Adenosine Contributes to Blood Flow Regulation in the Exercising Human Leg by Increasing Prostaglandin and Nitric Oxide Formation

Stefan P. Mortensen, Michael Nyberg, Pia Thaning, Bengt Saltin, Ylva Hellsten

Abstract—Adenosine can induce vasodilation in skeletal muscle, but to what extent adenosine exerts its effect via formation of other vasodilators and whether there is redundancy between adenosine and other vasodilators remain unclear. We tested the hypothesis that adenosine, prostaglandins, and NO act in synergy to regulate skeletal muscle hyperemia by determining the following: (1) the effect of adenosine receptor blockade on skeletal muscle exercise hyperemia with and without simultaneous inhibition of prostaglandins (indomethacin; 0.8 to 1.8 mg/min) and NO (N\textsuperscript{\text{\textregistered}}-mono-methyl-L-arginine; 29 to 52 mg/min); (2) whether adenosine-induced vasodilation is mediated via formation of prostaglandins and/or NO; and (3) the femoral arterial and venous plasma adenosine concentrations during leg exercise with the microdialysis technique in a total of 24 healthy, male subjects. Inhibition of adenosine receptors (theophylline; 399±9 mg, mean ± SEM) or combined inhibition of prostaglandins and NO formation inhibited the exercise-induced increase in leg blood flow by 14±1% and 29±2% (P<0.05), respectively, but combined inhibition of prostaglandins, NO, and adenosine receptors did not result in an additive reduction of leg blood flow (31±5%). Femoral arterial infusion of adenosine increased leg blood flow from ≈0.3 to ≈2.5 L/min. Inhibition of prostaglandins or NO, or prostaglandins and NO combined, inhibited the adenosine-induced increase in leg blood flow by 51±3%, 39±8%, and 66±8%, respectively (P<0.05). Arterial and venous plasma adenosine concentrations were similar at rest and during exercise. These results suggest that adenosine contributes to the regulation of skeletal muscle blood flow by stimulating prostaglandin and NO synthesis. (Hypertension. 2009;53:993-999.)

Key Words: skeletal muscle ■ regional blood flow ■ theophylline ■ indomethacin ■ 1-NMMA

Extracellular adenosine can function as a signaling molecule by matching blood flow with alterations in tissue oxygen supply or demand.\textsuperscript{1-3} In vivo evidence support a role for adenosine in skeletal muscle blood flow regulation by demonstrating the following: (1) femoral arterial adenosine infusion can induce vasodilation similar to that observed during intense exercise\textsuperscript{4}; (2) adenosine receptor blockade reduces limb blood flow during exercise\textsuperscript{4,5}; and (3) the interstitial adenosine concentration is tightly coupled to the increase in skeletal muscle blood flow during muscle contractions.\textsuperscript{6}

Adenosine sensitive P1 receptors (A\textsubscript{1}, A\textsubscript{2A}, and A\textsubscript{2B}) are located on vascular smooth muscle cells and in the endothelium of skeletal muscle.\textsuperscript{7} Reports regarding the mechanisms by which adenosine induces vasodilation are controversial. On one hand, adenosine has been suggested to induce vasodilation independent of the endothelium by direct action of P1 receptors on smooth muscle cells.\textsuperscript{8,9} On the other hand, in vitro and in vivo data also suggest a close interaction between endothelial P1 receptor stimulation and NO release.\textsuperscript{10-13} Furthermore, in vitro data have suggested that prostaglandins could be involved as a second messenger of adenosine by stimulating NO production.\textsuperscript{14} Single inhibition of NO\textsuperscript{15,16} or prostaglandin\textsuperscript{17-19} formation has little or no effect on exercising limb blood flow, but simultaneous inhibition of NO and prostaglandin formation lowers blood flow to exercising limbs by ≈30\%,\textsuperscript{19,20} suggesting that there is an interaction between the prostaglandin and NO systems. It remains unknown whether adenosine contributes to blood flow regulation via direct stimulation of smooth muscle cell P1 receptors and/or by stimulation of endothelial second messengers systems, eg, prostaglandins and NO. Furthermore, the possible interaction between prostaglandins and NO in response to adenosine stimulation has not been examined in humans.

