48.
Hanna, M.A., Taylor, C.R., Chen, B., La HS, Maraj J.J., Kilar, C.R., Behnke, B.J., Delp, M.D., Muller-Delp, J.M., 1985. Structural remodeling of coronary resistance arteries: effects of age and exercise training. J. Appl. Physiol. 2014 (117), 616–623.
Thijssen, D.H., Carter, S.E., Green, D.J., 2016. Arterial structure and function in vascular ageing: are you as old as your arteries? J. Physiol. (Paris) 594, 2275–2284.
Demer, L.L., Tintut, Y., 2008. Vascular calcification: pathobiology of a multifaceted disease. Circulation 117, 2938–2948.
Niederhoffer, N., Lartaud-Idjouadiene, I., Giummelly, P., Duvivier, C., Peslin, R., Atkinson, J., 1997. Calcification of medial elastic fibers and aortic elasticity. Hypertension 29, 999–1006.
Demer, L.L., 2002. Vascular calcification and osteoporosis: inflammatory responses to oxidized lipids. Int. J. Epidemiol. 31, 737–741.
Kim, K.M., 1976. Calcification of matrix vesicles in human aortic valve and aortic media. Fed. Proc. 35, 156–162.
Reynolds, J.L., Joannides, A.J., Skepper, J.N., McNair, R., Schurgers, L.J., Proudfoot, D., Jahnen-Dechent, W., Weissberg, P.L., Shanahan, C.M., 2004. Human vascular smooth muscle cells undergo vesicle-mediated calcification in response to changes in extracellular calcium and phosphate concentrations: a potential mechanism for accelerated vascular calcification in ESRD. J. Am. Soc. Nephrol. 15, 2857–2867.
Alexopoulos, N., Raggi, P., 2009. Calcification in atherosclerosis. Nat. Rev. Cardiol. 6, 681–688.
Vengrenyuk, Y., Carlier, S., Xanthos, S., Cardoso, L., Ganatos, P., Virmani, R., Einav, S., Gilchrist, L., Weinbaum, S., 2006. A hypothesis for vulnerable plaque rupture due to stress-induced debonding around cellular microcalcifications in thin fibrous caps. Proc Natl Acad Sci U S A 103, 14678–14683.
Maldonado, N., Kelly-Arnold, A., Vengrenyuk, Y., Laudier, D., Fallon, J.T., Virmani, R., Cardoso, L., Weinbaum, S., 2012. A mechanistic analysis of the role of microcalcifications in atherosclerotic plaque stability: potential implications for plaque rupture. Am. J. Physiol. Heart Circ. Physiol. 303, H619–628.
Cardoso, L., Kelly-Arnold, A., Maldonado, N., Laudier, D., Weinbaum, S., 2014. Effect of tissue properties, shape and orientation of microcalcifications on vulnerable cap stability using different hyperelastic constitutive models. J. Biomech. 47, 870–877.
Kelly-Arnold, A., Maldonado, N., Laudier, D., Aikawa, E., Cardoso, L., Weinbaum, S., 2013. Revised microcalcification hypothesis for fibrous cap rupture in human coronary arteries. Proc Natl Acad Sci U S A 110, 10741–10746.
Rambhia, S.H., Liang, X., Xenos, M., Alemu, Y., Maldonado, N., Kelly, A., Chakraborti, S., Weinbaum, S., Cardoso, L., Einav, S., Bluestein, D., 2012. Microcalcifications increase coronary vulnerable plaque rupture potential: a patient-based micro-CT fluid-structure interaction study. Ann. Biomed. Eng. 40, 1443–1454.
Mauriello, A., Servadei, F., Zoccai, G.B., Giacobbi, E., Anemona, L., Bonanno, E., Casella, S., 2013. Coronary calcification identifies the vulnerable patient rather than the vulnerable Plaque. Atherosclerosis 229, 124–129.
Duca, L., Floquet, N., Alix, A.J., Haye, B., Debelle, L., 2004. Elastin as a matrikine. Crit. Rev. Oncol. Hematol. 49, 235–244.
Yasmin, McEniery, C.M., O'Shaughnessy, K.M., Harnett, P., Arshad, A., Wallace, S., Maki-Petaja, K., McDonnell, B., Ashby, M.J., Brown, J., Cockcroft, J.R., Wilkinson, I.B., 2006. Variation in the human matrix metalloproteinase-9 gene is associated with arterial stiffness in healthy individuals. Arterioscler. Thromb. Vasc. Biol. 26, 1799–1805.
Yasmin, McEniery, C.M., Wallace, S., Dakham, Z., Pulsalkar, P., Maki-Petaja, K., Ashby, M.J., Cockcroft, J.R., Wilkinson, I.B., 2005. Matrix metalloproteinase-9 (MMP-9), MMP-2, and serum elastase activity are associated with systolic hypertension and arterial stiffness. Arterioscler. Thromb. Vasc. Biol. 25, 372.
Schwartz, S.M., Reidy, M.A., 1987. Common mechanisms of proliferation of smooth muscle in atherosclerosis and hypertension. Hum. Pathol. 18, 240–247.
Zieman, S.J., Kass, D.A., 2004. Advanced glycation endproduct crosslinking in the cardiovascular system: potential therapeutic target for cardiovascular disease. Drugs 64, 459–470.
Nenna, A., Spadaccio, C., Lusini, M., Ulianich, L., Chello, M., Basic, Nappi F., 2015. Clinical research against advanced glycation end products (AGEs): new compounds to tackle cardiovascular disease and diabetic complications. Recent Adv. Cardiovasc. Drug Discov. 10, 10–33.
Aronson, D., 2003. Cross-linking of glycated collagen in the pathogenesis of arterial and myocardial stiffening of aging and diabetes. J. Hypertens. 21, 3–12.
Mauriello, A., Orlandi, A., Palmieri, G., Spagnoli, L.G., Oberholzer, M., Christen, H., 1992. Age-related modification of average volume and anisotropy of vascular smooth muscle cells. Pathol. Res. Pract. 188, 630–636.
Nilsson, P.M., 2014. Hemodynamic aging as the consequence of structural changes associated with early vascular aging (EVA). Aging Dis. 5, 109–113.
Sell, D.R., Monnier, V.M., 2012. Molecular basis of arterial stiffening: role of glycation - a mini-review. Gerontology 58, 227–237.
Ferro, G., Duilio, C., Spinelli, L., Liucci, G.A., Mazza, F., Indolfi, C., 1995. Relation between diastolic perfusion time and coronary artery stenosis during stress-induced myocardial ischemia. Circulation 92, 342–347.
Moriya, J., Minamino, T., 2017. Angiogenesis, Cancer, and Vascular Aging. Front. Cardiovasc. Med. 4, 65.
Rivard, A., Fabre, J.E., Silver, M., Chen, D., Murohara, T., Kearney, M., Magner, M., Asahara, T., Isner, J.M., 1999. Age-dependent impairment of angiogenesis. Circulation 99, 111–120.
Bosch-Marce, M., Okuyama, H., Wesley, J.B., Sarkar, K., Kimura, H., Liu, Y.V., Zhang, H., Strazza, M., Rey, S., Savino, L., Zhou, Y.F., McDonald, K.R., Na, Y., Vandiver, S., Rabi, A., Shaked, Y., Kerbel, R., Lavallee, T., Semenza, G.L., 2007. Effects of aging and hypoxia-inducible factor-1 activity on angiogenic cell mobilization and recovery of perfusion after limb ischemia. Circ. Res. 101, 1310–1318.
Dai, X., Faber, J.E., 2010. Endothelial nitric oxide synthase deficiency causes collateral vessel rarefaction and impairs activation of a cell cycle gene network during arteriogenesis. Circ. Res. 106, 1870–1881.
Hoffmann, J., Haendeler, J., Aicher, A., Rossig, L., Vasa, M., Zeiher, A.M., Dimmeler, S., 2001. Aging enhances the sensitivity of endothelial cells toward apoptotic stimuli: important role of nitric oxide. Circ. Res. 89, 709–715.
Xin, Y., Wang, Y.M., Zhang, H., Li, J., Wang, W., Wei, Y.J., Hu, S.S., 2010. Aging adversely impacts biological properties of human bone marrow-derived mesenchymal stem cells: implications for tissue engineering heart valve construction. Artif. Organs 34, 215–222.
Faber, J.E., Zhang, H., Lassance-Soares, R.M., Prabhakar, P., Najafi, A.H., Burnett, M.S., Epstein, S.E., 2011. Aging causes collateral rarefaction and increased severity of ischemic injury in multiple tissues. Arterioscler. Thromb. Vasc. Biol. 31, 1748–1756.

