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Repository Citation

Fleenor, Bradley S.; Eng, Jason S.; Sindler, Amy L.; Pham, Bryant T.; Kloor, Jackson D.; and Seals, Douglas R., "Superoxide Signaling in Perivascular Adipose Tissue Promotes Age-Related Artery Stiffness" (2014). *Graduate Center for Nutritional Sciences Faculty Publications*. 7.  
https://uknowledge.uky.edu/nutrisci_facpub/7

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Notes/Citation Information
Published in Aging Cell, v. 13, issue. 3, 576-578.


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Digital Object Identifier (DOI)
http://dx.doi.org/10.1111/acel.12196

This article is available at UKnowledge: https://uknowledge.uky.edu/nutrisci_facpub/7
Superoxide signaling in perivascular adipose tissue promotes age-related artery stiffness

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Summary
We tested the hypothesis that superoxide signaling within aortic perivascular adipose tissue (PVAT) contributes to large elastic artery stiffening in old mice. Young (4–6 months), old (26–28 months), and old treated with 4-Hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPOL), a superoxide scavenger (1 μM in drinking water for 3 weeks), male C57BL6/N mice were studied. Compared with young, old had greater large artery stiffness assessed by aortic pulse wave velocity (aPWV, 436 ± 9 vs. 344 ± 5 cm s⁻¹) and intrinsic mechanical testing (3821 ± 427 vs. 1925 ± 271 kPa) (both P < 0.05). TEMPOL treatment in old reversed both measures of arterial stiffness. Aortic PVAT superoxide production was greater in old (P < 0.05 vs. Y), which was normalized with TEMPOL. Compared with young, old controls had greater pro-inflammatory proteins in PVAT-conditioned media (P < 0.05). Young recipient mice transplanted with PVAT from old compared with young donors for 8 weeks had greater aPWV (409 ± 7 vs. 342 ± 8 cm s⁻¹) and intrinsic mechanical properties (3197 ± 647 vs. 1889 ± 520 kPa) (both P < 0.05), which was abolished with TEMPOL supplementation in old donors. Tissue-cultured aortic segments from old in the presence of PVAT had greater mechanical stiffening compared with old cultured in the absence of PVAT and old with PVAT and TEMPOL (both, P < 0.05). In addition, PVAT-derived superoxide was associated with arterial wall hypertrophy and greater adventitial collagen I expression with aging that was attenuated by TEMPOL. Aging or TEMPOL treatment did not affect blood pressure. Our findings provide evidence for greater age-related superoxide production and pro-inflammatory proteins in PVAT, and directly link superoxide signaling in PVAT to large elastic artery stiffness.

Key words: fat; oxidative stress; peri-aortic; TEMPOL.

Introduction
Aging is the major risk factor for cardiovascular diseases (CVD), as nearly 90% of incident CV events occur in adults over 55 years of age (Go et al., 2013). Stiffening of the large elastic arteries (aorta and carotid arteries) is a strong, independent predictor of cardiovascular events with aging (Sutton-Tyrrell et al., 2005; Mitchell et al., 2010), and superoxide-dependent oxidative stress and inflammation are key mechanisms by which large elastic arteries stiffen with age (Kim et al., 2009; Sindler et al., 2011; Fleenor et al., 2012).

Perivascular adipose tissue (PVAT) surrounds large elastic arteries and may exert an important influence on arterial stiffness. Visceral white adipose tissues from older mice demonstrate greater oxidative stress, which may lead to greater pro-inflammatory cytokine and chemokine secretion (Findeisen et al., 2011; Padilla et al., 2013). However, it is unknown whether the production of superoxide production and pro-inflammatory proteins from PVAT is increased with aging, and whether superoxide signaling in PVAT contributes to large artery stiffening with age.

Results
Large elastic artery stiffness was greater in old compared with young control mice based on aortic pulse wave velocity (aPWV), the clinical gold standard measure (Sutton-Tyrrell et al., 2005; Mitchell et al., 2010) (Fig. 1A), and ex vivo intrinsic mechanical stiffness (Fig. 1B) (both, P < 0.05). Treatment with TEMPOL, a superoxide dismutase mimetic (Simonsen et al., 2009), reduced aortic stiffness in old mice to levels not different from young control animals (Fig. 1A,B). Aortic wall thickness, lumen diameter, and adventitial collagen I expression were greater in old compared with young control mice, and these differences were abolished with TEMPOL (Table S1 and Fig. S1) (all, P < 0.05). Arterial systolic, diastolic, and mean blood pressures were not different with aging or TEMPOL treatment (Table S1) as previously shown (Fleenor et al., 2012).

