Effects of stimulation of nitric oxide synthesis on large artery stiffness in patients with peripheral arterial disease

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Abstract

The role of endothelium-derived nitric oxide (NO) in modulation of large artery stiffness in patients with peripheral arterial disease (PAD) is unexplored. The aim of this study was to evaluate, using pulse wave analysis (PWA), changes in aortic and systemic arterial stiffness following administration of nitroglycerin and β2-agonist salbutamol in PAD patients (n = 24) and in healthy controls (n = 24). Changes in estimated aortic pulse wave velocity (T α) and in augmentation index (AIx), following administration of nitroglycerin and salbutamol, were assessed using PWA. Salbutamol-induced changes in T α and in AIx were significantly reduced in PAD patients (P < 0.001 and <0.001, respectively), while nitroglycerin-produced changes were not different (P = 0.25 and 0.35, respectively). Changes in T α after salbutamol administration were independent of changes in mean arterial pressure (MAP) (r = −0.21, P = 0.16). This study shows that stimulation of NO synthesis fails to modify stiffness of the large arteries in PAD patients and changes in aortic stiffness are independent of changes in MAP. Our data support the utility of PWA as a non-invasive method for assessment of NO-mediated vascular changes.

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I. Introduction

An intact endothelium releases several biologically active substances, including nitric oxide (NO), for regulation of vascular tone, homeostasis and thrombosis, as well as for prevention of various vascular pathologies, especially, atherosclerosis [1,2]. Endothelial dysfunction has been shown in several clinical conditions including hypercholesterolemia [2,3], peripheral arterial disease (PAD) [4] and coronary artery disease (CAD) [5], but also in smokers [2]. Although only limited prospective studies have been performed to estimate the relationship between endothelial function and cardiovascular risk, there is clear evidence that endothelial function predicts outcome in subjects with atherosclerosis [6], e.g. patients with PAD [7].

Besides several invasive and non-invasive methods for measuring endothelial function, pulse wave analysis (PWA) is a novel non-invasive, simple and reproducible method for assessing endothelial function in large-scale studies [3,5]. Endothelium-dependent vasodilation (EDV) due to stimulation of NO synthesis by the β2-agonist salbutamol (Salb) is a marker of endothelial function in case PWA is used. There are only preliminary results of endothelial function obtained by this method [3,5]. Moreover, patients with PAD have presumably endothelial dysfunction and are, therefore, appropriate candidates for basic mechanistic studies using PWA as a novel method for evaluating vascular changes.

PWA is also a validated and reproducible method to measure aortic and systemic arterial stiffness [8–10]. Increased
arterial stiffness is an important independent determinant of cardiovascular risk [11] and is also involved in atherogenesis [12]. Whether arterial stiffening represents a marker of atheroma or endothelial dysfunction is still unclear as is the role of assessment of arterial stiffness in detecting preclinical atherosclerosis.

We hypothesized that patients with PAD have increased aortic and systemic arterial stiffness and blunted response to β2-agonist stimulation in the aorta and large arteries as well as in the peripheral arteries. Moreover, data about NO and regulation of large artery stiffness in humans have been contradictory [8,13].

The aim of this study was to evaluate changes in aortic and systemic arterial stiffness following nitroglycerin (NTG) and Salb administration in patients with PAD. Furthermore, using a model of dysfunctional endothelium, we attempted to elucidate if impaired endothelial function is correlated with increased arterial stiffness and to test the hypothesis that stimulation of NO synthesis affects estimated aortic pulse wave velocity (PWV) and provides a measure of systemic arterial stiffness. The AIx was defined as the difference between the second and first systolic peaks of the central arterial waveform, expressed as a percentage of the total pulse pressure.

