Mitochondria-targeted antioxidant therapy with MitoQ ameliorates aortic stiffening in old mice

Rachel A. Gioscia-Ryan,1 Micah L. Battson,1 Lauren M. Cuevas,1 Jason S. Eng,1 Michael P. Murphy,2 and Douglas R. Seals1

1Department of Integrative Physiology, University of Colorado, Boulder, Boulder, Colorado; and 2MRC Mitochondrial Biology Unit, University of Cambridge, Cambridge, United Kingdom

Submitted 19 July 2017; accepted in final form 20 October 2017

Mitochondria-targeted antioxidant therapy with MitoQ ameliorates aortic stiffening in old mice. J Appl Physiol 124: 1194–1202, 2018. First published October 26, 2017; doi:10.1152/japplphysiol.00670.2017.---Aortic stiffening is a major independent risk factor for cardiovascular diseases, cognitive dysfunction, and other chronic disorders of aging. Mitochondria-derived reactive oxygen species are a key source of arterial oxidative stress, which may contribute to arterial stiffening by promoting adverse structural changes—including collagen overabundance and elastin degradation—and enhancing inflammation, but the potential for mitochondria-targeted therapeutic strategies to ameliorate aortic stiffening with primary aging is unknown. We assessed aortic stiffness [pulse-wave velocity (aPWV)], ex vivo aortic intrinsic mechanical properties [elastic modulus (EM) of collagen and elastin regions], and aortic protein expression in young (~6 mo) and old (~27 mo) male C57BL/6 mice consuming normal drinking water (YC and OC) or water containing mitochondria-targeted antioxidant MitoQ (250 μM; YMQ and OMQ) for 4 wk. Both baseline and postintervention aPWV values were higher in OC vs. YC (post: 482 ± 21 vs. 420 ± 5 cm/s, P < 0.05). MitoQ had no effect in young mice but decreased aPWV in old mice (OMQ, 426 ± 20, P < 0.05 vs. OC). MitoQ did not affect age-associated increases in aortic collagen-region EM, collagen expression, or proinflammatory cytokine expression, but partially attenuated age-associated decreases in elastin region EM and elastin expression. Our results demonstrate that MitoQ reverses in vivo aortic stiffness in old mice and suggest that mitochondria-targeted antioxidants may represent a novel, promising therapeutic strategy for decreasing aortic stiffness with primary aging and, possibly, age-related clinical disorders in humans. The destiffening effects of MitoQ treatment may be at least partially mediated by attenuation/reversal of age-related aortic elastin degradation.

NEW & NOTEWORTHY

We show that 4 wk of treatment with the mitochondria-specific antioxidant MitoQ in mice completely reverses the age-associated elevation in aortic stiffness, assessed as aortic pulse-wave velocity. The destiffening effects of MitoQ treatment may be at least partially mediated by attenuation of age-related aortic elastin degradation. Our results suggest that mitochondria-targeted therapeutic strategies may hold promise for decreasing arterial stiffening with aging in humans, possibly decreasing the risk of many chronic age-related clinical disorders.

INTRODUCTION

Advancing age is a primary risk factor for the development of numerous chronic degenerative diseases, which are the leading causes of morbidity and mortality in the United States and other developed nations (20, 30, 41). A key event underlying the etiology of many chronic age-related disorders is stiffening of the large elastic arteries, specifically, the aorta. Elevated aortic stiffness increases the pulsatile shear and pressure experienced by the heart, blood vessels, and other organs, which can have numerous pathophysiological effects contributing to the development of disease (23, 32, 34, 35, 38, 62). Indeed, aortic pulse-wave velocity (aPWV), the gold-standard measure of arterial stiffness, is a strong independent risk factor for incident cardiovascular events among older adults (34, 50), and it also predicts the development of chronic kidney disease, stroke, cognitive impairment, and Alzheimer disease (2, 7, 18, 21, 43, 53). Current demographic trends forecast a major increase in the number of older adults in the coming decades, which will be accompanied by attendant increases in disease prevalence and health care costs (19, 22, 56). As such, a top biomedical research priority is to identify strategies that prevent or reverse aortic stiffening with advancing age, as this may help prevent, reduce, or delay the development of multiple common disorders of aging.

A key mechanism underlying the development of age-related arterial stiffening may be vascular mitochondrial oxidative stress and associated excessive production of mitochondria-derived reactive oxygen species (mtROS). Mitochondria are now recognized as a primary source of arterial oxidative stress with aging and cardiovascular diseases (1, 4, 5, 16, 31, 38, 55, 61), and evidence from genetic models indicates that experimental modulation of mtROS affects large elastic artery stiffening. For example, age-related arterial stiffening, pathological remodeling, and vascular disease are accelerated in mice deficient in the mitochondrial antioxidant protein manganese superoxide dismutase (SOD2) (61). In support of a role specifically for mitochondria-derived oxidative stress, selective deletion of a cytosolic isoform of prooxidant enzyme NADPH oxidase (NOX1/2)—with the mitochondrial isoform (NOX4) intact—does not prevent age-related arterial stiffening in the setting of atherosclerosis (55), implicating mtROS as a key driver of age-related arterial pathology.