Ghebre, Y.T., Yakubov, E., Wong, W.T., Krishnamurthy, P., Sayed, N., Sikora, A.G., Bonnen, M.D., 2016. Vascular aging: implications for cardiovascular disease and therapy. Transl. Med. Sunnyvale (Sunnyvale) 6.
Erikson, G.A., Bodian, D.L., Rueda, M., Molparia, B., Scott, E.R., Scott-Van Zeeland, A.A., Topol, S.E., Wineinger, N.E., Niederhuber, J.E., Topol, E.J., Torkamani, A., 2016. Whole-genome sequencing of a healthy aging cohort. Cell 165, 1002–1011.
Cencioni, C., Spallotta, F., Mai, A., Martelli, F., Farsetti, A., Zeiher, A.M., Gaetano, C., 2015. Sirtuin function in aging heart and vessels. J. Mol. Cell. Cardiol. 83, 55–61.
Ota, H., Akishita, M., Eto, M., Iijima, K., Kaneki, M., Ouchi, Y., 2007. Sirt1 modulates premature senescence-like phenotype in human endothelial cells. J. Mol. Cell. Cardiol. 43, 571–579.
Tang, Y., Zhao, W., Chen, Y., Zhao, Y., Gu, W., 2008. Acetylation is indispensable for p53 activation. Cell 133, 612–626.
Donato, A.J., Magerko, K.A., Lawson, B.R., Durrant, J.R., Lesniewski, L.A., Seals, D.R., 2011. SIRT-1 and vascular endothelial dysfunction with ageing in mice and humans. J. Physiol. (Paris) 589, 4545–4554.
Zarzuelo, M.J., Lopez-Sepulveda, R., Sanchez, M., Romero, M., Gomez-Guzman, M., Ungvary, Z., Perez-Vizcaino, F., Jimenez, R., Duarte, J., 2013. SIRT1 inhibits NADPH oxidase activation and protects endothelial function in the rat aorta: implications for vascular aging. Biochem. Pharmacol. 85, 1288–1296.
Vasko, R., Xavier, S., Chen, J., Lin, C.H., Ratliff, B., Rabadi, M., Maizel, J., Tanokuchi, R., Zhang, F., Cao, J., Goligorsky, M.S., 2014. Endothelial sirtuin 1 deficiency perpetrates nephrosclerosis through downregulation of matrix metalloproteinase-14: relevance to fibrosis of vascular senescence. J. Am. Soc. Nephrol. 25, 276–291.
Moyzis, R.K., Buckingham, J.M., Cram, L.S., Dani, M., Deaven, L.L., Jones, M.D., Meyne, J., Ratliff, R.L., Wu, J.R., 1988. A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. Proc Natl Acad Sci U S A 85, 6622–6626.
d'Adda di Fagagna, F., Reaper, P.M., Clay-Farrace, L., Fiegler, H., Carr, P., Von Zglinicki, T., Saretzki, G., Carter, N.P., Jackson, S.P., 2003. A DNA damage checkpoint response in telomere-initiated senescence. Nature 426, 194–198.
Morgan, R.G., Donato, A.J., Walker, A.E., 2018. Telomere uncapping and vascular aging. Am. J. Physiol. Heart Circ. Physiol. 315 H1-h5.
Walker, A.E., Morgan, R.G., Ives, S.J., Cawthon, R.M., Andtbacka, R.H., Noyes, D., Lesniewski, L.A., Richardson, R.S., Donato, A.J., 2016. Age-related arterial telomere uncapping and senescence is greater in women compared with men. Exp. Gerontol. 73, 65–71.
Hunt, S.C., Kimura, M., Hopkins, P.N., Carr, J.J., Heiss, G., Province, M.A., Aviv, A., 2015. Leukocyte telomere length and coronary artery calcium. Am. J. Cardiol. 116, 214–218.
Diaz, V.A., Mainous 3rd, A.G., Everett, C.J., Schoepf, U.J., Codd, V., Samani, N.J., 2010. Effect of healthy lifestyle behaviors on the association between leukocyte telomere length and coronary artery calcium. Am. J. Cardiol. 106, 659–663.
Osburn, W.O., Kensler, T.W., 2008. Nrf2 signaling: an adaptive response pathway for protection against environmental toxic insults. Mutat. Res. 659, 31–39.
Pickering, A.M., Linder, R.A., Zhang, H., Forman, H.J., Davies, K.J., 2012. Nrf2-dependent induction of proteasome and Pa28alphabeta regulator are required for adaptation to oxidative stress. J. Biol. Chem. 287, 10021–10031.
Zhou, S., Sun, W., Zhang, Z., Zheng, Y., 2014. The role of Nrf2-mediated pathway in cardiac remodeling and heart failure. Oxid. Med. Cell. Longev. 2014, 260429.
Lytton, J., 2007. Na+/Ca2+ exchangers: three mammalian gene families control Ca2+ transport. Biochem. J. 406, 365–382.
Antoons, G., Willems, R., Sipido, K.R., 2012. Alternative strategies in arrhythmia therapy: evaluation of Na/Ca exchange as an anti-arrhythmic target. Pharmacol. Ther. 134, 26–42.
Svensson, K.J., Kucharzewska, P., Christianson, H.C., Skold, S., Lofstedt, T., Johansson, M.C., Morgelin, M., Bengzon, J., Ruf, W., Belting, M., 2011. Hypoxia triggers a proangiogenic pathway involving cancer cell microvesicles and PAR-2-mediated heparin-binding EGF signaling in endothelial cells. Proc Natl Acad Sci U S A 108, 13147–13152.
Osanai, T., Tanaka, M., Mikami, K., Kitajima, M., Tomisawa, T., Magota, K., Tomita, H., Okumura, K., 2018. Novel anti-aging gene NM_026333 contributes to proton-induced aging via NCX1-pathway. J. Mol. Cell. Cardiol. 125, 174–184.
Kannel, W.B., Cupples, L.A., D'Agostino, R.B., 1987. Sudden death risk in overt coronary heart disease: the Framingham Study. Am. Heart J. 113, 799–804.
Strait, J.B., Lakatta, E.G., 2012. Aging-associated cardiovascular changes and their relationship to heart failure. Heart Fail. Clin. 8, 143–164.
Nguyen, T.P., Qu, Z., Weiss, J.N., 2014. Cardiac fibrosis and arrhythmogenesis: the road to repair is paved with perils. J. Mol. Cell. Cardiol. 70, 83–91.
Cieslik, K.A., Trial, J., Crawford, J.R., Taffet, G.E., Entman, M.L., 2014. Adverse fibrosis in the aging heart depends on signaling between myeloid and mesenchymal cells; role of inflammatory fibroblasts. J. Mol. Cell. Cardiol. 70, 56–63.
Dobaczewski, M., de Haan, J.J., Frangogiannis, N.G., 2012. The extracellular matrix modulates fibroblast phenotype and function in the infarcted myocardium. J. Cardiovasc. Transl. Res. 5, 837–847.
Kovacs, G., Berghold, A., Scheidl, S., Olschewski, H., 2009. Pulmonary arterial pressure during rest and exercise in healthy subjects: a systematic review. Eur. Respir. J. 34, 888–894.
Hees, P.S., Fleg, J.L., Lakatta, E.G., Shapiro, E.P., 2002. Left ventricular remodeling with age in normal men versus women: novel insights using three-dimensional magnetic resonance imaging. Am. J. Cardiol. 90, 1231–1236.
Downes, T.R., Nomeir, A.M., Smith, K.M., Stewart, K.P., Little, W.C., 1989. Mechanism of altered pattern of left ventricular filling with aging in subjects without cardiac disease. Am. J. Cardiol. 64, 523–527.
