Enhanced endothelin-1-mediated leg vascular tone in healthy older subjects

Dick H. J. Thijssen,1 Gerard A. Rongen,2,3 Arie van Dijk,3 Paul Smits,2,3 and Maria T. E. Hopman1

1Department of Physiology, Institute of Fundamental and Clinical Movement Sciences, Departments of 2Pharmacology and Toxicology, 3Internal Medicine, and 4Cardiology, Radboud University Nijmegen Medical Centre, The Netherlands

Submitted 2 April 2007; accepted in final form 5 June 2007

OBJECTIVES: to assess the augmented contribution of an ET-1-mediated vasoconstriction. To address this hypothesis we examined the vasodilator response to combined ET_A- and ET_B-receptor antagonism in sedentary older men as well as in younger controls.

Aging is often associated with physical inactivity (40). Parallel to this, exercise training in older men improves vascular function (8), reduces plasma levels of ET-1 (27), and decreases vascular tone (6). To further explore the causal role of inactivity in age-related changes of the ET pathway, we repeated the experiments in the sedentary older men after 8 wk of cycling training. We hypothesize that cycling training in sedentary older men will increase leg baseline blood flow and partly decrease the ET-1-mediated vascular tone.

METHODS

Subjects

Eight healthy sedentary older men (67–76 yr; Table 1), who were classified as sedentary (no regular exercise), and eight healthy control subjects (7 men and 1 woman, 19–50 yr; Table 1), participated in the study. The female subject was examined in the follicular phase. Sedentary older participants were included if they participated in <1 h exercise/wk for at least the past year or longer. Subjects never smoked or stopped smoking at least 15 yr ago. All subjects were screened prior to participation in the morning after an overnight fast. Subjects were included when they fulfilled the inclusion criteria: normotensive (blood pressure ≤ 140/90 mmHg, auscultatory measurement after at least 5 min seated rest), no hypercholesterolemia (total cholesterol < 6.0 mM), and free of overt cardiovascular disease as assessed by medical history, physical examination, ankle-brachial pressure index, and ECG at rest. None of the subjects used medication known to interfere with the cardiovascular system (1 subject 400 mg beclometason daily, 1 subject 3,000 mg masalazine daily). The study was approved by the hospital ethics committee. All subjects gave their written informed consent before participation.

Experimental Design

The study was designed in two separate parts. First, control subjects and older men were studied to quantify the vasodilator response to antagonizing ET receptors in the leg. Second, at least 2 wk thereafter, the older men started with an 8-wk cycling training program to examine whether the contribution of ET to leg vascular tone is reversible by training. For this purpose, exactly the same experimental protocol was repeated 4–5 days after the final training session.

Protocol

ET-receptor inhibition. Experiments for both control subjects and the older men started at 8:30 AM after a 12-h overnight fast. All subjects refrained from caffeine-containing food and beverages, alcohol, and vitamin C for at least 18 h and did not perform any strenuous activities at least 48 h before testing. All tests were performed in a constant environmen...
Table 1. Physical characteristics and the incremental maximal test of controls and older men before and after 8 wk of exercise training

