Basic Research in Cardiology (2020) 115:41
https://doi.org/10.1007/s00395-020-0802-6

REVIEW

# Vascular autophagy in health and disease

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Received: 19 December 2019 / Accepted: 26 May 2020 / Published online: 6 June 2020


## Abstract

Homeostasis is maintained within organisms through the physiological recycling process of autophagy, a catabolic process that is intricately involved in the mobilization of nutrients during starvation, recycling of cellular cargo, as well as initiation of cellular death pathways. Specific to the cardiovascular system, autophagy responds to both chemical (e.g. free radicals) and mechanical stressors (e.g. shear stress). It is imperative to note that autophagy is not a static process, and measurement of autophagic flux provides a more comprehensive investigation into the role of autophagy. The overarching themes emerging from decades of autophagy research are that basal levels of autophagic flux are critical, physiological stressors may increase or decrease autophagic flux, and more importantly, aberrant deviations from basal autophagy may elicit detrimental effects. Autophagy has predominantly been examined within cardiac or vascular smooth muscle tissue within the context of disease development and progression. Autophagic flux within the endothelium holds an important role in maintaining vascular function, demonstrated by the necessary role for intact autophagic flux for shear-induced release of nitric oxide however the underlying mechanisms have yet to be elucidated. Within this review, we theorize that autophagy itself does not solely control vascular homeostasis, rather, it works in concert with mitochondria, telomerase, and lipids to maintain physiological function. The primary emphasis of this review is on the role of autophagy within the human vasculature, and the integrative effects with physiological processes and diseases as they relate to the vascular structure and function.

**Keywords** Macroautophagy · Flow-mediated dilation · Endothelium · Telomerase · Mitochondria

## Abbreviations

| | |
|---|---|
| AMPK | 5′ Adenosine monophosphate-activated protein kinase |
| CAD | Coronary artery disease |
| eNOS | Endothelial nitric oxide synthase |
| FMD | Flow-mediated dilation |
| SIRT | Sirtuins |
| $H_2O_2$ | Hydrogen peroxide |
| LC3 | Microtubule-associated protein 1A/1B-light chain 3 |
| mTOR | Mammalian target of rapamycin |
| NO | Nitric oxide |
| ROS | Reactive oxygen species |
| SOD | Superoxide dismutase |
| TERT | Telomerase reverse transcriptase |
| TFEB | Transcription factor EB |
| VSM | Vascular smooth muscle |

## Introduction

Autophagy is a cellular recycling process in response to various stressors (e.g. oxidative stress, hypoxia, and starvation) by which cells attempt to maintain homeostasis by providing recycled metabolic substrates, particularly during times of nutrient shortage/starvation. Functionally, autophagy facilitates switches in cellular phenotypes, such as the transition of smooth muscle cells from contractile to proliferative phenotypes [127], and the conversion of circulating monocytes to macrophages [166]. The process and signaling cascade of autophagy has been well described across many cell types in various species ranging from yeast to mammals, is relatively well-conserved evolutionarily, and importantly, disruption or excessive autophagy underlies the pathology of numerous chronic diseases (for reviews, see: [27, 40, 49, 103, 123]). Broadly, damaged or superfluous intracellular components are encapsulated within double-membraned

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autophagosomes, which fuse with lysosomes, and are subsequently degraded by acidic hydrolases into cellular metabolites that are then recycled for use in other physiological processes. Within these constraints and throughout this review, the term autophagy refers to macro-autophagy, being more prevalent than other forms of autophagy- micro-autophagy and chaperone-mediated autophagy. Micro-autophagy directly envelops vesicles via invaginations within the membrane, while chaperone-mediated autophagy occurs in mammalian cells and involves direct targeting and transportation of organelles that express a specific molecular target [5, 142]. The focus of this review is on macro-autophagy and cardiovascular disease with special emphasis on the role of autophagy in vascular health and function.

Autophagy is a key regulator of cardiovascular homeostasis, responding to physiological and pathophysiological stimuli (Fig. 1). Autophagy has been studied within cardiac tissue (e.g. cardiomyocytes), vascular smooth muscle (VSM), and cultured endothelial cells [162]. While recycling of cellular organelles is generally viewed as a beneficial process, insufficient and excessive levels of autophagy can lead to premature cell death (apoptosis). VSM and endothelial cells are plastic tissues responding to environmental factors that elicit changes in phenotypes [108, 137] therefore autophagy is critical in the maintenance of cellular homeostasis. Surprisingly, far less is known about how autophagy influences vascular function, specifically the microvasculature, or how it contributes to vascular pathologies. The primary focus of this review is to provide evidence on novel roles for autophagy within the vasculature from a functional perspective, and relevance to other physiological processes.

## Mechanisms of autophagy

Autophagy is highly sensitive to nutrient conditions and ATP levels [30]. Under conditions where nutrients are depleted or ATP diminished, autophagy is activated to conserve and regenerate metabolic substrates, thereby sustaining homeostasis. The most widely recognized regulators of autophagy are the mechanistic target of rapamycin complex 1 (mTORC1), which acts as a negative regulator of autophagy, sensing amino acid levels and inhibiting autophagy when levels are high, and 5′ adenosine monophosphate-activated protein kinase (AMPK), an energy sensing pathway that regulates autophagy by detecting the intra-cellular ratio of adenosine triphosphate (ATP) to adenosine monophosphate (AMP). Protein kinase B (AKT) regulates mTOR via class 1 phosphoinositide 3-kinase (PI3K). An integral part of the autophagy signaling cascade is the interaction between various autophagy related proteins to elongate the double-membrane autophagosome that encapsulates damaged or superfluous cells/organelles. It should be stressed that autophagy is not a static process, but rather dynamic, responding to various physiological stressors. Many of the methods to investigate autophagy are predicated on measuring snapshots of this dynamic process such as genetic markers or protein quantification in response to pharmacological/physiological interventions. With developing imaging technology, autophagic flux has become more easily measured and is a more robust indicator of the autophagy process. Readers are referred to recent reviews on measuring autophagic flux in various tissues [43, 72].

