Endothelin-1 vasoconstriction and the age-related decline in endothelium-dependent vasodilatation in men

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ABSTRACT

ET (endothelin)-1, a potent vasoconstrictor peptide released by the endothelium, plays an important role in vasomotor regulation and has been linked to diminished endothelial vasodilator capacity in several pathologies associated with human aging, including hypertension, Type 2 diabetes and coronary artery disease. However, it is currently unknown whether the decline in endothelial vasodilatation with advancing age is due to elevated ET-1 vasoconstrictor activity. Accordingly, we tested the hypothesis that the age-related impairment in ACh (acetylcholine)-mediated endothelium-dependent vasodilatation is due, at least in part, to increased ET-1-mediated vasoconstrictor tone. FBF (forearm blood flow) responses to ACh, SNP (sodium nitroprusside) and BQ-123 (ETA receptor blocker) were determined in 14 young (age, 25 ± 1 years) and 14 older (age, 61 ± 2 years) healthy non-obese men. Additionally, FBF responses to ACh were determined in the presence of ET A blockade. Vasodilatation to ACh was lower (approx. 25 %; P < 0.05) in the older men (from 4.9 ± 0.2 to 13.9 ± 0.9 ml · 100 ml⁻¹ of tissue · min⁻¹) compared with the young men (4.6 ± 0.3 to 17.2 ± 1.0 ml · 100 ml⁻¹ of tissue · min⁻¹). There were no differences in FBF responses to SNP between the young (4.8 ± 0.3 to 18.5 ± 0.3 ml · 100 ml⁻¹ of tissue · min⁻¹) and older (5.1 ± 0.3 to 17.3 ± 0.8 ml · 100 ml⁻¹ of tissue · min⁻¹) men. In the young men, resting FBF was not significantly altered by BQ-123, whereas, in the older men, FBF increased approx. 25 % in response to BQ-123 infusion (P < 0.05). Co-infusion of ACh with BQ-123 resulted in an approx. 20 % increase in the ACh-induced vasodilatation in older men compared with saline. In contrast, FBF responses to ACh were not significantly altered by ETA blockade in the young men. In conclusion, these results demonstrate that ET-1 vasoconstrictor activity contributes, at least in part, to diminished endothelium-dependent vasodilatation in older men.

INTRODUCTION

The incidence and prevalence of cardiovascular diseases and their clinical consequences increase steeply with advancing age in humans [1]. The cardiovascular complications associated with aging are by and large the result of endothelial dysfunction, particularly vasomotor dysregulation [2–4]. Results from our laboratory [5], and others [6], have shown that aging, independent of other risk factors, is associated with impaired ACh (acetylcholine)-mediated endothelium-dependent vasodilatation. Although much of the research addressing

Key words: aging, blood flow, endothelial function, endothelin, forearm blood flow, vasodilatation.

Abbreviations: ACh, acetylcholine; BMI, body mass index; BP, blood pressure; ET, endothelin; FBF, forearm blood flow; LDL, low-density lipoprotein; SNP, sodium nitroprusside.

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the decline in endothelium-dependent vasodilation with advancing age has focused on the role of NO [7], oxidative stress [8] and inflammatory factors [9], the exact mechanisms underlying the age-related impairment in endothelium-dependent vasodilatation are not fully understood.

ET (endothelin)-1 is the most potent vasoconstrictor peptide released by the endothelium, and increased ET-1 system activity has been linked to a number of cardiovascular pathologies associated with aging, including hypertension [10,11] and coronary artery disease [12,13]. We have reported previously that ET-1-mediated vasoconstrictor tone is greater in older compared with young men and that most of the ET-1 vasoconstrictor activity is mediated by the ET_A receptor [14]. It has been suggested that increased ET-1 system activity may play a key role in endothelial vasodilator dysfunction [15]. For example, Cardillo et al. [16] have shown that blockade of ET-1 receptors improves endothelium-dependent vasodilatation in hypertensive patients. Currently, it is unknown whether the age-related decline in ACh-mediated endothelium-dependent vasodilatation is due to elevated ET-1-mediated vasoconstrictor tone.

Accordingly, the aim of the present study was to determine whether ET-1 vasoconstrictor tone contributes to the age-related impairment in endothelium-dependent vasodilatation. To address this aim, we examined the effects of ET_A receptor blockade on ACh-mediated endothelium-dependent vasodilatation in young and older adult men. We hypothesized that the age-related impairment in ACh-mediated endothelium-dependent vasodilatation is due, at least in part, to increased ET-1-mediated vasoconstrictor tone.

