



Published in final edited form as:

Hypertension. 2012 April ; 59(4): 818–824. doi:10.1161/HYPERTENSIONAHA.111.189456.

Acute Reversal of Endothelial Dysfunction in the Elderly Following Antioxidant Consumption

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Abstract

Aging is associated with a pro-oxidant state and a decline in endothelial function. Whether acute, enteral antioxidant treatment can reverse this decrement in vascular function is not well known. Flow-mediated vasodilation and reactive hyperemia were evaluated following consumption of either placebo or an oral antioxidant cocktail (Vitamin C, 1000mg; Vitamin E, 600 I.U.; Alpha-lipoic acid, 600 mg) in 87 healthy volunteers (42 young, 25 ± 1 yrs; 45 older, 71 ± 1 yrs) using a double-blind, crossover design. Blood velocity and brachial artery diameter (ultrasound Doppler) were assessed before and after 5-min forearm circulatory arrest. Serum markers of lipid peroxidation, total antioxidant capacity, endogenous antioxidant activity, and Vitamin C were assayed, and plasma nitrate, nitrite, and 3-nitrotyrosine were determined. In the placebo trial, an age-related reduction in brachial artery vasodilation was evident (young: 7.4 ± 0.6 %; older: 5.2 ± 0.4 %). Following antioxidant consumption, flow-mediated vasodilation improved in older subjects (5.2 ± 0.4 %, placebo: 8.2 ± 0.6 %, antioxidant) but declined in the young (7.4 ± 0.6 %, placebo; 5.8 ± 0.6 %, antioxidant). Reactive hyperemia was reduced with age, but antioxidant administration did not alter the response in either group. Together, these data demonstrate that antioxidant consumption acutely restores endothelial function in the elderly, while disrupting normal endothelium-dependent vasodilation in the young, and suggest that this age-related impairment is due, at least in part, to free radicals.

Keywords

Aging; Endothelium; Free Radicals; Nitric Oxide; Vascular Function

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Financial Disclosures: None.

INTRODUCTION

In recent years, considerable effort has been expended to evaluate the relationship between age, oxidative stress, and vascular health, with largely equivocal results. However, there is convincing evidence of an overall increase in plasma free radical concentration with advancing age^{1,2} and a subsequent reduction in nitric oxide (NO) bioavailability which has been implicated in the age-related decline in endothelial function³⁻⁷. As endothelial dysfunction and increased vascular oxidative stress have been identified as predictors for risk of cardiovascular events⁸, antioxidant (AO) supplementation as a non-invasive approach to further understand vascular function, and the potential of this approach in the prevention and treatment of vascular dysfunction, is particularly attractive in the elderly population.

Despite the accepted association between oxidative stress and vascular health, chronic, large scale (>1000 subjects) clinical trials have failed to demonstrate a beneficial effect of long-term AO consumption on cardiovascular disease morbidity and mortality⁹. However, smaller, interventional studies focused on acute AO-mediated changes in vascular reactivity have provided more promising results. Infused, supra-physiologic doses of vitamin C have been documented to transiently restore endothelium-dependent vasodilation^{10,11} and skeletal muscle blood flow¹²⁻¹⁴ in the elderly, and BH₄ administration (a cofactor for endothelial NO production) acutely improves endothelial function¹⁵. However, studies utilizing more practical interventions, such as oral vitamin C at over-the-counter doses, have failed to demonstrate an improvement in age-related endothelial dysfunction¹⁰. Moreover, the effect of combined AOs administered concomitantly on vascular function in the elderly remains largely unknown. Thus, despite the myriad studies that have examined the role of oxidative stress on cardiovascular health with healthy aging, mechanistic studies designed to abruptly reduce plasma oxidative stress and subsequently determine the acute effects of this intervention on AO capacity, oxidative stress, and vascular function have not been undertaken.