The origin of intraluminal adenosine is thought to be the endothelium, degradation of intraluminal ATP, and/or the interstitial compartment. In vitro evidence from studies on laboratory animals suggests that adenosine is increased in the venous efflux of contracting muscle,\textsuperscript{21,22} as well as during

Received February 20, 2009; first decision March 12, 2009; revision accepted April 8, 2009.
From the Copenhagen Muscle Research Centre (S.P.M., M.N., P.T., B.S., Y.H.), Rigshospitalet; and the Institute of Exercise and Sports Sciences (M.N., Y.H.), University of Copenhagen, Denmark.
Correspondence to Stefan P. Mortensen, Copenhagen Muscle Research Centre, Rigshospitalet, Sector 7652, Blegdamsvej 9, 2100 Copenhagen Ø, Denmark. E-mail stefan@sport.dk
© 2009 American Heart Association, Inc.
Hypertension is available at http://hyper.ahajournals.org

DOI: 10.1161/HYPERTENSIONAHA.109.130880
hypoxia and ischemia. However, because of rapid uptake by red blood cells, as well as degradation in plasma, the half-life of adenosine is estimated to be <1 second. Therefore, despite the use of stop solutions and rapid centrifugation, adenosine determinations from blood samples are difficult to interpret, and the actual adenosine concentrations in the vascular beds supplying and draining resting and contracting muscles in humans remain unclear. Intravascular microdialysis probes allow in vivo determinations of actual adenosine levels in plasma without the problem of blood sampling and centrifugation. In the current study, this technique was applied to assess femoral arterial and femoral venous concentrations of adenosine.

Accordingly, the purpose of this investigation was to determine the following: (1) whether there is interaction or redundancy of adenosine with prostaglandins and NO during exercise in the control of blood flow; (2) whether adenosine-induced vasodilation is mediated by the action of prostaglandins and/or NO; and (3) arterial and venous plasma adenosine concentrations at rest and during exercise with the use of intravascular microdialysis probes. We hypothesized that adenosine, prostaglandins, and NO act in synergy to contribute to skeletal muscle hyperemia.

Materials and Methods

Twenty-four physically active male subjects with a mean (±SD) age of 24±1 years, body weight of 80±2 kg, height of 185±1 cm, and VO₂max of 48±1 mL/min per kilogram participated in 3 studies, whereas 6 of the subjects participated in 2 of the studies separated by 2 to 4 weeks. The subjects were informed of any risks and discomforts associated with the experiments before giving their informed, written consent to participate. The study was approved by the Ethics Committee of Copenhagen and Frederiksberg (KF 01-0139/6 and KF 11289201), and all of the procedures followed were in accordance with institutional guidelines.

The subjects refrained from caffeine, alcohol, and exercise for 24 hours before the study. On the day of the experiment, they arrived at the laboratory 1 hour before the experiment after a light breakfast. Catheters were placed into the femoral artery and vein of the experimental leg under local anesthesia, and a thermistor was temperature obtained in the femoral vein. Leg mass was calculated using an ABL725 analyzer (Radiometer) and were corrected for probe recovery, as described previously. Dalteparin (25 IU/mL; Fragmin, Pfizer) was also added to the perfusate to avoid clotting on the membrane. Adenosine and theophylline in perfusate and dialysate were analyzed with high-pressure liquid chromatography without previous treatment of samples.