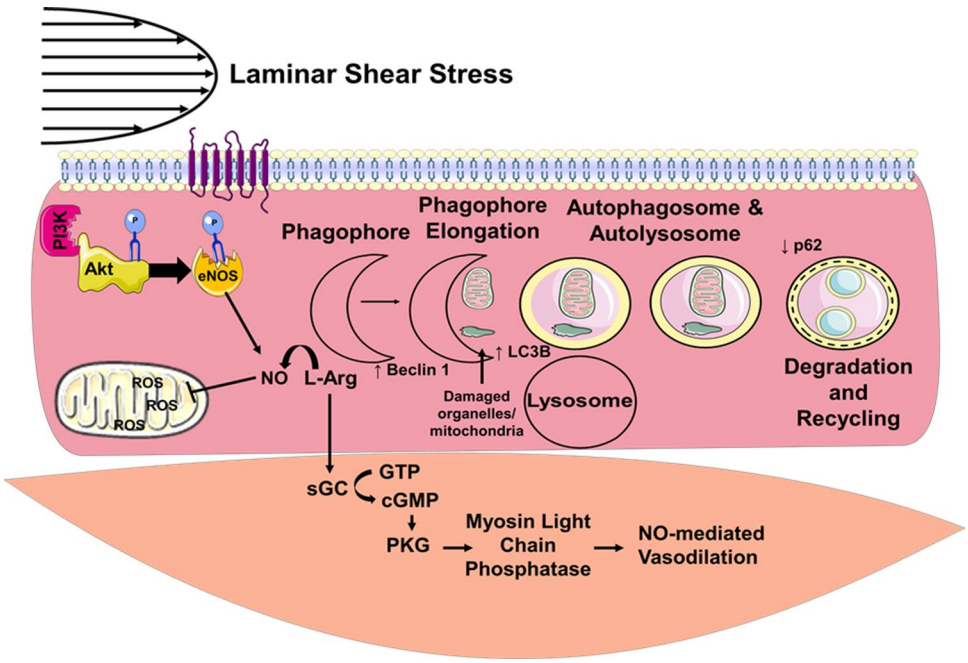


**Fig. 1** Conceptual overview of shear-induced signaling pathways to elicit vasodilation in health. Laminar shear stress confers adaptive autophagy within the endothelium and vascular smooth muscle by enhancing production of NO from eNOS, minimizing mitochondria-derived ROS, ultimately eliciting NO-mediated vasodilation. *NO* nitric oxide, *ROS* reactive oxygen species, *PI3K* Phosphoinositide 3-kinase, *eNOS* endothelial nitric oxide synthase, *L-Arg* l-arginine, *sGC* soluble guanylate cyclase, *GTP* guanosine triphosphate, *cGMP* cyclic guanosine monophosphate, *PKG* protein kinase g

## Autophagy and cardiovascular health and disease

Autophagy holds an essential role within development and progression of cardiovascular disease [12, 38, 39, 78, 122]. Excessive or insufficient levels of autophagic flux contribute to cardiovascular disease pathologies. Data from experimental animal models utilizing genetic deletions of various autophagy-related genes demonstrate structural and functional changes within the developing cardiovascular system, including defective development of valves and chambers of the heart, as well as development of atherosclerotic plaques within coronary arteries [81]. Various cardiovascular-related stressors such as aging, ischemia–reperfusion injury [93], biological and lifestyle factors (such as genetics, smoking, hypertension, and low physical activity) impact autophagy related genes, and proteins (e.g. LC3B, Atg12, Atg3), ultimately contributing to cardiovascular disease development and progression. Collectively, these factors are associated with an increase in reactive oxygen species (ROS), which is holds an important role in cardiovascular function in health and disease.

## Free radical species, reactive oxygen species and regulation of autophagy

Under healthy basal conditions, ROS and reactive nitrogen species (RNS) are maintained at physiological levels by anti-oxidants, and by superoxide dismutases (SOD's) [153]. NO quenches superoxide in an almost diffusion-limited manner, and much faster than spontaneous or enzymatically facilitated conversion to hydrogen peroxide ($H_2O_2$) by SOD [55]. Elevations in NADPH oxidase expression also generate elevated levels of ROS [33]. Overproduction of, prolonged exposure to ROS, or insufficient production of anti-oxidants ultimately results in oxidative stress, altering mitochondrial structure (e.g. membrane potential) and function (e.g. respiration, fission/fusion) resulting in protein modifications/aggregation and ultimately cell death [67, 141]. Free radical species are well known to modify mitochondrial proteins and function creating a precipitous cycle wherein cytosolic or mitochondria-derived ROS generate further ROS release from the mitochondria [172].

Under pathological conditions where cellular organelles are damaged by free radical species, autophagy/mitophagy is activated to degrade and recycle damaged organelles. If the damaged organelle is efficiently degraded and recycled, this results in cellular survival and maintenance of homeostasis. If the organelle is only partially, or incompletely degraded, this can result in further oxidative stress, accelerated by ROS-induced ROS-release cycle [99, 145, 162]. Autophagy is also activated by exposure to endothelial shear stress, enhancing phosphorylation of endothelial nitric oxide synthase (eNOS) and production of NO, but also generating ROS [9]. Thus free radicals and autophagy influence the vasculature resulting in adaptive and maladaptive outcomes, highlighting the role of free radicals as critical regulators of autophagy specific to the vasculature.

## Role of autophagy and vascular function with health and disease

### Autophagy within vascular smooth muscle

The vascular media is a plastic tissue as vascular smooth muscle cells (VSMC) may exhibit multiple phenotypes in response to environmental factors contributing to the development and progression of atherosclerotic plaque [6]. Readers are referred to a recent review by Salabei and Hill [127] for a comprehensive molecular overview of the role of autophagy in VSM.

#### Autophagy and atherosclerosis

Development of atherosclerosis is associated with VSM phenotype switch, smooth muscle cell death, plaque instability of arterial wall lesions, and importantly, vascular calcification [45]. Activation of autophagy in VSM is generally adaptive promoting VSMC survival, plaque stabilization [84] and reducing vascular calcification [23, 89]. Osonoi, et. al [110] demonstrated that within murine cultured smooth muscle cells, genetic deletion of Atg7 (involved autophagosome formation) increases atherosclerotic burden and results in maladaptive arterial remodeling with descending aortic ruptures being the most common cause of death [110]. Macrophage autophagy plays a protective role within the early phase of atherosclerosis as genetic deletion of Atg5 is demonstrated to accelerate atherosclerosis progression within murine models [87, 117]. Inefficient autophagy as a result of Atg5 deletion may result in further foam cell development and exaggerated inflammatory markers. Conversely, enhancement of autophagy within VSM and macrophages via trehalose or overexpression of transcription factor EB (TFEB) exerts athero-protective effects, reducing plaque burden and reductions in inflammatory markers [32, 149].

An important factor predisposing to cardiovascular events is the propensity for an atherosclerotic plaque to rupture vs. remain stable [4]. Basal autophagy has been demonstrated to be important in maintaining the integrity of the fibrous cap and restricting lipid accumulation [45, 44, 132, 144, 146]. Insufficient or inhibition of autophagy accelerates plaque

burden or renders it unstable, while activation of autophagy generally maintains stability [32, 44, 65, 87, 132, 147, 159]. In this context, arteries continuously exposed to laminar or high shear stress are protected against plaque formation [65, 147], and intact autophagy (that is, not insufficient nor hyperactive) is required for limiting plaque formation within these areas, attenuating cell death and release of inflammatory markers [157]. Blood flow within an artery may modulate plaque stability through autophagy via endothelial cell to VSM transmission [65, 147]. For example, shear-induced secretion of platelet derived growth factor (PDGF) isoforms by the endothelium act on PDGF receptors in VSM to determine proliferative/migratory activity and VSM phenotype in an autophagy dependent manner [48, 113].

