Exercise

Endothelin-1 Vasoconstrictor Tone Increases With Age in Healthy Men But Can Be Reduced by Regular Aerobic Exercise

Gary P. Van Guilder, Christian M. Westby, Jared J. Greiner, Brian L. Stauffer, Christopher A. DeSouza

Abstract—Increased endothelin-1–mediated vasoconstrictor tone has been linked to the etiology of a number of pathologies associated with human aging, including hypertension, congestive heart failure, and coronary artery disease. However, it is currently unclear whether aging, per se, is associated with enhanced endothelin-1 system activity. We hypothesized that endothelin-1 vasoconstrictor activity is greater in healthy older compared with young men and that regular aerobic exercise is an effective intervention for reducing endothelin-1 vasoconstrictor tone in older previously sedentary men. Forearm blood flow (plethysmography) responses to intra-arterial infusion of endothelin-1 (5 pmol/min; for 20 minutes) and selective (BQ-123; 100 nmol/min; for 60 minutes) and nonselective (BQ-123+BQ-788; 100 nmol/min; for 60 minutes) endothelin-1 receptor blockade were determined in 28 healthy, sedentary men: 13 younger (age: 27±1 years) and 15 older (age: 62±2 years). The vasoconstrictor response to endothelin-1 was significantly blunted (~65%) in the older versus younger men. In response to BQ-123, resting forearm blood flow increased (~20%; P<0.05) in the older but not in the younger men. The addition of BQ-788 to BQ-123 did not significantly affect the blood flow responses to BQ-123 in either group. Eight of the 15 older sedentary men completed a 3-month aerobic exercise intervention. After the intervention, the vasoconstrictor response to endothelin-1 was markedly increased (225%; P<0.05), whereas BQ-123 resulted in modest vasoconstriction in the previously sedentary older men. These results demonstrate that endothelin-1–mediated vasoconstrictor tone increases with age in healthy men but can be alleviated with regular aerobic exercise. (Hypertension. 2007;50:403-409.)

Key Words: elderly ■ exercise ■ endothelin ■ blood flow regulation

Many of the cardiovascular complications associated with aging (eg, hypertension, arterial spasm, and myocardial infarction) are attributable, at least in part, to endothelial dysfunction, particularly vasomotor dysregulation.1–3 Impaired vasomotor function occurs early in the atherosclerotic process, contributes to disease development and progression, and can trigger acute cardiovascular events.4–6 In addition to the synthesis and release of relaxing factors, such as NO, the vascular endothelium also produces contracting factors, the most potent of which is endothelin (ET)-1. Produced by the proteolytic cleavage of big ET-1 by ET converting enzyme, endothelial ET-1 is predominantly (>80%) released abluminally toward the vascular smooth muscle.7 Binding of ET-1 to ET receptor 1 (ET-A) and ET receptor 2 (ET-B) receptors on vascular smooth muscle cells activates the phospholipase C-inositol triphosphate pathway resulting in an increase in intracellular calcium causing phosphorylation of myosin kinase and, in turn, long-lasting smooth muscle cell contraction.7,8 Importantly, increased ET-1–mediated vasoconstriction has been linked to the etiology of a number of cardiovascular pathologies, including hypertension, vasospasm, coronary artery disease, and chronic heart failure.8–10 There is strong evidence in animal models that aging is associated with elevated ET-1 system activation.11,12 However, data regarding the influence of aging on ET-1 system activity in adult humans are limited. Kumazaki et al13 reported that cultured endothelial cells from the aorta of adults over the age of 50 years produce and release more ET-1 compared with cells from younger adults. Some studies have reported age-related increases in circulating levels of ET-114,15; however, the pathophysiological significance of these elevated values is unclear. Indeed, although ET-1 concentrations have been reported to be higher in older adults, values are generally in the normal physiological range (<5 pg/mL).16 Moreover, circulating plasma concentrations of the peptide may not necessarily reflect its vascular effects.
but rather variable spillover into, and clearance from, the bloodstream. Several studies have shown greater vasodilator response to ET receptor blockade in a number of pathological states despite no observed differences in circulating plasma levels of the peptide. The aims of the present study were to determine whether endogenous ET-1 vasoconstrictor activity increases with age in healthy, sedentary adult men and, if so, whether regular aerobic exercise training reduces ET-1-mediated vasoconstrictor tone in older men. We hypothesized that ET-1 vasoconstrictor activity is greater in healthy older compared with younger men and that regular aerobic exercise would reduce ET-1 vasoconstrictor tone in older, previously sedentary men. To address these aims we used both a cross-sectional study design and an intervention model to determine the influence of aging and the effects of exercise training on the ET-1 system.