MATERIALS AND METHODS

Subjects
A total of 28 healthy sedentary men participated in the study: 14 young (range, 21–34 years) and 14 older (range, 55–68 years). Subjects were excluded from the study if they presented a history or evidence of hepatic, renal or haematologic disease; peripheral vascular disease; stroke; diabetes (fasting plasma glucose >7.0 mmol/l) [17]; dyslipidaemia [total cholesterol ≥6.2 mmol/l, triacylglycerols (triglycerides) ≥3.5 mmol/l] [18] and hypertension (arterial BP (blood pressure) ≥140/90 mmHg) [19]. The older men were further evaluated for clinical evidence of coronary artery disease with electrocardiograms and BP 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 at least 1 year before the start of the study. Prior to 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.

Measurements

Body composition
Body mass was measured to the nearest 0.1 kg using a medical beam balance (Detecto). Percentage body fat was determined by dual energy X-ray absorptiometry (Lunar Radiation). BMI (body mass index) was calculated as weight (in kg) divided by height (in m) squared. Minimal waist circumference was measured according to published guidelines [20].

Metabolic measurements
Fasting plasma lipid and lipoprotein, glucose and insulin concentrations were determined using standard techniques by the clinical laboratory affiliated with the Clinical and Translational Research Center at the University of Colorado at Boulder.

Intra-arterial infusion protocol
All studies were performed between 07:00 and 10:00 hours after a 12-h 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 non-dominant arm under local anaesthesia (1% lidocaine). Heart rate and arterial BP were measured continuously throughout the infusion protocol. FBF (forearm blood flow) at rest and in response to each pharmacological agent was measured using strain-gauge venous occlusion plethysmography (D. E. Hokanson), as described previously by our laboratory [5]. Baseline FBF was measured for 5 min and 5 min prior to each drug infusion thereafter. Following the measurement of resting blood flow, FBF was assessed in response to infusions of ACh (IOLAB Pharmaceuticals) at 4.0, 8.0 and 16.0 μg·100 ml⁻¹ of tissue·min⁻¹ and SNP (sodium nitroprusside, Nitropress; Abbott Laboratories) at 1.0, 2.0 and 4.0 μg·100 ml⁻¹ of tissue·min⁻¹. Each dose of ACh and SNP was infused for approx. 5 min, and sufficient time (approx. 20 min) was allowed for FBF to return to resting levels between each vasoactive agent. To avoid an order effect, the sequence of drug administration was randomized. After the initial infusion of ACh and SNP, BQ-123 (Clinalfa), a selective ET_A receptor antagonist, was infused at a rate of 100 nmol/min for 60 min and FBF was measured every 10 min as described previously [14]. The selected dose of BQ-123 has been shown to completely inhibit the ET_A-mediated vasoconstrictor effect of ET-1 in the human forearm of healthy adults [21,22]. After 60 min, infusion of BQ-123 was continued at the same dose, and FBF was reassessed during co-administration of ACh as performed earlier.
Table 1  Selected subject characteristics

Values are means ± S.E.M. *P < 0.05 compared with young. HDL, high-density lipoprotein.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Young men (n = 14)</th>
<th>Older men (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (year)</td>
<td>25 ± 1</td>
<td>61 ± 2*</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>77.3 ± 2.4</td>
<td>81.1 ± 2.3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.7 ± 0.6</td>
<td>25.6 ± 0.5*</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>16.4 ± 1.2</td>
<td>25.7 ± 1.1*</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>84.0 ± 1.8</td>
<td>93.9 ± 1.7*</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>114 ± 3</td>
<td>125 ± 2.9*</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>66 ± 2</td>
<td>78 ± 2*</td>
</tr>
<tr>
<td>FBF (ml · 100 ml⁻¹ of tissue · min⁻¹)</td>
<td>3.0 ± 0.3</td>
<td>3.5 ± 0.2</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>4.1 ± 0.2</td>
<td>5.3 ± 0.2*</td>
</tr>
<tr>
<td>LDL-cholesterol (mmol/l)</td>
<td>2.4 ± 0.2</td>
<td>3.4 ± 0.2*</td>
</tr>
<tr>
<td>HDL-cholesterol (mmol/l)</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>Triacylglycerols (mmol/l)</td>
<td>1.1 ± 0.2</td>
<td>1.4 ± 0.1</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.7 ± 0.1</td>
<td>5.3 ± 0.1*</td>
</tr>
<tr>
<td>Insulin (pmol/l)</td>
<td>38.5 ± 3.8</td>
<td>55.6 ± 11.5</td>
</tr>
</tbody>
</table>

Figure 1  FBF responses to ACh (A) and SNP (B) in young and older men

Values are means ± S.E.M. *P < 0.05 compared with young.