Determination of endothelial function following an acute AO intervention is most easily achieved by assessing the degree of brachial artery vasodilation in response to an acute increase in shear stress. This flow-mediated vasodilation (FMD) of the brachial artery subsequent to a period of vascular occlusion provides a relevant index of endothelium-dependent vasodilation which reflects NO bioavailability¹⁶ and correlates well to coronary artery reactivity¹⁷. Importantly, both coronary¹⁸ and peripheral^{19,20} vasodilator function have been convincingly documented to predict future cardiovascular events, and as such, assessment of endothelium-dependent vasodilation may provide valuable prognostic and diagnostic patient information. Likewise, the magnitude of reactive hyperemia relates inversely to traditional cardiovascular disease risk factors²¹ and has also emerged as a clinically relevant tool in predicting future cardiovascular events. However, the role of NO in this response is minimal^{22,23}, and thus reactive hyperemia remains a clinically relevant, though somewhat non-specific, indicator of microvascular reactivity. Together, FMD and reactive hyperemia may therefore be seen as complimentary measurements that allow an opportunity to compare endothelial and microvascular function, as well as determination of the specific effect of oxidative stress and AO administration on these distinct regions of the vasculature.

In the current study, we sought to fill a void in the clinical literature by administering an oral AO cocktail (vitamin C, E, and alpha-lipoic acid) to abruptly reduce plasma free radical concentration, and assess the acute vascular responses to this reduction in oxidative stress in young and older subjects. Two hypotheses were tested; 1) We hypothesized that AO consumption would improve FMD in the elderly, but decrease FMD in their young, healthy

counterparts, and 2) We hypothesized that reactive hyperemia would not be affected by AO consumption in either young or older subjects.

METHODS

Subjects and General Procedures

42 young (25 ± 1 yrs) and 45 older (71 ± 1 yrs) healthy volunteers participated in the current study. All subjects were non-smokers, normotensive ($<140/90$ mmHg), and free of overt cardiovascular disease as determined by health history questionnaire and physical examination. Exclusion criteria included subjects with diagnosed cardiovascular disease, diabetes, hypercholesterolemia, hypertension, and women who were pregnant. Subjects were not taking any prescription medication, and were asked to abstain from vitamin supplements for 10 days prior to enrollment and during the course of the study. Subjects arrived to the laboratory at 0800 in a fasted state, having abstained from exercise and caffeine for 12 hours prior, in accordance with recently published guidelines²⁴. Premenopausal females were studied within the first five days of their menstrual cycle, as FMD measurements of the brachial artery have been documented to fluctuate with menstrual phase. Protocol approval and written informed consent were obtained according to the University of Utah and Veteran Affairs Institutional Review Board requirements. All data collection took place with subjects supine, in a thermoneutral environment.

Antioxidant Supplementation

All subjects reported to the laboratory twice within one week (>48 hrs apart) and received the antioxidant (AO) cocktail or placebo (PL) in a balanced, double-blind, crossover design. Supplements were taken in two doses, separated by 30 minutes to improve absorption, consumed 90 and 60 minutes prior to the FMD protocol. The first dose consisted of 300 mg of alpha-lipoic acid, 500 mg Vitamin C, and 200 I.U. Vitamin E, and the second dose was 300 mg alpha-lipoic acid, 500 mg Vitamin C, and 400 I.U. Vitamin E. Placebo microcrystalline cellulose capsules of similar taste, color, and appearance were likewise consumed in two doses within the same time frame. We have previously reported the efficacy of this antioxidant cocktail to reduce carbon- and O₂-centered free radical levels, as measured by electron paramagnetic resonance (EPR) spectroscopy, in both young²⁵ and older²⁶ subject populations.

Measurements

Details of the FMD procedure have been described previously²⁷, and were performed in accordance with current recommendations²⁴. Briefly, a blood pressure cuff was placed on the right arm proximal to the elbow and distal to the placement of the ultrasound Doppler probe on the brachial artery (BA). The BA was insonated approximately midway between the antecubital and axillary regions, and measurements of BA diameter and blood velocity (V_{mean}) measurements were obtained continuously at rest and for 2 minutes following cuff deflation (Logiq 7, GE Medical Systems, Milwaukee, WI).