Fleg, J.L., Strait, J., 2012. Age-associated changes in cardiovascular structure and function: a fertile milieu for future disease. Heart Fail. Rev. 17, 545–554.
Chantler, P.D., Lakatta, E.G., 2012. Arterial-ventricular coupling with aging and disease. Front. Physiol. 3, 90.
Spadaccio, C., Rainer, A., Mozetic, P., Trombetta, M., Dion, R.A., Barbato, R., Nappi, F., Chello, M., 2015. The role of extracellular matrix in age-related conduction disorders: a forgotten player? J. Geriatr. Cardiol. 12, 76–82.
Hakim, F.A., Shen, W.K., 2014. Atrial fibrillation in the elderly: a review. Future Cardiol. 10, 745–758.
Lin, Y.K., Chen, Y.A., Lee, T.I., Chen, Y.C., Chen, S.A., Chen, Y.J., 2018. Aging modulates the substrate and triggers remodeling in atrial fibrillation. Circ. J. 82, 1237–1244.
Andrade, J., Khairy, P., Dobrev, D., Nattel, S., 2014. The clinical profile and pathophysiology of atrial fibrillation: relationships among clinical features, epidemiology, and mechanisms. Circ. Res. 114, 1453–1468.
Rahman, F., Kwan, G.F., Benjamin, E.J., 2014. Global epidemiology of atrial fibrillation. Nat. Rev. Cardiol. 11, 639–654.
da Silva, R.M., 2015. Atrial fibrillation: epidemiology and peculiarities in the elderly. Cardiovasc. Hematol. Agents Med. Chem. 13, 72–77.
Morin, D.P., Bernard, M.L., Madias, C., Rogers, P.A., Thihalolipavan, S., Estes 3rd, N.A., 2016. The State of the Art: Atrial Fibrillation Epidemiology, Prevention, and Treatment. Mayo Clin. Proc. 91, 1778–1810.
Scioli, M.G., Bielli, A., Arcuri, G., Ferlosio, A., Orlandi, A., 2014. Ageing and microvasculature. Vasc Cell 6, 19.
Barton, M., 2014. Aging and endothelin: determinants of disease. Life Sci. 118, 97–109.
Camici, G.G., Savarese, G., Akhmedov, A., Luscher, T.F., 2015. Molecular mechanism of endothelial and vascular aging: implications for cardiovascular disease. Eur. Heart J. 36, 3392–3403.
Fontana, L., 2018. Interventions to promote cardiometabolic health and slow cardiovascular ageing. Nat. Rev. Cardiol. 15, 566–577.
Nowak, K.L., Rossman, M.J., Chonchol, M., Seals, D.R., 2018. Strategies for achieving healthy vascular aging. Hypertension 71, 389–402.
Ungvari, Z., Parrado-Fernandez, C., Csiszar, A., de Cabo, R., 2008. Mechanisms underlying caloric restriction and lifespan regulation: implications for vascular aging. Circ. Res. 102, 519–528.
Ashor, A.W., Lara, J., Siervo, M., Celis-Morales, C., Oggioni, C., Jakovljevic, D.G., Mathers, J.C., 2015. Exercise modalities and endothelial function: a systematic review and dose-response meta-analysis of randomized controlled trials. Sports Med. 45, 279–296.
Laughlin, M.H., Bowles, D.K., Duncker, D.J., 2012. The coronary circulation in exercise training. Am. J. Physiol. Heart Circ. Physiol. 302, H10–23.
Riordan, M.M., Weiss, E.P., Meyer, T.E., Ehsani, A.A., Racette, S.B., Villareal, D.T., Fontana, L., Holloszy, J.O., Kovacs, S.J., 2008. The effects of caloric restriction- and exercise-induced weight loss on left ventricular diastolic function. Am. J. Physiol. Heart Circ. Physiol. 294, H1174–1182.
Leibowitz, D., Jacobs, J.M., Gilon, D., Lande-Stessman, I., Ein-Mor, E., Stessman, J., 2016. Cardiac Structure and Function and Frailty in Subjects Aged 85 and 86 Years. Am. J. Cardiol. 118, 760–764.
Abou, R., Leung, M., Tonsbeek, A.M., Podlesnikar, T., Maan, A.C., Schalij, M.J., Ajmone Marsan, N., Delgado, V., Bax, J.J., 2017. Effect of aging on left atrial compliance and electromechanical properties in subjects without structural heart disease. Am. J. Cardiol. 120, 140–147.
Moreau, K.L., Donato, A.J., Seals, D.R., DeSouza, C.A., Tanaka, H., 2003. Regular exercise, hormone replacement therapy and the age-related decline in carotid arterial compliance in healthy women. Cardiovasc. Res. 57, 861–868.
Tanaka, H., Dinenno, F.A., Monahan, K.D., Clevenger, C.M., DeSouza, C.A., Seals, D.R., 2000. Aging, habitual exercise, and dynamic arterial compliance. Circulation 102, 1270–1275.
Whipple, M.O., Schorr, E.N., Talley, K.M.C., Lindquist, R., Bronas, U.G., Treat-Jacobson, D., 2018. Variability in individual response to aerobic exercise interventions among older adults. J. Aging Phys. Act. 26, 655–670.
Fontana, L., Partridge, L., 2015. Promoting health and longevity through diet: from model organisms to humans. Cell 161, 106–118.
Bevilacqua, L., Ramsey, J.J., Hagopian, K., Weindruch, R., Harper, M.E., 2005. Long-term caloric restriction increases UCP3 content but decreases proton leak and reactive oxygen species production in rat skeletal muscle mitochondria. Am. J. Physiol. Endocrinol. Metab. 289, E429–438.
Sohal, R.S., Weindruch, R., 1996. Oxidative stress, caloric restriction, and aging. Science 273, 59–63.
Jablonski, K.L., Racine, M.L., Geolfos, C.J., Gates, P.E., Chonchol, M., McQueen, M.B., Seals, D.R., 2013. Dietary sodium restriction reverses vascular endothelial dysfunction in middle-aged/older adults with moderately elevated systolic blood pressure. J. Am. Coll. Cardiol. 61, 335–343.
Weiss, E.P., Racette, S.B., Villareal, D.T., Fontana, L., Steger-May, K., Schechtman, K.B., Klein, S., Holloszy, J.O., 2006. Improvements in glucose tolerance and insulin action induced by increasing energy expenditure or decreasing energy intake: a randomized controlled trial. Am. J. Clin. Nutr. 84, 1033–1042.
Hofer, T., Fontana, L., Anton, S.D., Weiss, E.P., Villareal, D., Malayappan, B., Leeuwenburgh, C., 2008. Long-term effects of caloric restriction or exercise on DNA and RNA oxidation levels in white blood cells and urine in humans. Rejuvenation Res. 11, 793–799.
Fontana, L., Meyer, T.E., Klein, S., Holloszy, J.O., 2004. Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans. Proc Natl Acad Sci U S A 101, 6659–6663.
Meydani, S.N., Das, S.K., Pieper, C.F., Lewis, M.R., Klein, S., Dixit, V.D., Gupta, A.K., Villareal, D.T., Bhapkar, M., Huang, M., Fuss, P.J., Roberts, S.B., Holloszy, J.O., Fontana, L., 2016. Long-term moderate calorie restriction inhibits inflammation without impairing cell-mediated immunity: a randomized controlled trial in non-obese humans. Aging (Albany NY) 8, 1416–1431.