Methods

Subjects

 Twenty-eight healthy, sedentary men participated in the cross-sectional study: 13 younger (age range: 21 to 34 years) and 15 older (age range: 52 to 70 years). All of the subjects were normotensive (arterial blood pressure ≤140/90 mm Hg) and free of overt cardiovascular and metabolic disease as assessed by medical history, physical examination, and fasting blood chemistries. The older men were further evaluated for clinical evidence of coronary artery disease with electrocardiograms and blood pressure at rest and during incremental exercise performed to exhaustion. None of the subjects smoked, were taking medications (including vitamins), or performed regular physical exercise for ≥1 year before the start of the study. Eight older men from the cross-sectional investigation enrolled in the exercise intervention and were studied before and after 3 months of aerobic exercise training. Before participation, all of the subjects had the research study and its potential risks and benefits explained fully before providing written informed consent according to the guidelines of the University of Colorado at Boulder. All of the procedures were performed per institutional guidelines.

Body Composition

Body mass was measured to the nearest 0.1 kg with a medical beam balance. Percentage of body fat was determined by dual energy X-ray absorptiometry (Lunar Corp.). Body mass index (BMI) was calculated as weight (kilograms) divided by height (meters) squared. Minimal waist circumference was measured according to published guidelines.

Maximal Oxygen Consumption ($V_{\text{O}_2}$ max)

To assess aerobic fitness, subjects performed incremental treadmill exercise with a modified Balke protocol. Maximal oxygen consumption ($V_{\text{O}_2}$ max) was measured with on-line computer-assisted open circuit spirometry as described previously. In addition, heart rate was measured throughout the protocol, and the total exercise time to exhaustion was recorded.

Metabolic Measurements

Fasting plasma lipid, lipoprotein, glucose, and insulin concentrations were determined with standard techniques. Plasma concentrations of C-reactive protein (CRP) and oxidized low-density lipoprotein (ox-LDL) were determined by high-sensitivity enzyme immunoassay (ALPCO Diagnostics, R&D Systems). Intra-assay and interassay coefficients of variation were 7.8% and 3.0%, respectively, for CRP and 5.3% and 3.3%, respectively, for ox-LDL.

Intra-Arterial Infusion Protocol

All of the studies were performed between 7 AM and 10 AM after a 12-hour overnight fast in a temperature-controlled room. Under strict aseptic conditions, a 5-cm, 20-gauge catheter was inserted into the brachial artery of the nondominant arm under local anesthesia (1% lidocaine). Heart rate and arterial blood pressure were continuously measured throughout the infusion protocol. Forearm blood flow (FFB) at rest and in response to each pharmacological agent was measured with strain-gauge venous occlusion plethysmography (D.E. Hokanson, Bellevue, Wash), as described previously. Baseline FFB was measured for 5 minutes and for 5 minutes before each drug infusion thereafter. To rule out the possibility of nonspecific differences to vasoconstrictor agents with aging, vascular responses to norepinephrine were determined. Norepinephrine was infused at a rate of 260 pmol/min for 5 minutes, and FFB was measured during the last 3 minutes. After a 20-minute rest period to allow FFB to return to baseline levels, ET-1 (Clinalfa, AG) was infused at a rate of 5 pmol/min for 20 minutes, and FFB was measured during the last 3 minutes. After a 30-minute rest period to allow resting FFB to return to baseline, BQ-123 (Clinalfa, AG), a selective ET$_\alpha$ receptor antagonist, was infused at a rate of 100 nmol/min for 60 minutes, and FFB was measured every 10 minutes. The selected dose of BQ-123 has been shown to completely inhibit the vasoconstrictor effect of ET-1 in the human forearm of healthy adults. After 60 minutes of BQ-123 infusion, the FFB response to nonselective ET-1 receptor blockade was assessed by the coadministration of BQ-123 and BQ-788 (Clinalfa, AG) for an additional 60 minutes. FFB was measured every 10 minutes during the combined BQ-123 and BQ-788 infusion. BQ-788, a specific antagonist of ET$_\beta$ receptors, was infused at a rate 50 pmol/min, a dose shown to effectively inhibit ET$_\beta$ receptors. Because of drug availability, studies involving BQ-788 were only performed in the cross-sectional study.