Analyses

V_{mean} was automatically calculated using commercially available software (Logiq 7). End-diastolic, ECG R-wave gated images were collected via video output from the Logiq 7 for off-line analysis of BA vasodilation using automated edge-detection software (Medical Imaging Applications, Coralville, Iowa). Flow-mediated vasodilation (FMD) was quantified as the maximal percent change in BA diameter following cuff release. Shear rate was calculated as: $\text{Shear Rate } (s^{-1}) = V_{\text{mean}} \bullet 8 / \text{vessel diameter}$. Blood flow was calculated as: $\text{Blood flow } (ml \bullet \text{min}) = (V_{\text{mean}} \bullet \pi \bullet [\text{Vessel Diameter} / 2]^2 \bullet 60)$. For both shear rate and blood

flow, cumulative area-under-curve (AUC) values were integrated with the trapezoidal rule and calculated as $\sum \{y_i[x_{(i+1)} - x_i] + (1/2)[y_{(i+1)} - y_i][x_{(i+1)} - x_i]\}$. Reactive hyperemia was quantified as cumulative BA blood flow for two minutes (AUC) following cuff occlusion. Normalized FMD was calculated by dividing FMD (%) by the cumulative shear rate AUC at the time of peak BA vasodilation.

Assays

In a subset of subjects ($n = 18$ young, $n = 30$ older), blood samples were obtained from the antecubital vein immediately prior to FMD testing on both PL and AO visits. Total AO capacity was assessed by the ferric reducing ability of plasma (FRAP) assay, and endogenous AO activity was assessed by determining superoxide dismutase (SOD, Cayman Chemical Company, Ann Arbor, MI). Plasma ascorbate concentration was also determined (Cosmo Bio, Carlsbad, CA). Quantitative determination of thiobarbituric acid reactive substances (TBARS) was performed to assess lipid peroxidation, a marker of oxidative stress (Bioassay Systems, Hayward, CA). Total nitrate and nitrite were measured using a standard colorimetric nitrate reductase/Greiss reaction assay (Cayman Chemical, Ann Arbor, MI). Measurement of nitrotyrosine (3-NT) was performed using competitive ELISA (Cell Biolabs, San Diego, CA). A lipid panel was obtained for all subjects by standard techniques.

Statistics

Statistics were performed with the use of commercially available software (SigmaPlot 11, Systat Software Inc., Point Richmond, California, USA). Repeated measures two-way analysis of variance (ANOVA) was used to identify significant changes in measured variables between conditions and groups, with the Bonferroni test used for post-hoc analysis when a significant main effect was found. All group data are expressed as mean \pm standard error. Significance was established at $P < 0.05$.

RESULTS

Subject characteristics are presented in Table 1, and assay results are documented in Table 2.

In the PL trial, FMD was significantly attenuated in older subjects compared to their younger counterparts ($5.2 \pm 0.4\%$, older PL; $7.4 \pm 0.6\%$, younger PL) (Figure 1). In the older group, AO administration significantly improved FMD ($5.2 \pm 0.4\%$ to $8.2 \pm 0.6\%$, PL vs. AO). In contrast, AO administration in the young group had a detrimental effect on FMD ($7.4 \pm 0.6\%$ to $5.8 \pm 0.6\%$, PL vs. AO) (Figure 1). When normalized for shear stimulus, the age-associated decrement in FMD was still evident ($0.22 \pm 0.03\%/s^{-1}\cdot s$, older PL; $0.31 \pm 0.03\%/s^{-1}\cdot s$, young PL). AO administration significantly improved normalized FMD in the older group (0.22 ± 0.03 to $0.37 \pm 0.03\%/s^{-1}\cdot s$) and reduced normalized FMD in the young (0.31 ± 0.03 to $0.23 \pm 0.02\%/s^{-1}\cdot s$, PL vs. AO). The time to peak dilation was significantly greater in the older group, but was not affected by AO administration (61 ± 3 s, older PL; 50 ± 3 s, young PL; 62 ± 4 s, older AO; 52 ± 3 s, young AO). In both the PL and antioxidant AO trials, a significant age-related decrement in reactive hyperemia was evident; however, AO treatment did not alter the hyperemic response in either group (Figure 2).