Excessive levels of arterial mtROS may promote arterial stiffening via redox-related alterations in structural protein turnover and through induction of proinflammatory signaling.
Changes in arterial wall structure are a major mechanism by which the large elastic arteries stiffen with age (9, 17, 24, 32, 62); specific structural alterations include increased deposition of the load-bearing protein collagen and degradation and fragmentation of elastin (17, 24, 42). Oxidative stress, including that derived specifically from mitochondria, alters the activity of the enzymes involved in structural protein turnover and shifts the balance of synthesis and breakdown toward collagen deposition and elastin degradation (9, 17, 24, 38, 55, 61, 62), contributing to dysregulation of structural protein homeostasis and consequent arterial stiffening.

Mitochondria-derived ROS are also emerging as important for promoting and sustaining arterial inflammation, a hallmark of arterial aging and critical mediator of arterial stiffening (24, 38, 39, 57). A proinflammatory environment in the vasculature, secondary to excessive mtROS production, may contribute to arterial stiffening through many mechanisms, including induction of gene expression patterns that alter structural protein turnover, impairment of vascular endothelial function, increases in vascular smooth muscle cell tone, and further invasiveness of the vascular wall by proinflammatory mediators that also reinforce oxidative stress (24, 31, 32, 39, 57, 61).

Our laboratory recently demonstrated that treating old mice with the mitochondria-targeted antioxidant MitoQ to lower mitochondrial oxidative stress completely reversed the age-related impairment in arterial endothelial function in old mice (15). However, the effects of mitochondria-targeted antioxidants on arterial stiffness with primary aging have never been investigated. Therefore, in this study, we tested the hypothesis that 4 wk of MitoQ supplementation in the drinking water would decrease arterial stiffness (as assessed in vivo by aPWV) and consequent arterial stiffening.

In vivo assessment of arterial stiffness: aortic pulse-wave velocity. In vivo arterial stiffness was assessed at baseline and after 4 wk of MitoQ treatment by aortic pulse-wave velocity (aPWV) using Doppler ultrasound, as previously described by our laboratory (11, 28). Briefly, mice were anesthetized via inhaled isoflurane (1.5–2%) and positioned supine on a warmed platform with paws secured to electrocardiogram leads. Doppler probes were placed at the transverse aortic arch and abdominal aorta to detect pulse waves. Three consecutive 2-s recordings were made for each animal and used to determine time delay between the electrocardiogram R-wave and the foot of the Doppler signal for each site (Δtime\text{abdominal} and Δtime\text{transverse}). aPWV was then calculated as aPWV = (physical distance between the two probes)/(Δtime\text{abdominal} − Δtime\text{transverse}) and reported in centimeters per second.

To examine the potential role of changes in blood pressure to treatment-related differences in aPWV, we assessed systolic and diastolic blood pressure at baseline and after 4 wk of MitoQ or normal drinking water consumption using the CODA noninvasive tail-cuff system, as previously described (11, 28). The pressure measurements from 20 collection cycles (following five acclimation cycles) on each of three consecutive days were averaged for each mouse at each time point.

**Ex vivo assessment of arterial stiffness: intrinsic mechanical stiffness.** After all in vivo assessments were completed, mice were euthanized and aortas were harvested for measurements of ex vivo intrinsic mechanical stiffness and protein expression. Two 1-mm aortic rings from the thoracic region (dissected free of surrounding connective tissue) were used to assess intrinsic arterial stiffness via wire myography, as described previously by our laboratory (6, 10, 14, 28). Aortic rings were loaded into heated myograph chambers (Danish Myo Technology, Aarhus, Denmark) with calcium-free PBS. After three cycles of prestretching were completed, ring diameter was increased to achieve 1 mN force and then incrementally stretched by ~10% every 3 min until failure. The force corresponding to each stretching interval was recorded and used to calculate stress and strain, defined as follows: 

\[
\text{Strain} = \Delta d/d_i = (d - d_i)/d_i,
\]

where \(d\) is diameter, \(d_i\) is initial diameter; 

\[
\text{Stress} = (\Delta F)/HD,
\]

where \(F\) is load, \(H\) is wall thickness determined by histology, and \(D\) is vessel length.

The slope of the stress-strain curve was used to determine the elastic modulus in the collagen-dominant and elastin-dominant regions of the curve, as described below.