Exercise Intervention

The 3-month home-based aerobic exercise training program used in the present study has been described previously by our laboratory. Briefly, subjects were asked to exercise 5 to 7 days per week, 40 to 50 minutes per day, at 65% to 75% of their individual maximum heart rate, determined during maximal exercise testing. Most subjects walked, but some incorporated jogging into their exercise session as their fitness improved, to maintain their heart rate within the prescribed range. Compliance with the exercise program was documented every 2 weeks with data downloaded directly from heart rate monitors (Polar Electro) and from exercise logs. Subjects who completed the 3-month exercise intervention were studied 20 to 24 hours after their last exercise training session to avoid the immediate (acute) effects of exercise, while still representing their normal physiological state (ie, habitually exercising).

Statistical Analysis

Differences in subject baseline characteristics and the magnitude of change in FFB to norepinephrine and ET-1 were determined by between-group ANOVA. Group differences in FFB responses to BQ-123 and BQ-123 combined with BQ-788 were determined by repeated-measures ANOVA. Changes in the dependent variables resulting from the exercise intervention were assessed by repeated-measures ANOVA. Relations between variables of interest were assessed by linear and stepwise regression analysis. Plasma CRP concentration data were log transformed to satisfy basic assumptions of parametric testing. However, the absolute values for CRP are presented to facilitate clinical interpretation. All of the data are expressed as means±SE. Statistical significance was set a priori at P<0.05.

Results

Cross-Sectional Study

Selected subject characteristics are presented in Table 1. The mean age difference between the younger and older men was 36 years. Although none of the subjects were obese, body mass, BMI, percentage of body fat, and waist circumference were significantly higher in the older compared with the
Age and FBF Responses to Norepinephrine and ET-1

The vasoconstrictor response to norepinephrine was not significantly different between groups (data not shown). FBF was reduced by 32% in both groups (data not shown). In contrast, the vasoconstrictor response to ET-1 was significantly blunted (≈65%) with age (Figure 1). In the older men, ET-1 produced a 7% reduction in resting FBF compared with a 19±5% reduction in the younger control subjects. In the overall study population, the magnitude of vasoconstriction in response to ET-1 was inversely related to the following (all P<0.05): BMI (r = −0.38), percentage of body fat (r = −0.47), total cholesterol (r = −0.43), low-density lipoprotein cholesterol (r = −0.43), and CRP (r = −0.50).

Age and FBF Responses to Selective and Nonselective ET Receptor Blockade

The FBF responses to selective ET\textsubscript{A} receptor blockade with BQ-123 were markedly different (P<0.01) between the groups (Figure 2). In the younger men, resting FBF was not significantly altered by BQ-123, whereas a significant vasodilator response was observed in the older men. Indeed, in older men, FBF increased ≈20% in response to BQ-123 infusion (Figure 2). Percentage of body fat (r = 0.42; P<0.05) and VO\textsubscript{2} max (r = −0.46; P<0.01) were the only correlates of the vasodilator response to BQ-123 in the overall study population. The addition of BQ-788 to BQ-123 did not significantly affect the FBF responses to BQ-123 in either group (Figure 2). As a result, distinct age-related differences (P<0.05) remained in the FBF responses with nonselective ET\textsubscript{A}/ET\textsubscript{B} blockade. There were no significant correlates with the vascular response to nonselective blockade.

