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Endogenous endothelin-1 and femoral artery shear rate: impact of age and implications for atherosclerosis

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Abstract

Background—Both altered shear rate and endothelin-1 (ET-1) are associated with the age-related development of atherosclerosis. However, the role of ET-1, a potent endogenous vasoconstrictor, in altering shear rate in humans, especially in the atherosclerotic-prone vasculature of the leg, is unknown. Therefore, this study examined the contribution of ET-1 to the age-related alterations in common femoral artery (CFA) shear rate.

Method—BQ-123, a specific endothelin type A (ET_A) receptor antagonist, was infused into the CFA, and diameter and blood velocity were measured by Doppler ultrasound in young ($n = 8$, 24 ± 2 years) and old ($n = 9$, 70 ± 2 years) study participants.

Results and conclusion—The old had greater intima-media thickening in the CFA, indicative of a preatherogenic phenotype. Prior to infusion, the old study participants exhibited reduced mean shear rate ($27 \pm 3/s$) compared with the young study participants ($62 \pm 9/s$). This difference was likely driven by attenuated antegrade shear rate in the old as retrograde shear rate was similar in the young and old. Inhibition of ET_A receptors, by BQ-123, increased leg blood flow in the old, but not in the young, abolishing age-related differences. Older study participants had a larger CFA (young: 0.82 ± 0.03 cm, old: 0.99 ± 0.03 cm) in which BQ-123 induced significant vasodilation ($5.1 \pm 1.0\%$), but had no such effect in the young ($-0.8 \pm 0.8\%$). Interestingly, despite the age-specific, BQ-123-induced increase in leg blood flow and CFA diameter, shear rate patterns remained largely unchanged. Therefore, ET-1, acting through the ET_A receptors, exerts a powerful age-specific vasoconstriction. However, removal of this vasoconstrictor stimulus does not augment mean shear rate in the old.

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Conflicts of interest

There are no conflicts of interest.

Keywords

aging; blood flow; endothelial function; shear stress

INTRODUCTION

Disturbances in blood flow and subsequent alterations in shear rate evoke profound modifications in endothelial cell phenotype [1–6] and vascular function [7–10] that may directly contribute to the development of age-related vascular disease, including atherosclerosis [2,5]. Clearly, the magnitude and direction of shear rate contributes to peripheral vascular adaptations [9,10]; however, the mechanisms responsible for these hemodynamically driven changes and their role in aging humans are not well understood.

Recent mechanistic investigations revealed important roles for nitric oxide (NO) and α -adrenergic vasoconstriction in the altered shear rate patterns in the arms of older individuals [8,11]. However, the development of atherosclerosis is far greater in the leg than the arm [12–14] which may be explained by the two to three-fold lower mean shear rate in the legs [8,11,15–17]. Indeed, in our previous report [18], the age-related reduction in mean shear rate in the leg was driven by reduced antegrade shear rate as there was no difference in retrograde shear rate with advancing age. Additionally, in an attempt to elucidate the mechanisms contributing to this age-related reduction in mean shear rate in the leg, we pharmacologically inhibited NO synthase with N^G-monomethyl-L-arginine (L-NMMA) and determined that the age-related reduction in mean shear rate persisted [18]. Thus, mechanisms other than alterations in NO bioavailability must account for the attenuated mean shear rate in the legs of older, healthy individuals.

Endothelin-1 (ET-1), a potent endogenous vasoconstrictor and modulator of vascular tone [19], may contribute to the age-related differences in shear rate. ET-1 exhibits mitogenic and proinflammatory properties when bound to the endothelin type A (ET_A) receptor, implicating this pathway in the development and progression of atherosclerosis [20–23]. Additionally, ET-1 has been linked to endothelial dysfunction that accompanies normal aging [23–30] and to the development and progression of several age-related cardiovascular diseases including hypertension, coronary artery disease, and congestive heart failure [31–34]. An abundance of evidence suggests that ET-1 is capable of contributing to age-related alterations in shear rate, but this has yet to be actually documented.

