CALL FOR PAPERS Small Vessels–Big Problems: Novel Insights into Microvascular Mechanisms of Diseases

Effect of PDE5 inhibition on the modulation of sympathetic α-adrenergic vasoconstriction in contracting skeletal muscle of young and older recreationally active humans

Michael Nyberg,1 Peter Piil,1 Jon Egelund,1 Randy S. Sprague,2 Stefan P. Mortensen,3,4 and Ylva Hellsten1

1Department of Nutrition, Exercise and Sports, University of Copenhagen, Copenhagen, Denmark; 2Department of Pharmacological and Physiological Science, St. Louis University School of Medicine, St. Louis, Missouri; 3Department of Cardiovascular and Renal Research, Institute of Molecular Medicine, University of Southern Denmark, Odense, Denmark; and 4The Centre of Inflammation and Metabolism and the Centre for Physical Activity Research, Department of Infectious Diseases, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark

Submitted 17 August 2015; accepted in final form 28 September 2015

CONTRACTILE ACTIVITY markedly increases the O2 demand in skeletal muscle, thus requiring an increase in blood flow and O2 delivery to the tissue. This increase in blood flow to the exercising muscle is the result of a local attenuation of the vasoconstrictor effect of sympathetic nervous activity, termed functional sympatholysis, and vasodilation induced by local vasoactive mechanisms (6, 15). Since the first report on the hemodynamic response to exercise in older individuals in 1974 (42), evidence has accumulated for an insufficient blood flow to contracting skeletal muscle in aging (3, 5, 17, 18, 23, 26, 28, 30, 31). This altered regulation with aging could be related to a diminished ability for functional sympatholysis (10, 20, 36) and/or reduced efficacy of local vasodilator pathways (20, 38, 40).

ATP has been proposed to play a role in skeletal muscle vasodilatation (11–13) which is supported by its potent vasodilator capacity (20, 22, 34) and the observation that the concentration of this substance increases in the venous effluent of contracting skeletal muscle of young subjects (17, 21). In contrast, ATP has been reported not to increase in venous plasma draining the exercising forearm of older subjects (17). One major source of plasma ATP has been suggested to be erythrocytes as these cells release ATP in response to increased shear stress (39, 43), mechanical deformation (39, 43), and deoxygenation (11). Hence, one mechanism underlying the lower plasma ATP levels in aging could be a reduced release of ATP from erythrocytes in response to low Po2 (17). In humans with type 2 diabetes, a condition in which ATP release from erythrocytes is impaired, inhibition of cGMP-binding phosphodiesterase 5 (PDE5) effectively rescues low O2-induced ATP release in isolated erythrocytes (32). Hence, systemic inhibition of PDE5 in older humans could poten-
tially lead to an increase in plasma [ATP] in conditions of low \( \text{PO}_2 \) such as during skeletal muscle contractions which could explain the increased exercise hyperemia in older subjects following PDE5 inhibition (26). Importantly, when infused into the arterial vasculature supplying skeletal muscle, ATP can significantly blunt \( \alpha \)-adrenergic vasoconstriction in both young (34) and older (16, 20) subjects. Therefore, an increase in plasma [ATP] through PDE5 inhibition could lead to an improved ability for functional sympatholysis, which would serve to improve perfusion of the contracting skeletal muscle fibers (36).

The vasodilator effect of nitric oxide (NO) is mediated via elevation of cGMP concentrations (35) and it has been shown that aging is associated with a reduced vascular response to infusion of the endothelium-dependent vasodilator ACh (20, 40) as a consequence of a lower NO bioavailability (40). Consequently, the increase in exercise hyperemia with PDE5 inhibition in older subjects (26) could also reflect that a lower cGMP level during exercise was, at least in part, related to a diminished NO bioavailability. As opposed to the reports on the vascular effects of ATP, consistent evidence has been provided to support that NO is neither sufficient nor essential for functional sympatholysis in humans (9, 31, 33, 41). Consequently, an NO-mediated increase in local vasodilation in the contracting leg muscles would, therefore, be expected to be independent of an improved ability for functional sympatholysis.