2. Methods

2.1. Subjects

The study group consisted of 24 patients with PAD having stages II–IV as defined by Fontaine: stage II—intermittent claudication, stage III—leg pain at rest and stage IV—tissue loss due to ischemic ulcer or gangrene. All patients were recruited from the Clinic of Cardiovascular and Thoracic Surgery, University Clinics of Tartu, Estonia. The subjects were all male with angiographically proven PAD, i.e. with stenosis or occlusion of the arteries of the lower extremities. The ankle brachial pressure index (ABPI) was less than 0.81 (range 0.2–0.8) in patients with PAD. The patients’ exclusion criteria were the following: myocardial infarction, coronary revascularization or cerebrovascular events during the last 6 months, earlier revascularization procedures at the wrist employing a high fidelity micromanometer (SCOR 301B; Millar Instruments® , TX, USA). Using a validated transfer function [15], the corresponding ascending aortic waveform was generated, from which central hemodynamics, augmentation index (Alx) and T\textsubscript{r} were calculated.

2.2. Study protocol

The subjects were studied and plasma samples were collected between 8:00 and 10:00 a.m. after an overnight fast and abstinence from any medications, tobacco, alcohol and tea or coffee. After 15 min of rest in a quiet, temperature-controlled room, ABPI and blood pressure were measured and PWA was performed with provocative pharmacological tests. All hemodynamic and PWA recordings were made in duplicate for each time point. Thereafter, venous blood samples were drawn from the antecubital fossa for the measurement of glucose, total cholesterol, LDL cholesterol, HDL cholesterol, triglycerides and creatinine. Height and weight were recorded and body mass index (BMI) was calculated.

2.3. Measurement of ABPI

The ABPI was measured using Mini Dopplex D900 (Huntleigh Healthcare Ltd.®, Cardiff, UK). The ABPI value was calculated as an average of two resting measurements.

2.4. Hemodynamics, arterial stiffness and endothelial function

Peripheral blood pressure was measured in the dominant arm using a validated oscillometric technique (OMRON M4-1, Omron Healthcare Europe BV®, Hoofddorp, The Netherlands). MAP was calculated from the integration of the radial pressure waveform using the Sphygmocor software (SCOR Pxs, 7.0; AlCor Medical®, Sydney, Australia).

Arterial stiffness [8,9] and endothelial function [3] were assessed by PWA using a Sphygmocor apparatus as described previously. The peripheral pressure waveforms were recorded from the radial artery of the dominant arm at the wrist employing a high fidelity micromanometer (SPT-301B; Millar Instruments®, TX, USA). Using a validated transfer function [15], the corresponding ascending aortic waveforms were then generated, from which central hemodynamics, augmentation index (Alx) and T\textsubscript{r} were calculated. The Alx was defined as the difference between the second and first systolic peaks of the central arterial waveform, expressed as the percentage of central pulse pressure (CPP). The Alx, a predominant determinant of wave reflections, depends also on several factors, including ventricular ejection, HR, MAP and aortic pulse wave velocity (PWV) and provides a measure of systemic arterial stiffness. The T\textsubscript{r} represents the composite travel time of the pulse wave to the periphery, the main reflectance site (aortic bifurcation) and its return to the ascending aorta, thus providing aortic stiffness [8,16].
Table 1
Baseline characteristics