Recently, we reported that inhibition of the ET_A receptor abolished the age-related reduction in leg blood flow (LBF) in healthy older individuals [35], revealing an important role of endogenous ET-1 in the regulation of the vasculature of the leg. Given this previous finding and the established role of ET-1 in atherosclerosis and endothelial dysfunction, this study sought to determine whether ET-1, acting through the ET_A receptor, contributes to the proatherogenic shear rate pattern in the legs of older adults. We directly tested the hypothesis that antagonism of the ET_A receptor accomplished by the infusion of BQ-123 into the common femoral artery (CFA) would eliminate differences in conduit vessel shear rate between young and old study participants by preferentially augmenting mean shear rate in the old and remove in not the young. If confirmed, this finding would reveal an important

role of ET-1-mediated vasoconstriction in the age-related reduction in mean shear rate in the leg, and provide a potential therapeutic target to impede the progression of atherosclerosis with advancing age.

MATERIALS AND METHODS

Eight young (24 ± 2 years) and nine older (70 ± 2 years) healthy individuals volunteered to participate in the present study. A subset of these data focusing on the role of ET_A receptor inhibition and blood flow regulation during basal conditions and knee extension exercise in these study participants has been reported previously [35]. Study participants were not taking any prescription medications and were free of overt cardiovascular disease. Protocol approval and written informed consent were obtained according to the University of Utah and Salt Lake City Veterans Affairs Medical Center Institutional Review Boards, in accordance with the principles outlined in the Declaration of Helsinki. All data collection took place at the Salt Lake City Veterans Affairs Medical Center's Geriatric Research, Education, and Clinical Center in the Utah Vascular Research Laboratory.

Experimental protocol

Study participants reported to the Utah Vascular Research Laboratory at 0800 h on the experimental day. Upon arrival at the laboratory, body mass and height were recorded, and the right femoral artery was catheterized (18-gauge central venous catheter; Arrow International, Reading, Pennsylvania, USA) using the Seldinger technique. After the catheter placement, study participants rested for ~30 min and then performed knee extension exercise as previously described for a separate investigation [35]. After knee extension exercise, study participants rested for at least 60 min to ensure the return to baseline central and peripheral hemodynamics, including LBF, mean arterial pressure (MAP), and heart rate. Following attainment of stable baseline hemodynamics, BQ-123 infusion was initiated. All measures were made with the study participant in the upright seated posture.

BQ-123 infusion

Thigh volume was determined anthropometrically and then used for the calculation of drug dosing. A selective ET_A receptor antagonist (BQ-123 Clinalfa, Calbiochem-Novabiochem, Laufelfingen, Switzerland) was prepared in physiological saline (0.9% NaCl) and administered intraarterially at 10 nmol/min per l of thigh volume (infusion rates of 0.8–1.5 ml/min). This dose has been reported to establish a plateau in both forearm [36,37] and quadriceps [35,38] vasodilation without altering MAP. BQ-123 was infused for 45 min with measures of CFA diameter and blood velocity taken prior to and at 5 min intervals during the 45-min infusion.

Measurements

CFA measures were obtained distal to the inguinal ligament and proximal to the deep and superficial femoral bifurcation. The ultrasound system was equipped with a linear transducer operating at an imaging frequency of 10 MHz. Vessel lumen diameter and intima-media thickening (IMT) were determined at a perpendicular angle along the central axis of the scanned area. IMT was measured in duplicate on the far wall from the interface between

blood and intima and the interface between media and adventitia, and then averaged [39]. ECG R-wave-gated images were collected via video output from the Logic 7 for off-line analysis of IMT using automated edge-detection software (Medical Imaging Applications, Coralville, Iowa, USA). IMT normalized for vessel lumen diameter was also calculated.

Blood velocity was measured using the same transducer with a frequency of 5MHz. All blood velocity measurements were obtained with the probe appropriately positioned to maintain an insonation angle of 60° or less. The sample volume was maximized according to the vessel size and was centered within the vessel. Arterial diameter was measured and mean blood velocity (V_{mean}) (angle-corrected and intensity-weighted area under the curve) was automatically calculated (Logic 7). Using arterial diameter and V_{mean} , LBF in the CFA was calculated as $\text{LBF} = V_{\text{mean}} \times \pi (\text{vessel diameter}/2)^2 \times 60$, where blood flow is in milliliters per minute. It should be noted that basal LBF with and without ET_A receptor inhibition, as previously reported [35], is presented to give context to the novel shear rate findings, which are the focus of this investigation. Additionally, all Doppler ultrasound data (blood velocity and CFA diameter) were reanalyzed to determine antegrade, retrograde, and mean blood flow and shear rates for the current investigation.