Exercise Intervention

All 8 of the older men (65±2 years) completed the 3-month exercise intervention study. Subjects exercised an average of 5.0±0.3 days per week for 54±4 minutes per day at an intensity of 68±1% of maximal heart rate. Table 2 displays

| Table 1. Selected Subject Characteristics of the Cross-Sectional Study |
|---|---|---|
| Variable | Young (n=13) | Older (n=15) |
| Age, y | 26±1 | 62±2* |
| Body mass, kg | 74.2±2.4 | 84.2±3.2* |
| BMI, kg/m\textsuperscript{2} | 23.5±0.7 | 26.8±0.8* |
| Body fat, % | 14.3±1.3 | 27.3±1.4* |
| Waist circumference, cm | 82.0±1.9 | 95.7±2.6* |
| Systolic BP, mm Hg | 113±3 | 128±1.6* |
| Diastolic BP, mm Hg | 70±3 | 78±1* |
| VO\textsubscript{2} max, mL/kg per minute | 50.6±1.6 | 32.0±1.6* |
| FBF, mL/100 mL tissue per minute | 3.0±0.4 | 3.1±0.5 |
| Total cholesterol, mmol/L | 4.3±0.1 | 5.1±0.2* |
| LDL cholesterol, mmol/L | 2.6±0.2 | 3.5±0.2* |
| HDL cholesterol, mmol/L | 1.2±0.1 | 1.1±0.1 |
| Triglycerides, mmol/L | 1.0±0.2 | 1.1±0.1 |
| Glucose, mmol/L | 4.8±0.1 | 5.2±0.1* |
| Insulin, pmol/L | 30.0±3.2 | 33.9±4.2 |
| C-reactive protein, mg/L | 0.7±0.1 | 3.1±0.8* |
| oxLDL, U/L | 44.3±3.3 | 56.2±4.0* |

*P<0.05 vs younger men.
The selected subject characteristics of men who participated in the exercise intervention. There were no significant differences in body mass, percentage of body fat, arterial blood pressure, maximal oxygen consumption, and plasma CRP and oxLDL concentrations after exercise training. However, aerobic exercise training increased exercise time to exhaustion by 20% \((P<0.01)\) and significantly decreased heart rate and rating of perceived exertion (Borg scale) at the same absolute submaximal level of exercise (70% of baseline \(VO_2\) max).

**Exercise Training and FBF Responses to Norepinephrine, ET-1, and BQ-123**

The vasoconstrictor response to norepinephrine was not affected by aerobic exercise training (data not shown). However, the vasoconstrictor response to ET-1 was 3-fold greater after versus before exercise training \((19.4\pm4.4\% \text{ versus } 6.0\pm4.7\%; \ P \leq 0.05; \text{ Figure 3})\). Of note, there was no difference in the vasoconstrictor response to ET-1 in the older men who completed the exercise intervention and the young men who participated in the cross-sectional study. In addition, regular aerobic exercise training significantly reduced ETA receptor–mediated vasoconstrictor tone. After the exercise intervention, BQ-123 elicited modest vasodilation in the forearm of previously sedentary older men compared with marked vasodilation before the exercise-training program (Figure 4). There were no significant correlates of the change in the response to BQ-123 with exercise training.

**Discussion**

The primary new findings of the present study are as follows: (1) older men demonstrated a blunted forearm vasoconstrictor response to exogenous ET-1 compared with younger men; (2) selective ETA receptor blockade elicited a significant forearm vasodilator response in the older but not the younger men; moreover, this response was not affected by concomitant ETB receptor blockade in either group; and (3) a relatively brief (3-month) period of habitual aerobic exercise training enhanced forearm vasoconstriction to exogenous ET-1 and abolished the vasodilator response to ETA receptor blockade in previously sedentary older men. Collectively, these findings indicate that endogenous ET-1–mediated vasoconstrictor tone is elevated in older sedentary men; however, it can be alleviated by regular aerobic exercise.