Accordingly, the aim of the current study was to determine whether the increase in exercise hyperemia with PDE5 inhibition was associated with an improved ability for functional sympatholysis in older adults during exercise engaging the knee extensors. We hypothesized that PDE5 inhibition with the highly selective inhibitor sildenafil would increase circulating ATP levels and improve functional sympatholysis in the exercising leg.

METHODS

A total of 23 healthy male subjects of which 9 were young (23 \( \pm \) 1 yr) and 14 were older (72 \( \pm \) 1 yr) participated in the study (Table 1). All subjects were recreationally active, but none participated in moderate- or high-intensity exercise for 3 days per week during the past 12 mo. The two groups were matched for physical activity level according to age group norm for maximal oxygen uptake (l/min, \( \text{V}\text{O}_2 \text{max} \)) (2). The subjects underwent screening by means of a medical examination, 12-lead electrocardiogram (ECG), and blood sampling from the antecubital vein. Exclusion criteria were history or symptoms of cardiovascular disease, renal dysfunction, insulin resistance, diabetes, or hypercholesterolemia. All subjects were nonsmokers, and none of the subjects were taking prescription medicine. Subjects refrained from caffeine, alcohol, and exercise for 24 h before each experimental day. On the day of the experiment the subjects arrived at the laboratory after eating breakfast. The study was approved by the Ethics Committee of the Region of Capital of Copenhagen (H-3-2012-176) and conducted in accordance with the guidelines of the Declaration of Helsinki. Written informed consent was obtained from all subjects before enrollment into the study. Baseline characteristics and measurements obtained during control exercise without and with tyramine (1 \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{kg leg mass}^{-1} \); Sigma Aldrich, St. Louis, MO) were placed in the femoral artery and vein of the experimental leg and in the femoral artery of the nonexperimental leg 2 cm below the inguinal ligament and advanced 10 cm in the proximal direction. Following 80 min of rest, subjects were positioned in a supine position where they received femoral arterial infusion of tyramine (1 \( \mu \text{mol} \cdot \text{min}^{-1} \cdot \text{kg leg mass}^{-1} \)). Tyramine was infused into the femoral artery for 2.5 min and
Table 1. **Subject characteristics**

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Young (n=6)</th>
<th>Older (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>23 ± 1</td>
<td>72 ± 1*</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.83 ± 0.01</td>
<td>1.80 ± 0.02</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>75.8 ± 3.3</td>
<td>79.1 ± 2.3</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>13.7 ± 1.4</td>
<td>24.6 ± 1.5*</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>117 ± 3</td>
<td>140 ± 5*</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>60 ± 1</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>Mean arterial blood pressure, mmHg</td>
<td>80 ± 2</td>
<td>90 ± 2*</td>
</tr>
<tr>
<td>VO2max, l/min</td>
<td>3.42 ± 0.24</td>
<td>2.48 ± 0.88*</td>
</tr>
<tr>
<td>VO2max, relative to body weight, l/min†kg⁻¹</td>
<td>45.9 ± 2.7</td>
<td>31.5 ± 1.0*</td>
</tr>
<tr>
<td>Experimental leg mass, kg</td>
<td>11.9 ± 0.6</td>
<td>11.3 ± 0.3</td>
</tr>
<tr>
<td>Experimental lean leg mass, kg</td>
<td>10.0 ± 0.5</td>
<td>8.7 ± 0.2*</td>
</tr>
<tr>
<td>Peak workload during knee-extensor exercise, W</td>
<td>48 ± 3</td>
<td>42 ± 6</td>
</tr>
<tr>
<td>Glucose, average (from HbA1c), mmol/mol</td>
<td>5.4 ± 0.1</td>
<td>6.1 ± 0.1*</td>
</tr>
<tr>
<td>Total cholesterol, mmol/l</td>
<td>4.3 ± 0.3</td>
<td>5.3 ± 0.2*</td>
</tr>
<tr>
<td>HDL-C, mmol/l</td>
<td>1.4 ± 0.1</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>LDL-C, mmol/l</td>
<td>2.6 ± 0.2</td>
<td>3.3 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. VO2max, maximal oxygen uptake; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. Different from young: *P < 0.05.