Shear rate and oscillatory shear index were calculated according to the following equations; shear rate = $(4 \times V_{\text{mean}}/\text{arterial diameter})$ and oscillatory shear index = $|\text{retrograde shear}|/(|\text{antegrade shear}| + |\text{retrograde shear}|)$ [15,16]. It should be noted that for the purpose of comparison with previous investigations [8,11,15,17], V_{mean} was multiplied by a factor of 4 and not 8, as is common for the calculation of shear rate when sample volume is maximized according to vessel size [40]. MAP was collected continuously by an indwelling catheter connected to a pressure transducer (Transpac IV; Abbot Laboratories, Chicago, Illinois, USA). The pressure transducer was placed at the level of the catheter, reflecting MAP in the femoral artery. Leg vascular conductance (LVC) was calculated as LBF/MAP .

Plasma ET-1 concentrations were assessed by quantitative enzyme immunoassay (R&D Systems, Minneapolis, Minnesota, USA). Intra and interassay coefficients were 4.5 and 5.5%, respectively. As previously described and reported by Barrett-O'Keefe *et al.* [35], net ET-1 release was calculated according to the following equation: net ET-1 release (ng/min) = $(C_V - C_A) \times [\text{LBF} \times ((101 - (\text{hct}/100)))]$, where C_V and C_A are venous and arterial plasma concentration, respectively, and hct is hematocrit.

Data and statistical analysis

Ultrasound images and Doppler velocity spectra were recorded prior to infusion (baseline) and at regular intervals throughout the 45 min BQ-123 infusion. For the purpose of this investigation, only preinfusion and 45 min of infusion are reported as this was the time point by which a plateau in LBF was evident in all study participants [35]. During each 60 s ultrasound Doppler segment, V_{mean} was averaged across five intervals of 12 s each, which were matched to intima-to-intima CFA diameter measurements evaluated during diastole. Statistics were performed with the use of commercially available software (SigmaPlot 11.0; Systat Software, Point Richmond, California, USA). Sample size calculations were performed using the previously documented effect size and standard deviation for BQ-123-induced changes in LBF and vascular conductance [35]. For these outcome measures, it was

estimated that six participants/group were needed to achieve the desired power of 0.8 ($\alpha = 0.05$). A two-way repeated-measures analysis of variance was used to identify significant changes in measured variables within and between conditions and groups. After a significant main effect or interaction, pair-wise comparisons were made using Fischer least significant difference. Pearson moment correlations were assessed for IMT, IMT normalized for diameter, and shear rate parameters. Significance was set at an α level of 0.05, and data are presented as mean $\pm$ SEM.

RESULTS

Study participant characteristics

Young and old study participants were similar in terms of height, weight, BMI, and blood characteristics (Table 1). Older study participants had a larger caliber CFA than the young (young: 0.82 ± 0.03 , old: 0.99 ± 0.03 cm). Absolute IMT and IMT normalized for CFA diameter were greater in the old than the young (Table 1). MAP tended to be higher in the old (115 ± 4 mmHg) compared with the young (101 ± 6 mmHg, $P = 0.053$) and was not altered by BQ-123 (old: 113 ± 4 , young: 97 ± 5 mmHg). HR was not different between young (67 ± 4 bpm) and old (66 ± 2 bpm) and was not altered by BQ-123 (young: 71 ± 3 bpm, old: 70 ± 3 bpm).

BQ-123 evoked changes in common femoral artery and endothelin-1 release

BQ-123 evoked a significant increase in CFA diameter in the old ($5.1 \pm 1.0\%$, 1.04 ± 0.04 cm), but not in the young ($-0.8 \pm 0.8\%$, 0.82 ± 0.03 cm). Net ET-1 release across the limb was similar between young and old prior to BQ-123 infusion (young: 26 ± 3 , old: 21 ± 4 ng/min); however, following BQ-123 administration, net ET-1 release was greater in the old (37 ± 5 ng/min) than in the young (22 ± 3 ng/min).

Leg blood flow and leg vascular conductance

The old study participants exhibited a 30% lower baseline LBF and 44% lower LVC (Fig. 1) than the young. The age-associated reduction in LBF was driven by a lower CFA blood velocity (young: 12 ± 3 m/s, old: 7 ± 1 m/s) as CFA diameter was larger in the older study participants (Table 1). BQ-123 increased LBF ($27 \pm 5\%$) and LVC ($30 \pm 5\%$) in the old such that age-related reductions were eliminated (Fig. 1). In contrast, BQ-123 had no effect on LBF and LVC in the young.