Study 1: The Effect of Adenosine Receptor Blockade and/or NO and Prostaglandin Inhibition on Skeletal Muscle Hyperemia

In study 1, the subjects completed 1 minute of passive one-leg knee-extensor exercise followed by 4 minutes of active exercise (24±1 watts [W]; ie, 30% of peak workload) under the following conditions: saline (control) and theophylline (P1 receptor blocker; Sygehus Apotekerne; n=7) or control, indomethacin (INDO; cyclooxygenase inhibitor)+N⁵-mono-methyl-L-arginine (l-NMMA; NO synthase [NOS] inhibitor), and INDO+ l-NMMA+theophylline (n=7; Figure 1). Saline, INDO (120 μg/min per kilogram of leg mass; Confortid, Alphapharma), and/or l-NMMA (4 mg/min per kilogram of leg mass; Confortid, Alphapharma), and/or L-NMMA (4 mg/min per kilogram of leg mass) were infused into the femoral artery and vein before and during (2.5 and 5.5 minutes) exercise. LBF was measured 5 minutes before exercise, 5 minutes into exercise, and 5 minutes into the recovery period.

Study 2: Role of Prostaglandin and NO in Adenosine-Induced Vasodilation

In study 2, adenosine (ITEM development AB) was infused into the femoral artery during 2 stepwise 3-minute infusion periods (0.15±0.1 and 0.30±0.1 μmol/min per kilogram of leg mass, respectively) under the following conditions: (1) control, (2) INDO (n=7) or l-NMMA (n=8), and (3) INDO+l-NMMA (n=6). All of the tests were separated by a 30-minute rest period. Saline, INDO, and/or l-NMMA were infused into the femoral artery for 5 minutes before adenosine infusion (loading dose) and at a lower rate (maintenance dose) during the 6 minutes of adenosine infusion. Blood samples (1 to 5 mL) were drawn simultaneously from the femoral artery (nonexperimental leg) and vein before and during (2.5 and 5.5 minutes) adenosine infusion.

Study 3: Leg Plasma Adenosine Concentrations Before, During, and After Exercise

In study 3, the subjects (n=8) performed one-leg knee-extensor exercise for 10 minutes at 16±1 and 32±2 W (ie, 20% and 40% of peak workload). Microdialysate was collected for 10 minutes before the start of exercise, during exercise, and during the 10-minute recovery period. LBF was measured 5 minutes before exercise, 5 minutes into exercise, and 5 minutes into the recovery period.

In study 2, LBF was measured with ultrasound Doppler (Vivid 7, GE Healthcare), whereas LBF in the 2 studies that involved exercise (studies 1 and 3) was measured by the constant-infusion thermodilution method. Heart rate was obtained from an ECG, whereas arterial pressures were monitored with transducers positioned at the level of the heart (Pressure Monitoring kit, Baxter). Blood gases, hemoglobin, and lactate concentrations were measured using anABL725 analyzer (Radiometer) and were corrected for temperature obtained in the femoral vein. Leg mass was calculated from whole-body dual-energy x-ray absorptiometry scanning (Prodigy, GE Healthcare). The microdialysis probes were perfused with isotonic saline (0.9% NaCl) at a rate of 5 μL/min. [2-14C]adenosine (<0.1 mCi/mL) was added to the perfusate, and the obtained data were corrected for probe recovery, as described previously. Dalteparin (25 IU/mL; Fragmin, Pfizer) was also added to the perfusate to avoid clotting on the membrane. Adenosine and theophylline in perfusate and dialysate were analyzed with high-pressure liquid chromatography without previous treatment of samples.

Statistical Analysis

A 2-way, repeated-measures ANOVA was performed to test significance within and between trials. After a significant F test, pairwise differences were identified using Tukey’s honestly significant difference posthoc procedure. Differences between the single- and double-blockade trials during adenosine infusion were assessed with a t test. The significance level was set at P<0.05, and data are mean±SEM unless otherwise indicated.
**Study 1: The effect of adenosine receptor blockade and/or prostaglandin and nitric oxide inhibition on skeletal muscle hyperemia.**

![Image](https://hyper.ahajournals.org/)

**Study 2: Role of prostaglandins and nitric oxide in adenosine induced vasodilation**

![Image](https://hyper.ahajournals.org/)

**Study 3: Leg plasma adenosine concentrations before, during and after exercise**

![Image](https://hyper.ahajournals.org/)

**Results**

**Effect of Theophylline on Leg and Systemic Variables Before and During Exercise**

Theophylline infusion increased resting LBF, vascular conductance, and O2 delivery (P<0.05), whereas there was no difference in mean arterial pressure (MAP; Figure 2). During exercise, LBF, vascular conductance, and leg O2 delivery were lower after theophylline infusion (P<0.05), whereas there was no difference in MAP. The lower O2 delivery during exercise was paralleled by an increase in a-vO2 difference (P<0.05), resulting in a maintained leg VO2. Blood variables are displayed in Table S1 (available in the online data supplement at http://hyper.ahajournals.org).