Cai, W., He, J.C., Zhu, L., Peppa, M., Lu, C., Uribarri, J., Vlassara, H., 2004. High levels of dietary advanced glycation end products transform low-density lipoprotein into a potent redox-sensitive mitogen-activated protein kinase stimulant in diabetic patients. Circulation 110, 285–291.
Estruch, R., Ros, E., Salas-Salvado, J., Covas, M.I., Corella, D., Aros, F., Gomez-Gracia, E., Ruiz-Gutierrez, V., Fiol, M., Lapetra, J., Lamuela-Raventos, R.M., Serra-Majem, L., Pinto, X., Basora, J., Munoz, M.A., Sorli, J.V., Martinez, J.A., Fito, M., Gea, A., Hernan, M.A., Martinez-Gonzalez, M.A., 2018. Primary prevention of cardiovascular disease with a mediterranean diet supplemented with extra-virgin olive oil or nuts. N. Engl. J. Med. 378, e34.
Katz, D.L., Meller, S., 2014. Can we say what diet is best for health? Annu. Rev. Public Health 35, 83–103.
Jani, B., Rajkumar, C., 2006. Ageing and vascular ageing. Postgrad. Med. J. 82, 357–362.
Virdis, A., Ghiadoni, L., Taddei, S., 2011. Effects of antihypertensive treatment on endothelial function. Curr. Hypertens. Rep. 13, 276–281.
Papakatsika, S., Stabouli, S., Antza, C., Kotsis, V., 2016. Early vascular aging: a new target for hypertension treatment. Curr. Pharm. Des. 22, 122–126.
Kampoli, A.M., Tousoulis, D., Tentolouris, C., Stefanadis, C., 2012. Novel agents targeting nitric oxide. Curr. Vasc. Pharmacol. 10, 61–76.
Buikema, H., Monnink, S.H., Tio, R.A., Crijns, H.J., de Zeeuw, D., van Gilst, W.H., 2000. Comparison of zofenopril and lisinopril to study the role of the sulfhydryl-group in improvement of endothelial dysfunction with ACE-inhibitors in experimental heart failure. Br. J. Pharmacol. 130, 1999–2007.
Pasini, A.F., Garbin, U., Nava, M.C., Stranieri, C., Pellegrini, M., Boccioletti, V., Luchetta, M.L., Fabrizzi, P., Lo Cascio, V., Cominacini, L., 2007. Effect of sulfhydryl and non-sulfhydryl angiotensin-converting enzyme inhibitors on endothelial function in essential hypertensive patients. Am. J. Hypertens. 20, 443–450.
Istvan, E.S., Deisenhofer, J., 2001. Structural mechanism for statin inhibition of HMG-CoA reductase. Science 292, 1160–1164.
Biasucci, L.M., Biasillo, G., Stefanelli, A., 2010. Inflammatory markers, cholesterol and statins: pathophysiological role and clinical importance. Clin. Chem. Lab. Med. 48, 1685–1691.
Ghaffari, N., Ball, C., Kennedy, J.A., Stafford, I., Beltrame, J.F., 2011. Acute modulation of vasoconstrictor responses by pravastatin in small vessels. Circ. J. 75, 1506–1514.
Mason, R.P., Walter, M.F., Jacob, R.F., 2004. Effects of HMG-CoA reductase inhibitors on endothelial function: role of microdomains and oxidative stress. Circulation 109 Ii34-41.
Akira, K., Amano, M., Okajima, F., Hashimoto, T., Oikawa, S., 2006. Inhibitory effects of amlodipine and fluvastatin on the deposition of advanced glycation end products in aortic wall of cholesterol and fructose-fed rabbits. Biol. Pharm. Bull. 29, 75–81.
Ishibashi, Y., Yamagishi, S., Matsui, T., Ohta, K., Tanoue, R., Takeuchi, M., Ueda, S., Nakamura, K., Okuda, S., 2012. Pravastatin inhibits advanced glycation end products (AGEs)-induced proximal tubular cell apoptosis and injury by reducing receptor for AGEs (RAGE) level. Metabolism 61, 1067–1072.
Wang, J., Xu, J., Zhou, C., Zhang, Y., Xu, D., Guo, Y., Yang, Z., 2012. Improvement of arterial stiffness by reducing oxidative stress damage in elderly hypertensive patients after 6 months of atorvastatin therapy. J. Clin. Hypertens. Greenwich (Greenwich) 14, 245–249.
Hong, H., Zeng, J.S., Kreulen, D.L., Kaufman, D.I., Chen, A.F., 2006. Atorvastatin protects against cerebral infarction via inhibition of NADPH oxidase-derived superoxide in ischemic stroke. Am. J. Physiol. Heart Circ. Physiol. 291, H2210–2215.
Manfredini, V., Biancini, G.B., Vanzin, C.S., Dal Vesco, A.M., Wayhs, C.A., Peralba Mdo, C., Vargas, C.R., 2010. Apolipoprotein, C-reactive protein and oxidative stress parameters in dyslipidemic type 2 diabetic patients treated or not with simvastatin. Arch. Med. Res. 41, 104–109.
Hakamada-Taguchi, R., Uehara, Y., Kuribayashi, K., Numabe, A., Saito, K., Negoro, H., Fujita, T., Toyo-oka, T., Kato, T., 2003. Inhibition of hydroxymethylglutaryl-coenzyme a reductase reduces Th1 development and promotes Th2 development. Circ. Res. 93, 948–956.