Statistical analysis

Differences in subject baseline characteristics were determined by one-way ANOVA. Group differences in FBF responses to ACh, SNP and BQ-123 were determined by repeated-measures ANOVA. All results are expressed as means ± S.E.M. Statistical significance was set a priori at P < 0.05.

RESULTS

Selected subject characteristics are presented in Table 1. The mean age difference between the young and older men was 36 years. Although none of the subjects were obese, BMI, percentage body fat and waist circumference were significantly higher (P < 0.05) in the older compared with young men. The older men also demonstrated higher (P < 0.05) resting systolic and diastolic BP as well as higher fasting plasma total cholesterol, LDL (low-density lipoprotein)-cholesterol and glucose concentrations; however, all values were within clinically normal limits. FBF in the non-infused arm and mean arterial BP remained constant throughout the infusion protocols in all of the subjects (results not shown).

FBF responses to ACh and SNP

The FBF responses to ACh between the young and older men are shown (Figure 1). Infusion of ACh resulted in an increase in FBF compared with baseline in both groups; however, as expected, the increase in FBF in response to ACh is approx. 25% lower (P < 0.05).
in the older (from 4.9 ± 0.2 to 13.9 ± 0.9 ml·100 ml⁻¹ of tissue·min⁻¹) compared with young (from 4.6 ± 0.3 to 17.2 ± 1.0 ml·100 ml⁻¹ of tissue·min⁻¹) men. There were no significant differences between the groups in the FBF responses to SNP (Figure 1).

**FBF responses to BQ-123 and ACh+BQ-123**

The FBF responses to selective ETA receptor blockade with BQ-123 were markedly different (P < 0.05) between the groups (Figure 2). In the young men, resting FBF was not significantly altered by BQ-123, whereas in the older men, FBF increased approx. 25% in response to BQ-123 infusion. The co-infusion of BQ-123 with ACh did not significantly increase ACh-mediated vasodilatation in the young subjects. However, co-infusion of BQ-123 with ACh resulted in an approx. 20% increase in ACh-induced vasodilatation in older men compared with saline (Figure 3). Of note, the co-infusion of BQ-123 abolished the age-related difference in ACh-mediated vasodilatation between the groups. Indeed, the FBF response to ACh in the presence of BQ-123 was not different between the young (from 4.4 ± 0.2 to 18.8 ± 1.0 ml·100 ml⁻¹ of tissue·min⁻¹) and the older (from 4.5 ± 0.2 to 17.6 ± 0.8 ml·100 ml⁻¹ of tissue·min⁻¹) men.

**DISCUSSION**

The primary new finding of the present study is that ET-1-mediated vasoconstriction contributes to the age-related decline in endothelium-dependent vasodilatation in healthy men. Indeed, ACh-mediated endothelium-dependent vasodilatation in older men was restored to levels similar to that of young men with co-infusion of the ETA receptor antagonist BQ-123.

Consistent with previous reports from our laboratory [5,23] and others [6,24], ACh-mediated endothelium-dependent vasodilatation was markedly blunted (approx. 25%) in the older compared with young men. The older men also demonstrated, in contrast with their young counterparts, a significant vasodilator response to the selective ETA receptor antagonist BQ-123, confirming
our previous finding, in a comparable population of adult men, that ETA receptor-mediated ET-1 vasoconstrictor tone increases with age [14]. The results of the present study extend these findings by demonstrating that blockade of the ETA receptor improves ACh-mediated endothelium-dependent vasodilatation in older men. This indicates that the age-related reduction in vasodilator function is due, at least in part, to elevated ETA receptor-mediated ET-1 vasoconstriction. To our knowledge, this is the first study to determine the influence of ET-1-mediated vasoconstriction on the decline in endothelial vasodilatation with advancing age.