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Controls (n = 8)</th>
<th>Older Men (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>34±12†</td>
<td>70±3</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>71±7†</td>
<td>76±7</td>
</tr>
<tr>
<td>Body height, cm</td>
<td>177±10</td>
<td>173±8</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>22.7±2.3†</td>
<td>25.4±2.8</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>73±5</td>
<td>77±6</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>123±9</td>
<td>129±9</td>
</tr>
<tr>
<td>Cholesterol, mM</td>
<td>4.4±1.2</td>
<td>4.8±0.4</td>
</tr>
<tr>
<td>Triglycerides, mM</td>
<td>1.0±0.6</td>
<td>1.0±0.3</td>
</tr>
<tr>
<td>HDL, mM</td>
<td>1.3±0.1</td>
<td>1.2±0.2</td>
</tr>
<tr>
<td>LDL, mM</td>
<td>2.6±1.0</td>
<td>3.2±0.3</td>
</tr>
<tr>
<td>Endothelin-1, pg/ml</td>
<td>0.91±0.20</td>
<td>0.73±0.14</td>
</tr>
<tr>
<td>Incremental maximum test</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VO₂ max, ml·kg⁻¹·min⁻¹</td>
<td>30.8±4.8</td>
<td>33.3±5.5</td>
</tr>
<tr>
<td>Maximal workload, W</td>
<td>171±32</td>
<td>201±39*</td>
</tr>
<tr>
<td>Maximal lactate, mM</td>
<td>7.7±2.1</td>
<td>7.6±1.2</td>
</tr>
<tr>
<td>Maximal heart rate, beats/min</td>
<td>165±7</td>
<td>166±8</td>
</tr>
<tr>
<td>RQ</td>
<td>1.20±0.13</td>
<td>1.17±0.06</td>
</tr>
<tr>
<td>Workload at RQ 1, W</td>
<td>102±45</td>
<td>135±36*</td>
</tr>
<tr>
<td>VO₂ at RQ 1, ml·kg⁻¹·min⁻¹</td>
<td>21.5±2.3</td>
<td>25.8±1.7*</td>
</tr>
<tr>
<td>Heart rate at RQ 1, beats/min</td>
<td>137±3</td>
<td>136±3</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD. Values from controls for lipid profile represents 7 subjects and endothelin (ET-1) levels represent 6 subjects. Blood pressure values are derived from the screening procedure. BP, blood pressure; VO₂max, maximal oxygen consumption; RQ, respiratory quotient. *Indicates a significant difference between before and after training using a paired Student’s t-test. †Indicates a significant difference between controls and older men using an unpaired Student’s t-test.

quiet, temperature-controlled room (23.5 ± 0.5°C) with the subjects in the supine position.

A modified Seldinger technique was used to introduce an intra-arterial cannula (Angiocath 16 gauge, Becton Dickinson, Sandy, UT) into the right femoral artery at the level of the inguinal ligament under local anesthesia (0.4 ml lidocaine 20 mg/ml). This cannula was used for the intra-arterial administration of ET-receptor antagonists by an automatic syringe infusion pump (Type P4000, Welmed, Hampshire, UK; Ref. 23) and for blood pressure measurement (monitor: Type 78353B, Hewlett-Packard, Houston, TX; transducer: Type PX600, Edwards Lifesciences, Unterschleissheim, Germany). Heart rate was recorded continuously with ECG (lead II). After complete instrumentation and at least 30 min after cannulation of the femoral artery, an arterial blood sample was collected in nonheparinized tubes and stored at −80°C for later analysis. Plasma ET-1 levels were determined using a chemoluminescent immunoassay with sensitivity for ET-1 of <0.16 pg/ml. The intra- and interassay variation coefficient is 2.5 and 10.2%, respectively (QuantiGlo, R&D Systems, Abingdon, UK). Subsequently, a 30-min measurement of baseline leg blood flow was performed, while saline was infused into the femoral artery. Bilateral leg blood flow was measured three to four times per minute, using ECG-triggered venous occlusion strain-gauge plethysmography as previously described (41). Briefly, an occlusion cuff (12-cm width) was placed proximally around the upper leg and was connected to a rapid cuff inflator (Hokanson, Bellevue, WA), which inflated the cuff (ECG triggered) to a pressure of 50 mmHg. This cuff pressure has been shown to be optimal to examine baseline blood flow using venous occlusion plethysmography (14). This pressure was sustained for nine heart beats, after which the cuff was instantaneously deflated (for 10 heart beats). A mercury-in-silastic strain gauge (Hokanson) was placed at midhigh, at least 10 cm above the patella and at least 4 cm below the cuff (41). The lower legs were supported 10 cm above heart level to facilitate venous outflow between the venous occlusion periods.