**Effect of INDO, L-NMMA, and Theophylline on Leg and Systemic Variables Before and During Exercise**

At rest and during exercise, LBF, vascular conductance, and leg O2 delivery were lower with double and triple blockade compared with control (P<0.05), whereas there was no difference between double and triple blockade. Blood pressures remained unchanged between trials. The lower O2 delivery during double and triple blockade was paralleled by an increase in a-vO2 difference, but leg VO2 remained lower during exercise in both trials compared with the control (P<0.05).

**Effect of INDO on Leg and Systemic Variables at Baseline and During Adenosine Infusion**

Resting LBF was 0.30±0.03 L/min during control conditions and did not change with infusion of INDO (0.23±0.01 L/min; Figure 3). Adenosine infusion increased LBF to 1.75±0.22 and 2.58±0.27 L/min, but it was ≈48% lower with coinfusion of INDO (0.89±0.10 and 1.31±0.16 L/min; P<0.05). There was no change in arterial pressure when adenosine was infused alone (90±2 versus 88±3 and 89±3 mm Hg) or in combination with INDO (93±2 versus 93±2 and 93±2 mm Hg). Therefore, leg vascular conductance (LVC) was lower when adenosine was coinflused with INDO during the low (20±2 versus 9±1 mL/min per mm Hg in control versus INDO, respectively) and high (29±3 versus 14±2 mL/min per mm Hg, respectively) adenosine infusion rates. Adenosine infusion increased leg VO2 during INDO (P<0.05), but there was no difference between trials (Table S2).

**Effect of L-NMMA on Leg and Systemic Variables at Baseline and During Adenosine Infusion**

Infusion of L-NMMA lowered basal LBF from 0.32±0.04 to 0.22±0.04 L/min (P<0.05). Adenosine infusion increased
LBF to 1.93±0.40 and 2.71±0.46 L/min during control conditions, but it was lower with coinfusion of l-NMMA (0.85±0.11 and 1.58±0.22 L/min; P<0.05). Accounting for the change in blood flow during basal conditions, the adenosine-induced increase in LBF was reduced by 44±10% and 35±7% with coinfusion of l-NMMA during the low and high adenosine infusion rates, respectively. MAP increased from 89±3 mm Hg during baseline conditions to 91±3 and 93±3 mm Hg when adenosine was coinfused with l-NMMA and was higher than when adenosine was infused during control conditions (P<0.05). LVC was lower with l-NMMA inhibition during basal conditions (4±0 versus 3±1 mL/min per mm Hg; P<0.05). During adenosine infusion, LVC was lower with l-NMMA (9±2 and 18±3 mL/min per mm Hg, respectively) compared with control (25±4 and 32±3 mL/min per mm Hg, respectively; P<0.05). There was no difference in leg \( V_o_2 \) with adenosine and/or l-NMMA infusion.

**Effect of Combined INDO and l-NMMA on Leg and Systemic Variables at Baseline and During Adenosine Infusion**

Combined infusion of INDO and l-NMMA lowered basal LBF from 0.36±0.02 to 0.20±0.01 L/min (P<0.05). Aenosine infusion increased LBF to 1.80±0.26 and 2.42±0.26 L/min during control conditions, but it was lower with coinfusion of INDO and l-NMMA (0.50±0.04 and 0.94±0.13 L/min; P<0.05). Accounting for the change in blood flow during basal conditions during combined INDO and l-NMMA infusion, the adenosine-induced increase in LBF was reduced by 69±6% and 59±7% compared with control. MAP increased from 89±3 mm Hg at baseline to 91±3 and 93±3 mm Hg when adenosine was coinfused with l-NMMA (P<0.05) and was higher compared to when adenosine was infused during control conditions (P<0.05). l-NMMA infusion reduced LVC at baseline (4±0 versus 3±1 mL/min per mm Hg; P<0.05) and was lower during adenosine infusion (9±2 and 18±3 mL/min per mm Hg) compared with control conditions (25±4 and 32±3 mL/min per mm Hg; P<0.05). There was no difference in leg \( V_o_2 \) with adenosine and/or INDO+1-L-NMMA infusion.