Common femoral artery shear rate

Mean shear rate was approximately 80% lower in the old than the young (Fig. 2a). This attenuated mean shear rate in the old was driven by a reduction in antegrade shear rate (Fig. 2b) as there was no age-related difference in retrograde shear rate (Fig. 2c). Antegrade and mean shear rate were significantly and negatively correlated with IMT ($r = -0.65$, $P < 0.03$) and IMT normalized for CFA diameter ($r = -0.51$, $P < 0.03$). BQ-123 increased antegrade shear rate in both the young and the old ($P = 0.05$); however, a corresponding increase in retrograde shear rate resulted in a lack of change in mean shear rate. Oscillatory shear index was slightly, albeit significantly, increased in response to BQ-123 in the young but not in the old (Fig. 2d).

DISCUSSION

The age-related development of atherosclerosis has been linked to both ET-1 and altered conduit artery shear rate patterns. However, the role of ET-1 in altering shear rate in the atherosclerotic-prone vasculature of the lower limbs with age is not known. Previously, we reported that the selective inhibition of the ET_A receptor abolished the 30% reduction in LBF observed in the elderly. This finding served as the impetus for the current investigation which sought to determine whether ET-1, acting through the ET_A receptor, contributes differentially to the modulation of shear rate patterns in the CFA of young and old individuals. As anticipated, the old study participants exhibited a reduced mean shear rate, driven primarily by reduced antegrade shear, as there was no age-related difference in retrograde shear rate. With these initial observations as a back drop, this is the first mechanistic evaluation of the contribution of ET-1 to age-related differences in shear rate patterns in humans, and several important findings highlight the role of ET-1, acting through the ET_A receptor, in the regulation of the vasculature with age. First, endogenous ET-1 contributes to the regulation of vasomotor tone in the CFA of the old, but not in the young, as evidenced by the significant vasodilation of the CFA in the old in response to ET_A receptor inhibition. Second, despite significant increases in LBF and LVC in the old, mean shear rate remained unchanged during ET_A receptor inhibition. Collectively, these findings reveal that endogenous ET-1 contributes to the regulation of vascular tone in an age-specific manner, but does not account for the age-related reduction in mean shear rate observed in the CFA.

Shear rate and age: the role of endothelin-1

Blood flow through peripheral conduit arteries is characterized by an oscillatory pattern consisting of both forward (antegrade) and backward (retrograde) flow that correspond to ventricular systole and diastole, respectively [41,42]. The direction and magnitude of blood flow and subsequent shear rate through the conduit artery influences vascular function and the development of atherosclerosis by directly impacting endothelial cell phenotype and function [2,5]. As previously reported by our group [18] and others [15,17], and further confirmed by the present findings, the reduction in mean shear in the CFA with age is driven primarily by reduced antegrade shear rate (Fig. 2). Of note, this finding may be limb-specific, as previous reports implicate elevated retrograde shear rate as the driving force for reduced mean shear rate in the arm of older individuals [8,11]. However, it should also be noted that the potential for low shear-induced atherosclerosis is far greater in the legs, as mean shear rate is two to three-fold lower than that of the arms [8,11,15,16]. Indeed, this attenuated mean shear rate has been postulated as being at least partially responsible for the elevated occurrence of atherosclerosis in the legs with age [16]. Furthermore, based on the current findings, it appears that reduced antegrade shear rate, and not augmented retrograde shear rate, recognized in the arms of the elderly, likely plays an essential role in the age-related reduction in mean shear rate and subsequent development of atherosclerosis in the legs. In support of this contention, antegrade shear rate was significantly and negatively correlated with IMT and IMT normalized for CFA, revealing an association between antegrade shear rate and a recognized atherosclerotic risk factor [43] in these healthy individuals.