**Collagen elastic modulus.** When aortic rings are subjected to stress-strain testing, the region of the stress-strain curve corresponding to the highest forces represents the stretching of predominantly collagen fibers (25, 47). The elastic modulus of the collagen-dominant region was defined as the slope of the linear regression fit to the final four points of the stress-strain curve, as described previously (6, 14, 28). See (Fig. 2) for representative stress-strain curve.

**Elastin elastic modulus.** During stress-strain testing in aortic rings, the region of the stress-strain curve corresponding to the stretching of exclusively elastin fibers is a lower-force region before collagen fiber engagement that can be identified as the portion of the stress-strain curve where curvature (determined from the second derivative of the stress-strain curve) is approximately zero; the engagement of collagen fibers is indicated by an elevation in the curvature (nonzero second derivative) (25). To determine the boundaries of the elastin region of our stress-strain curves, we calculated the roots of the second derivative of a 7th-order polynomial fit to the data (\(R^2 > 0.99\)). The first root was considered the boundary between the very low-force region and the elastin region, and the second root was considered the boundary between the elastin region and the onset of collagen fiber engagement (25). The elastic modulus of the elastin region was then determined as the slope of the linear regression fit to the stress-strain data between the two points. See Fig. 2 for the representative stress-strain curve.
Aortic protein expression. Aortic expression of structural proteins collagen-I and α-elastin was determined in aortic homogenates by standard Western blot techniques and immunohistochemistry (IHC) in aortic sections, as previously described (6, 11, 28). Aortic protein expression of inflammatory cytokines was determined using a custom multiplex ELISA (Ciraplex, Aushon Biosystems, Billerica, MA), as previously described (27, 29).

Before Western blot analysis and cytokine multiplex, aortas were homogenized in radioimmunoprecipitation assay lysis buffer, and protein concentration was determined using the Pierce BCA assay kit (ThermoFisher Scientific, Fremont, CA). For Western blot analysis, 15 μg of aortic protein were loaded onto 4–12% polyacrylamide gels and then transferred onto nitrocellulose membranes (Criterion System; Bio-Rad, Hercules, CA). Membranes were incubated (overnight at 4°C) with primary antibodies: collagen-I (1:1,000; Millipore, Burlington, MA), α-elastin (1:200; Abcam, Cambridge, MA), and glyceraldehyde 3-phosphate dehydrogenase (1:1,000, normalizer, GAPDH; Cell Signaling, Danvers, MA). Proteins were visualized on a digital acquisition system (ChemiDoc-It, UVP, Upland, CA) using chemiluminescence with horseradish peroxidase polymer secondary for 30 min. Slides were dehydrated and cover-slipped after a 10-min or 1-min exposure to diaminobenzidine (elastin polymer secondary for that at 4 °C with primary antibodies for collagen-I (1:200; Millipore) or collagen-I (1:200; Millipore) and then incubated with the labeled secondary antibody for 1 h at 4°C with primary antibodies for α-elastin (1:200; Abcam) or collagen-I (1:200; Millipore) and then incubated with the labeled secondary antibody for 1 h at 4°C with primary antibodies for α-elastin (1:200; Abcam) or collagen-I (1:200; Millipore) and then incubated with the labeled secondary antibody for 1 h at 4°C with primary antibodies for α-elastin (1:200; Abcam) or collagen-I (1:200; Millipore) and then incubated with the labeled secondary antibody for 1 h at 4°C with primary antibodies for α-elastin (1:200; Abcam) or collagen-I (1:200; Millipore) and then incubated with the labeled secondary antibody. Images were captured using Cirascan imager (Aushon), and results were analyzed with Cirasoft software (Aushon). If levels of a given cytokine were undetectable (e.g., fell below the limit of detection of the assay), samples were excluded from the analysis.

For IHC, ~1-mm thoracic aortic segments were frozen in OCT compound in liquid nitrogen-cooled isopentane before sectioning. Aortic sections (7 μm) were fixed in acetone, washed in Tris buffer, and stained using the Dako EnVision+ System-HRP-DAB kit, as performed previously in our laboratory (11). Sections were incubated for 1 h at 4°C with primary antibodies for α-elastin (1:50; Abcam) or collagen-I (1:200; Millipore) and then incubated with the labeled polymer secondary for 30 min. Slides were dehydrated and cover-slipped after a 10-min or 1-min exposure to diaminobenzidine (elastin and collagen, respectively).

Stained aortic sections were imaged using a Nikon Eclipse TS100 photomicroscope under identical conditions. Quantification of the integrated density of the stain was performed using ImageJ software by a single investigator blinded to the group assignment of each sample. Collagen-I expression was assessed in the whole artery sections, comprising both the medial and adventitial layers, whereas elastin expression was assessed in the medial layer, the primary site of age-related changes in elastin expression (9, 10). Integrated density values from four sections were averaged to provide a single value for each protein per aorta, which are expressed relative to the mean of the young control group.