Autophagy is a potent target for the treatment of atherosclerosis, as well as for limiting the detrimental effects of plaque rupture and release of plaque debris and thrombotic factors. This concept is demonstrated by the anti-atherosclerotic beneficial effects (e.g. reduced VSM proliferation) of drug-eluding stents containing first and second generation mTOR inhibitors such as sirolimus and everolimus [76, 95]. Some data suggests that mTOR inhibitor-coated stents result in favorable clinical outcomes (reduced neointimal hyperplasia or repeat revascularization) when compared to bare metal stents chronically [11, 42, 102, 138]. In the short term, however, drug-eluting stents may promote thrombosis at the level of stent placement, necessitating dual anti-platelet therapy. This is due to delayed endothelialization which is associated with an increase in platelet aggregation [35, 41, 58]. Stent placement within blocked arteries is designed to limit ischemia of downstream tissue, however deployment of the stent may induce localized endothelial injury from balloon inflation resulting in plaque rupture and release of particles eliciting downstream coronary microvascular dysfunction [52, 70, 71]. Thus, there appears to be both atheroprotective and detrimental effects of autophagy stimulation. The specific role of autophagy in mediating these events is unclear, and represents a future area of investigation.

Disease severity may play a role in the effectiveness of autophagy in drug-eluting stents, however direct evidence for this is lacking. For example, Zhao, et al. [168]. utilizing peripheral blood monocytes demonstrated that acute myocardial infarction decreased beclin-1 and LC3II levels relative to control patients, or stable angina pectoris. In this study, patients with unstable angina pectoris also demonstrate reductions in beclin-1 and LC3II relative to stable angina and controls, but greater than those with acute myocardial infarction. It should be noted that this study did not investigate autophagic flux per se and requires further investigation. Given this evidence it appears that acute cardiovascular events and heightened disease severity result in reductions in autophagy. On the other hand, it is well established that autophagy is beneficial for survival in response to ischemia, while reperfusion stimulates excess autophagy resulting in more cell death. Overall, the effects of autophagy appear to be context dependent [160].

It should be noted that while current generation drug eluting stents containing mTOR inhibitors confer some beneficial effect through autophagy, systemic administration of mTOR inhibitors result in side-effects such as dyslipidemia, hyperglycemia, hypertension and immuno-suppressive effects [77, 95, 132]. Targeting macrophage autophagy for treatment of atherosclerosis has emerged as a promising approach. Activation of autophagy via TFEB in macrophages reverses plaque-induced reductions in autophagy and protects against plaque development within animal models [32, 135]. Other cardiovascular drugs such as statins and calcium channel blockers may induce autophagy. For a review on current cardiovascular drugs and their potential use in autophagy, readers are directed to a review by Salabei and Conklin [124].

### Autophagy and hypertension

Hypertension shares an etiological relationship with cardiovascular disease. Evidence links autophagy to the development and progression of systemic arterial hypertension independent of effects on cardiac tissue [24, 109, 131, 133, 170, 171]. Specific to the VSM, increases in autophagy have been demonstrated to promote the switch in VSM phenotype from contractile to macrophage-like and synthetic/proliferative. At the same time, hypertrophy, proliferation and calcification VSM increase contractile tone, subsequently increasing peripheral resistance and systemic blood pressure [127, 126]. In obese, hypertensive Zucker diabetic fatty rats, excessive autophagy is associated with hypertension and endothelial dysfunction, while administration of a resveratrol analogue ameliorated these detrimental effects, which was blocked by co-administration of rapamycin [29]. Induction of hypertension with angiotensin II activates mTORC1 leading to hypertrophic responses in vascular smooth muscle [47, 140], while, inhibition of mTORC1 in rats fed a high-salt diet ameliorates salt-induced hypertension [75]. More recently, McCarthy, et al. [98]. demonstrated that autophagic activity is reduced in spontaneously hypertensive relative to normotensive Wistar rats which was associated with endothelial dysfunction. Interestingly, restoration of autophagy with trehalose improved endothelial function in SHR independent of improvements in blood pressure. Most data examining autophagy in hypertension has been collected in large vessels, however as blood pressure and organ perfusion are regulated by small resistance vessels. As such, the role of autophagy in arterial hypertension within the vasculature remains on ongoing area of study.

Pulmonary hypertension is characterized by hyperproliferation of VSM within pulmonary arteries (resistance

arteries) leading to reductions in lumen diameter, dramatic increases in pulmonary artery pressure, eventually resulting in right heart failure. These VSM and microcirculatory changes are a result of an over-activation of autophagy. Indeed, markers of autophagy are elevated in the lungs of patients with pulmonary hypertension, as well as within various animal models of pulmonary hypertension [82]. Inhibition of autophagy by blocking lysosomal degradation within these various models impedes the development and progression of pulmonary hypertension [91]. Additionally, pulmonary hypertension results in mitochondrial fragmentation, inducing a hyperproliferative state within VSM [121]. Thus autophagy contributes to both systemic and pulmonary hypertension through modulation of VSM phenotypes within both large and smaller arteries, although divergent effects further highlight the complex, and dichotomous nature of autophagy.

## Influence of autophagy on endothelial function with health and disease

Endothelial function is a critical barometer of cardiovascular health influenced by numerous factors including age, oxidative stress, genetics, and lifestyle factors. "Conduit artery" (i.e. macrovasculature) refers to the large elastic capacitance arteries that conduct blood under relatively high pressure to the distal vessels, and include the aorta, and primary arterial branches to visceral organs and somatic tissues. More distal to the heart, the composition of arteries changes from elastic to more muscular in content, allowing for greater vasomotion and regulation of perfusion and blood pressure. As arteries branch, both the number and summed cross-sectional area increases, peaking with capillaries characterized by low flow, low pressure, but high volume of distribution (e.g. microvasculature) [21]. Both the macro- and microvasculature exhibit vasodilation to various pharmacological stimuli, as well as shear stress (flow-mediated dilation, FMD) with the primary endothelium-dependent mechanism of dilation under physiological conditions being NO in health (Fig. 1) [57]. Extensive data from our lab indicates that development of coronary artery disease [88, 116], physical (increased intraluminal pressure) [7, 31] or chemical stressors (e.g. increase in ceramide, or lysophosphatidic acid) [14, 36] switches the primary mechanism of microvascular dilation from NO to the $H_2O_2$ largely due to an increase in mitochondria-derived ROS (Fig. 2) [88, 116]. The specific role of autophagy on microvascular function in health and disease is an emerging area of research.

### Autophagy and endothelial-(dys)function

The endothelium is a critical regulator of vascular health and function and is constantly exposed to varying levels of shear stress via blood flow which in turn releases various vasoactive compounds to regulate vascular tone and vascular cell phenotype. The autophagy signaling cascade has typically been studied within cultured endothelial cells. Cultured endothelial cells are proliferative, and have divergent responses to physical and chemical stressors when compared to quiescent cells within the vasculature, and thus may exhibit differential autophagy responses as well. Nevertheless, systemically circulating compounds/chemicals, as well as processes within endothelial cells themselves regulate autophagy and promote endothelial dysfunction. As discussed previously, atherosclerosis is a hallmark of vascular aging, with endothelial dysfunction as a precursor within the development of atherosclerosis. Indeed, oxidized low-density lipoprotein [165], advanced glycation end-products [158], and various lipid compounds may promote autophagosome formation within endothelial cells which are mediated in part by ROS [56].