Kwak, B., Mulhaupt, F., Myit, S., Mach, F., 2000. Statins as a newly recognized type of immunomodulator. Nat. Med. 6, 1399–1402.
Shen, W., Shi, H.M., Fan, W.H., Luo, X.P., Jin, B., Li, Y., 2011. The effects of simvastatin on angiogenesis: studied by an original model of atherosclerosis and acute myocardial infarction in rabbit. Mol. Biol. Rep. 38, 3821–3828.
Tziakas, D.N., Chalikias, G.K., Stakos, D., Tentes, I.K., Thomaidi, A., Chatzikyriakou, S., Mitrousi, K., Kortsaris, A.X., Kaski, J.C., Boudoulas, H., Konstantinides, S., 2009. Statin use is associated with a significant reduction in cholesterol content of erythrocyte membranes. A novel pleiotropic effect? Cardiovasc. Drugs Ther. 23, 471–480.
Assmus, B., Urbich, C., Aicher, A., Hofmann, W.K., Haendeler, J., Rossig, L., Spyridopoulos, I., Zeiher, A.M., Dimmeler, S., 2003. HMG-CoA reductase inhibitors reduce senescence and increase proliferation of endothelial progenitor cells via regulation of cell cycle regulatory genes. Circ. Res. 92, 1049–1055.
Satoh, M., Minami, Y., Takahashi, Y., Tabuchi, T., Itoh, T., Nakamura, M., 2009. Effect of intensive lipid-lowering therapy on telomere erosion in endothelial progenitor cells obtained from patients with coronary artery disease. Clin. Sci. 116, 827–835.
Olivieri, F., Mazzanti, I., Abbatecola, A.M., Recchioni, R., Marcheselli, F., Procopio, A.D., Antonicelli, R., 2012. Telomere/Telomerase system: a new target of statins pleiotropic effect? Curr. Vasc. Pharmacol. 10, 216–224.
Mahmoudi, M., Gorenne, I., Mercer, J., Figg, N., Littlewood, T., Bennett, M., 2008. Statins use a novel Nijmegen breakage syndrome-1-dependent pathway to accelerate DNA repair in vascular smooth muscle cells. Circ. Res. 103, 717–725.
Wassmann, S., Laufs, U., Muller, K., Konkol, C., Ahlbory, K., Baumer, A.T., Linz, W., Bohm, M., Nickenig, G., 2002. Cellular antioxidant effects of atorvastatin in vitro and in vivo. Arterioscler. Thromb. Vasc. Biol. 22, 300–305.
Chen, J.W., Zhou, S.B., Tan, Z.M., 2010. Aspirin and pravastatin reduce lectin-like oxidized low density lipoprotein receptor-1 expression, adhesion molecules and oxidative stress in human coronary artery endothelial cells. Chin Med J (Engl) 123, 1553–1556.
Puccetti, L., Santilli, F., Pasqui, A.L., Lattanzio, S., Liani, R., Ciani, F., Ferrante, E., Ciabattoni, G., Scarpini, F., Ghezzi, A., Auteri, A., Davi, G., 2011. Effects of atorvastatin and rosuvastatin on thromboxane-dependent platelet activation and oxidative stress in hypercholesterolemia. Atherosclerosis 214, 122–128.
Anisimov, V.N., 2013. Metformin: do we finally have an anti-aging drug? Cell Cycle 12, 3483–3489.
Barzilai, N., Crandall, J.P., Kritchevsky, S.B., Espeland, M.A., 2016. Metformin as a tool to target aging. Cell Metab. 23, 1060–1065.
Liu, B., Fan, Z., Edgerton, S.M., Yang, X., Lind, S.E., Thor, A.D., 2011. Potent anti-proliferative effects of metformin on trastuzumab-resistant breast cancer cells via inhibition of erbB2/IGF-1 receptor interactions. Cell Cycle 10, 2959–2966.
Batandier, C., Guigas, B., Detaille, D., El-Mir, M.Y., Fontaine, E., Rigoulet, M., Leverve, X.M., 2006. The ROS production induced by a reverse-electron flux at respiratory-chain complex 1 is hampered by metformin. J. Bioenerg. Biomembr. 38, 33–42.
Bridges, H.R., Jones, A.J., Pollak, M.N., Hirst, J., 2014. Effects of metformin and other biguanides on oxidative phosphorylation in mitochondria. Biochem. J. 462, 475–487.
Zheng, Z., Chen, H., Li, J., Li, T., Zheng, B., Zheng, Y., Jin, H., He, Y., Gu, Q., Xu, X., 2012. Sirtuin 1-mediated cellular metabolic memory of high glucose via the LKB1/AMPK/ROS pathway and therapeutic effects of metformin. Diabetes 61, 217–228.
Algire, C., Moiseeva, O., Deschenes-Simard, X., Amrein, L., Petruccelli, L., Birman, E., Viollet, B., Ferbeyre, G., Pollak, M.N., 2012. Metformin reduces endogenous reactive oxygen species and associated DNA damage. Cancer Prev. Res. Phila. (Phila) 5, 536–543.
Agarwal, N., Rice, S.P., Bolusani, H., Luzio, S.D., Dunseath, G., Ludgate, M., Rees, D.A., 2010. Metformin reduces arterial stiffness and improves endothelial function in young women with polycystic ovary syndrome: a randomized, placebo-controlled, crossover trial. J. Clin. Endocrinol. Metab. 95, 722–730.