Table 2. **Blood variables at rest and during tyramine infusion**

<table>
<thead>
<tr>
<th>Blood Variable</th>
<th>Young</th>
<th>Sildenafil</th>
<th>Older</th>
<th>Sildenafil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CON</td>
<td>CON</td>
<td>Older</td>
<td>Sildenafil</td>
</tr>
<tr>
<td>P02, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>102 ± 2</td>
<td>105 ± 1</td>
<td>102 ± 3</td>
<td>103 ± 2</td>
</tr>
<tr>
<td>v</td>
<td>40 ± 1</td>
<td>32 ± 2†</td>
<td>44 ± 3</td>
<td>35 ± 2†</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>14.0 ± 0.3</td>
<td>14.0 ± 0.3</td>
<td>13.8 ± 0.2</td>
<td>14.0 ± 0.2</td>
</tr>
<tr>
<td>v</td>
<td>13.8 ± 0.3</td>
<td>13.8 ± 0.3</td>
<td>13.3 ± 0.2</td>
<td>13.2 ± 0.2</td>
</tr>
<tr>
<td>O2 saturation, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>98.1 ± 0.2</td>
<td>98.2 ± 0.1</td>
<td>98.1 ± 0.2</td>
<td>98.1 ± 0.1</td>
</tr>
<tr>
<td>v</td>
<td>72.3 ± 1.1</td>
<td>57.3 ± 3.4†</td>
<td>77.9 ± 2.3</td>
<td>64.4 ± 3.4†</td>
</tr>
<tr>
<td>O2 content, ml/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>186 ± 3</td>
<td>187 ± 4</td>
<td>184 ± 3</td>
<td>186 ± 3</td>
</tr>
<tr>
<td>v</td>
<td>135 ± 6</td>
<td>107 ± 7†</td>
<td>140 ± 6</td>
<td>115 ± 7†</td>
</tr>
<tr>
<td>Leg VO2, ml/min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>15 ± 3</td>
<td>5 ± 1†</td>
<td>18 ± 3</td>
<td>6 ± 1†</td>
</tr>
<tr>
<td>v</td>
<td>0.9 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>0.9 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
<tr>
<td>pH</td>
<td>7.394 ± 0.006</td>
<td>7.393 ± 0.006</td>
<td>7.399 ± 0.004</td>
<td>7.398 ± 0.004</td>
</tr>
<tr>
<td>v</td>
<td>7.375 ± 0.004</td>
<td>7.368 ± 0.004†</td>
<td>7.383 ± 0.003</td>
<td>7.380 ± 0.002*</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>61 ± 2</td>
<td>61 ± 3</td>
<td>61 ± 3</td>
<td>58 ± 3</td>
</tr>
<tr>
<td>FVP, mmHg</td>
<td>3.4 ± 0.7</td>
<td>4.0 ± 0.6</td>
<td>3.1 ± 0.8</td>
<td>3.4 ± 0.8</td>
</tr>
</tbody>
</table>

Values are means ± SE. a, femoral arterial; v, femoral venous; CON, control setting; FVP, femoral venous pressure. Significant difference from rest within same condition: †P < 0.05; significant difference from young within same condition: #P < 0.05; significant difference from CON within same condition: *P < 0.05.
sildenafil intake, infusion of SNP, SNP + tyramine, and knee-extensor exercise were repeated in the same order as during the control condition.

Measurements and calculations. Femoral arterial blood flow (FABF) was measured with ultrasound Doppler (Vivid E9, GE Healthcare, Denmark) equipped with a linear probe operating at an imaging frequency of 11 MHz and Doppler frequency of 5.0 MHz and as previously described (24).

Intra-arterial and intravenous pressure was monitored with transducers (Pressure Monitoring Set, Edwards Lifesciences) positioned at the level of the catheters. Blood gases, hemoglobin, glucose, and lactate were measured using an ABL800 FLEX analyzer (Radiometer, Denmark). Total cholesterol, low-density lipoprotein (LDL-C), and high-density lipoprotein (HDL-C) were analyzed using an automatic analyzer using enzymatic kits (Modular P-Module), HbA1c using HPLC, and norepinephrine using ELISA (Research ELISA, LDN, Nordhorn, Germany). Leg mass was calculated from whole body dual-energy x-ray absorptiometry scanning (Prodigy, GE Healthcare, Denmark), leg vascular conductance (LVC) as FABF/(mean FAP/mean FVP), where FAP is femoral arterial pressure and FVP is femoral venous pressure; leg O2 uptake as arteriovenous O2 difference × FABF; and leg lactate release as arteriovenous lactate difference × FABF.