**Pharmacological agents.** After the baseline measurements, the combined infusion of a selective ETA- and ETB-receptor antagonist (BQ-123 and BQ-788, respectively, Clinalfa, Calbiochem-Novabiochem, Lützelfingen, Switzerland) was started to inhibit the actions of the ET receptors of the right leg. BQ-123 has a high affinity for the ETA receptor (20) and is demonstrated to effectively counteract the vasoconstrictor effect of ET-1 infusion in the human forearm (18). BQ-788 is highly selective for the ETB receptor and antagonizes the vasoactive responses induced by an ETB-selective agonist (21). BQ-123 (10 nmol·min⁻¹·liter leg volume⁻¹) and BQ-788 (1 nmol·min⁻¹·liter leg volume⁻¹) were dissolved in saline, immediately prior to administration. During the whole protocol, infusion rate was kept constant at 0.1 ml·min⁻¹·liter tissue⁻¹. Infusion rate was adjusted to total leg volume, which was determined by anthropometry as described and validated by Jones and Pearson (22). The ET-receptor antagonists were infused for 75 min (13, 43). Previous studies showed that a 60-min coinfusion of BQ-123 and BQ-788 (at, respectively, 10 and 1 nmol·min⁻¹·liter tissue⁻¹ into the brachial artery) resulted in maximal vasodilation in the forearm of healthy subjects without triggering any systemic blood pressure effects (13, 19).

**Physical training program.** The older men trained under supervision for 8 wk (3 sessions/wk) with at least 1 day between subsequent exercise bouts. A cycling ergometer (Lode, Angiolo300, Groningen, The Netherlands) was used for leg cycling exercise. Each session started with a 10-min warm-up at ~55% of the individual heart rate reserve (HRR), followed by 20-min cycling exercise at 65% HRR. Intensity of the exercise bouts was increased throughout the training period to a maximum of 85%HRR. In the first training session the average workload was 157 ± 48 kJ and in the 24th (i.e., last) training session, 234 ± 50 kJ.

**Physical fitness.** In older men, physical fitness was assessed before and after the training by an incremental maximal exercise test on a cycling ergometer (Lode, Angiolo300) using a multistage protocol (workload increased by 10 W/min, starting at 10 W). Subjects were familiar with cycling exercise and were instructed to keep a constant pedaling rate of 60–80 revolutions/min (11). Oxygen consumption was measured continuously by a gas analyzer (Jaeger Benelux, Breda, The Netherlands) and averaged over 30-s intervals. Maximal oxygen consumption was determined as the mean of the last minute of the test. The criteria used to assess the quality of the maximal cycling test included J) heart rate in excess of 90% of age predicted maximum (220–age); 2) respiratory exchange ratio ≥1.10, and J) identification of a plateau (≥150 ml increase in 1 min) in oxygen consumption despite an additional increase in workload, which has been used previously (12). In all tests, at least two of the three criteria were met.

**Data Analysis**

Plhysiological data of both legs were digitalized with a sample frequency of 100 Hz and analyzed offline by a customized computer program (Matlab, Mathworks). Leg blood flow (ml·min⁻¹·dl⁻¹ leg volume⁻¹) was calculated as the slope of the volume change curve over a 4-s interval, starting directly after the inflation-induced cuff artifact (41). Leg vascular resistance was calculated as the mean arterial pressure [mmHg; calculated as diastolic pressure + (1/3-systolic pressure – diastolic pressure)] divided by leg blood flow (ml·min⁻¹·dl⁻¹) and expressed in arbitrary units (AU; mmHg·ml⁻¹·min⁻¹·dl⁻¹). In addition, the ratio between the infused and control (contralateral) leg was calculated to correct for possible systemic changes (32).

Because of the anticipated differences in baseline leg blood flow and vascular resistance between controls and older men, the response to intra-arterial drug infusion was expressed as percentage changes
from baseline. For this purpose, baseline blood flow, vascular resistance, and flow ratio were calculated by averaging registrations over a 10-min interval prior to the infusion of the ET-receptor antagonists. Likewise, blood flow, vascular resistance, and blood flow ratio were analyzed over the last 10 min of the intra-arterial drug infusion, where ET-receptor inhibition reached its maximal effect (36).