**Plasma Adenosine Concentrations Before, During, and After One-Leg Knee-Extensor Exercise**

Exercise increased LBF from 0.2±0.0 to 2.5±0.2 L/min (16 W) and 4.1±0.3 L/min (32 W) and was 0.4±0.0 L/min during the recovery period. Arterial plasma adenosine concentration was 31±3 nmol/L at rest, 30±6 and 38±13 nmol/L during exercise (16 and 32 W, respectively), and 28±9 nmol/L during the recovery period (Figure 4). Venous plasma adenosine concentration was 18±6, 30±12, 28±12, and 26±7 nmol/L, respectively. There was no difference between arterial and venous adenosine concentrations.

**Discussion**

There were several novel findings in the present study that support a role for adenosine in regulating skeletal muscle
blood flow by increasing prostaglandin and NO formation: (1) adenosine receptor blockade alone, or inhibition of prostaglandin and NO formation combined, lowered vascular conductance during exercise, whereas simultaneous adenosine receptor blockade with inhibition of prostaglandin and NO synthesis did not induce an additive reduction in vascular conductance during exercise; (2) the vasodilatory response to adenosine was inhibited by single blockade of either prostaglandins synthesis or NO synthesis; and (3) combined inhibition of prostaglandin and NO formation produced an additive reduction in the vasodilatory response to adenosine. Furthermore, the unchanged plasma adenosine concentration during exercise indicates that interstitial adenosine may be a more important contributor than luminal adenosine to blood flow regulation.

Adenosine Contributes to Skeletal Muscle Blood Flow Regulation by Stimulating Prostaglandin and NO Formation

The present findings that inhibition of P1 receptors lowered exercise hyperemia by 14% and that simultaneous inhibition of the NO and prostaglandin systems lowered exercise hyperemia by 29% are generally consistent with previous findings. Adenosine could, however, explain ≤30% of the exercise hyperemia, because the dose of theophylline used in the present study has been found to inhibit the vasodilatory response to adenosine by 55%. A major novel observation was that adding P1 receptor blockade to simultaneous inhibition of prostaglandins and NO did not further reduce vascular conductance. The lack of additive effect suggests that there is not redundancy but rather an interaction among the adenosine, prostaglandin, and NO systems by which the vasodilatory effect of adenosine is mediated by prostaglandins and NO.

Adenosine Vasodilator Response Is Mainly Prostaglandin and NO Mediated

Further investigation into the mechanisms by which adenosine induces vasodilatation demonstrated that the prostaglandin and NO systems are mediators of adenosine-induced vasodilation. Our finding of a role of NO in mediating adenosine-induced vasodilation is in agreement with previous findings.

Figure 3. Change in LBF, MAP, and leg vascular conductance from resting conditions to intra-arterial adenosine infusion with and without coinfusion of INDO and/or L-NMMA. Data are mean±SEM for 7 to 8 subjects. *Significantly different from control, P<0.05.

Figure 4. Femoral arterial and venous plasma adenosine concentrations before, during, and after one-leg knee extensor exercise. Data are mean±SEM for 8 subjects.
In the human forearm, but this is the first study to demonstrate a close interaction between adenosine and prostaglandins in humans. Interestingly, the inhibition in the adenosine-induced increase in conductance when prostaglandins and NO were inhibited simultaneously (≈71%) was lower than the combined effect of single prostaglandin (≈56%) and single NO (≈41%) inhibition, suggesting that there is an interaction between the prostaglandin and NO systems. Consistent with this observation, an in vitro study clearly demonstrated that prostaglandin inhibition reduced NO formation in response to adenosine stimulation. This observation, which suggests that prostaglandins promote NO synthesis, is also supported by the greater reduction in vascular conductance with inhibition of prostaglandins compared with inhibition of NOS. Therefore, the present findings suggest that the vasodilator response to adenosine, to a large extent, is mediated by prostaglandins and NO and that part of the increased NO formation could be prostaglandin mediated (Figure 5). The proposed direct action of interstitial adenosine on adenosine receptors on the smooth muscle cells consequently appears to be less important.