Mechanical forces are an important modulator of endothelial autophagy [68]. It is important to note that autophagy within endothelial cells serves as a renewal function, protecting against endothelial cell injury, and plays an integral role in mediating the progression of vascular diseases. Shear stress generated by the frictional forces (blood flow) along the endothelium induces autophagy in a reversible manner via mechano-transduction, and is critical for endothelial cell alignment [85, 147] NO production, and excessive ROS mitigation (Figs. 1, 2) [15, 54]. Shear stress along endothelial cells may be laminar (smooth flow) or turbulent/oscillatory, and is usually pulsatile in larger vessels that dampens with transmission to the microvasculature. High levels of laminar shear stress ($> 15$ dynes/cm$^2$) promote autophagy within endothelial cells, and up-regulate expression of endothelial NO synthase (eNOS) while inhibiting expression of the potent vasoconstrictor endothelin-1 (ET-1) [46]. This physiological response to shear is sustained suggesting a chronic beneficial effect on the vasculature [90]. Pre-treatment with rapamycin, an mTOR-dependent autophagy activator, further enhances eNOS expression and suppresses ET-1 expression, while pre-treatment with an inhibitor of autophagy (3-MA), enhances ET-1 expression. In contrast, low levels of shear stress (e.g. regions of artery curvature, arterial segments distal to carotid artery ligation) reduce autophagy and promote pro-atherogenic responses [147, 167]. Disturbed/turbulent flow increases autophagosome formation but impair p62-mediated clearance of autophagosomes and promotes endothelial dysfunction [85, 147]. Bharath et al. demonstrated that within bovine aortic endothelial cells exposed to physiological levels of shear stress, autophagy markers increased due to an increased phosphorylation of AMPK [9]. These elevations in autophagy indicators and phosphorylated AMPK regulate mTOR signaling and thereby autophagy [9, 163]. Guo et al. [46]. demonstrated that physiological levels

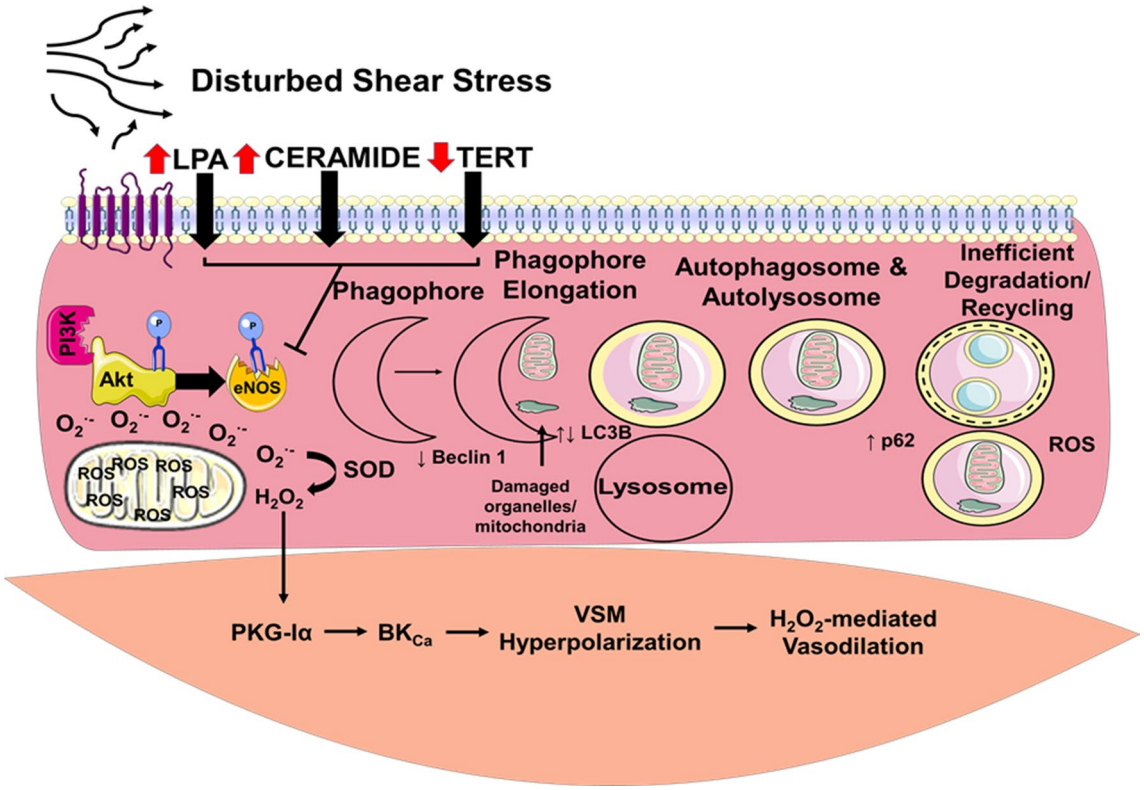


**Fig. 2** Disturbed shear stress, decreases in TERT, as well as elevations in LPA and ceramide confer maladaptive autophagy. Both excessive and insufficient, minimizes NO formation from eNOS, preferentially producing $H_2O_2$ and ultimately driving the primary mechanism of vasodilation to $H_2O_2$ in response to shear stress. Maladaptive autophagy may not sufficiently degrade the cellular cargo, ultimately eliciting further elevations in ROS. *LPA* lysophosphatidic acid, *TERT* telomerase reverse transcriptase, *NO* nitric oxide, *ROS* reactive oxygen species, *PI3K* Phosphoinositide 3-kinase, *eNOS* endothelial nitric oxide synthase, $O_2^-$ superoxide, $H_2O_2$ hydrogen peroxide, $BK_{Ca}$ large conductance calcium activated potassium channel, *VSM* vascular smooth muscle

of laminar shear stress increase markers of autophagy which were associated with up-regulation of eNOS expression, and parallel down-regulation of the vasoconstrictor ET-1. Importantly, autophagy activation (rapamycin) enhanced eNOS expression and reduced ET-1 expression in response to shear, while inhibition of autophagy with 3-MA exerted the opposite effect. Inhibition of mTOR via rapamycin treatment in transgenic mice (designed to express fluorescent labeled human eNOS isoforms), increased eNOS expression in regions of the carotid artery exposed to low shear stress, while reducing maximal eNOS expression at regions of high shear stress [19]. The authors attributed the paradoxical reduction in maximal eNOS expression in areas of high shear stress to rapamycin having a shear-stress dependent impact on protein synthesis, however this was not tested directly [19].