**Statistics**

The primary end point of this study is the vasodilator response to ET-receptor inhibition in the leg in controls vs. older men, as well as pre- vs. postcycling training in older men. In a pilot study (n = 8), we found a SD of 13% of the average vasodilator response (between groups) and 12% for within group comparison (before vs. after training). With a mean relevant effect of the ET inhibition of 20% (control vs. older men) or 15% (before vs. after training), we calculated that (α = 0.05), six to eight subjects would be needed to achieve a power of 80%. Kolmogorov-Smirnov tests indicated a normal distribution of data. Results are expressed as means ± SE, unless stated otherwise. To assess differences between controls and older men (unpaired) and between the pre- and posttraining measurement (paired), the effect of ET-receptor antagonism was compared using a *t*-test. To examine possible correlations between body mass index and the contribution of ET-1 to regulate vascular tone, Pearson correlation coefficient was used. Differences were considered to be statistically significant at a two-sided probability value of 0.05.

**RESULTS**

**Baseline Characteristics**

All older men successfully completed the training with a compliance with the training sessions of 100%. Leg volume, resting heart rate, and baseline leg blood flow and vascular resistance were not different between controls and healthy older men (Table 2). Diastolic and systolic blood pressure, ET-1 plasma levels, and cholesterol levels were not different between controls and healthy older men (Table 1). Body weight and body mass index were significantly larger in the older men compared with controls (Table 1).

**ET-Receptor Inhibition in Controls and Older Men**

**Controls.** Inhibition of the ET receptors did not affect mean arterial pressure, heart rate, nor blood flow and vascular resistance in the noninfused leg in the control subjects (Table 2). The ET-receptor inhibition induced a small, but significant change in blood flow (10 ± 4%) and vascular resistance (−10 ± 4%) of the infused leg. As a consequence, the blood flow ratio between both legs increased significantly by 9 ± 4% (Figs. 1 and 2).

**Older men.** During ET-receptor antagonism, heart rate and mean arterial pressure did not change significantly in older men (Table 2). However, blood flow increased (29 ± 9%) and vascular resistance decreased (−23 ± 7%) in the infused leg of the older men (Fig. 2). In the noninfused leg, no significant change in blood flow or vascular resistance was observed (Table 2). Inhibition of the ET receptors induced a significant increase in the blood flow ratio (26 ± 8%: Fig. 2).

**Controls vs. older men.** In aged men, the increase in blood flow and in blood flow ratio in response to ET antagonism was more pronounced than in controls (Table 2, Fig. 2).

**Effects of Cycling Training**

In the group of sedentary older men, maximal workload during the cycling test was significantly increased after training (Table 1). Although not significant, maximal oxygen consumption tended to increase (P = 0.09). Submaximal oxygen consumption and power output at respiratory quotient 1.00 were significantly increased after training in older men. Heart rate was not changed at respiratory quotient 1.00 (Table 1). Systolic and diastolic blood pressure, leg volume, ET-1 plasma levels, and body mass did not change in the older men by training. Resting heart rate and baseline leg vascular resistance decreased significantly, while baseline leg blood flow increased by training (Tables 1 and 2).

After training, ET-receptor inhibition did not change mean arterial pressure, but was accompanied by a significant increase

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**Table 2. Vascular characteristics are presented for controls and older men before start of infusion (baseline) and at the end of the infusion of ET-receptor antagonists (last 10 min, end infusion)**