Microdialysis procedure. The microdialysis probes were perfused at a rate of 5 µl/min with isotonic saline (0.9% NaCl). To determine

![Graphs](http://ajpheart.physiology.org/)

**Fig. 2.** Femoral arterial blood flow (A), leg arteriovenous O2 difference (B), mean femoral arterial blood pressure (C), leg O2 uptake (D), leg vascular conductance (E), and femoral venous norepinephrine (F) in young (n = 9) and older (n = 8) subjects during knee-extensor exercise performed at 40% Wmax (19 ± 1 and 15 ± 1 W: young and older) without (CON) and with sildenafil. Significant difference from CON within same condition: *P < 0.05; significant difference from without tyramine within same condition: †P < 0.05.
the relative exchange of ATP over the dialysis membrane, a small amount (2.7 nM) of [3-3H]ATP (<0.1 μCi/ml) was added to the perfusate for the calculation of probe recovery. Dalteparin (12,500 anti-Xa IE/ml; Fragmin, Pfizer) was added to the perfusate to avoid blood clotting on the membrane. The molecular probe recovery (PR) was calculated as PR = (dpmperfusate − dpmdialysate)/dpmperfusate, where dpm denotes disintegrations per minute (14, 37). The 3H activity (in dpm) was measured (in triplicate) on a liquid scintillation counter (Tri-Carb 2910 TR; Perkin Elmer) after addition of the infused/dialysate (5 μl each) to 3.0 ml of Ultima Gold scintillation liquid (Perkin Elmer). After collection of samples, the microdialysate was weighed, and the actual flow rate was calculated to estimate any loss of fluid or abnormal decrease in perfusion rate. The relative loss (recovery) of ATP was 27 ± 2% (rest, CON), 31 ± 2% (exercise, CON), 19 ± 2% (rest, sildenafil), and 27 ± 3% (exercise, sildenafil).

Plasma ATP concentrations in the microdialysis dialysate were measured in duplicates with the luciferin-luciferase technique. Briefly, 200 μl of a diluted sample was injected into a cuvette containing 100 μl of firefly lantern extract (10 mg/ml distilled water, FLE 250; Sigma, St. Louis, MO) and 100 μl of n-luciferin solution (10 mg/20 ml distilled water; Research Products International, Mount Prospect, IL). The light emitted from the reaction with ATP was quantified using a luminometer (TD 20/20; Turner Designs, Sunnyvale, CA). A standard curve was generated for each experiment. The coefficient of variation between duplicate measurements was 9.0 ± 1.5%.

Statistical analysis. Specific hypothesis testing during rest, infusion, and exercise was performed with two-way repeated-measures ANOVA. After a significant F test, pairwise differences were identified using a Student-Newman-Keuls post hoc test. Baseline differences between young and older subjects were assessed with unpaired t-tests. Statistical significance was set at a priori at 0.05 and data are presented as means ± SE.

RESULTS

Arterial tyramine infusion at rest. In the control setting, infusion of tyramine at rest reduced (P < 0.05) FABF (78 ± 5 and 50 ± 6%, young and older) and LVC (78 ± 5 and 50 ± 6%) whereas FAP remained unchanged (79 ± 1 vs. 83 ± 2 mmHg and 92 ± 3 vs. 94 ± 3 mmHg) in both groups (Fig. 1). Sildenafil increased (P < 0.05) FABF (67 ± 24 and 83 ± 25%, young and older) and LVC (67 ± 26 and 136 ± 40%) but this effect of sildenafil was abolished with tyramine. Femoral venous norepinephrine increased (P < 0.05) to a similar extent with tyramine in both groups without and with sildenafil. There was no difference between groups in the reduction in LVC for a given increase in norepinephrine in the control setting and with sildenafil. Blood variables, heart rate, FVP, and leg O2 uptake are presented in Table 2.