These findings in cells and animals have been recently translated into humans utilizing novel approaches. Park et al. [114] demonstrated that the hyperemic response to dynamic handgrip exercise elevated primary endothelial cell markers of autophagy in parallel with NO production within the radial artery. In subjects with diabetes, Fetterman et al. [34] reported reductions in autophagy along with endothelial dysfunction, and impaired eNOS activation) at the level of autophagosome-lysosome fusion. The same investigators demonstrated within cultured endothelial cells that exposure to high glucose concentrations impaired autophagy similar to that observed in vivo, and inhibiting autophagy with bafilomycin (inhibits lysosome acidification) abrogated insulin-mediated eNOS activation, but did not change basal eNOS phosphorylation. Activation of autophagy with spermidine improved insulin-mediated eNOS phosphorylation in both primary and cultured endothelial cells, but had no effect in cells exposed to high glucose [34]. These results demonstrate a prominent role for autophagy in vascular function both in health and disease, and highlight the role for mechanical (shear) and chemical (high glucose) stress in contributing to function of the endothelium at least in part through autophagy.

While the endothelium releases NO in response to shear stress to elicit vasodilation, ROS are also released, particularly from within diseased endothelium. $H_2O_2$, an endothelial derived hyperpolarizing factor, is preferentially formed and released from the endothelium of arterioles from

subjects with CAD, or from non-CAD arterioles subjected to telomerase reverse transcriptase inhibition (TERT), ceramide, LPA, as well as acute pressure stress in response to flow [7, 8, 13, 14, 31, 36, 88, 116]. Shear-induced $H_2O_2$ can damage mitochondrial DNA and enhance superoxide generation which sequesters NO by forming peroxynitrite a potent inhibitor of endothelial NOS and prostacyclin synthase [112]. Deletion/knockdown of Atg3 in endothelial cells reduces NO release and exaggerates ROS production [9]. Liu et al. [90] demonstrated using gain/loss of function experiments that shear-induced autophagy markers are sensitive to redox status. The authors further demonstrated that under flow conditions, endothelial cells were more resistant to oxidant-induced injury, but this protective effect was abolished with inhibition of autophagy [90]. Similarly, Bharath et al. demonstrated that shear-induced autophagy within endothelial cells maintains NO generation via glycolytic dependent purinergic signaling [10].

Data from pre-hypertensive spontaneously hypertensive rats and obese Zucker diabetic fatty rats suggest that excessive autophagy may contribute to microvascular endothelial dysfunction (e.g. reduced endothelium- dependent dilation to acetylcholine) demonstrated by reduced phosphorylation of mTOR concomitant with increased ratio of LC3 II/I and p62 within the microvasculature (3$^{rd}$ order mesenteric arterioles). Increases in LC3 II/I and elevated p62 indicate a high rate of formation of autophagosomes, but impaired fusion of the autophagosome with the lysosome, impeding the clearance of damaged cargo. These effects were ameliorated via activation of mTOR, subsequently suppressing autophagy, suggesting at least pre-hypertension is associated with impaired autophagic flux [28, 29]. These data highlight and provide proof of concept concerning the critical role of autophagy within endothelial cells in response to shear stress as well as the dual nature of autophagy within the development of cardiovascular disease. Future investigations should examine the role of autophagic flux in determining the primary mechanism of microvascular FMD within health and disease, as well as the role of autophagic flux in development of vascular disease pathologies.

Sirtuins (SIRT) have a broad range of physiological effects ranging from cell survival to mediation of signaling pathways, with several SIRT proteins identified in humans [66]. SIRT1 exerts a variety of cellular effects such as regulation of cell cycle, and longevity effects as a positive regulator of autophagy [69]. SIRT1 may directly deacetylate specific autophagy related proteins critical for the autophagy signaling cascade, directly activating the AMPK-mTOR-autophagy axis, as well as deacetylate eNOS, enhancing NO production and promoting NO-mediated endothelium dependent vasodilation [96]. Laminar shear stress enhances SIRT1-induced eNOS deacetylation; however, it appears that a basal level of phosphorylated eNOS via AMPK is necessary for this interaction [17]. Within the context of vascular regulation by autophagy, Liu et al. demonstrated that physiological levels of flow on cultured endothelial cells promotes autophagy via SIRT1 activation in a redox sensitive manner [90], while administration of the exogenous activator of autophagy, spermidine, exerts its protective effects on the vasculature independent of SIRT1 activity [105]. Loss or impairment of another NAD-dependent deacetylase sirtuin, SIRT3, results in cardiac hypertrophy, accelerated development of angiotensin II induced hypertension, along with elevated mitochondrial superoxide production concomitant with reduced eNOS production of NO [26]. SIRT3 deficiency or deletion does not impact native endothelial function, but does exacerbate angiotensin II induced endothelial and cardiac dysfunction [26, 151]. Interestingly, loss of SIRT3 impairs autophagic flux as demonstrated by reduced autophagosome formation and reduction in autolysosome degradation [86]. Conversely, overexpression of SIRT3 enhances autophagy, specifically, mitophagy and results in reduced mitochondrial ROS, and decreased fibrosis [151]. Collectively, these findings further highlight the role of autophagy as a critical regulator of vascular function, and highlight the role for upstream modulators of autophagy on vascular function. The interplay and role for SIRT and autophagy on cardiovascular function remains an ongoing area of investigation.

### Aging, endothelial dysfunction and autophagy

Advancing age is associated with exaggerated ROS concomitant with reductions in NO bioavailability, directly impacting the health and function of the vasculature [134]. Vascular aging is characterized by stiffening of conduit arteries, endothelial dysfunction, atherosclerosis, and increases in cardiovascular disease risk [100]. Several lines of evidence demonstrate that autophagy mediates some of these age-associated maladaptive responses. Within primary endothelial cells from older adults, autophagy markers are reduced relative to young adults [79]. Furthermore, atherosclerotic plaques are found in regions of low shear stress, or within the curvature of arteries, and enhancing autophagy, or increasing shear stress in these areas is generally beneficial [10, 9, 19, 46, 65, 114, 147]. Enhancing autophagy within the vasculature is an attractive target for treatment of cardiovascular disease. LaRocca et al. [80] demonstrated that four weeks of supplementation with the autophagy inducer spermidine reduced large artery arterial stiffness (assessed by carotid-femoral pulse-wave velocity), improved endothelial function (enhanced dilation to intra-arterial acetylcholine), and reduced markers of oxidative stress in aged rats. These improvements in older rats paralleled elevations in proteins associated with markers of autophagy suggesting that enhancing autophagy in a model of cardiovascular

aging improves conduit vascular responses, effectively recapitulating a younger cardiovascular phenotype. This same group demonstrated that primary endothelial cells harvested from the brachial artery of older adults exhibit reductions in autophagy markers that are associated with markedly reduced endothelial function [79]. In a parallel mouse model, trehalose, a non-reducing natural disaccharide that induces autophagy through nuclear translocation of TFEB and inhibition of cellular glucose transport [25], restored endothelium-dependent dilation and reduced markers of oxidative stress [79]. Kaplon et al. [63] translated these findings into humans, demonstrating that trehalose supplementation improved both endothelium-dependent and independent dilation within the microvasculature (reactive hyperemia), but not within conduit arteries. Although these studies demonstrate improvement in vascular health through enhancement of autophagy, they should be interpreted with caution as concentrations of trehalose used exceeded 100 mmol/L, while animal data utilizing i.p. administration demonstrates that as little as 1 mM exerts beneficial effects [135]. Additionally, in the study by Kaplon et al. vascular improvements were restricted to subjects who gained less than 5 lb [63]. In contrast to these studies, Headland et al [51]. examined endothelial function in response to short-term energy restriction (a potent inducer of autophagy), demonstrating that brachial artery FMD exhibited no changes in response to consecutive days of calorie restriction. It should be emphasized that involvement of autophagy within this experimental design is only implied, and examination of conduit artery function requires further investigation. Collectively, trehalose and caloric restriction represent potential avenues for improvement of vascular function, however this area requires further investigation.