Saisho, Y., 2015. Metformin and Inflammation: Its Potential Beyond Glucose-lowering Effect. Endocr. Metab. Immune Disord. Drug Targets 15, 196–205.
Song, Y.M., Lee, Y.H., Kim, J.W., Ham, D.S., Kang, E.S., Cha, B.S., Lee, H.C., Lee, B.W., 2015. Metformin alleviates hepatosteatosis by restoring SIRT1-mediated autophagy induction via an AMP-activated protein kinase-independent pathway. Autophagy 11, 46–59.
Xie, Z., Lau, K., Eby, B., Lozano, P., He, C., Pennington, B., Li, H., Rathi, S., Dong, Y., Tian, R., Kem, D., Zou, M.H., 2011. Improvement of cardiac functions by chronic metformin treatment is associated with enhanced cardiac autophagy in diabetic OVE26 mice. Diabetes 60, 1770–1778.
Jadhav, K.S., Dungan, C.M., Williamson, D.L., 2013. Metformin limits ceramide-induced senescence in C2C12 myoblasts. Mech. Ageing Dev. 134, 548–559.
Moiseeva, O., Deschenes-Simard, X., St-Germain, E., Igelmann, S., Huot, G., Cadar, A.E., Bourdeau, V., Pollak, M.N., Ferbeyre, G., 2013. Metformin inhibits the senescence-associated secretory phenotype by interfering with IKK/NF-kappaB activation. Aging Cell 12, 489–498.
Tentolouris, C., Tousoulis, D., Goumas, G., Stefanadis, C., Davies, G., Toutouzas, P., 2000. L-Arginine in coronary atherosclerosis. Int. J. Cardiol. 75, 123–128.
Tousoulis, D., Antoniades, C., Tentolouris, C., Goumas, G., Stefanadis, C., Toutouzas, P., 2002. L-arginine in cardiovascular disease: dream or reality? Vasc. Med. 7, 203–211.
Gronros, J., Kiss, A., Palmer, M., Jung, C., Berkowitz, D., Pernow, J., 2013. Arginase inhibition improves coronary microvascular function and reduces infarct size following ischaemia-reperfusion in a rat model. Acta Physiol. Oxf. (Oxf) 208, 172–179.
Antoniades, C., Tousoulis, D., Vasiliadou, C., Marinou, K., Tentolouris, C., Ntarladimas, I., Stefanadis, C., 2004. Combined effects of smoking and hypercholesterolemia on inflammatory process, thrombosis/fibrinolysis system, and forearm hyperemic response. Am. J. Cardiol. 94, 1181–1184.
Ohara, Y., Peterson, T.E., Harrison, D.G., 1993. Hypercholesterolemia increases endothelial superoxide anion production. J. Clin. Invest. 91, 2546–2551.
Heart Protection Study Collaborative G, 2002. MRC/BHF Heart Protection Study of antioxidant vitamin supplementation in 20,536 high-risk individuals: a randomised placebo-controlled trial. Lancet 360, 23–33.
Armour, J., Tyml, K., Lidington, D., Wilson, J.X., 1985. Ascorbate prevents microvascular dysfunction in the skeletal muscle of the septic rat. J. Appl. Physiol. 2001 (90), 795–803.
Brass, E.P., Koster, D., Hiatt, W.R., Amato, A., 2013. A systematic review and meta-analysis of propionyl-L-carnitine effects on exercise performance in patients with claudication. Vasc. Med. 18, 3–12.
Csiszar, A., Labinskyy, N., Pinto, J.T., Ballabh, P., Zhang, H., Losonczy, G., Pearson, K., de Cabo, R., Pacher, P., Zhang, C., Ungvari, Z., 2009. Resveratrol induces mitochondrial biogenesis in endothelial cells. Am. J. Physiol. Heart Circ. Physiol. 297, H13–20.
Csiszar, A., Labinskyy, N., Podlutsky, A., Kaminski, P.M., Wolin, M.S., Zhang, C., Mukhopadhyay, P., Pacher, P., Hu, F., de Cabo, R., Ballabh, P., Ungvari, Z., 2008. Vasoprotective effects of resveratrol and SIRT1: attenuation of cigarette smoke-induced oxidative stress and proinflammatory phenotypic alterations. Am. J. Physiol. Heart Circ. Physiol. 294, H2721–2735.
Ungvari, Z., Orosz, Z., Rivera, A., Labinskyy, N., Xiangmin, Z., Olson, S., Podlutsky, A., Csiszar, A., 2007. Resveratrol increases vascular oxidative stress resistance. Am. J. Physiol. Heart Circ. Physiol. 292, H2417–2424.
Banks, A.S., Kon, N., Knight, C., Matsumoto, M., Gutierrez-Juarez, R., Rossetti, L., Gu, W., Accili, D., 2008. SirT1 gain of function increases energy efficiency and prevents diabetes in mice. Cell Metab. 8, 333–341.
Carrizzo, A., Forte, M., Damato, A., Trimarco, V., Salzano, F., Bartolo, M., Maciag, A., Puca, A.A., Vecchione, C., 2013. Antioxidant effects of resveratrol in cardiovascular, cerebral and metabolic diseases. Food Chem. Toxicol. 61, 215–226.
Zhu, Y., Tchkonia, T., Pirtskhalava, T., Gower, A.C., Ding, H., Giorgadze, N., Palmer, A.K.,