DISCUSSION

The present study sought to evaluate the efficacy of acute AO administration to abruptly reduce plasma oxidative stress, and to subsequently determine the acute effects of this intervention on AO capacity, oxidative stress, and vascular function in young and older individuals. In the elderly, we identified a reduction in FMD that was accompanied by

elevated oxidative stress, and demonstrated the ability of an acute AO intervention to reverse this age-associated decrement. In contrast, AO administration did not prove beneficial in younger individuals who had lower levels of oxidative stress, but rather resulted in a reduction in FMD. Reactive hyperemia was also lower in the elderly, but was not altered by AO in either group, supporting the NO-specific nature of the AO intervention. Together, these findings support the important role of free radicals in age-associated endothelial dysfunction, and identify the efficacy of AOs available over the counter as capable of acutely improving endothelial function in the elderly.

FMD, Age, and Antioxidants

Brachial artery FMD testing has emerged as a simple, non-invasive means of evaluating endothelial function in a variety of healthy and disease populations^{24, 28, 29}. Vasodilation of the brachial artery subsequent to a period of vascular occlusion provides an index of endothelium-dependent vasodilation which reflects NO bioavailability¹⁶ and is thought to be predictive of future cardiovascular events^{19, 20}. These previous studies have led to the concept that results of endothelial function testing may serve as a “barometer for cardiovascular health”³⁰ and promoted interest in interventions with the prospect of improving endothelial health and thereby limiting cardiovascular risk.

We observed an age-related decline in FMD in the elderly (Figure 1), which is in agreement with the majority of studies that have collectively identified a decline in endothelium-dependent vasodilation with advancing age, often as early as the fourth decade^{31–33}. However, this age-related decrement appeared readily reversible, as evidenced by improved FMD following AO administration. Indeed, AO consumption acutely improved brachial artery FMD in the elderly to that of their younger counterparts (Figure 1), suggesting that an AO cocktail at modest, enteral doses is sufficient to acutely reverse age-related endothelial dysfunction. This profound improvement in endothelial function extends former studies demonstrating the capacity of high-dose, infused AOs to acutely improve endothelium-dependent vasodilation^{4, 10, 15} in the elderly, now revealing a similar beneficial effect using a typical, over-the-counter combination of AOs available to the general public. As discussed above, FMD is generally thought to provide a bioassay of NO¹⁶, and as such it is likely that this AO-mediated improvement in FMD in the elderly was achieved through an acute increase in NO bioavailability.

In contrast to the positive outcome in the elderly, the AO intervention proved detrimental to endothelium-dependent vasodilation in young, healthy individuals (Figure 1). Previous studies from our group have identified a similar pattern of “paradoxical” age-specific AO effects, which appears to be related to the underlying level of oxidative stress³⁴. Specifically, during handgrip exercise, BA vasodilation is impaired in the elderly, but this age effect is restored to that of the young following AO consumption³⁵; however, AO administration had an opposite effect in the young, resulting in an impaired vasodilatory capacity during exercise²⁵. Findings from the present study thus complement these previous exercise studies through the use of BA FMD measurements, a widely accepted method for examining endothelial function²⁸.

Evidence of negative responses to AO in the young appears to support the emerging concept of a beneficial role for free radicals in the peripheral circulation. Indeed, others have documented that free radicals such as H₂O₂ and reactive oxygen species (i.e. ONOO⁻) may also act as potent vasodilators³⁶ and thus may, in fact, contribute to FMD³⁷. In addition to direct vasodilatory properties, free radicals may also play an important role in upregulation of endogenous AO capacity through increased expression of several enzymes known to “detoxify” free radicals³⁸. Considering these former studies and the present data, we speculate that in the young, where pro and antioxidant forces are somewhat balanced, the

aggressive reduction in free radical concentration following AO administration may have removed or suppressed certain oxidative species which possess some beneficial properties in terms of vascular regulation, resulting in the observed decrement in FMD.