Caution is warranted when interpreting the influence of shear stress on autophagy in cultured endothelial cells as this experimental design does not truly mimic the arterial system, which is comprised of both straight segments (exposed to laminar flow) as well as bifurcations and bulges (e.g. carotid sinus). Pestana et al. [115] demonstrated that inhibition of starvation-induced autophagy via chloroquine promoted NO-mediated vasodilation in a dose–response manner, and protected against superoxide generation in HUVEC's and rat aortic rings. These results are in direct contrast to previous studies discussed above showing that enhancing autophagy elicits NO-mediated vasodilation. Reconciliation of these divergent results may depend on how autophagy is activated/inhibited. In this regard, proximal inhibition of autophagy with the class III PI3K inhibitor 3 methyladenine (3-MA) (inhibition of elongation of phagophore) may paradoxically induce autophagy under nutrient rich conditions, while suppressing autophagy under starvation conditions [62, 154]. Conversely, bafilomycin A and chloroquine act more distal within the autophagy signaling cascade to inhibit fusion of the autophagosome with the lysosome, neutralizing the acidic hydrolases within the lysosome, respectively [107]. Collectively, these divergent findings of pharmacological modulation of autophagy highlight the dichotomous nature of autophagy and the necessity to interpret these results within the appropriate experimental design. Recently, more specific sensors/probes have been developed to more accurately reflect autophagic flux [43, 62, 72].

## Integration of autophagy, signaling pathways, and future directions as they relate to vascular function

### Autophagy, mitophagy and the mitochondria

Mitochondrial biogenesis is regulated by peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), while recycling is mediated by mitochondrial specific autophagy (mitophagy), which has a distinct signaling cascade [12, 150]. Mitochondrial function is a key regulator of cardiovascular health, as mitochondrial disturbances contribute to development and progression of disease. As mitochondria are the main source of cellular ATP, optimal function is of key importance to maintaining homeostasis, particularly in response to physiological stressors. Upon exposure to shear stress, cultured endothelial cell mitochondria release ATP and enhance intracellular calcium signaling [161], ultimately eliciting vasodilation. Mitochondria continuously undergo fluctuations in structure and function. Mitochondrial fission induced by activation of cellular stress pathways and cytosolic proteins permeabilize the mitochondrial membrane, changing the morphological structure to a more spherical shape and fragmented organization, forming a small bud that is then tagged for encapsulation by an autophagosome. Mitochondrial specific proteins mark damaged mitochondria for encapsulation and degradation by mitophagy. PTEN-induced kinase 1 (PINK1) and Parkin are integral synergistic regulators of mitophagy. Depolarized mitochondria causes PINK1 to accumulate on the outer membrane, recruiting Parkin, which marks damaged mitochondria for mitochondrial specific autophagy [53, 106]. Conversely, mitochondrial fusion activated by various GTPases prevents autophagic degradation of mitochondria and are associated with repair of damaged mitochondria, however the exact mechanism for this is unclear.

Damage to mitochondria, whether structural or functional, alters mitochondrial respiration in favor of production of ROS such as superoxide, $H_2O_2$, as well as reactive aldehydes such as 4-hydroxy-2-nonenal through lipid peroxidation [59]. Mitochondrial damage starts a vicious cycle of ROS-induced ROS-release [172]. Aging itself is associated with elevated basal levels of ROS [59], as well as alterations

to mitochondria structure and function [143]. Mitophagy selectively degrades damaged mitochondria, thus limiting ROS production and accumulation in response to pathophysiological stressors. Attenuated or impaired formation of new mitochondria, and accumulation of damaged mitochondria are a cornerstone in the development of chronic diseases associated with aging, such as heart failure [64, 73].

## Vascular effects of mitophagy

Mitochondria are critical in the development and function of the cardiovascular system, providing ATP for maintenance of vascular homeostasis. PGC1α is expressed in endothelial cells and helps protect against oxidative stress, limiting inflammation and maintaining/improving NO bioavailability [22]. Given that mitochondrial dysfunction is associated with vascular dysfunction and atherosclerosis [12, 83, 88, 152] autophagy/mitophagy generally has a protective role in response to stress in VSM and the endothelium (Tables 1, 2). Mitochondria-derived ROS promote damage and degradation of mitochondrial DNA, contributing to inflammation and progression of atherosclerosis. For example, within VSM, mitochondrial fission has been demonstrated to play a critical role in limiting intimal hyperplasia weakening mitochondrial membrane integrity and enhancing mitochondria-derived ROS formation [148]. In further support of this, expression of the key regulator of mitochondrial fission, dynamin-related protein 1 (DRP-1) is upregulated within vascular calcification formations, while inhibition of DRP-1 in VSMC limited the phenotype transition [118]. Mitochondrial fusion as a result of phosphorylation of the outer mitochondrial membrane protein mitofusion-2 suppresses VSMC proliferation and phenotype switching, ultimately mitigating ROS-induced damage [169].

Elevations in mitochondria-derived ROS have been demonstrated to contribute to endothelium-dependent vasodilation [16, 88, 152]. Mitochondria-derived $H_2O_2$ is the primary mechanism for FMD in patients with CAD, and loss of PGC1α is implicated in contributing to CAD pathology. Data from our laboratory demonstrate that upregulating PGC1α in isolated arterioles from patients with CAD creates a unique FMD phenotype mediated by both NO and $H_2O_2$, protects against acute exposure to increased intraluminal pressure and is important for exercise-induced protection against vascular stress. Conversely, in non-CAD tissue inhibition of PGC1α switches the primary mechanism of FMD to $H_2O_2$ [61, 60]. Inhibition of mitochondrial fission within rat aorta and mesenteric arteries, attenuates the vasoconstrictor effects of ET-1 [18], while exposure to a high glucose induces mitochondria fission and fragmentation. These increases in fission were associated with enhanced mitochondria-derived ROS concomitant to reductions in NO signaling [136]. Ultimately, mitochondrial dynamics regulate mitochondrial turnover that is intimately involved in not only autophagic/mitophagy flux, but also strongly influence vascular function.