<table>
<thead>
<tr>
<th>Variables</th>
<th>PAD patients (n=24)</th>
<th>Controls (n=24)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>57.5 ± 8.7</td>
<td>57.2 ± 6.4</td>
<td>0.88</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>72.6 ± 13</td>
<td>77.2 ± 8.7</td>
<td>0.16</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>174.6 ± 6.1</td>
<td>173.4 ± 6.1</td>
<td>0.65</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.3 ± 3.8</td>
<td>25.1 ± 2.7</td>
<td>0.26</td>
</tr>
<tr>
<td>ABI</td>
<td>0.5 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PSBP (mmHg)</td>
<td>125.7 ± 11.4</td>
<td>122.1 ± 11.7</td>
<td>0.1</td>
</tr>
<tr>
<td>PDBP (mmHg)</td>
<td>75.8 ± 7.1</td>
<td>74.3 ± 6.1</td>
<td>0.45</td>
</tr>
<tr>
<td>Peripherale PP (mmHg)</td>
<td>52.7 ± 8.7</td>
<td>47.9 ± 9.4</td>
<td>0.12</td>
</tr>
<tr>
<td>Mean arterial pressure (mmHg)</td>
<td>94.3 ± 8.8</td>
<td>91.5 ± 7.9</td>
<td>0.26</td>
</tr>
<tr>
<td>CSBP (mmHg)</td>
<td>120.1 ± 12.6</td>
<td>113.8 ± 11.7</td>
<td>0.08</td>
</tr>
<tr>
<td>CDPP (mmHg)</td>
<td>76.7 ± 7.3</td>
<td>75.5 ± 6.3</td>
<td>0.39</td>
</tr>
<tr>
<td>Central PP (mmHg)</td>
<td>45.3 ± 8.2</td>
<td>38.8 ± 9.2</td>
<td>0.07</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>64.5 ± 7.8</td>
<td>60 ± 7.8</td>
<td>0.051</td>
</tr>
<tr>
<td>Augmentation index (%)</td>
<td>34.4 ± 7.9</td>
<td>27.3 ± 6.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AIx@75 (%)</td>
<td>29.3 ± 8.2</td>
<td>20 ± 6.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Tₘ (ms)</td>
<td>135.5 ± 14.1</td>
<td>147.5 ± 8.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Height/Tₘ (cm/ms)</td>
<td>1.3 ± 0.12</td>
<td>1.19 ± 0.08</td>
<td>0.001</td>
</tr>
<tr>
<td>Total-cholesterol (mmol/L)</td>
<td>5.6 ± 1</td>
<td>5.3 ± 1.3</td>
<td>0.43</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.3 ± 0.3</td>
<td>1.4 ± 0.4</td>
<td>0.13</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.9 ± 1</td>
<td>3.7 ± 0.7</td>
<td>0.52</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.6 ± 0.6</td>
<td>1.3 ± 0.5</td>
<td>0.07</td>
</tr>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.1 ± 0.5</td>
<td>5.3 ± 0.4</td>
<td>0.16</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>81.9 ± 17.9</td>
<td>86.5 ± 12.0</td>
<td>0.31</td>
</tr>
<tr>
<td>Medication, n (%)</td>
<td>24(100)</td>
<td>0(0)</td>
<td>–</td>
</tr>
<tr>
<td>Pentoxifylline</td>
<td>24(100)</td>
<td>0(0)</td>
<td>–</td>
</tr>
<tr>
<td>Aspirin</td>
<td>20(83.3)</td>
<td>0(0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Calcium channel blockers</td>
<td>6(25)</td>
<td>0(0)</td>
<td>0.008</td>
</tr>
<tr>
<td>Current smoking, n (%)</td>
<td>23 (95.8)</td>
<td>3(12.5)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. unless otherwise indicated. ABI: ankle brachial pressure index; PSBP: peripheral systolic blood pressure; PDBP: peripheral diastolic blood pressure; PP: pulse pressure; CSBP: central systolic blood pressure; CDPP: central diastolic blood pressure.

After the baseline measurements of hemodynamics, AIx and Tₘ, a 500 μg tablet of NTG (Nycodem®, Roskilde, Denmark) was placed under the tongue for 3 min. Pulse wave recordings were performed 3, 5, 10, 15 and 20 min after NTG administration. Next, after restoration of hemodynamics, 400 μg of the β₂-agonist Salb (Glaxo Wellcome Production®, Evreux, France) was given by inhalation and recordings were made 5, 10, 15 and 20 min after administration. A maximum change in AIx following Salb administration was defined as EDV, while a maximum change in AIx after NTG was interpreted as a marker of endothelium-independent vasodilation.

2.5. Data analysis

The response to NTG and Salb was defined as a maximum change in each parameter after drug administration. The data were expressed as mean ± S.D. or n (%) and the relevant parameters were tested for normality using the Kolmogorov–Smirnov test. The comparisons between the patients and the controls were analyzed using unpaired two-tailed Student’s t-test. The effects of NTG and Salb on hemodynamics were investigated by paired t-test. The correlations between the variables were examined using multiple linear regression analysis (software R, Version 1.9.0 for Windows). For multiple regression model building, forward and backward stepwise variable selection procedures were applied.