Statistical analysis. All statistical analyses were performed using SPSS 23.0 software (Armonk, NY). Data were first assessed for outliers and normality/homogeneity of variance. Between-group differences in morphological characteristics and aortic protein expression (Western blot, immunohistochemistry, and multiplex ELISA) were determined using one-way ANOVA. Between-group differences in elastic modulus (collagen and elastin regions) were determined using a linear mixed model with age (young vs. old) and treatment (control vs. MitoQ) as factors, whereas within-group differences in aPWV and blood pressure were examined using a linear mixed model that also included a repeated factor (preintervention vs. postintervention period). When a significant main effect was observed, Fisher’s least significant difference post hoc tests were performed to determine specific pair-wise differences.

RESULTS

MitoQ consumption across the 4-wk treatment period was similar to our previous report and not different between young and old mice (~1 mmol/day; Ref. 15). Select morphological
characteristics and blood pressure are shown in Table 1. Consistent with our previous study (15), 4 wk of MitoQ treatment did not influence overall morphology; although there were age-associated differences in body mass, heart mass, and quadriceps mass, these were not different between mice receiving MitoQ vs. normal drinking water. There were no age- or treatment-related differences in aortic diameter or systolic and diastolic blood pressure.

MitoQ treatment reverses aortic stiffening in old mice. At baseline, aPWV was significantly higher in old compared with young mice, and aPWV was not significantly different from baseline to postintervention in either young or old control mice receiving normal drinking water (Fig. 1). In contrast, 4 wk of MitoQ treatment significantly decreased aPWV in old mice to levels similar to young mice following the intervention period.

MitoQ treatment had no effect on aPWV in young mice. These results indicate that 4 wk of MitoQ treatment specifically reverses aortic stiffening in old mice.

Potential mechanisms underlying the destiffening effects of MitoQ treatment in old mice. In our previous study using MitoQ treatment in old mice (15), the same dose and duration of treatment as used in the present study normalized the age-related elevation in aortic whole cell and mitochondria-specific superoxide production, indicating a profound antioxidant effect of MitoQ in arteries. To investigate further how decreased levels of mtROS in aging arteries may contribute to the destiffening effects of MitoQ, in the present study, we investigated key mechanisms that have been implicated downstream of mitochondrial oxidative stress in the development of age-related arterial stiffening, namely, changes in arterial structural proteins and inflammation.

Ex vivo aortic stiffness—collagen- and elastin-mediated mechanical properties of aortic rings. The elastic modulus of the collagen region of stress-strain curves was significantly greater in old control vs. young control mice (Fig. 3A), whereas the elastic modulus of the elastin region was significantly lower in old control compared with young control mice (Fig. 3B), indicating an age-related increase in intrinsic arterial stiffness mediated by increased collagen and reduced elastin. MitoQ treatment had no effect on the collagen elastic modulus, such that the values in old and young MitoQ-treated mice were not significantly different from old and young control mice, respectively. However, in arteries from old mice treated with MitoQ, the elastic modulus in the elastin region was significantly greater than that of old control but remained significantly lower than the elastin elastic modulus of young MitoQ-treated mice, indicating attenuation of the age-related decline in elastin.

Aortic expression of structural proteins. Consistent with our intrinsic mechanical stiffness observations, aortic collagen protein expression was significantly greater (Fig. 4, A and B), and aortic elastin expression was lower (Fig. 4, C and D, \( P = 0.074 \) and 0.086, respectively) in old control versus young control mice. MitoQ treatment did not affect aortic collagen content, such that collagen expression in old MitoQ-treated mice was not significantly different than that of old control mice.
whether assessed in whole artery homogenate by Western blot analysis or in aortic sections via IHC. When measured in whole artery homogenate by Western blot analysis, aortic elastin levels in old MitoQ-treated mice were intermediate between (and not significantly different from) those of young control or old control mice. However, when assessed via IHC in the medial layer of aortas—the primary site of age-related elastin degradation (9, 10)—elastin content in old MitoQ-treated mice was greater than that of old control mice (\( P < 0.07 \)). Together with our observations of intrinsic mechanical properties, these results suggest that the reduction in in vivo aortic stiffening in old mice after MitoQ treatment was mediated not by effects on aortic collagen, but possibly by partial preservation of elastin.

Aortic inflammatory cytokine expression. Aortic expression of proinflammatory cytokines IL-6, IL-10, and IFN-γ (Fig. 5, A–C) was significantly higher, and expression of IL-1β (Fig. 5D) tended to be higher, in old compared with young control mice, consistent with previous investigations demonstrating elevated levels of arterial cytokines with aging and association with vascular dysfunction (3, 27, 29, 44). Cytokine levels were not affected by 4 wk of MitoQ treatment (\( P > 0.05 \) OMQ vs. OC for all cytokines), suggesting that the destiffening effects of MitoQ were not mediated by changes in these aortic cytokines. However, these results do not preclude the possibility that MitoQ treatment may have influenced other components of inflammatory signaling pathways.