## Autophagy and telomerase

While the vasculature is typically non-replicative, mechanical (e.g. oscillatory/turbulent shear stress) and chemical (e.g. oxLDL) injury to the vascular wall elicits replication and proliferation that precede atherosclerosis. Continued replication or division of cells is mediated by specialized structures called telomeres, located on the end of chromosomes that act to maintain stability and functionality while undergoing replication or division [111]. Subsequent release of ROS due to telomere dysfunction (shortening/uncapping) necessitates that autophagy responds to damaged cellular organelles. Telomerase is comprised of an RNA component, TERC and a catalytic component, telomerase reverse transcriptase (TERT), that work in concert to counteract telomere shortening and maintaining telomere length. Telomerase and its subunits have been described as nuclear-specific, however more recent evidence has demonstrated non-canonical roles for TERT within the mitochondria [128, 129]. TERT may be reversibly shuttled from the nucleus and translocated to the mitochondria where it exerts a protective effect in response to oxidative stress [1, 139]. Administration of antioxidants and statins prevent this shuttle [1]. This movement of TERT from the nucleus to mitochondria appears to be dependent upon mTOR signaling as dietary restriction and administration of rapamycin (activate autophagy via mTOR suppression) results in mitochondrial localization of TERT and decreased ROS within mouse brain homogenates and fibroblasts [101]. Taken together, TERT and autophagy hold integral supportive roles within intracellular signaling and cell survival in response to physiological stressors and there appears to be significant intersections between them (Tables 3, 4).

## Telomerase and autophagy in the vasculature

In the vasculature, telomere shortening/uncapping induces mitochondria DNA damage, ROS production and is inversely related to forearm endothelium-dependent dilation [104, 120], inflammation, and reductions in NO [130]. Beyer et al. [8] examined whether telomerase activity influences flow-mediated dilation within the microvasculature in subjects with and without coronary artery disease (CAD). Inhibition of telomerase activity with BIBR 1532 in non-CAD vessels had no effect on the magnitude of FMD, but switched the mechanism of dilation from NO to $H_2O_2$, similar to that seen with CAD. Conversely, in CAD vessels treated with an activator of telomerase, the primary mechanism of dilation

**Table 1** Mitochondria in vascular smooth muscle

| Organism | Model/cell line | Mechanism of action/intervention | Target-relation to autophagy | Vascular outcomes | References |
|---|---|---|---|---|---|
| Human and rat | Atherosclerotic carotid arteries; aortic VSMC; C57B6 aorta VSMC | ↑DRP-1 | Mitochondrial fission | Vascular calcification, ↓DRP-1 restricts VSM phenotype transition and ↓oxidative stress | [118] |
| Mouse and cell | Thoracic aorta VSMC; NIH/3T3; MEF | Platelet-derived Growth Factor (PDGF) | PDGF → fission via mitochondrial localization of DRP-1$^{Ser616}$. ↓of fission blocks PDGF-induced cell migration | ↑↑ Fission and ROS indicate excessive mitophagy. Inhibition of fission ↓intimal hyperplasia | [148] |
| Human and cell | Primary VSMC from mesenteric arteries; HeLa; HeLa-Parkin | oxLDL-induced apoptosis | oxLDL depolarizes mitochondria and induces fission via DRP-1$^{Ser616}$. Damaged mitochondria removed via PINK1-Parkin ubiquination | Mitophagy is protective against oxLDL injury | [141] |
| Human | Human carotid atherosclerotic plaques and normal aortas from aortic valve surgery or root replacement | oxLDL | PINK1-Parkin; | ↑↑Autophagy in atherosclerotic plaque samples; ↑PINK1 expression in plaque VSMC associated with ↓in mitochondrial respiration and mtDNA damage | [164] |
| Rat | Aortic VSMC | PDGF Stimulation | Drp-1, Opa1, Fis1, LC3II/I; Inhibition of DRP-1 preserved mitochondrial morphology, no Δ in LC3II. Hyperactivation of mitophagy | Switch in VSMC phenotype from contractile to secretory, ↑VSM proliferation | [125] |
| Human and mouse | Aortic VSM; ApoE$^{-/-}$; Pink$^{-/-}$mice aorta | Apelin-13 induced VSM proliferation | Mitochondrial fission, ↑LC3II/I, beclin-1 and ↓p62 upon apelin-13 stimulation; ↑PINK1-Parkin, VDAC1 and Tom20 | ↓of PINK1-Parkin and mitochondrial fission ameliorated VSM proliferation | [50] |

**Table 2** Mitochondria in the endothelium

| Organism | Model/cell line | Mechanism of action/intervention | Target-relation to autophagy | Vascular Outcomes | References |
|---|---|---|---|---|---|
| Human | Adipose resistance arterioles | Coronary artery disease, FMD | PGC1α, mitochondrial biogenesis/fusion | PGC1α involved in switch in the mechanism of dilation with CAD; ↑ PGC1α protects against pressure-induced stress | [60] |
| Rat | Sprague–Dawley thoracic aorta and mesenteric arteries | Endothelial-dependent vasodilation and mitochondrial membrane integrity | Mitochondrial fission via DRP-1 | ↓ Mitochondrial fission vasodilates pre-constricted arteries and limits the vasoconstrictor effects of ET-1; ↓ of DRP-1 ↓ ET-1-induced mitochondrial fission in aorta VSM | [18] |
| Human | Primary venous endothelial cells and HAEC | Diabetes mellitus, high glucose (30 mM) | Mitochondria fission; expression of Fis1, DRP-1, mitochondrial ROS; siRNA suppression of fission-related markers | ↓Mitochondrial networks and ↑ expression of fission, ROS production, ↓eNOS and cGMP production; ↓ mitochondrial fission prevents HG mitochondrial disruption, ROS, eNOS activation and cGMP | [136] |
| Cell | HUVEC | VEGF-mediated angiogenesis | Mitofusin (MFN) expression in endothelial cells influences mitochondrial networks | VEGF-A ↑ expression of MFN 1/2; ↓in MFN expression sensitizes mitochondria to oxidative stress, ↓membrane potential, enhancing susceptibility to autophagy | [92] |
| Rat and cell | Male C57BL/6 and HAEC | High fat (HF) diet and palmitic acid (PA) | Pink1-Parkin and mitochondria membrane potential | ↑PINK1-Parkin, LC3, and co-localization with LAMP1. Mitophagy is protective in maintaining mitochondria membrane integrity in response to PA, preventing dysfunction. HF diet ↑PINK1-Parkin expression in aorta | [155] |

**Table 3** Telomerase and the vascular smooth muscle and endothelium

| Organism | Model/cell line | Mechanism of action/intervention | Target-relation to autophagy | Vascular Outcomes | References |
|---|---|---|---|---|---|
| Human | VSM and atherosclerotic plaques | Atherosclerosis | Senescence | ↓Telomere length associated with atherosclerotic severity, ↓proliferation and early senescence; ↑ telomerase expression rescued senescence | [97] |
| Human | Adipose and atrial resistance arterioles | Coronary artery disease, flow-mediated dilation | Not specific | Modulation of TERT activity directly alters the mechanism of dilation in health and disease. Activation of TERT in CAD supports NO-mediated dilation, inhibition of TERT in non-CAD alters the mechanism of dilation to $H_2O_2$ | [8] |
| Human | Skeletal muscle feed arteries | Age-related telomere uncapping | ↑Cellular senescence and inflammation associated with greater telomere uncapping but not shortening | Telomere uncapping associated with p53/p21-induced senescence | [104] |
| Human | Primary venous endothelial cells; brachial artery | Age-related reduction in telomerase, flow-mediated dilation | Age-associated ↓in telomerase activity | Aging ↑cellular senescence in sedentary adults and endothelial-dependent dysfunction, habitual exercise rescues age-related reduction in FMD | [120] |

switched from $H_2O_2$ to NO identifying a critical role for telomerase in flow-mediated dilation phenotype (Fig. 2) [8].