In all analyses the values of AIx, corrected for the HR 75 beats/min (AIx@75), were used. Raw AIx was inserted in the multiple linear regression model. Significance was defined as P < 0.05.

3. Results

3.1. Characteristics of the patients with PAD and the controls

The clinical characteristics of the 24 patients and 24 matched controls are summarized in Table 1. There was no significant difference between the groups in age, height, BMI, peripheral and central blood pressures, MAP, HR, glucose, total cholesterol, HDL cholesterol, LDL cholesterol, triglyceride and creatinine level. However, there occurred a difference in AIx, AIx@75 and Tₘ (also in Tₘ corrected for body height) between the groups (P = 0.001, <0.001 and <0.001, respectively).

3.2. Hemodynamic changes after administration of NTG and Salb

There was no significant difference between the groups in the response to Salb for any parameter except for AIx.
Table 2
The maximum changes from baseline in each parameter after NTG and Salb administration

<table>
<thead>
<tr>
<th>Variables</th>
<th>PAD patients (n=24)</th>
<th>Controls (n=24)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Salb</td>
<td>NTG</td>
</tr>
<tr>
<td>AIx (%)</td>
<td>−3.5 ± 4.6</td>
<td>−22.5 ± 6.6</td>
</tr>
<tr>
<td>AIx@75 (%)</td>
<td>−3.4 ± 3.8</td>
<td>−19 ± 5.6&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>T&lt;sub&gt;r&lt;/sub&gt; (ms)</td>
<td>1.1 ± 4.5</td>
<td>18.6 ± 9.7&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>−1.7 ± 8.1</td>
<td>−7.5 ± 7.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>−0.3 ± 5.8</td>
<td>5.8 ± 5.1&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Data are presented as means ± S.D. and significance from baseline are indicated by *P*< 0.05, **P**< 0.01 and ***P***< 0.001. Significant differences in the response after both drugs between groups are indicated in column by **P**< 0.001.

and T<sub>r</sub> (Table 2). The Salb-produced absolute changes in AIx and T<sub>r</sub> from baseline were significant only in healthy subjects (*P*< 0.001 and <0.006, respectively), however, Salb did not affect significantly HR or MAP in either group.

After the administration of NTG, MAP decreased both in the patient group and in the control group (*P* = 0.004 and 0.001, respectively), while NTG caused an increase in HR only in the patient group (*P* = 0.01). Nevertheless, there was no significant difference in the changes in MAP and HR between the groups after the administration of either drug. The Salb-induced changes in T<sub>r</sub> and AIx were significantly reduced in the PAD patients compared with the control subjects (*P*< 0.001 and <0.001, respectively), while the NTG-produced changes were not significantly different (*P* = 0.25 and 0.35, respectively).

### 3.3. Relationships between endothelial function, arterial stiffness and MAP

Linear regression analysis was used to establish whether endothelial function correlated with arterial stiffness. There was an inverse association between the improvement in AIx after Salb administration, i.e. EDV and baseline AIx (Fig. 1) as well as between EDV and CPP (*R* = −0.32, *P* = 0.03). No association was found between EDV and peripheral pulse pressure (data not shown).

Additional linear regression analysis was performed to compare the effects of the drugs on the AIx and T<sub>r</sub> with reference to MAP, a key determinant of large artery stiffness. The regression line for AIx and MAP shows linear correlation between the changes in AIx and MAP after the administration of NTG and Salb (Fig. 2). In contrast, there was no correlation between the changes in T<sub>r</sub> and MAP produced by either drug (Fig. 3). There occurred a significant linear correlation between the drug-produced changes in AIx and T<sub>r</sub> as shown in Fig. 4.