Ikeno, Y., Hubbard, G.B., Lenburg, M., O'Hara, S.P., LaRusso, N.F., Miller, J.D., Roos, C.M., Verzosa, G.C., LeBrasseur, N.K., Wren, J.D., Farr, J.N., Khosla, S., Stout, M.B., McGowan, S.J., Fuhrmann-Stroissnigg, H., Gurkar, A.U., Zhao, J., Colangelo, D., Dorronsoro, A., Ling, Y.Y., Barghouthy, A.S., Navarro, D.C., Sano, T., Robbins, P.D., Niedernhofer, L.J., Kirkland, J.L., 2015. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. Aging Cell 14, 644–658.

Roos, C.M., Zhang, B., Palmer, A.K., Ogrodnik, M.B., Pirtskhalava, T., Thalji, N.M., Hagler, M., Jurk, D., Smith, L.A., Casaclang-Verzosa, G., Zhu, Y., Schafer, M.J., Tchkonia, T., Kirkland, J.L., Miller, J.D., 2016. Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. Aging Cell 15, 973–977.

Malavolta, M., Pierpaoli, E., Giacconi, R., Costarelli, L., Piacenza, F., Basso, A., Cardelli, M., Provinciali, M., 2016. Pleiotropic effects of Tocotrienols and quercetin on cellular senescence: introducing the perspective of senolytic effects of phytochemicals. Curr. Drug Targets 17, 447–459.