Recently, we examined the contribution of NO bioavailability to altered shear rate patterns with age to better elucidate the mechanisms involved in this process [18]. In this previous study, NO synthase inhibition evoked a significant, yet similar, reduction in mean shear rate in both young and old, suggesting that a non-NO derived pathway must be involved in the attenuated mean shear rate observed with age. Given this previous finding, the current investigation focused on ET-1, as NO and ET-1 possess opposing properties with regards to atherogenesis and the regulation of vasomotor tone [27,28,44–48]. Specifically, although NO is a potent antiatherogenic molecule associated with cardiovascular health and good endothelial function [49], ET-1, when acting through the ET_A receptor, possesses proatherogenic properties linked to cardiovascular disease and endothelial dysfunction [50]. Importantly, and particularly relevant to the current study, under conditions of reduced NO bioavailability, such as with advanced age, the proatherogenic and vasoconstrictive effects of ET-1 may be relatively unopposed, resulting in significant alterations in vessel tone, function, and disease progression [28]. Indeed, in the current investigation, inhibition of ET_A receptor activation achieved by the intraarterial infusion of BQ-123 evoked a significant (5%) vasodilation of the CFA only in the old. The mechanism responsible for the increase in vasodilation and blood flow during BQ-123 administration in the old is not entirely clear, but may be due to an age-associated increase in ET_A receptor density [51] and/or an increase in receptor sensitivity [52]. Interestingly, at rest, net ET-1 release (measured across the leg) was similar between young and old study participants; however, following ET_A receptor inhibition, net ET-1 release in leg increased in the old, but not in the young [35]. This finding suggests an increased displacement of previously bound ET-1 from the ET_A receptor and may support the notion of increased ET_A receptor density with advancing age. Overall, the current observations are indicative of an age-specific augmentation of endogenous ET-1-mediated regulation of CFA diameter which will play a role in determining shear rate. Additional research in humans is required to confirm the potential role of ET_A receptor density and sensitivity in the augmented ET-1 regulation of vasomotor tone in the elderly.

Shear rate and blood flow: the role of endothelin-1

Our group recently identified the importance of endogenous ET-1 in the regulation of resting skeletal muscle perfusion as ET_A receptor inhibition ameliorated the ~30% reduction in LBF often observed in old study participants [35], which agrees with previous work by Desouza *et al.* [53] and Thijssen *et al.* [38]. The current study has expanded these hemodynamic findings by carefully examining the changes in CFA diameter and blood velocity induced by such ET_A receptor inhibition, revealing increased LBF and concomitant dilation of the CFA. This differential regulation of vascular tone (LBF and CFA diameter) and shear rate by ET-1 was unexpected and contrary to our hypothesis.

Upon further examination, the dissociated regulation of LBF and shear rate by ET-1 may be explained by the differing mathematical relationships between shear rate and vessel diameter (shear rate = $4 \times V_{\text{mean}}/\text{vessel diameter}$) and blood flow and vessel diameter [blood flow = $V_{\text{mean}} \times \pi (\text{vessel diameter}/2)^2$]. Though the relationship between shear rate and diameter is curvilinear, the vessel diameters commonly observed in the CFA for healthy adults (range 0.6–1.1 cm) [54] are on a relatively ‘flat’ portion of the curve, such that relatively large changes in diameter within this range do not translate to substantial alterations in shear rate.

In contrast, small changes in vessel diameter are associated with relatively large alterations in calculated blood flow, as changes across the physiologic range fall on the 'steeper' portion of the curvilinear relationship between diameter and flow. Additionally, shear rate is a consequence of the hemodynamic environment at the level of the conduit artery, whereas vascular resistance (or conductance) of downstream resistance vessels and blood pressure acting in accordance to Ohm's law govern LBF. Thus, unique and somewhat complex hemodynamic parameters contribute to the discordant regulation of LBF and shear rate by ET-1.

Shear rate and age: beyond endothelin-1

The mechanisms accounting for the attenuated antegrade and mean shear rate in the leg with age remain elusive. Indeed, the current investigation reveals that ET-1, although being an important regulator of vascular tone and LBF with age, does not account for the age-related reduction in mean shear rate. In the current study, BQ-123 was administered to selectively inhibit ET_A receptors which have been linked in the development of atherosclerosis and are upregulated in response to reduced shear stress [46,55–58]. Further rationale for focusing on the ET_A receptor subtype comes from recent work reporting that inhibition of the endothelin type B (ET_B) receptor following selective ET_A receptor did not further augment blood flow or vascular conductance in arm, suggesting a primary role for the ET_A receptor in the regulation of vasomotor tone [24]. However, since the ET_B receptor was not inhibited in the current study, we are unable to draw conclusions regarding a role of ET-1 acting through the ET_B receptor on shear rate patterns in the leg. Additionally, because of the recognized crosstalk between ET-1 and NO-mediated vasodilation, ET-1 may be indirectly contributing to enhanced vasoconstriction in the old by reducing NO bioavailability [47,51]. Although we cannot rule out this potential scenario, in the current study, the importance of such a phenomenon is likely minimal because in the old, ET_A receptor inhibition resulted in significant vasodilation in the femoral artery and a 30% microcirculatory-mediated increase in blood flow despite the increased ET-1 release across the leg.