Although l-NMMA alone or in combination with INDO alters baseline flow, we did not infuse a vasodilator to correct for this response, because it has clearly been demonstrated that baseline correction with the NO donor sodium nitroprusside does not alter the blood flow response to adenosine during NOS inhibition. This is consistent with observations during exercise and NOS inhibition, where a change in baseline flow does not alter blood flow during exercise.

**Arterial and Venous Plasma Adenosine Concentrations During Exercise**

To avoid the confounding factors of adenosine degradation and uptake in blood samples, a novel type of intravascular microdialysis probe was used to determine plasma adenosine in vessels supplying and draining active muscle in vivo. The similar arterial and venous adenosine concentrations suggest that adenosine is not released from the interstitium of exercising muscle. This observation is in contrast to observations in the isolated dog limb but in agreement with the observation that the endothelium is an effective barrier for adenosine. Furthermore, plasma ATP increases during exercise, but adenosine does not play a role in ATP-induced vasodilation, and the breakdown products of ATP are, therefore, likely to be rapidly taken up by erythrocytes. A methodological limitation may be that the rapid uptake and degradation of adenosine require sampling from veins closer to the active muscle.

**Perspectives**

The current finding that adenosine regulates the formation of prostaglandins and NO in the skeletal muscle vasculature and that prostaglandins regulate NO formation suggests that the interaction among these 3 vasodilators may be of importance for proper regulation of peripheral vascular tone. The present results also suggest that interstitial adenosine may be more important than luminal adenosine, and an essential task in the future is to reveal the cellular origin of interstitial adenosine, as well as what physiological stimuli that lead to adenosine formation.

The current finding that the vasodilatory effect of adenosine is mainly mediated by the formation of prostaglandins and NO suggests that, when the formation of NO is impaired in hypertension, this may affect also the ability of adenosine to induce vasodilation. In future studies it will be important to examine the mechanism underlying the impaired vasodilator response to adenosine in patients with essential hypertension, including the aspect of whether formation of NO and prostaglandins in response to adenosine is impaired.
Sources of Funding
This study was supported by a grant from the Lundbeck Foundation and the Danish Medical Research Council. S.P.M. was supported by a grant from the Copenhagen Hospital system.

Disclosures
None.

References
Adenosine Contributes to Blood Flow Regulation in the Exercising Human Leg by Increasing Prostaglandin and Nitric Oxide Formation

Stefan P. Mortensen, Michael Nyberg, Pia Thaning, Bengt Saltin and Ylva Hellsten

Hypertension. 2009;53:993-999; originally published online May 11, 2009; doi: 10.1161/HYPERTENSIONAHA.109.130880

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2009 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/53/6/993

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2009/05/04/HYPERTENSIONAHA.109.130880.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/
ONLINE SUPPLEMENT

ADENOSINE CONTRIBUTES TO BLOOD FLOW REGULATION IN THE
EXERCISING HUMAN LEG BY INCREASING
PROSTAGLANDIN AND NITRIC OXIDE FORMATION

Stefan P. Mortensen¹, Michael Nyberg¹,², Pia Thaning¹,
Bengt Saltin¹ & Ylva Hellsten¹,²

¹The Copenhagen Muscle Research Centre, Rigshospitalet, Denmark
²Institute of Exercise and Sports Sciences, University of Copenhagen, Denmark