To the best of our knowledge, this is the first study to use well-established pharmacological approaches to demonstrate age-related increases in ET-1 production and vasoconstrictor
tone in healthy adults. In response to exogenous ET-1, the forearm vasoconstrictor response was markedly blunted (≈65%) in the older compared with younger men. The reduced vasoconstrictor effect of the peptide in the older men suggests greater endogenous ET-1 bioavailability and continuous receptor activation with aging, as well as a potential concomitant downregulation of ET-1 receptors. Chronic exposure to ET-1 has been shown to induce a reduction in ET-1 receptors on vascular smooth cells. It is important to note that it is unlikely that the observed differences in the ET-1 response were because of a nonspecific age-related decline in contractile function of the vascular smooth muscle, because the vasoconstrictor response to norepinephrine was similar between the older and younger men.

In addition to a blunted FBF response to ET-1, the older men demonstrated a significant vasodilator response to ET_A receptor blockade, whereas FBF was largely unchanged in the younger subjects. Interestingly, nonsselective ET_A,B receptor antagonism did not result in a notable change in FBF above that observed with ET_A blockade alone in the older men. These findings suggest that aging is associated with an increase in ET-1 vasoconstrictor tone that is mediated primarily via the ET_A receptor. The vasodilator response to the ET_A receptor antagonist, BQ-123, in our older men, whereas modest in magnitude (≈20%), is comparable with previous findings in healthy middle-aged and older adults by other investigators with similar methodology. It is possible that the modest increase in FBF in response to BQ-123 may reflect some degree of ET-1–induced ET_A receptor down-regulation. The lack of an effect, either an increase or decrease, in the FBF responses to BQ-123 with the addition of BQ-788 supports the notion of a balanced overall contribution of ET_A receptors (endothelial and smooth muscle) to vascular tone in healthy adults and that this balance is not disrupted with age. Interestingly, similar findings have been reported in type 2 diabetes, whereas the vasodilator response to BQ-123 is augmented with BQ-788 in hypertensive subjects but blunted in subjects with hypercholesterolemia, demonstrating unique pathology-related differences in endothelial and smooth muscle ET-A receptor action.

The mechanisms responsible for the apparent age-related increase in ET-1 system activity are not clear. A number of factors may play a role, including oxidative stress and inflammatory cytokines. Aging is associated with increased oxidative stress that has been linked to impaired endothelial function. Reactive oxidant species, such as hydrogen peroxide and superoxide, as well as oxLDL, have been shown to upregulate the synthesis of ET-1 in cultured endothelial and vascular smooth muscle cells. Interestingly, ET-1 has also been reported to increase superoxide production. Thus, it is plausible that aging is associated with a vicious cycle of oxidative stress, levels of circulating inflammatory cytokines, such as CRP and tumor necrosis factor-α, also tend to increase with age. Both CRP and tumor necrosis factor-α have been connected to increased ET-1 production. In the present study, we observed significant age-related increases in circulating concentrations of oxLDL and CRP. Of note, there was a strong inverse correlation between CRP concentrations and the vasoconstrictor responses to ET-1, suggesting a link between inflammation and ET-1 production with aging. The lack of a relation between oxLDL and the vascular response to ET-1 does not dismiss a potential additional influence of oxidative stress on the ET-1 system with aging.