Superoxide production was increased in whole tissue samples of PVAT surrounding the thoracic aorta (Fig. 1C), and in adipocytes isolated from PVAT of old control compared with young control mice (Fig. S2) (both, P < 0.05). TEMPOL normalized aortic PVAT superoxide production in whole tissue samples from old mice to young control levels (Fig. 1C) (P < 0.05).

Because excessive superoxide production induces inflammation, we assessed the secretion of pro-inflammatory cytokines/chemokines in PVAT-conditioned media. The pro-inflammatory cytokines/chemokines GM-CSF, IL-6, CXCL1, CCL2/MCP-1, CXCL2 were greater in PVAT-conditioned media from old compared with young control mice (Fig. S3) (all, P < 0.05). CS/C5a and TIMP-1 were also detected in conditioned media from old mice, but not from young controls (Fig. S3).

Thoracic aorta PVAT was removed from young control, old control, and old TEMPOL-treated donors and transplanted directly onto the abdominal aorta of young recipient mice for 8 weeks (Fig 2A). Young recipient mice transplanted with PVAT from old animals had greater aortic stiffness as indicated by increased aPWV and ex vivo intrinsic mechanical stiffness compared with those transplanted with PVAT from young donors (Fig. 2B,C) (both, P < 0.05). TEMPOL treatment in old donors abolished the increases in aPWV and ex vivo intrinsic mechanical stiffness observed with transplantation into young recipient mice (Fig 2B, C)(both, P < 0.05).
Wall thickness and adventitial collagen I expression were greater in young recipient mice transplanted with PVAT from old control compared with young control PVAT donors (both, \( P < 0.05 \)), an effect that was not observed with PVAT donated from TEMPOL-treated old animals (Table S2 and Fig. S4). Lumen diameter and arterial systolic, diastolic, and mean blood pressures were not significantly different between groups (Table S2).

To further determine the effects of PVAT on arterial stiffness, aortic segments from additional young and old control mice were cultured in the presence (+) or absence (−) of PVAT for 72 h. Intrinsic mechanical stiffness was greater in aortic segments from old (+) PVAT compared with all aortic segments from young control mice (Fig. 2D) (all, \( P < 0.05 \)). Compared with aortic segments from old (−) PVAT, samples from old (+) PVAT had greater intrinsic stiffness (Fig. 2D) (\( P < 0.05 \)). TEMPOL reversed the intrinsic mechanical properties in arterial segments (+) PVAT to levels similar to old (−) PVAT (Fig. 2D) (\( P < 0.05 \)).

**Discussion**

The present study provides the first evidence directly linking PVAT with large elastic artery stiffness. The effects of PVAT from old mice in the promotion of arterial stiffening were demonstrated in vivo using a fat transplant model and in an in vitro tissue culture model. Importantly, superoxide production in PVAT from older animals was shown to be greater, and this was normalized with TEMPOL treatment, which, in turn, reversed PVAT-mediated arterial stiffening. The age-related increase in PVAT superoxide oxide production was associated with increased cytokine and chemokine secretion, indicating superoxide signaling may promote inflammation in PVAT. We also confirm the superoxide-lowering effect of TEMPOL to improve arterial function in old mice, suggesting excessive superoxide signaling is an important process in arterial stiffening (Fleenor et al., 2012). Arterial blood pressure can influence arterial stiffening; however, blood pressure was not...
different with aging or TEMPOL. Finally, our results suggest that superoxide signaling within PVAT may play an important role in the increase in aortic wall thickness and adventitial collagen I expression with aging.

Perivascular adipose tissue surrounding the thoracic aorta has been shown to resemble the phenotype of brown adipose tissue (Cannon & Nedergaard, 2004; Padilla et al., 2013). Assessing the phenotype, including inflammatory pathways, of thoracic aorta PVAT and whether the phenotype changes with transplantation is of interest and should be examined in future investigations.

In conclusion, our results provide the first evidence for aortic PVAT as a novel mechanism and potential therapeutic target in large elastic artery stiffening and increased CVD risk with aging.

**Acknowledgments**

BSF, JSE, ALS, and DRS contributed to the conception, experimental design, and interpretation of the data. BSF, JSE, BTP, and JDK collected the data. All authors were involved with the preparation and final approval of the manuscript. The authors are grateful to Drs. Frederique Yiannikouris and Sean Thatcher for providing the adipocyte isolation protocol, and Wilson S. Eng for providing insight into the development of the mechanical testing protocol.

**Sources of funding**

This study was supported by the National Institutes of Health Grants R01 AG013038, T32 HL007822 and T32 AG000279.

**Conflict of interest**

None declared.

**References**