Together, these data in young and older individuals demonstrate a dichotomous effect of AO consumption on endothelial function with age; consumption of an AO cocktail at over-the-counter doses acutely improved endothelial function in the elderly, while disrupting normal endothelium-dependent vasodilation in the young.

Reactive Hyperemia, Age, and Antioxidants

Following circulatory occlusion, resistance vessel vasodilation provokes an increase in arm vascular conductance, and thus acts as the downstream stimulus for upstream increases in BA shear rate and subsequent BA vasodilation. However, the factors which contribute to reactive hyperemia *per se* are thought to be somewhat more complex than BA FMD. Indeed, this response may be attributed to a combination of endothelium-dependent dilation, inherent myogenic action, and vasodilatory metabolites produced in response to tissue ischemia, including adenosine and non-endothelium dependent vasodilators.

Like FMD, the magnitude of reactive hyperemia relates inversely to traditional cardiovascular disease risk factors²¹ and has thus emerged as a clinically relevant tool in predicting future cardiovascular events³⁹. However, the role of NO in this response appears modest^{22, 23}, and thus reactive hyperemia remains a clinically relevant, though somewhat non-specific, indicator of microvascular reactivity. In the present study, these complimentary measurements allowed inter- and intra-subject comparison of the effects of AO administration on both endothelial and microvascular function, as well as an opportunity to further define the specific effect of acute AO consumptions on NO bioavailability. As illustrated in Figure 2, reactive hyperemia was significantly reduced in older subjects in the PL trial. The mechanisms for this age-related difference remain unknown, but it is speculated that an age-related decline in sensitivity to ischemia or production of vasodilatory metabolites may at least partially explain these intriguing findings. This supposition is supported by the recent observation that the muscle metaboreflex becomes less sensitive with advancing age⁴⁰. AO administration did not alter reactive hyperemia in either group, which was anticipated based on former work from our group²⁷ and others²² which failed to identify NO as a significant contributor to post-occlusion reactive hyperemia. These findings are in line with other AO studies which have been unable to document a change in reactive hyperemia following intra-arterial ascorbic acid¹⁰ or BH₄¹⁵ in elderly subjects. When viewed in conjunction with the marked AO effect on FMD, the lack of a treatment effect in the microvasculature further supports the assertion that the AO cocktail consumed in the present study likely improved endothelial function in the elderly through a reduction in oxidative stress and subsequent improvement in conduit artery NO bioavailability. It is also tempting to speculate that free radicals may play a proportionally bigger role than NO in governing reactive hyperemia through vascular smooth muscle hyperpolarization or the oxidative activation of the cGMP-dependent kinase via disulfide-dimerization, though discerning these mechanistic pathways is beyond the scope of the present study.

Quantitative Assessment of Antioxidant Efficacy

One essential component of any study examining the efficacy of an AO intervention is detecting increases in circulating AO concentrations and documenting a subsequent reduction in free radical concentration and NO production. In terms of AO, we determined that vitamin C concentration and markers of AO capacity increased in both young and older groups (Table 2), confirming the equally efficacious effect of the AO treatment between groups. It is thus apparent that AO-mediated changes in endothelial function cannot be

attributed to differences in absorption of the AO cocktail. It is also noteworthy that administration of exogenous AOs increased superoxide dismutase (SOD) activity (Table 2), one of the most ubiquitous endogenous AOs. It thus seems that AO consumption resulted in a two-tier effect, including both direct scavenging effects of free radicals and a secondary decrease in oxidative stress via “sparing” of endogenous AOs, which collectively improved endothelial function in the elderly.