Knee-extensor exercise. When tyramine was infused during exercise performed at 40% Wmax, FABF and LVC were lower (P < 0.05) compared with without tyramine in both groups in the control setting and with sildenafil (Fig. 2). The reduction in FABF and LVC with tyramine was unaltered with sildenafil in young subjects whereas there was an additional reduction in LVC (16 ± 2 vs. 9 ± 3%, P < 0.05) with sildenafil in the older subjects, in whom tyramine reduced the sildenafil induced increase in LVC by 38 ± 9% (Fig. 3). Leg arteriovenous O2 difference increased (P < 0.05) when tyramine was infused in both groups without and with sildenafil, thereby maintaining leg O2 uptake within the same condition. FAP increased (P < 0.05) with tyramine infusion in the young subjects without sildenafil but this effect of tyramine was not detected with sildenafil (Fig. 2). In contrast, FAP did not change with tyramine infusion in the older subjects in the control setting but FAP increased (113 ± 5 vs. 108 ± 4 mmHg, P < 0.05) with tyramine in the older subjects with sildenafil. Femoral venous norepinephrine increased (P < 0.05) with tyramine infusion in both groups without and with sildenafil. In both the young and older subjects, femoral venous norepinephrine was higher (P < 0.05) with sildenafil than without in both conditions. Femoral venous norepinephrine increased (P < 0.05) to a similar extent with tyramine in both groups without and with sildenafil (Fig. 3). Blood variables, heart rate, and FVP are presented in Table 3.

Femoral venous plasma ATP in the older subjects. In the older subjects, femoral venous plasma [ATP] was 76 ± 14 nmol/l at rest and increased (P < 0.05) to 289 ± 99 nmol/l during exercise performed at 6 W (Fig. 4). Following sildenafil, femoral venous plasma [ATP] was increased (P < 0.05) ~4-fold at rest (286 ± 79 nmol/l) to a level comparable to that
Blood variables during knee-extensor exercise

<table>
<thead>
<tr>
<th>Blood Variable</th>
<th>Young</th>
<th>Sildenafil</th>
<th>Young</th>
<th>Sildenafil</th>
</tr>
</thead>
<tbody>
<tr>
<td>P02, mmHg</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>104 ± 2</td>
<td>105 ± 3</td>
<td>103 ± 2</td>
<td>104 ± 3</td>
</tr>
<tr>
<td>v</td>
<td>25 ± 1</td>
<td>22 ± 1†</td>
<td>25 ± 1</td>
<td>23 ± 1†</td>
</tr>
<tr>
<td>Hemoglobin, g/dl</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>14.7 ± 0.3</td>
<td>14.8 ± 0.2</td>
<td>14.7 ± 0.2</td>
<td>14.9 ± 0.2</td>
</tr>
<tr>
<td>v</td>
<td>14.2 ± 0.3</td>
<td>14.7 ± 0.3</td>
<td>14.2 ± 0.3</td>
<td>14.5 ± 0.3</td>
</tr>
<tr>
<td>O2 saturation, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>98.1 ± 0.1</td>
<td>98.2 ± 0.1</td>
<td>98.0 ± 0.1</td>
<td>98.1 ± 0.2</td>
</tr>
<tr>
<td>v</td>
<td>38.2 ± 1.8</td>
<td>31.0 ± 2.9†</td>
<td>38.5 ± 1.5</td>
<td>32.6 ± 2.1†</td>
</tr>
<tr>
<td>O2 content, ml/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>196 ± 3</td>
<td>197 ± 3</td>
<td>196 ± 3</td>
<td>198 ± 3</td>
</tr>
<tr>
<td>v</td>
<td>74 ± 5</td>
<td>62 ± 6†</td>
<td>74 ± 3</td>
<td>64 ± 5†</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>2.1 ± 0.4</td>
<td>2.2 ± 0.4</td>
<td>1.6 ± 0.4</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>v</td>
<td>2.6 ± 0.6</td>
<td>3.0 ± 0.6</td>
<td>2.1 ± 0.6</td>
<td>2.3 ± 0.8</td>
</tr>
<tr>
<td>Lactate release, mmol/min</td>
<td>1.2 ± 0.7</td>
<td>1.7 ± 0.6</td>
<td>1.1 ± 0.6</td>
<td>1.1 ± 0.6</td>
</tr>
<tr>
<td>pH</td>
<td>7.380 ± 0.005</td>
<td>7.390 ± 0.007</td>
<td>7.394 ± 0.007</td>
<td>7.394 ± 0.008</td>
</tr>
<tr>
<td>a</td>
<td>7.289 ± 0.011</td>
<td>7.284 ± 0.012</td>
<td>7.306 ± 0.012*</td>
<td>7.303 ± 0.014*</td>
</tr>
<tr>
<td>v</td>
<td>99 ± 6</td>
<td>102 ± 8</td>
<td>100 ± 6</td>
<td>105 ± 8†</td>
</tr>
<tr>
<td>FVP, mmHg</td>
<td>17.5 ± 0.8</td>
<td>17.6 ± 0.9</td>
<td>18.0 ± 1.0</td>
<td>17.5 ± 0.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. a, femoral arterial; v, femoral venous; CON, control setting; EX, exercise. Significant difference from EX within same condition: †P < 0.05; significant difference from young within same condition: #P < 0.05; significant difference from CON within same condition: *P < 0.05. CON: control setting; EX: exercise.