### 3.4. Multivariate relationship between the changes in AIx and T<sub>r</sub> following β<sub>2</sub>-stimulation and the other variables

To investigate further the factors influencing the response of AIx to Salb, a multiple linear regression model was developed with the change in AIx after Salb as the dependent variable. Age, weight, smoking status (defined as 0: non-smoker; 1: smoker), baseline MAP and AIx, LDL and HDL cholesterol, triglycerides, glucose and change in HR and MAP were entered in the model. The final model (Table 3) accounted for about 50% of the variability in the response of AIx to Salb. The improvement in AIx, i.e. EDV, was negatively correlated with smoking, age and change in MAP.

Fig. 1. The effect of Salb administration on improvement in augmentation index, i.e. EDV and baseline AIx (Fig. 1) as well as between EDV and CPP (*R* = −0.32, *P* = 0.03). No association was found between EDV and peripheral pulse pressure (data not shown).

Fig. 2. The relationship between maximum change in augmentation index (AIx@75) and mean arterial pressure (MAP) after NTG (○) and Salb (●) administration in all study subjects (n = 48). The linear regression lines are shown for NTG (—) (*R* = 0.4, *P* = 0.005) and Salb (——) (*R* = 0.3, *P* = 0.04).
Fig. 3. The relationship between maximum change in estimated aortic pulse wave velocity (Tr) and in mean arterial pressure (MAP) after NTG (○) and Salb (■) administration in the all study subjects (n=48). The linear regression lines are shown for NTG (—) (R = −0.03; P = 0.84) and Salb (——) (R = −0.21; P = 0.16).

Fig. 4. The relationship between maximum change in augmentation index (AIx@75) and in estimated aortic pulse wave velocity (Tr) after NTG (○) and Salb (■) administration in the all study subjects (n=48). The linear regression lines are shown for NTG (—) (R = −0.31, P = 0.03) and Salb (——) (R = −0.5, P < 0.001).

The data for either group were used to develop another multiple linear regression model with the changes in Tr after Salb as the dependent variable. Age, weight, smoking status, baseline MAP and Tr, LDL and HDL cholesterol, triglycerides, glucose and change in HR and MAP were entered into the model. The final model only accounted for about 25% (R² = 0.27, P < 0.02) of the variability in Tr, indicating that the change in Tr after Salb administration was significantly inversely correlated with plasma triglyceride only (P = 0.03).

4. Discussion

The current study describes for the first time the effects of NTG and Salb on aortic and systemic arterial stiffness in patients with PAD using PWA. Our main novel findings were that: (1) Salb-produced changes in aortic and systemic arterial stiffness are reduced in patients with PAD; (2) endothelial dysfunction is accompanied with increased arterial stiffness; (3) systemic stimulation of NO synthesis decreases aortic PWV independently of changes in MAP.

4.1. Endothelium as a key factor for cardiovascular diseases

Endothelial dysfunction precedes development of atherosclerosis [2] and plays an important role in the clinical course of cardiovascular diseases [6,7]. As PAD is a manifestation of generalized atherosclerosis, these patients frequently suffer from myocardial infarction and stroke. Therefore, assessment of preoperative cardiovascular risk in patients with PAD is a significant clinical challenge. Previously, it has been reported that endothelial function in patients undergoing vascular surgery independently predicts postoperative [17] and long-term [7] cardiovascular events. A possible future approach would be a preoperative combination of non-invasive endothelial assessment with other clinical scoring systems to better determine individual surgical risk [4,7,17].

We demonstrated that the systemic stimulation of NO synthesis by the β2-agonist Salb changed AIx and Tr significantly in healthy subjects but failed to modulate the aortic pulse waveform in patients with PAD (Table 2). In agreement with other authors [3,5], our finding that PWA is a useful method for detecting pathological changes in the vasculature allows to attach great importance to non-invasive techniques in monitoring of disease progression as well as in identifying subjects with preclinical atherosclerosis. Interventions for reversing endothelial dysfunction [18,19] could limit cardiovascular risk and enhancement of NO production may help maintain bypass patency and limb salvage in patients with PAD [20].

We found that EDV was inversely correlated with smoking status, age and change in MAP (Table 3). This is quite expected as endothelial function declines with age and with tobacco use, a strong risk factor for PAD. It is also assumed [16] that changes in AIx are related to MAP, a major determinant of large artery stiffness (Fig. 2).