Mechanistic evidence for the cross-talk between autophagy and telomerase stems from convergence of signaling pathways regarding metabolism. Data from animal models of telomerase deficiency indicate telomere shortening impairs glucose tolerance and insulin secretion [74]. Overexpression of TERT induces autophagy via inhibition of mTOR complex 1 kinase activity under basal and nutrient deprivation conditions, while TERT deficiency impairs autophagic flux [3]. Miwa et al. [101] demonstrated that within the brain of aged mice, TERT protein expression is reduced concomitant with excessive mitochondria-derived ROS, while administration of rapamycin, or dietary restriction ameliorated these effects. These autophagic effects elicited mitochondrial localization of TERT to reduce mitochondria-derived ROS, and more importantly had beneficial effects on memory and learning [101]. Caloric restriction increases both telomerase activity and autophagy resulting in improvements in cardiac function within the heart of diabetic rats [94]. Taken together, autophagy and extranuclear telomerase activity appear to share common metabolic pathways with hexokinase 2, the rate limiting catalyst for glucose metabolism, demonstrated to be the molecular transducer connecting these two processes [119]. Future investigations examining the cross-talk between telomerase and autophagy within the context of vascular function would reveal insight into whether these two pathways run in parallel (e.g. both effect vascular function) or whether telomerase acts upstream of autophagy to exert its vascular effects.

## Questions, perspectives and future directions

Unanswered questions remain regarding autophagy and vascular function. These include defining the mechanisms by which physiological stressors and pharmacological agents positively and negatively influence vascular autophagic flux, as modulation of autophagic flux in vascular diseases (macro and microvascular disease) may ameliorate disease pathology [37, 107]. As previously highlighted, one challenge in targeting autophagy is its dichotomous nature, whereby the goal is to target maladaptive autophagy while maintaining basal cell survival levels to optimize the beneficial effect. For example, trehalose, a natural disaccharide, is implicated in reducing age and disease-associated endothelial dysfunction and atherosclerotic plaque burden in human and animal models, possibly through nuclear translocation of TFEB [32, 63, 98]. How trehalose/TFEB exert vascular protective effects remains to be investigated. In addition, whether pharmacologically activating autophagy exerts an additive

**Table 4** Telomerase in other organs

| Organism | Model/cell line | Mechanism of action/intervention | Target-relation to autophagy | Vascular outcomes | References |
|---|---|---|---|---|---|
| Mouse | C57BL/6 and TERT$^{-/-}$mice brain and MCF-7 cells | Aging, dietary restriction | Aging brain | Aging associated with ↑in mitochondrial ROS and TERT expression; offset by caloric restriction; mTOR inhibition ↑mitochondrial localization of TERT in brain and ↓mitochondrial ROS in WT but not TERT$^{-/-}$ mice | [101] |
| Rat | Otsuka Long-Evans Tokushima fatty rat (OLETF) hearts | Spontaneous type II diabetes mellitus, caloric restriction | Diabetes ↓ telomerase activity, caloric restriction restored telomerase activity in diabetic rats, resulting in an ↑in LC3II/LC3I | Diabetes ↑ blood pressure and ↓ LV diastolic function, ↓ expression of MnSOD and eNOS. Caloric restriction improved blood pressure and LV diastolic function, restoring expression of MnSOD and eNOS | [94] |
| Cell | 293 T/17; HepG2; U2OS; MEF generated from TERT transgenic and TERT knockout mice | Genetic overexpression or knockout of TERT | TERT overexpression ↓ mTORC1 kinase activity and ↑ LC3II, while knockout of TERT ↑ phosphorylation of p70S6K and ↓ expression of LC3II | Not addressed, however provides mechanistic evidence of cross-talk between telomerase and autophagy | [3] |
| Mouse | TerT/TerC$^{-/-}$ and C57BL/J6 mice Kidney | Ischemia–reperfusion | I/R in TERT and TERC$^{-/-}$ delayed autophagosome formation, ↑↑p62 accumulation; ↑p-mTOR, p70S6K; rapamycin ↑Lc3II/I and ↓p62 | Telomerase deficiency impairs renal recovery to I/R due to reduction in autophagy via increase mTOR activation | [20] |
| Rat | TERT$^{-/-}$ WKY rat heart | Angiotensin II and Ischemia–reperfusion | None | ANG-II ↓ LV function, ↑infarct size | [2] |

effect in conjunction with a physiological stressor that induces autophagy remains to be interrogated. Activation of autophagy via trehalose ameliorates age-induced endothelial dysfunction. Whether exercise-induced autophagy influences vascular structure and function is a future area of exploration, with one group providing compelling evidence that handgrip-induced increases in shear rate activates endothelial cell autophagy, resulting in increases in NO as well as ROS in young adult males [114]. How this is altered with aging, disease, or exercising training or whether further activation of autophagy enhances the vaso-protective effects of exercise remains to be investigated. Given the evidence that a basal level of autophagy is critical to maintain homeostasis, it is unclear whether activation of autophagy in healthy individuals would impart maladaptive effects. Interrogation of these questions would shed light onto the adaptive vs. maladaptive role of autophagy within the vasculature.

Within skeletal muscle, autophagy holds a critical role in adaptions to exercise. Given that the beneficial effects of exercise and physical activity on the vasculature are mediated by shear stress, it would be interesting to examine whether vascular autophagy (endothelial or VSM) is involved in the structural and functional adaptations to physical activity and exercise training. Cardiac-specific exercise induced protection was recently reviewed elsewhere, concluding that exercise training would enhance basal autophagy and preventing cardiovascular disease in otherwise healthy populations, while exercise training in diseased populations may mitigate excessive autophagy, thereby decreasing the severity of disease [156]. Future directions within the field of autophagy in the vasculature should focus on the functional role of autophagy, specifically delineating the role of autophagic flux in vascular health and function in response to physiological stressors.

## Conclusions

Autophagy is an evolutionarily conserved recycling process critical in the maintenance of homeostasis across organ systems. The autophagy signaling cascade is complex, and historically, autophagy has been examined with a focus on cardiomyocytes. Novel translational evidence is emerging on the role of autophagy and the functional ramifications specific to the vasculature system. While autophagy is generally viewed as beneficial, excessive as well as insufficient levels of autophagy may exert detrimental effects within the cardiovascular system. Specific to the vasculature, autophagy may be the crux in determining vascular health and function due to its numerous interactions with other physiological pathways in determining cell fate and survival.

**Funding** This work was supported by the National Institutes of Health Grants T32GM089586 and AHA 20POST35050017 (W.E.H); R01-HL-133029 (A.M. Beyer); R01-HL-135901-01 (D.D. Gutterman).

## Compliance with ethical standards

**Conflict of interest** The authors have nothing to disclose.

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