measured during exercise in the control condition. Plasma [ATP] did not change further with exercise in the sildenafil trial.

Arterial infusion of SNP and tyramine in the older subjects. In the older subjects, SNP induced an increase (P < 0.05) in FABF (1.45 ± 0.19 l/min) and LVC (17.88 ± 1.79 ml·min⁻¹·mmHg⁻¹) in the control setting (Fig. 5). Infusion of tyramine reduced the SNP-induced change in FABF and LVC by 52 and 55%. Sildenafil augmented (P < 0.05) the SNP-induced increase in LVC but this effect of sildenafil was abolished with tyramine since the percent reduction in the SNP-induced increase in LVC was similar (~50%) without and with sildenafil. Infusion of tyramine increased (P < 0.05) FAP to a similar extent without (84 ± 4 vs. 89 ± 4 mmHg) and with sildenafil (66 ± 3 vs. 71 ± 2 mmHg).

DISCUSSION

The aim of the current study was to determine the mechanism underlying the effect of PDE5 inhibition on exercise hyperemia in older recreationally active individuals. The primary finding was that the higher blood flow to the exercising leg with PDE5 inhibition in older subjects was not associated with an increase in plasma [ATP] in the venous drainage of active skeletal muscle or with an improved ability for functional sympatholysis. Furthermore, NO-stimulated vasodilation in the leg did not blunt α-adrenergic vasoconstriction and this was unaffected by PDE5 inhibition. Collectively, these findings suggest that PDE5 inhibition improves the efficacy of local nonsympatholytic vasodilator pathways in older subjects during skeletal muscle contractions.