DISCUSSION

The primary, novel finding of this study is that 4 wk of treatment with the mitochondria-targeted antioxidant MitoQ in old mice completely reverses the age-associated increase in aortic stiffness, assessed in vivo as aPWV. Our observation that MitoQ treatment decreases aortic stiffness in old mice extends previous work with general antioxidant compounds and adds to the evidence from transgenic and disease models
that specifically implicate mitochondrial oxidative stress as a key contributor to aortic stiffening. A previous preclinical intervention study from our laboratory using the general antioxidant compound TEMPOL established oxidative stress as a key mechanism underlying age-related aortic stiffening (12), and other strategies that decrease arterial oxidative stress also ameliorate arterial stiffness (11, 13, 14, 28, 49). Recent work with genetic and disease models indicates that mitochondria are a major source of the vascular oxidative stress contributing to arterial stiffness. Mice with genetic deletion of mitochondrial antioxidant enzyme SOD2, a model of excess mitochondrial oxidative stress, demonstrate exacerbation of age-related aortic stiffening (61), and progression of age-related arterial stiffening is unaffected in mice with genetic deletion of cytosolic prooxidant NADPH oxidase (NOX1/2) but intact mitochondria-localized NADPH oxidase (NOX4) (55). Our finding here that in vivo treatment with the mitochondria-targeted antioxidant MitoQ in old mice decreases aortic stiffness provides further support for mitochondrial oxidative stress as a key mediator of arterial dysfunction with primary aging. Most importantly, our results extend previous observations from genetic and disease models (55) by demonstrating that a pharmacological intervention targeting excessive mtROS production reverses aortic stiffening in the setting of primary aging in mice, thus establishing an essential platform for translation to humans.

To gain initial mechanistic insight into the destiffening effects of MitoQ treatment, we assessed intrinsic mechanical stiffening ex vivo in aortic rings and examined both the collagen- and elastin-predominant regions of the stress-strain curves. In contrast to previous studies showing that the destiffening effects of late-life interventions, including those associated with decreased whole cell and mitochondrial oxidative stress, are primarily mediated by decreases in arterial collagen content (9, 11, 12, 14, 37, 55), we observed that MitoQ treatment had no significant effect on the collagen region elastic modulus or aortic collagen expression but instead attenuated the age-related decline in aortic elastin region elastic modulus and tended to preserve elastin expression. Our finding of partial elastin preservation with MitoQ treatment is consistent with the observations that heterozygous SOD2-deficient mice, a model of excess mtROS, show marked exacerbation of age-associated declines in arterial elastin content (61) and that lifelong caloric restriction, a setting of lower mtROS (26), preserves arterial elastin content with aging (8). Collectively, our results suggest that decreasing mitochondrial oxidative stress may at least partially preserve elastin content in the aorta, contributing to lower levels of stiffness.

Future studies are warranted to elucidate the mechanisms by which decreased mitochondrial oxidative stress (via MitoQ treatment) may preserve aortic elastin content in aging. One possible link may be mtROS-mediated regulation of enzy-

---

**Fig. 5.** MitoQ treatment does not affect the age-related increase in aortic inflammatory cytokines. Expression of inflammatory cytokines IL-6 (n = 7–10/group) (A), IL-10 (n = 7–9/group) (B), IFN-γ (n = 7–10/group) (C), and IL-1β (n = 4–10/group) (D) in aortic homogenates from young and old control (YC and OC) and young and old MitoQ-treated (YMQ and OMQ) mice. Sample sizes reflect all aortic homogenates for which cytokine levels were detectable; samples were excluded when cytokine levels were undetectable/ below the limit of quantification of the assay. Error bars represent means ± SE. *P < 0.05 vs. YC. ^P > 0.05 vs. YC. *0.10 > P > 0.05 vs. YC (P = 0.08, OC vs. YC; P = 0.06, OMQ vs. YC).
mes that govern elastin turnover, including matrix metalloproteinasises (MMP; Refs. 36 and 62)—changes in the activity of which are associated with arterial stiffening in both mouse models and human aging (32, 33, 58). For example, increased levels of MMP-2, a key enzyme involved in elastin degradation (9, 17, 59), accompany the loss of arterial elastin in heterozygous SOD2 knockout mice (61). Further, primary aging in preclinical models is associated with increased arterial MMP-2 expression (9, 59), and elevated aortic MMP-2 levels are also observed in human aging (33). Collectively, these previous studies suggest that age-related increases in mtROS may contribute to arterial elastin degradation via increased MMP-2 activity and that targeting excess mtROS, e.g., via MitoQ treatment, may attenuate elastin degradation, preserving elastin content in large elastic arteries and contributing to lower levels of stiffness. Although our results do not support a role for MitoQ in decreasing total arterial collagen content, future studies could examine not only arterial content of this key structural protein, but also changes in collagen fiber orientation (17) and formation of cross-links among proteins, both of which have the potential to influence arterial stiffness (9, 23, 62).