<table>
<thead>
<tr>
<th></th>
<th>Younger Subjects (n = 8)</th>
<th>Olders Men Pretraining (n = 8)</th>
<th>Olders Men Posttraining (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>End infusion</td>
<td>P</td>
</tr>
<tr>
<td>Leg volume, liter</td>
<td>9.3 ± 0.3</td>
<td>8.8 ± 0.2</td>
<td>0.05</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>57 ± 4</td>
<td>60 ± 4</td>
<td>0.25</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>84 ± 3</td>
<td>103 ± 4*</td>
<td>0.05</td>
</tr>
<tr>
<td>Infused leg</td>
<td>3.6 ± 0.4</td>
<td>3.1 ± 0.3</td>
<td>0.05</td>
</tr>
<tr>
<td>BF, ml·min⁻¹·dl⁻¹</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>0.008</td>
</tr>
<tr>
<td>VR, AU</td>
<td>27 ± 4</td>
<td>36 ± 4</td>
<td>0.02</td>
</tr>
<tr>
<td>VR, %</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>0.009</td>
</tr>
<tr>
<td>Noninfused leg</td>
<td>3.5 ± 0.4</td>
<td>3.1 ± 0.3</td>
<td>0.05</td>
</tr>
<tr>
<td>BF, ml·min⁻¹·dl⁻¹</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>0.008</td>
</tr>
<tr>
<td>VR, AU</td>
<td>28 ± 3</td>
<td>34 ± 3</td>
<td>0.85</td>
</tr>
<tr>
<td>VR, %</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>0.97</td>
</tr>
<tr>
<td>Infused and noninfused</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>0.007</td>
</tr>
<tr>
<td>Flow ratio, %</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
<td>0.007</td>
</tr>
</tbody>
</table>

*Data are presented as means ± SE. Because of technical problems in one control subject, the data of the noninfused leg were not available for analysis. Blood pressure values are derived from the intrafemoral pressure measurements. Values for the noninfused leg and ratio in controls, therefore, represent 7 subjects. The columns with *P* values refer to a paired Student’s *t*-test between “Baseline” and “End-infusion.” HR, heart rate; MAP, mean arterial pressure; BF, blood flow; VR, vascular resistance; AU, arbitrary units. *P ≤ 0.05 from controls (t-test); †P ≤ 0.05 from older men pretraining (t-test).*
in heart rate (Table 2). Blood flow increased (25 ± 8%) and vascular resistance decreased (−16 ± 5%) in the infused leg during the ET-receptor inhibition (Table 2). Blood flow and vascular resistance of the noninfused leg did not significantly change during ET-receptor inhibition (Fig. 2). The blood flow ratio showed a small, but significant, increase after inhibition of the ET receptors (7 ± 3%, Table 2).

**Pretraining vs. posttraining.** The response in heart rate and mean arterial pressure to ET antagonists did not differ between pre- and posttraining measurements. Also the changes in blood flow and vascular resistance in the infused and noninfused leg during ET-receptor inhibition did not change by training (Fig. 2). However, the increase in blood flow ratio during ET-receptor inhibition was significantly lower after training (Fig. 2).

**Correlations**

No correlation is present between the body mass index and the vasodilator response to ET-receptor antagonism in younger ($r^2 = 0.04, P = 0.66$) or older subjects ($r^2 = 0.15, P = 0.39$).

**DISCUSSION**

The present study demonstrates that 1) the relative contribution of ET-1 to vascular tone of the lower limbs is larger in older men compared with younger men, and 2) exercise training in sedentary older men increases basal leg blood flow, which may in part be mediated through the endothelin pathway. Thus the contribution of ET-1 to increased baseline leg vascular tone in older men may, at least in part, be reversible by endurance training.

Previous studies reported an age-related linear increase in leg vascular tone, resulting in a ~25% lower baseline leg blood flow in older compared with adolescent men (6, 7). Although not significant, the difference between controls and older men for blood flow (~15%) and vascular resistance (~30%) in the (non-)infused leg or pooled leg data are similar to previous studies (6, 7). Despite an age difference of 37 yr, the relatively large range in age in the younger group (19–50 yr) may contribute to the lack of difference in leg blood flow between both groups. Intensity of our exercise training is rather moderate compared with others (9, 38, 39), which may explain the nonsignificant change in maximal oxygen consumption. However, cycling training decreased leg baseline vascular tone, increased maximal oxygen consumption in 7 of 8 subjects, increased submaximal oxygen consumption and workload, and increased maximal workload during the cycling test. This indicates the effectiveness of our training program in the present study.