Effect of PDE5 inhibition on plasma [ATP] and functional sympatholysis. Aging is associated with an impaired ability for functional sympatholysis in both the forearm and leg vasculature (10, 20). In addition, whereas the venous concentration of the sympatholytic compound ATP increases in young subjects (17, 21), this effect of skeletal muscle contractions has been suggested to be absent in older subjects due to an attenuated release from deoxygenated erythrocytes (17). Interestingly, PDE5 inhibition has been shown to reduce low O2-induced ATP release in isolated erythrocytes, an effect likely to be mediated via cGMP-dependent inhibition of PDE3 and consequently elevated cAMP levels (32). In contrast to these previous observations, current results indicate that PDE5 inhibition did not increase the level of femoral venous plasma [ATP] in the older subjects in response to contractions nor did it improve the ability for functional sympatholysis. With regard to the
levels in response to skeletal muscle contractions in the older ing (19). The preserved capacity to increase circulating ATP level of physical activity has been shown to alter ATP signal-
group of older individuals was recreationally active since the
modality and sampling method (21). This finding is in contrast
to that observed in young subjects using the same exercise
during exercise in the control trial increased to a level similar
changes to tyramine were obtained. Nevertheless, the lower
than that used during the trial in which the hemodynamic
endure the whole exercise period, workload had to be lower
a sufficient volume of dialysate. For the older subjects to
technique did require a prolonged period of exercise to obtain
stable ATP concentrations (21). The use of the microdialysis
probe does not allow erythrocytes and ATP degrading enzymes
microdialysis technique, as the membrane cut-off of the used
membrane, which provides a sampling fluid with
half-life of this substance in blood is \( <1 \text{s} \) (21) due to the rapid
degradation by nucleotides (46) and uptake in erythrocytes and
other cells (44). Therefore, despite the use of stop solutions and
rapid centrifugation, ATP determinations from blood samples are
difficult to interpret. Consequently, we chose to use the
microdialysis technique, as the membrane cut-off of the used
probe does not allow erythrocytes and ATP degrading enzymes
to pass the membrane, which provides a sampling fluid with
stable ATP concentrations (21). The use of the microdialysis
technique did require a prolonged period of exercise to obtain
a sufficient volume of dialysate. For the older subjects to
endure the whole exercise period, workload had to be lower
than that used during the trial in which the hemodynamic
changes to tyramine were obtained. Nevertheless, the lower
workload still allowed for the detection of a potential effect of
PDE5 inhibition on the contraction-induced change in venous plasma [ATP].

The lack of effect of PDE5 inhibition on venous plasma [ATP] may have been related to the fact that plasma [ATP] during exercise in the control trial increased to a level similar
to that observed in young subjects using the same exercise
modality and sampling method (21). This finding is in contrast
to that of Kirby and co-workers (17) in the forearm of seden-
tary subjects and the discrepancy may reflect that the current
group of older individuals was recreationally active since the
level of physical activity has been shown to alter ATP signal-
ing (19). The preserved capacity to increase circulating ATP levels in response to skeletal muscle contractions in the older
subjects may partly explain the similar ability for functional
sympatholysis in the young and older subjects.

The current study demonstrating a similar ability for func-
tional sympatholysis in young and older subjects is in contrast
to that of previous studies demonstrating an impaired ability to
blunt \( \alpha \)-adrenergic vasoconstriction during exercise in older subjects (10, 20). This discrepancy is likely to reflect that the young and older subjects were matched for physical activity as
the ability for functional sympatholysis appears to be more
related to the training status of skeletal muscle than age per se
(25). Notably, aging has been shown to be associated with a
reduced post-junctional \( \alpha \)-adrenergic responsiveness (8, 20),
that could serve to reduce the vasoconstrictor response to
tyramine-induced release of norepinephrine. However, the re-
duction in leg vascular conductance for a given increase in
femoral venous norepinephrine during infusion of tyramine at
rest was not different between the young and older subjects,
indicating that the similar ability for functional sympatholysis
was not related to an altered \( \alpha \)-adrenergic responsiveness in the
older subjects.

Another interesting observation in the current study was that
plasma [ATP] at rest was markedly increased with PDE5
inhibition in the older subjects. The mechanism underlying this
pronounced effect of PDE5 inhibition on ATP levels may
include increased ATP release from deoxygenated erythrocytes
(32) or it may be that an increase in shear stress stimulated
endothelial cells to release ATP (45); however, an ACh-
induced increase in blood flow does not increase plasma ATP
levels (21). To rule out that the increase in plasma [ATP] was
an effect of the prior exercise bout, a time control was per-
formed in two subjects in which no increases were detected
(67 ± 33 vs. 13 ± 8 nM before and 1 h after the exercise bout).
This finding is also in agreement with the observation that
venous plasma [ATP] returns to baseline during the first 7 min
of recovery (21).

Although the level of plasma ATP at rest with sildenafil
reached levels comparable to that detected during exercise, the
sildenafil-induced increase in leg vascular conductance was
still abolished with infusion of tyramine. Although this would
seem to argue against a role for plasma ATP in the modulation
of sympathetic \( \alpha \)-adrenergic vasoconstriction in contracting
skeletal muscle it may be that contractile activity was needed
in order for plasma ATP to have its sympatholytic effect at this
concentration. Moreover, in the control setting and with silden-
afil, blood flow and venous PO\(_2\) reached similar levels during
tyramine infusion. This could indicate that vasodilator systems
may have partly counteracted the vasoconstrictor effect to
ensure that \( O_2 \) delivery and consequently tissue \( P_{O_2} \) did not
decrease below a critical level. These possibilities warrant
further investigation.