It is also important to consider mechanisms other than preservation of aortic elastin content that may have contributed to the dramatic decrease in aortic stiffness we observed with MitoQ treatment in old mice. In addition to structural changes, age-related arterial stiffening is also mediated by hemodynamic factors (including age-related reductions in vascular endothelial function) and increased vasomotor tone (17, 24, 62). Although our data indicate that changes in resting blood pressure did not contribute to the effects of MitoQ treatment, it is plausible that some of the destiffening that we observed in old mice was due to improvements in vascular endothelial function. Our previous study (15) demonstrated that MitoQ treatment increases endothelium-dependent dilation and nitric oxide bioavailability in old mice, both of which are important direct (e.g., effects on pulse pressure and smooth muscle tone) and indirect (e.g., nitric oxide, regulation of structural protein turnover) mediators of large elastic artery stiffness in vivo (17, 32, 38, 60, 62).

Aortic inflammatory cytokine levels were significantly elevated in aortic tissue of old vs. young mice, consistent with previous studies (27, 29, 44). Chronic low-grade arterial inflammation with aging, primarily mediated by NF-κB activation, can be triggered by excessive oxidative stress—including that derived from mitochondria—in a reciprocally reinforcing process that serves to impair arterial function (3, 29, 54). Although there is some evidence for a role of mtROS in mediating arterial inflammation and consequent dysfunction in atherosclerosis/disease models (31, 55), our observations in the present study do not support an anti-inflammatory role for MitoQ in reversing arterial stiffening in primary aging. After 4 wk of MitoQ treatment, there was no difference between old control and old MitoQ-treated aortic cytokine levels, despite the pronounced reversal of arterial stiffening in the latter. This suggests that the destiffening effects of MitoQ were mediated by a mechanism other than normalization of the aortic cytokines that we assessed here. However, it remains possible that MitoQ treatment influenced other components of inflammatory signaling, and future studies are warranted to investigate these possibilities.

Although the present study investigated the therapeutic efficacy of MitoQ in the setting of existing age-related aortic stiffness, it would also be of clinical relevance to determine whether targeting/decreasing mtROS earlier in life before the onset of aortic stiffening could prevent or slow the progression of pathological aortic remodeling and consequent cardiovascular sequelae. Given that excess mtROS are implicated as a key factor in the pathogenesis of numerous age-related conditions, including vascular dysfunction (1, 4, 5), it is possible that limiting an age-related increase in mtROS via treatment in early or midlife could prevent aortic stiffening. This possibility is supported by work from disease and senescence models, indicating that mitochondria-targeted therapeutics initiated before or at the onset of experimental insult or injury can prevent development or slow progression of dysfunction (45, 51, 52).

Because low, physiological levels of mtROS are critical for the maintenance of cellular homeostasis, any optimal long-term therapeutic strategy would likely need to maintain mtROS at physiological levels rather than eliminate them completely.

**Conclusion.** In conclusion, the present study demonstrates that late-life treatment with a mitochondria-targeted antioxidant, MitoQ, effectively reverses aortic stiffening in the setting of primary aging. Our results suggest that this effect is mediated at least partially by attenuation/reversal of the age-related reduction in aortic elastin content, but additional work is needed to conclusively determine the mechanism(s) underlying the destiffening effect of MitoQ. Importantly, these results indicate that mitochondria-targeted antioxidants may represent a novel, promising therapeutic strategy for decreasing aortic stiffness, and potentially decreasing the risk of multiple chronic age-associated conditions in humans.

**ACKNOWLEDGMENTS**

The authors thank Dr. Blair Dodson and Jesse Goodrich for study assistance.

**GRANTS**

This study was supported by National Institutes of Health Grants AG047784 (to R. Giosca-Ryan); AG-000279 (to R. Giosca-Ryan); HL-107120-04 (to D. R. Seals) and AG-0138038 (to D. R. Seals). Work in M. P. Murphy’s laboratory is supported by the Medical Research Council UK (MC_U105663142) and by a Wellcome Trust Investigator Award (110159/Z/15/Z).

**DISCLOSURES**

M. P. Murphy is on the scientific advisory board of Antipodean Pharmaceuticals. All other authors declare that they have no conflicts of interest.

**AUTHOR CONTRIBUTIONS**

R.A.G.-R., M.L.B., L.M.C., and J.S.E., M.P.M., and D.R.S. edited and revised manuscript; R.A.G.-R., M.L.B., L.M.C., J.S.E., M.P.M., and D.R.S. interpreted results of experiments; R.A.G.-R. prepared figures; R.A.G.-R.-drafted manuscript; R.A.G.-R., M.L.B., L.M.C., J.S.E., M.P.M., and D.R.S. edited and revised manuscript; R.A.G.-R., M.L.B., L.M.C., J.S.E., M.P.M., and D.R.S. approved final version of manuscript.