4.2. Arterial stiffness and endothelial function in patients with atherosclerosis

There is no clear evidence of the association between atherosclerosis and arterial stiffness or of the validity of the
indices of arterial stiffness in identification of persons with preclinical atherosclerosis. Avolio et al. showed that aortic PWV was virtually identical in populations with low and high prevalence of atherosclerosis [21] and others reported that arterial stiffening is not associated with atherosclerosis [22]. As atherosclerosis is predominantly an intimal disease, whose principal effect is local flow limitation, while the media may only be secondarily weakened [23], these findings are quite expected. On the contrary, association has been shown between carotid–radial PWV and extent of coronary atherosclerosis [24] and increased aortic PWV has been reported in patients with PAD [12]. Moreover, aortic stiffness is an independent predictor of cardiovascular events [11,25].

In contrast to atherosclerosis, the two forms of arteriosclerosis—hypertensive and senile—are diffuse and affecting mainly the media and increasing stiffness in the whole arterial tree [23]. As the process of arterial stiffening, especially, due to arteriosclerosis, is highly susceptible to aging and MAP, there were no significant differences in these parameters between the subgroups in our study. However, we demonstrated that the PAD patients had increased estimated aortic PWV and AIx, which evidently characterizes a combination of atherosclerotic and arteriosclerotic alterations both in the aorta and in the peripheral arteries. Stiffening throughout the arterial tree resulted in elevated CPP characterizing aortic stiffness, particularly when elucidating the specific action of NO in modulating arterial stiffness. Apart from findings that arterial stiffening is not associated with atherosclerosis [22], there is a link between NO and regulation of large artery stiffness.

We used an estimated measure of aortic stiffness instead of carotid–femoral PWV. Although London et al. found linear relationship between $T_e$ and carotid–femoral PWV [30], these parameters are not absolutely identical; $T_e$ is responsive, at least in part, to wave reflection, while carotid–femoral PWV characterizes more directly pulse wave transmission in the aorta. In agreement with a previous study [16], we found linear relationship between the drug-produced changes in $T_e$ and AIx (Fig. 4). Both drugs resulted in a later return of the reflected wave to the aorta, indicating reduced aortic and systemic arterial stiffness. It seems likely that absence of correlation between $T_e$ and MAP indicates that the decrease in aortic PWV after Salb and NTG administration reflects slower wave propagation in the aorta due to reduced aortic stiffness rather than changes in distending pressure.

4.4. Limitations

There exist limitations on the use of the indirect indices of arterial stiffness, particularly when elucidating the specific action of NO in modulating arterial stiffness. Apart from characterizing aortic stiffness, $T_e$ depends, in part, on wave reflection and we cannot exclude the influence of release of endogenous vasoactive substances on peripheral arterial stiffness. But, in patients with PAD, direct PWV measurement was frequently complicated owing to the stenosis or occlusions of the iliac, femoral and/or carotid arteries. In addition, information about cardiac index could be considered useful in elucidating the potential influence of ventricular-vascular interactions on arterial stiffness.

The limitation of the study is also the potential confounding long-term effects of the medications and smoking on endothelial reactivity. We were unable to withdraw chronically ill patients from their medications for extended periods of time. However, this mechanistic study was not intended to focus on analyzing the etiology of vascular damage, e.g. the effects of smoking and medications on vascular alterations.

Finally, we studied only males to avoid the complicating effects of endogenous hormones, considering also that patients with PAD are predominantly men. This makes it difficult to use the current findings for females.

4.5. Summary

We have shown for the first time that stimulation of NO synthesis by $\beta_2$-agonist Salb fails to modulate aortic and systemic arterial stiffness in patients with PAD. This study supports the hypothesis that Salb modulates arterial stiffness, in part via a direct effect of NO on large artery mechanics rather than changes in blood pressure. We also demonstrated that there may exist a link between endothelial function and arterial stiffness. Our data suggest that PWV could be a useful tool for assessment of alterations in NO-mediated vascular changes, which predict several cardiovascular pathologies.
Acknowledgments

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