In previous studies, using the perfused forearm model, forearm blood flow increased by 35–60% during infusion of the ET-receptor antagonists BQ-123 and BQ-788 (18, 43). In contrast, we observed only a slight vasodilator effect of ET-1 inhibition (9% increase in blood flow) in the leg of healthy subjects. The difference in response to ET-receptor inhibition between forearm and leg vascular bed suggests that ET-1 has a different physiological effect on the lower limbs than on the upper limbs in healthy subjects. Recently, significantly different responses are reported for human forearm and leg vascular beds to endothelium-dependent (acetylcholine, substance P) and -independent (sodium nitroprusside) stimuli (29). Moreover, infusion of ET-1 in the rat hindquarters skeletal muscle bed results in a significantly lower vasoconstrictor response compared with the mesenteric bed (15). It may be hypothesized that the level of activity explains the differences in contribution of ET-1 to vascular tone between both limbs. The average level of skeletal muscle activity varies markedly between the upper and lower extremities. As bipeds, the legs are far more active during daily life (i.e., locomotion, standing) and during sports activities (i.e., running, cycling) than the human forearm.

We observed in the present study that healthy older men demonstrate a significantly larger vasodilator response to ET-receptor inhibition compared with younger subjects. This indicates that ET-1 may be a major contributor to the elevated baseline leg vascular tone in older men. This finding agrees with two recent studies in animals (2, 10) that reported an enhanced contribution of ET-1 to the increased baseline leg vascular tone with advanced age. However, the observation in the present study represents the first human study that demonstrates the role of ET-1 to leg vascular tone in older men.

Given the inclusion of healthy physically inactive older men, the larger vasoconstrictor response to ET-1 in older men can be explained by 1) a decreased level of physical fitness, and/or 2) the physiological aging process. To extend our knowledge about the age-related change in regulation of the ET pathway, older men performed an 8-wk aerobic cycling training. After

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**Fig. 1.** Relative change in blood flow ratio in younger subjects ($n = 8, 19–50$ yr) and older men ($n = 8, 67–76$ yr), both before and after the training procedure during 75 min of endothelin (ET)-1 receptor inhibition.

**Fig. 2.** The mean (±SE) relative increase in blood flow ratio and vascular resistance (VR) during ET-receptor inhibition in younger subjects ($n = 8, 19–50$ yr) and older men ($n = 8, 67–76$ yr), both before and after the training procedure. *Significant relative increase from baseline. NS, not significant.
training, the decrease in vascular resistance of the infused leg during ET-receptor inhibition in older men was lower than before training, but not significant. Expressing the effect of ET antagonists on vascular tone as flow ratio, to exclude small systemic effects (33), a significantly lower contribution of ET-1 to leg vascular tone is observed in older men after training. However, blood flow in the noninfused leg in the older men after training tended to increase during ET antagonism (Table 2). Although vascular resistance and flow ratio are well preferred over blood flow data (30, 31), this change in blood flow indicates that the response in flow ratio to ET-receptor inhibition after training should be interpreted with caution. Possibly, this has underestimated the vasodilator response in the infused leg after training when expressed as flow ratio. Nevertheless, our observations suggest that exercise training in older men reduces the age-related increase in ET-1-mediated vascular tone, but does not normalize this response after 8 wk of cycling training.

Recently, the regulation of the ET pathway in sedentary and 12-wk trained older rats was examined (10) and demonstrated that advanced age enhances the ET-mediated vasoconstriction, whereas exercise training in older rats does not alter the ET-mediated vasoconstriction. The different finding regarding the effect of exercise training may be explained by the different species used (rats vs. humans). In addition, we examined older men before and after training (longitudinal), whereas Donato et al. (10) studied sedentary and trained older rats (cross-sectional). The latter design is less susceptible to detect small differences.