Our previous investigation suggests that NO, although playing a critical role in modulating shear rate patterns, does not account for the age-related attenuation of mean shear rate in the CFA [18]. Therefore, several other factors may account for the age-related reduction in mean shear rate including elevated sympathetically mediated α -adrenergic vasoconstriction, structural adaptations (enlargement of vessel diameter), augmented arterial stiffness, and myogenic regulation [59–63]. In the arm, α -adrenergic blockade reduced the accentuated retrograde and oscillatory shear in the old [11], though direct evidence supporting this mechanism in the leg is lacking. Structural adaptations with age may also help explain the altered shear rate, as the current old study participants exhibited classic signs of vascular aging including larger vessel diameter and increased IMT, a critical precursor for lesion development [43]. The increase in vessel diameter is likely because of the degeneration and subsequent remodeling of the arterial wall [54]. One possible stimulus for this change could be related to the increase in blood pressure and accompanying increase in distending force acting on the arterial wall. Unlike pathological conditions (e.g. arteriovenous fistula) where vessel diameter is augmented to sufficiently normalize shear stress [64], the increase in vessel diameter with age appears to be dissociated from blood flow, as blood flow,

particularly in the legs, tends to decrease with age [59,65–67]. Clearly, several factors beyond ET-1 may be contributing to attenuated mean shear rate with age, and therefore further investigation, especially in the atherosclerotic-prone vasculature of the leg, is warranted.

Perspective

In the atherosclerotic-prone vasculature of the leg, there is evidence that the reduction in mean shear rate with age is driven primarily by attenuated antegrade shear rate. However, ET_A receptor inhibition, by the infusion of BQ-123 into the CFA, resulted in significant vasodilation of the CFA and an increase in LBF without a concomitant alteration in mean shear rate. Therefore, despite a clear age-specific role in the regulation of vascular tone, endogenous ET-1, acting through the ET_A receptors, does not account for the age-related reduction in mean shear rate in the CFA.

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Abbreviations

CFA	common femoral artery
ET-1	endothelin-1
ET_A	endothelin type A receptor
ET_B	endothelin type B
IMT	intima–media thickening
LBF	leg blood flow
LVC	leg vascular conductance
MAP	mean arterial pressure
NO	nitric oxide
V_{mean}	mean blood velocity

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FIGURE 1.

(a) Common femoral artery blood flow and (b) vascular conductance during control and ET_A receptor inhibition via intraarterial infusion of BQ-123 in young and old study participants. * $P<0.05$, significant difference from young. † $P<0.05$, significant difference from control. ET_A , endothelin type A.

FIGURE 2.

(a) Mean shear rate, (b) antegrade shear rate, (c) retrograde shear rate, and (d) oscillatory shear index during control and ET_A receptor inhibition via intraarterial infusion of BQ-123 in young and old study participants. For the purpose of comparison, retrograde shear rate is presented with a positive y axis. * P <0.05, significant difference from young. † P <0.05, significant difference from control. ET_A, endothelin type A.

TABLE 1

Participant characteristics

	Young	Old
Age (year)	24 ± 2	70 ± 2 *
Height (cm)	172 ± 2	172 ± 3
Weight (kg)	71 ± 6	75 ± 3
BMI (kg/m ²)	24 ± 5	25 ± 1
IMT (cm)	0.037 ± 0.003	0.088 ± 0.005 *
IMT normalized for femoral artery diameter	0.045 ± 0.003	0.090 ± 0.006 *
Glucose (mg/dl)	68 ± 6	67 ± 2
Total cholesterol (mg/dl)	153 ± 10	173 ± 8
Triglycerides (mg/dl)	69 ± 12	96 ± 14
HDL (mg/dl)	60 ± 9	55 ± 8
LDL (mg/dl)	78 ± 9	83 ± 10

Values are means ± SEM, significant difference between young and old. HDL, high-density lipoprotein; IMT, intima-media thickening; LDL, low-density lipoprotein.

* $P < 0.05$.