Oxidative stress was significantly reduced following AO administration in the elderly, as documented by a reduction in lipid peroxidation end-products (TBARS) (Table 2). Interestingly, the same cannot be said for the young, in whom TBARS did not decrease as a consequence of the AO cocktail. In combination with our previous reports identifying the efficacy of this AO cocktail to reduce carbon- and O₂-centered free radical levels utilizing EPR spectroscopy in both young²⁵ and older²⁶ subject populations, confirms the ability of this AO treatment to acutely reduce oxidative stress when baseline levels are elevated, as in the elderly. Surprisingly, AO consumption did not affect 3-Nitrotyrosine in either group, and was reduced in the elderly compared to young in the placebo condition (Table 2). This was an unexpected finding, considering the age-related increase in TBARS and the subsequent reduction following AO consumption in the older group, but is in agreement with prior work reporting similar tissue 3-Nitrotyrosine levels in both young and older rats before and after chronic AO supplementation⁴¹.

Plasma Nitrite and Nitrite:Nitrate were assessed as a surrogate measure of vascular NO bioavailability (Table 2), as it has been demonstrated that circulating nitrite sensitively reflects acute changes in regional eNOS activity⁴². For both Nitrite and Nitrite:Nitrate, we observed an age-related decline that is consistent with previous studies⁷ and the functional measurements of FMD (Figure 2). However, we were unable to detect a significant decrease in either Nitrite or Nitrite:Nitrate following AO consumption. As with the 3-Nitrotyrosine, this lack of an AO effect on plasma Nitrite and Nitrite:Nitrate may reflect the somewhat subtle nature of the oral AO treatment utilized in the present study, an intervention that appears to provoke functional improvements in endothelium-dependent vasodilation without detectable increases in circulating nitrite levels.

Perspectives

In contrast to the largely disappointing findings from clinical trials concerning the beneficial effect of chronic AO administration on cardiovascular health⁹, the present study has identified a marked and acute improvement in endothelial-dependent vasodilation in the elderly following consumption of an oral AO cocktail. However, the difference in design between acute and long-term interventional studies precludes a direct comparison with data from these previous studies. Indeed, the present study administered a single AO load in order to provoke an abrupt reduction in plasma oxidative stress, and subsequently determine the acute effects of this intervention on AO capacity, oxidative stress, and vascular function in young and older individuals. Employing this short-term, interventional design has allowed us to demonstrate the ability of an oral AO cocktail to reverse the age-associated impairment in endothelial function in the elderly without the additional confounding variables associated with longer-term treatment studies. These findings provide important mechanistic insight regarding the link between endothelial dysfunction and oxidative stress in the elderly, and demonstrate a striking plasticity in endothelial function in response to a simple, oral AO intervention.

Experimental Considerations

It should be noted that the 3-Nitrotyrosine assay reflects peroxynitrite from multiple upstream sources, and is the combined consequence of basal NO bioavailability, superoxide

levels, and SOD activity⁴³. Thus, the reduction in 3-Nitrotyrosine in the elderly, and lack of an effect following AO administration, is somewhat difficult to interpret due to this assay's lack of specificity. We also acknowledge that the present data does not offer insight concerning the potential vascular benefits of a long-term AO treatment. It is anticipated that follow-up studies involving chronic administration of this oral AO cocktail will build upon these acute vascular measurements, allowing a more appropriate comparison to existing, larger-scale AO studies.

Summary

Administration of an AO cocktail documented to reduce plasma oxidative stress significantly improved endothelial function in the elderly, reversing the age-related decrement in FMD. In contrast, the same AO intervention proved detrimental to endothelium-dependent vasodilation in young, healthy individuals. AO administration did not alter reactive hyperemia in either group, supportive of an endothelium-dependent, NO-specific effect. These findings reveal a pivotal role of oxidative stress in age-associated endothelial dysfunction, and support the efficacy of oral AO administration in physiologic doses as a means to acutely improve conduit artery endothelial function in the elderly.

Acknowledgments

Funding Sources: Funded in part by NIH PO1 HL-091830 (R.S.R.), TRDRP 15RT-0100 (R.S.R.), VA RR&D E6910R (R.S.R.), AHA 0835209N (D.W.W), and AHA 10SDG305006 (R.A.H).

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NOVELTY AND SIGNIFICANCE

What is New?