**REFERENCES**

1. Ballinger SW, Patterson C, Knight-Lozano CA, Burow DL, Conklin CA, Hu Z, Reuf J, Horaist C, Lebovitz R, Hunter GC, McIntyre K, Runge MS. Mitochondrial integrity and function in atherogenesis. Circulation. 106: 544–549, 2002. doi:10.1161/01.CIR.0000023921.93743.89.
2. Cooper LL, Woodard T, Sigurdsdottir S, van Buchem MA, Torjersen AA, Inker LA, Aspeland T, Eiriksdottir G, Harris TB, Budnason V,
37. Nosaka T, Tanaka H, Watanabe I, Sato M, Matsuda M. Influence of regular exercise on age-related changes in arterial elasticity: mechanistic insights from wall compositions in rat aorta. *Can J Appl Physiol* 28: 204–212, 2003. doi:10.1139/h03-016.

38. Paneni F, Diaz Cañestro C, Libby P, Löscher TF, Camici GG. The aging cardiovascular system: understanding it at the cellular and clinical levels. *J Am Coll Cardiol* 69: 1952–1967, 2017. doi:10.1016/j.jacc.2017.01.064.

39. Park S, Lakatta EG. Role of inflammation in the pathogenesis of arterial stiffness. *Yonsei Med J* 53: 258–261, 2012. doi:10.3349/ymj.2012.53.2.258.

40. Rodriguez-Cuenca S, Cochené HM, Logan A, Abakumova I, Prime TA, Rose C, Vidal-Puig A, Smith AC, Rubinsztein DC, Fearnley IM, Jones BA, Pope S, Heales SJ, Lam BY, Neogi SG, McFarlane I, James AM, Smith RA, Murphy MP. Consequences of long-term oral administration of the mitochondria-targeted antioxidant MitoQ to wild-type mice. *Free Radic Biol Med* 48: 161–172, 2010. doi:10.1016/j.freeradbiomed.2009.10.039.

41. Roger VL, Go AS, Lloyd-Jones DM, Benjamin EJ, Berry JD, Borden WB, Bravata DM, Dai S, Ford ES, Fox CS, Fullerton HJ, Gillespie C, Hallpern SM, Heit JA, Howard VJ, Kjellstrand CK, Kittner SJ, Lackland DT, Lichtman MJ, Lisabeth LD, Makuc DM, Marcus GM, Marelli A, Matchar DB, Moy CS, Mozaffarian D, Mussolino ME, Nichol G, Paynter NP, Soliman EZ, Sorlie PD, Sotoodehnia N, Turan TN, Virani SS, Wong ND, Woo D, Turner MB; American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2012 update: a report from the American Heart Association. *Circulation* 125: e2–e220, 2012. doi:10.1161/CIR.0b013e3182a43e1c.

42. Sell DR, Monnier VM. Molecular basis of arterial stiffening: role of glycation—a mini-review. *Gerontology* 58: 227–237, 2012. doi:10.1159/000334668.

43. Selley ML. Increased concentrations of homocysteine and asymmetric dimethylarginine and decreased concentrations of nitric oxide in the plasma of patients with Alzheimer’s disease. *Neurobiol Aging* 24: 903–907, 2003. doi:10.1016/S0197-4580(03)00007-1.

44. Sindler AL, Fleenor BS, Calvert JW, Marshall KD, Zigler ML, Lefer DJ, Seals DR. Nitrite supplementation reverses vascular endothelial dysfunction and large elastic artery stiffness with aging. *Aging Cell* 10: 429–437, 2011. doi:10.1111/j.1474-9726.2011.00679.x.

45. Skulachev VP, Anisimov VN, Antonenko YN, Bakeeva LE, Chernyak SM, Sokikh MY, Yaguzhinsky LS, Zorov DB, Wilkinson IB, Franklin SS, Cockcroft JR. Animal and human studies with the mitochondria-targeted antioxidant MitoQ. *Biochim Biophys Acta* 1787: 437–461, 2009. doi:10.1016/j.bbbp.2008.12.008.

46. Smith RAJ, Murphy MP. Animal and human studies with the mitochondria-targeted antioxidant MitoQ. *Ann NY Acad Sci* 1201: 96–103, 2010. doi:10.1111/j.1749-6632.2010.05627.x.

47. Sokolis DP, Boudoulas H, Karayannacos PE. Assessment of the aortic stress-strain relation in uniaxial tension. *J Biomech* 35: 1213–1223, 2002. doi:10.1016/S0021-9290(02)00073-8.