Kirkland, J.L., Tchkonia, T., Zhu, Y., Niedernhofer, L.J., Robbins, P.D., 2017. The clinical potential of senolytic drugs. J. Am. Geriatr. Soc. 65, 2297–2301.

Kirkland, J.L., Tchkonia, T., 2017. Cellular senescence: a translational perspective. EBioMedicine 21, 21–28.

Minamino, T., Komuro, I., 2008. Vascular aging: insights from studies on cellular senescence, stem cell aging, and progeroid syndromes. Nat. Clin. Pract. Cardiovasc. Med. 5, 637–648.

Matsushita, H., Chang, E., Glassford, A.J., Cooke, J.P., Chiu, C.P., Tsao, P.S., 2001. eNOS activity is reduced in senescent human endothelial cells: preservation by hTERT immortalization. Circ. Res. 89, 793–798.

Minamino, T., Miyauchi, H., Yoshida, T., Ishida, Y., Yoshida, H., Komuro, I., 2002. Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction. Circulation 105, 1541–1544.

Bruunsgaard, H., Skinhoj, P., Pedersen, A.N., Schroll, M., Pedersen, B.K., 2000. Ageing, tumour necrosis factor-alpha (TNF-alpha) and atherosclerosis. Clin. Exp. Immunol. 121, 255–260.

Harris, T.B., Ferrucci, L., Tracy, R.P., Corti, M.C., Wacholder, S., Ettinger Jr., W.H., Heimovitz, H., Cohen, H.J., Wallace, R., 1999. Associations of elevated interleukin-6 and C-reactive protein levels with mortality in the elderly. Am. J. Med. 106, 506–512.

Csiszar, A., Labinskyy, N., Smith, K., Rivera, A., Orosz, Z., Ungvari, Z., 2007. Vasculoprotective effects of anti-tumor necrosis factor-alpha treatment in aging. Am. J. Pathol. 170, 388–398.

Watson, C.J., Horgan, S., Neary, R., Glezeva, N., Tea, I., Corrigan, N., McDonald, K., Ledwidge, M., Baugh, J., 2016. Epigenetic therapy for the treatment of hypertension-induced cardiac hypertrophy and fibrosis. J. Cardiovasc. Pharmacol. Ther. 21, 127–137.

Zhang, X., Hu, M., Lyu, X., Li, C., Thannickal, V.J., Sanders, Y.Y., 2017. DNA methylation regulated gene expression in organ fibrosis. Biochim Biophys Acta Mol Basis Dis 1863, 2389–2397.

Ghosh, A.K., Rai, R., Flevaris, P., Vaughan, D.E., 2017. Epigenetics in reactive and reparative cardiac fibrogenesis: the promise of epigenetic therapy. J. Cell. Physiol. 232, 1941–1956.

Grimaldi, V., De Pascale, M.R., Zullo, A., Soricelli, A., Infante, T., Mancini, F.P., Napoli, C., 2017. Evidence of epigenetic tags in cardiac fibrosis. J. Cardiol. 69, 401–408.