The mechanisms responsible for the improvement in endothelium-dependent vasodilatation following blockade of the ETA receptor in older men are not clear. However, there are several potential mechanisms that may be involved. First, endothelial regulation of vasomotor tone is the result of an intricate balance between endothelium-derived vasodilating factors, such as NO and endothelium-derived hyperpolarizing factor and vasoconstricting factors, such as ET-1 [25]. It is possible that ETA receptor antagonism inhibited the vasoconstrictor effect of ET-1, thereby allowing endothelium-derived relaxing factors to act largely unopposed and dilate the vessel appropriately in response to ACh stimulation. Second, it is thought that the age-related impairment in endothelium-dependent vasodilatation is due to a decline in endothelium-derived NO bioavailability [7,26]. Blockade of the ETA receptor may improve endothelium-dependent vasodilatation by directly, or indirectly, increasing the bioavailability of NO. This idea is supported by data from Mather et al. [27] showing that basal NO production was augmented during ETA antagonism in adults who exhibited enhanced ET-1-mediated vasoconstrictor tone. This augmentation in NO may result from increased ET-1 binding to ETA receptors on the endothelium and the subsequent production of NO [28]. Alternatively, ETA receptor blockade may indirectly improve NO bioavailability via reductions in local concentrations of reactive oxygen species. ET-1 binding to the ETA receptor elicits activation of NADPH oxidase, which promotes the production of reactive oxygen species such as superoxide anion, a highly reactive free radical that inactivates NO [29–31]. Thus several mechanisms may underlie ET-1’s influence on endothelial vasodilator function; future studies are needed to better clarify this complex interaction.

We have shown previously that regular aerobic exercise confers improvement in ACh-mediated endothelium-dependent vasodilatation in older adults [5]. The precise mechanisms underlying this improvement have not been elucidated, although enhanced NO bioavailability is thought to play a key role [32]. The current study provides insight into an additional potential mechanism by which aerobic exercise improves endothelial vasodilator function in middle-aged and older men. Given our previous finding that ET-1 vasoconstrictor tone in middle-aged and older men is reduced by regular aerobic exercise [14], it is not unreasonable to suggest that the exercise-induced decrease in ET-1-mediated vasoconstrictor tone may contribute to the concomitant increase in endothelium-dependent vasodilatation. Furthermore, it is tempting to speculate that the greater NO vasodilator action associated with regular aerobic exercise may be secondary to reductions in ET-1 system activity. This notion is supported by data demonstrating that ETA receptor blockade augments NO bioavailability [27,33,34]. Thus, the vasomotor benefits conferred by regular aerobic exercise may involve a reduction in ET-1 vasoconstrictor tone that mediates improvements in NO-mediated endothelium-dependent vasodilatation in older adults.

Although within clinical normal ranges, there were some differences in haemodynamic and metabolic variables between the young and older men in the present study. Considering there are emerging findings that both BP and cholesterol (total cholesterol and LDL-cholesterol) levels in the high normal range are associated with increased cardiovascular risk [35–38] and potentially endothelial dysfunction [39,40], we performed subgroup analyses matching for these variables to ensure that our results were not biased by these group differences. In a subgroup of men matched for systolic and diastolic BP (n = 9/group), a significant age-related difference in the vascular response to BQ-123 was observed, similar to that of the overall study population. Similarly, in a subgroup of men matched for total cholesterol and LDL-cholesterol (n = 8/group), the vascular response to BQ-123 was significantly different between the young and older men and almost identical to the responses observed in the overall study population. Collectively, these data provide further support for the main effect of age on endogenous ET-1-mediated vasoconstrictor tone.

It is important to emphasize that the present study involved only men. We have reported recently that postmenopausal middle-aged and older women are under less ETA receptor-mediated ET-1 vasoconstrictor tone than age-matched men [41]. Despite the lower ET-1-mediated vasoconstrictor tone, postmenopausal women exhibit impaired ACh-mediated endothelium-dependent vasodilatation [7]. Thus it is likely that additional mechanisms besides enhanced ET-1 system activity contribute to impaired endothelium-dependent vasodilatation in middle-aged and older women. Consequently, the present results should be viewed within the context of the study population.

In conclusion, the seminal finding of the present study is that blockade of the ETA receptor improved endothelium-dependent vasodilatation in older adult men. ET-1-mediated vasoconstrictor tone appears to be a key mechanism underlying the age-related impairment in endothelial vasodilator function observed in this population.
AUTHOR CONTRIBUTION

Christian Westby performed FBF measurements, was involved in data collection, data analysis, data interpretation and manuscript preparation, and contributed intellectually to the overall conduct of the study. Brian Weil was involved in data collection, data analysis, data interpretation and manuscript preparation, and contributed intellectually to the overall conduct of the study. Jared Greiner was involved in data collection, contributed intellectually to the overall conduct of the study. Christopher DeSouza directed the study and contributed intellectually to all aspects of the study, particularly the writing and editing of the manuscript prior to submission.

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