48. Sprott RL, Ramirez I. Current inbred and hybrid rat and mouse models. *ILAR J* 38: 104–109, 1997. doi:10.1093/ilar.38.3.104.

49. Stepan J, Tran H, Benjo AM, Pellakuru L, Barodka V, Ryoo S, Nyhan SM, Lussman C, Gupta G, White AR, Dahe JR, Shoukas AA, Levine BD, Berkowitz DE. Alagille syndrome in combination with exercise ameliorates age-associated ventricular and vascular stiffness. *Exp Gerontol* 47: 565–572, 2012. doi:10.1016/j.exger.2012.04.006.

50. Sutton-Tyrrell K, Najjar SS, Boudreau RM, Venkatchalam L, Kupelian V, Simonsick EM, Havlik R, Lakatta EG, Spurgue H, Kritchevsky S, Pahor M, Bauer D, Newman A; Health ABC Study. Elevated aortic pulse wave velocity, a marker of arterial stiffness, predicts cardiovascular events in well-functioning older adults. *Circulation* 111: 3384–3390, 2005. doi:10.1161/CIRCULATIONAHA.104.386326.

51. Sweetyne MT, Pippin JW, Eng DG, Hudkins KL, Chiao YA, Campbell MD, Marcinek DJ, Alpers CE, Szeto HH, Rabivinotch PS, Shankland SJ. The mitochondrial-targeted peptide, SS-31, improves glomerular architecture in mice of advanced age. *Kidney Int* 91: 1126–1145, 2017. doi:10.1016/j.kint.2016.10.036.

52. Szeto HH, Liu S, Soong Y, Alam N, Prusky GT, Seshan SV. Protection of mitochondria prevents high-fat diet-induced glomerulopathy and proximal tubular injury. *Kidney Int* 90: 997–1101, 2016. doi:10.1016/j.kint.2016.06.013.

53. Tso CW, Seshadri S, Beiser AS, Westwood JD, Decarli C, Ar A, Himali JJ, Hamburg NM, Vita JA, Levy D, Larson MG, Benjamin EJ, Wolf PA, Vasan RS, Mitchell GF. Relations of arterial stiffness and endothelial function to brain aging in the community. *Neurology* 81: 984–991, 2013. doi:10.1212/01.wnl.0000418247.3618.

54. Ungvari Z, Orosz Z, Labinszky N, Rivera A, Xiangxin Z, Smith K, Ciszar A. Increased mitochondrial H2O2 production promotes endothelial NF-κB activation in aged rat arteries. *Am J Physiol Heart Circ Physiol* 293: H37–H47, 2007. doi:10.1152/ajpheart.01344.2006.

55. Vendrov AE, Vendrov KC, Smith A, Yuan J, Sumida A, Robidoux J, Runge MS, Madamanchi NR. NOX4 NADPH oxidase-dependent mitochondrial oxidative stress in aging-associated cardiovascular disease. *Antioxid Redox Signal* 23: 1389–1409, 2015. doi:10.1089/ars.2014.6221.

56. Vincent GK, Velkoff VA. The Older Population in the United States: 2010 to 2050. Washington D.C: U.S. Census Bureau, 2010.

57. Wang M, Jiang L, Monticone RE, Lakatta EG. Proinflammation: the key to arterial aging. *Trends Endocrinol Metab* 25: 72–79, 2014. doi:10.1016/j.tem.2013.10.002.

58. Wang M, Kim SH, Monticone RE, Lakatta EG. Matrix metalloproteinases promote arterial remodeling in aging, hypertension, and atherosclerosis. *Hypertension* 65: 798–793, 2015. doi:10.1161/HYPTENSIONAHA.114.03618.

59. Wang M, Lakatta EG. Altered regulation of matrix metalloproteinase-2 in aortic remodeling during aging. *Hypertension* 39: 865–873, 2002. doi:10.1161/01.HTYP.0000145063.13322.66.

60. Wilkinson IB, Franklin SS, Cockcroft JR. Nitric oxide and the regulation of large artery stiffness: from physiology to pharmacology. *Hypertension* 44: 112–116, 2004. doi:10.1161/01.HYP.0000138068.03933.40.

61. Zhou R-H, Vendrov AE, Tehivile I, Niu XL, Moinar KC, Rojas M, Carter JD, Tong H, Stouffer GA, Madamanchi NR, Runge MS. Mitochondrial oxidative stress in aortic stiffening with age: the role of smooth muscle cell function. *Arterioscler Thromb Vasc Biol* 32: 745–755, 2012. doi:10.1161/ATVBAHA.111.243121.

62. Ziemken SJ, Melenovsky V, Kass DA. Mechanisms, pathophysiology, and therapy of arterial stiffness. *Arterioscler Thromb Vasc Biol* 25: 932–943, 2005. doi:10.1161/01.ATV.0000156048.78317.29.