- A single dose of antioxidant vitamins C, E and alpha-lipoic acid improved endothelium-dependent vasodilation in the elderly to a level comparable to that of their younger, healthy counterparts. This new finding suggests that acute antioxidant consumption at physiologic doses is sufficient to acutely reverse age-related endothelial dysfunction.
- In contrast to the positive outcome in the elderly, the AO intervention proved detrimental to endothelium-dependent vasodilation in young, healthy individuals. This “paradoxical” effect of AO on vascular function appears to be related to differences in the underlying level of oxidative stress between young and older populations.

What is Relevant?

- In contrast to the largely disappointing findings from clinical trials concerning the beneficial effect of chronic AO administration on cardiovascular health, the present study has identified a marked and acute improvement in endothelial-dependent vasodilation in the elderly following consumption of an oral AO cocktail. As oxidative stress and endothelial function commonly accompany geriatric hypertension, these findings in the elderly may represent a potential therapeutic target for improving disease-related decrements in endothelium-dependent vasodilation.

Summary

- Findings from the present study have identified a pivotal role of oxidative stress in age-associated endothelial dysfunction, and support the efficacy of oral AO administration in physiologic doses as a means to acutely improve conduit artery endothelial function in the elderly.

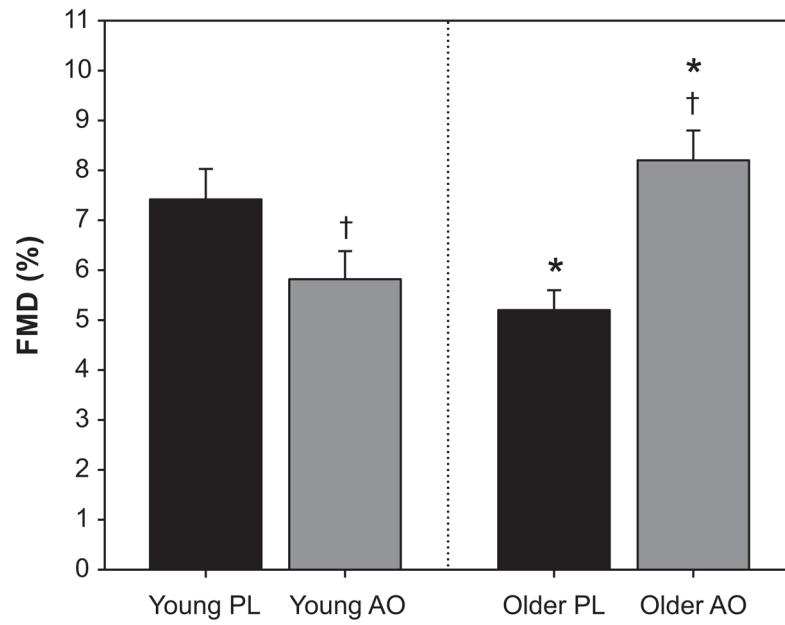


FIGURE 1. Brachial artery flow-mediated vasodilation (FMD) in young and older subjects following placebo (PL) or antioxidant (AO) administration. In the PL trial, an age-associated decline in FMD was apparent. Following AO consumption, FMD was reduced in young, but improved in the elderly. * Significant difference between young and older groups, $P < 0.05$; † Significant difference between PL and AO, $P < 0.05$.