Putative mechanisms underlying the effect of PDE5 inhibi-
tion on exercise hyperemia. In a recent study, the current group
of older subjects were reported to have a reduced vasodilator
response to ACh compared with that of young (26), which is in
line with previous observations (20, 40). Through inhibition of
enzymatic NO formation this reduced vascular response has
been shown to be an effect of a lower NO bioavailability (40).
As the vasodilator effect of NO is mediated via cGMP (35),
one mechanism underlying the increase in exercise hyperemia
with PDE5 inhibition in older subjects (26) could be an
improved cGMP signaling, which would serve to potentiate the

Fig. 5. Change in leg vascular conductance with femoral arterial SNP and
SNP + tyramine infusion (A) and change in leg vascular conductance with
tyramine (B) in older subjects (\( n = 7 \)) without (CON) and with sildenafil.
Significant difference from CON within same condition: *\( P < 0.05 \); significant
difference from without tyramine within same condition: †\( P < 0.05 \).

AJP-Heart Circ Physiol • doi:10.1152/ajpheart.00653.2015 • www.ajpheart.org
effect of NO and thereby compensate for a diminished NO bioavailability. Notably, pharmacological inhibition of NO synthesis does not impact the ability of contracting forearm muscles to blunt vasoconstrictor responses to tyramine as well as $\alpha_1$- and $\alpha_2$-adrenoceptor agonists (7, 9) and infusion of SNP also fails to blunt vasoconstrictor responses to tyramine and $\alpha_1$- and $\alpha_2$-adrenoceptor agonists in the forearm (33, 41). Furthermore, infusion of ascorbic acid increases NO-mediated vasodilation in the exercising forearm of older individuals without improving functional sympatholysis (31). Hence, an improved NO signaling would be expected to lead to vasodilation without improving the ability for functional sympatholysis. However, given the differences in $\alpha$-adrenergic responsiveness (27) and vascular responses to various vasodilators (29) between the upper and lower extremities, the ability of exogenous NO to blunt $\alpha$-adrenergic vasoconstriction in the leg was also assessed in the older subjects. In line with findings in the forearm vasculature (33), infusion of tyramine induced a substantial reduction of $\sim 50\%$ in SNP-induced vasodilation and this was not affected by sildenafil. Interestingly, tyramine was found to cause a similar reduction in the sildenafil-induced increase in leg vascular conductance during exercise, thus further supporting a role for an improved NO signaling in the increased exercise hyperemia.

In conclusion, the increase in blood flow to contracting skeletal muscle of older subjects with PDE5 inhibition was not associated with increased levels of venous plasma [ATP] during exercise or an improved ability for functional sympatholysis. Therefore, one putative mechanism underlying the effect of PDE5 inhibition on exercise hyperemia could be an improved efficacy of the NO system.

ACKNOWLEDGMENTS

R. Damsgaard is gratefully acknowledged for excellent medical assistance.

GRANTS

This study was supported by a grant from The Danish Council for Independent Research and the Lundbeck Foundation. The Centre of Inflammation and Metabolism (CIM) is supported by a grant from the Danish National Research Foundation (DNRF55). The Centre for Physical Activity Research (CFAS) is supported by a grant from Trygfonden.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.N., P.B.P., J.E., S.P.M., and Y.H. edited and revised manuscript; M.N., P.B.P., J.E., S.P.M., and Y.H. performed experiments; M.N. prepared figures; M.N. drafted manuscript; M.N. and P.B.P. analyzed data; M.N., P.B.P., R.S.S., S.P.M., and Y.H. designed research; M.N., P.B.P., J.E., S.P.M., and Y.H. interpreted results of experiments; M.N., P.B.P., J.E., S.P.M., and Y.H. approved final version of manuscript.

REFERENCES