Our results lead to an interesting question regarding the mechanism behind the adaptations of the ET pathway in the older men. In the present study, ET-1 plasma levels were not different between controls and older men, but also no difference was found between pre- and posttraining. This suggests that plasma ET-1 levels unlikely explain the increased contribution of ET-1 to leg vascular tone with aging. In contrast, some studies demonstrated elevated ET-1 plasma levels with advanced age (3, 28), which decreased after endurance training (27). Because clearance of ET-1 and sensitivity of the receptors for ET-1 is lacking in all studies, the value of ET-1 plasma levels can be argued. In addition, a change in sensitivity (and/or density) of the ET_A and/or ET_B receptors or changes in ET-receptor signaling may explain the age-related upregulation of the ET pathway. Parallel to this, Donato et al. (10) recently reported that the augmented constrictor response to ET-1 in older rat skeletal muscle arterioles is mediated through an enhanced ET_A-receptor signaling pathway. In contrast, Asai et al. (2) reported an impairment of the endothelial ET_B receptors to cause the enhanced ET-1-mediated vasoconstriction in older monkeys. Future studies in humans should further elucidate the underlying mechanism of the age-related change in the ET pathway.

Apart from the contribution of ET-1 to the elevated vascular tone in older men, other factors are known to contribute as well. For example, a reduced nitric oxide-mediated vasodilation (37) and elevated sympathetic α-adrenergic vasoconstriction (9) partially contribute to the age-related increased vascular tone.

Although younger and older subjects differed regarding body mass index, this unlikely explains the increased vasodilator response to ET-receptor inhibition in the older men. A previous study demonstrated that lean, overweight, and obese subjects without hypertension demonstrate similar vasodilator responses to ET-receptor inhibition (4). Moreover, we found no correlation between body mass index and the vasodilator response to ET-receptor inhibition. In addition, the decrease in blood flow ratio after training in the older men was not accompanied by a decrease in BMI.

Limitations

In this study we used local infusions of drugs into the femoral artery. Since the leg represents an ~8-fold larger vascular bed compared with the forearm, higher dosages were necessary, and therefore systemic spillover effects may have occurred. However, we observed no change in mean arterial pressure or heart rate during ET-receptor inhibition, except for an increase in heart rate in the posttraining sessions of the older men. This latter change in heart rate may have been mediated by baroreceptor unloading due to a more generalized vasodilator response to ET-receptor inhibition. Accordingly, although not significant, blood flow in the noninfused leg tended to increase. To exclude the influence of systemic changes in blood pressure and heart rate on the ultimate results as much as possible (33), we expressed the effects of ET-1 on vascular tone as vascular resistance and blood flow ratio. Nevertheless, using the flow ratio only, one may underestimate the actual contribution of ET-1 to leg vascular tone after training in older men.

Increasing evidence supports a pathophysiological role for ET-1 in the modulation of vascular tone in cardiovascular disease (26, 34). Based on the constrictor action of ET-1 in older men, an enhanced ET-1 signal transduction mechanism may contribute to the elevations in skeletal muscle vascular resistance and, consequently, systemic vascular resistance. These changes in the ET pathway may partially explain the underlying mechanism for the predisposition of older men to cardiovascular pathology such as hypertension. Upregulation of the ET pathway in the older men may also result in media hypertrophy (34). This vascular remodeling process could further contribute to the development of hypertension. The physiological effect of exercise training in older men on the ET pathway potentially has beneficial consequences for vascular structure and function, but also for reducing the cardiovascular risk. However, future research is necessary to elucidate the clinical relevance of our observed physiological effect.

In conclusion, the enhanced activity of the endothelin pathway in older men compared with younger controls, suggests that ET-1 is a key mediator in the increased leg vascular tone with advancing age. These adaptations may be the result of a change in ET-receptor sensitivity or signaling, rather than changes in plasma levels of ET-1. In addition, 8 wk of exercise training decreases baseline leg vascular tone. Results of the present study suggest that this could in part be explained by a reduced contribution of the endothelin pathway in older men.

ACKNOWLEDGMENTS

We thank Rebecca Paulus and Jos Evers for their enthusiastic assistance during the training and Dr. Frans Boomsma (Erasmus MC, Rotterdam, The Netherlands) for the measurement of ET-1 plasma levels.
REFERENCES