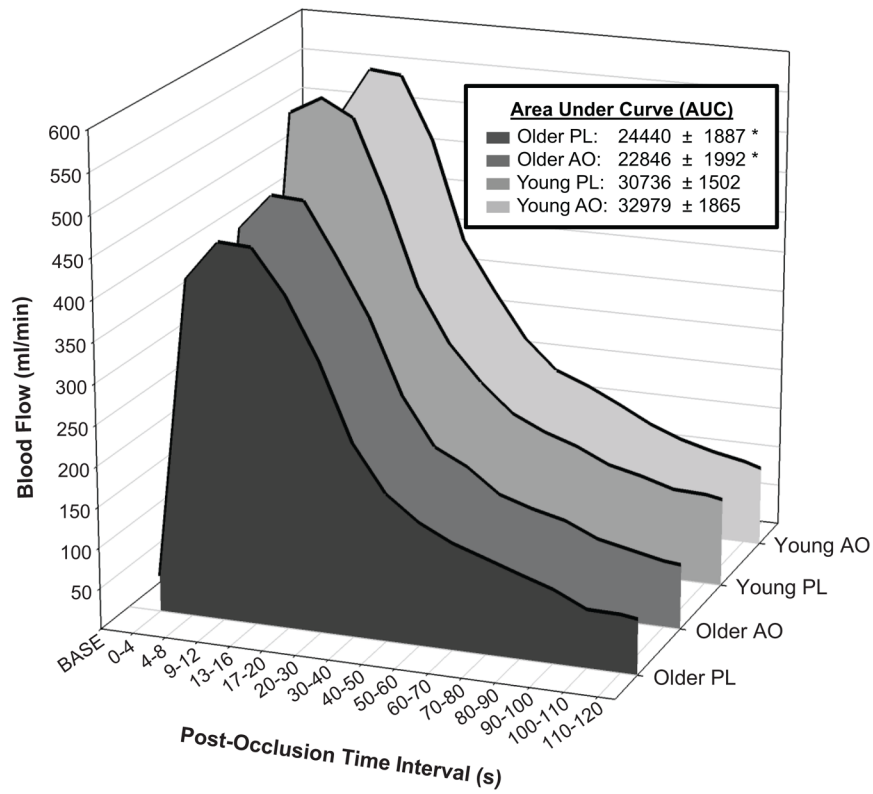


FIGURE 2. Reactive hyperemia following 5-min cuff occlusion in young and older subjects following placebo (PL) or antioxidant (AO) administration. Elderly subjects exhibited a decline in post-occlusion blood flow, but there was no effect of AO treatment. * Significant difference between young and older groups, $P < 0.05$. AUC, Area-under-curve.

TABLE 1

Subject Characteristics.

Variable	Young (n = 42)	Older (n = 45)
Age (yrs)	25 ± 1	71 ± 1*
Height (cm)	172 ± 2	168 ± 1
Weight (kg)	64 ± 2	73 ± 2*
Body Mass Index (kg/m ²)	22 ± 1	25 ± 1*
Total cholesterol (mg/dl)	157 ± 6	182 ± 8*
High-density lipids (mg/dl)	55 ± 3	52 ± 3
Low-density lipids (mg/dl)	93 ± 4	114 ± 7*
Triglycerides (mg/dl)	69 ± 6	100 ± 8
BA diameter (mm)	3.7 ± 0.1	4.0 ± 0.2*
BA blood flow (ml/min)	44 ± 3	73 ± 6*

BA, brachial artery.

* Significant difference between young and older groups, $P < 0.05$.

TABLE 2

Serum measurements of antioxidant capacity, oxidative stress, and antioxidant activity.

Variable	Young (n = 18)		Older (n = 30)	
	PL	AO	PL	AO
Vitamin C (µg/ml)	1.4 ± 0.2	2.2 ± 0.2 [†]	1.9 ± 0.2	3.3 ± 0.3 [†]
Thiobarbituric Acid Reactive Substances (TBARS, µM)	1.51 ± 0.19		2.70 ± 0.13*	2.19 ± 0.09 [†]
Ferric-reducing AO power (FRAP, µM/L)	803 ± 32	947 ± 41 [†]	1059 ± 30*	1194 ± 38 [†]
Superoxide Dismutase (SOD, U/ml)	9.4 ± 0.8	10.6 ± 0.8 [†]	8.3 ± 0.3	9.02 ± 0.3 [†]
3-Nitrotyrosine (nM)	242 ± 21	275 ± 32	197 ± 8*	198 ± 9*
Nitrite (µM)	8.0 ± 0.7	8.0 ± 0.7	6.8 ± 1.3*	5.3 ± 0.4*
Nitrite:Nitrate	0.09 ± 0.01	0.11 ± 0.01	0.06 ± 0.01*	0.05 ± 0.01*

PL, placebo; AO, antioxidant.

* Significant difference between young and older groups, $P < 0.05$;[†] Significant difference between PL and AO, $P < 0.05$.