Short title: Role of adenosine in blood flow regulation

Correspondence to:
Stefan P. Mortensen
The Copenhagen Muscle Research Centre
Rigshospitalet, Section 7652
Blegdamsvej 9, 2100 Copenhagen Ø, Denmark
Phone +45 35457552
Fax +45 35457634
E-mail: stefan@sport.dk
Tabel S1. Blood variables before and during exercise with and without inhibition of adenosine receptors, prostaglandins and/or nitric oxide

<table>
<thead>
<tr>
<th>Blood Variable</th>
<th>Adenosine receptor blockade trial</th>
<th>Prostaglandin, NO and adenosine receptor blockade trial</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control (0.9% NaCl)</td>
<td>TEO</td>
</tr>
<tr>
<td></td>
<td>Rest</td>
<td>Exercise</td>
</tr>
<tr>
<td>PO2 (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>97±2</td>
<td>101±3</td>
</tr>
<tr>
<td>v</td>
<td>31±1</td>
<td>26±2†</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>15.1±0.1</td>
<td>15.6±0.2</td>
</tr>
<tr>
<td>v</td>
<td>14.9±0.1</td>
<td>15.5±0.2</td>
</tr>
<tr>
<td>O2 saturation (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>98±1</td>
<td>98±0</td>
</tr>
<tr>
<td>v</td>
<td>54±4</td>
<td>35±3†</td>
</tr>
<tr>
<td>O2 content (ml/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>201±2</td>
<td>205±3</td>
</tr>
<tr>
<td>v</td>
<td>109±8</td>
<td>73±7†</td>
</tr>
<tr>
<td>PCO2 (mmHg)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>40±1</td>
<td>43±1</td>
</tr>
<tr>
<td>v</td>
<td>50±1</td>
<td>61±2†</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>1.0±0.2</td>
<td>1.8±0.1†</td>
</tr>
<tr>
<td>v</td>
<td>0.9±0.1</td>
<td>1.9±0.2†</td>
</tr>
</tbody>
</table>

a. femoral arterial. v. femoral venous. TEO: theophylline. PO2, PCO2 and pH values were corrected for changes in blood temperature.
* Different from control, † different from baseline conditions, ‡ different from double blockade, P<0.05.
### Tabel S2. Blood variables before and during adenosine infusion with and without inhibition of prostaglandins and/or nitric oxide

<table>
<thead>
<tr>
<th>Blood Variable</th>
<th>Indomethacin (Control (0.9% NaCl))</th>
<th>Indomethacin (L-NMMA)</th>
<th>L-NMMA (Control (0.9% NaCl))</th>
<th>L-NMMA (L-NMMA)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>1.9</td>
<td>3.5</td>
<td>Baseline</td>
</tr>
<tr>
<td>PO2 (mmHg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>101±1</td>
<td>107±4</td>
<td>105±2</td>
<td>100±1</td>
</tr>
<tr>
<td>v</td>
<td>35±3</td>
<td>63±2†</td>
<td>70±1†</td>
<td>34±1</td>
</tr>
<tr>
<td>Hemoglobin (g/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>14.6±0.3</td>
<td>14.7±0.2</td>
<td>14.9±0.2</td>
<td>14.3±0.3</td>
</tr>
<tr>
<td>v</td>
<td>14.7±0.3</td>
<td>14.5±0.2</td>
<td>14.7±0.1</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±0</td>
<td>98±0</td>
<td>98±0</td>
<td>98±0</td>
</tr>
<tr>
<td>v</td>
<td>61±4</td>
<td>91±1*</td>
<td>94±0*</td>
<td>62±2</td>
</tr>
<tr>
<td>O2 content (ml/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>194±4</td>
<td>196±3</td>
<td>198±2</td>
<td>191±4</td>
</tr>
<tr>
<td>v</td>
<td>121±10</td>
<td>178±2</td>
<td>187±3</td>
<td>121±6</td>
</tr>
<tr>
<td>Leg VO2</td>
<td>21±1</td>
<td>33±3</td>
<td>30±6</td>
<td>17±0</td>
</tr>
</tbody>
</table>

a. femoral arterial. v. femoral venous. PO2 values were corrected for changes in blood temperature.

* Different from control, † different from baseline conditions, \( P<0.05 \).