Considering the older men in the present study were free of many age-related cardiovascular and metabolic abnormalities, such as clinically overt coronary artery disease, hypertension, dyslipidemia, and type 2 diabetes, it is tempting to speculate that the observed increase in ET-1 system activity may be a primary consequence of human aging. However, the fact that our subjects were sedentary should not be overlooked. We have shown previously that a sedentary lifestyle contributes to impaired vascular endothelial health and function with advancing age. A seminal finding of the present study was the beneficial effects of moderate aerobic exercise training in reducing ET-1 production and vasoconstrictor tone in previously sedentary older men. Three months of regular aerobic exercise (primarily walking) resulted in a marked (≈225%) increase in the vasoconstrictor response to ET-1 administration and a modest vasoconstrictor, rather than vasodilator, response to ET_A receptor blockade in previously sedentary older men. In fact, the FBF responses to ET-1 and ET_A receptor blockade in the older men after the exercise intervention were similar to those of the younger sedentary men, suggesting that regular aerobic exercise can counteract the age-related increase in ET-1 system activation. Of note, the training program did not significantly alter body mass, arterial blood pressure, or selected cardiometabolic risk factors, suggesting a direct effect of aerobic exercise on the ET-1 system. We have shown previously that the same aerobic exercise training program used in the present study restored acetylcholine-mediated forearm endothelium-dependent vasodilation in a similar population of older men. The results of this study compliment these findings. Indeed, it is possible that the exercise-induced improvement in vasodilator function may be attributable, at least in part, to diminished ET-1–mediated vasoconstrictor tone. We are currently pursuing this hypothesis. Clinically, it is important to emphasize that our results were accomplished with a home-based, moderate-intensity, aerobic exercise training program that can be safely performed by most, if not all, sedentary healthy older adults. Reducing ET-1 system activity may contribute mechanistically to the cardioprotection conferred to older adults who engage in habitual aerobic exercise.

There are 4 important experimental considerations regarding the present study. First, although cross-sectional studies are often used to assess the effects of aging on vascular function, the inherent possibility that genetic and/or other lifestyle behaviors, independent of age, influenced our results cannot be ignored. In an effort to isolate the primary influence of aging, per se, we studied sedentary, nonmedicated men free of cardiometabolic abnormalities that commonly manifest with advancing age and are associated with increased ET-1 system activity, such as hypertension and type 2 diabetes. Second, our study involved only men; it is possible that women may demonstrate a different endothelial phenotype with respect to aging, exercise, and ET-1 system regulation. Indeed, we have previously reported gender-
related differences in several aspects of endothelial health and function.\textsuperscript{45,46} Third, the lack of a nonexercising control group is a shortcoming of our intervention study. However, the exercise training program used in the present study has consistently been shown to improve endovascular function in healthy adult humans varying in age and body composition.\textsuperscript{27-40,42} Thus, it is not unreasonable to assume that the exercise-induced change in the responses to ET-1 and ET\textsubscript{A} receptor blockade was a primary effect of the intervention. Finally, there is discrepancy in the literature regarding FBF responses to ET-1 receptor antagonism in healthy adult humans. Our findings, and the interpretation thereof, of a vasodilator response to BQ-123 in the experimental (ie, older men) but not the control (ie, younger men) group are consistent with seminal work by Cardillo et al\textsuperscript{18,25,32} studying other pathologic conditions, such as hypertension, hypercholesterolemia, and type 2 diabetes. In contrast, other investigators have reported marked vasodilator responses to BQ-123 and infusion times of BQ-123 across all of the studies are clear and are outside the scope of this study. Importantly, the results of the present study support the postulate that a vasodilator response to ET\textsubscript{A} receptor antagonism, coupled with evidence of increased ET-1 production, is indicative of enhanced ET-1 vasoconstrictor tone in vivo.\textsuperscript{18}

**Perspectives**

In conclusion, the results of this study demonstrate significant differences in FBF responses to exogenous ET-1 administration and ET-1 receptor antagonism between healthy, sedentary younger and older adult men. Age-related increases in ET-1 production and vasoconstrictor tone may contribute to the increased cardiovascular risk and morbidity in older men. Importantly, this is not an irreversible consequence of advancing age. Regular aerobic exercise is an effective lifestyle intervention strategy for reducing ET-1 system activity in previously sedentary older men.

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**Disclosures**

None.

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