Erythrocyte and the Regulation of Human Skeletal Muscle Blood Flow and Oxygen Delivery
Role of Circulating ATP
José González-Alonso, David B. Olsen, Bengt Saltin

Abstract—Blood flow to contracting skeletal muscle is tightly coupled to the oxygenation state of hemoglobin. To investigate if ATP could be a signal by which the erythrocyte contributes to the regulation of skeletal muscle blood flow and oxygen (O₂) delivery, we measured circulating ATP in 8 young subjects during incremental one-legged knee-extensor exercise under conditions of normoxia, hypoxia, hyperoxia, and CO+normoxia, which produced reciprocal alterations in arterial O₂ content and thigh blood flow (TBF), but equal thigh O₂ delivery and thigh O₂ uptake. With increasing exercise intensity, TBF, thigh vascular conductance (TVC), and femoral venous plasma [ATP] augmented significantly (P<0.05) in all conditions. However, with hypoxia, TBF, TVC, and femoral venous plasma [ATP] were (P<0.05) or tended (P=0.14) to be elevated compared with normoxia, whereas with hyperoxia they tended to be reduced. In CO+normoxia, where femoral venous O₂Hb and (O₂+CO)Hb were augmented compared with hypoxia despite equal arterial deoxygenation, TBF and TVC were elevated, whereas venous [ATP] was markedly reduced. At peak exercise, venous [ATP] in exercising and nonexercising limbs was tightly correlated to alterations in venous (O₂+CO)Hb (r²=0.93 to 0.96; P<0.01). Intrafemoral artery infusion of ATP at rest in normoxia (n=5) evoked similar increases in TBF and TVC than those observed during exercise. Our results in humans support the hypothesis that the erythrocyte functions as an O₂ sensor, contributing to the regulation of skeletal muscle blood flow and O₂ delivery, by releasing ATP depending on the number of unoccupied O₂ binding sites in the hemoglobin molecule. (Circ Res. 2002;91:1046-1055.)

Key Words: skeletal muscle blood flow ■ erythrocytes ■ oxygen sensor ■ oxygen delivery
hemoglobin in arterial and femoral venous blood during a to precisely manipulate the amount of O\textsubscript{2} and CO bound of hypoxia in the presence of hypercapnia, 2 hypoxia alone, 3 and ATP is released from red blood cells with exposure to

This mechanism has never been studied in intact humans.

Thirteen healthy recreationally active subjects (12 males and 1 female, age of 24 ± 3 years, body weight of 75 ± 9 kg, and height of 183 ± 7 cm). The subjects were fully informed of any risks and discomforts associated with the experiments before giving their informed written consent to participate. The studies conformed to the code of Ethics of the World Medical Association (Declaration of Helsinki) and were approved by the Ethics Committee of Copenhagen and Frederiksberg communities.

In the first study, 8 of the subjects completed 3 to 4 four-minute knee-extensor exercise bouts at 27 ± 1, 46 ± 1, 64 ± 2, and 85 ± 2% (±SEM) of their normoxic peak power output (78 ± 5 W; ~60 rpm) under the following conditions: (1) normoxia (inspiratory oxygen fraction, F\textsubscript{IO} \textsubscript{2} 21%); (2) systemic hypoxia (F\textsubscript{IO} \textsubscript{2} ~10%); (3) systemic hyperoxia (F\textsubscript{IO} \textsubscript{2} 100%); and (4) carbon monoxide (CO) breathing combined with normoxia (21 ± 1% circulating carboxyhemoglobin fraction (FCOHb); F\textsubscript{IO} \textsubscript{2} 21%). Each experimental condition was separated by ~1 hour of rest. The normoxic, hypoxic, and hyperoxic trials were counterbalanced across subjects, whereas the CO+normoxia trial was performed last, due to the relatively long elimination period required to return to baseline COHb levels.

The subjects reported to the laboratory at 8 AM, after the ingestion of a normal breakfast. On arrival, they rested in a supine position. Catheters were placed under local anesthesia into the femoral artery and vein of the exercising thigh, and the femoral vein or forearm vein of nonexercising limbs using the Seldinger technique. The femoral arterial and veins catheters were positioned 1 to 2 cm proximal or distal from the inguinal ligament. A thermistor to measure venous blood temperature was inserted through the femoral venous catheter into the closed-circuit system previously described.\textsuperscript{19} CO was administered into the closed-circuit system using calibrated plastic syringes (total CO administered 325 ± 36 mL). During exercise, heart rate and arterial blood pressure were recorded continuously. Thigh blood flow was measured after 60 and 150 seconds of exercise. Arterial and venous blood samples (1 to 3 mL) were withdrawn simultaneously before blood flow measurements.

The other five subjects participated in a second investigation aimed to determine whether intraarterial infusion of ATP in the femoral artery at rest (in the supine position) would increase skeletal muscle blood flow and vascular conductance to the same extent than exercise under normoxic conditions. ATP (Sigma A7699) dissolved in isotonic saline (1 mg/mL) was infused into the femoral artery (FaBF) using a constant infusion technique during stepwise 4 minutes infusion periods with 10 minutes washout intervals between doses. ATP infusion rates given were 200, 300, 400, 1000, 5000, 10000, 16000, and 25000 nmol/min. Furthermore, a bolus injection (1 mL) was used to determine the latency and duration of response to a 2000 nmol/mL dose.

In the first study, femoral venous blood flow (ie, thigh blood flow, TBF; largely indicative of quadriceps muscle blood flow and vascular conductance to the same extent than exercise under normoxic conditions) was determined by the constant infusion technique.\textsuperscript{29} TBF was calculated using a heat balance equation. During the ATP infusion study, TBF was measured in the femoral artery using an ultrasound Doppler.\textsuperscript{30} Femoral arterial blood flow (FaBF=V\textsubscript{mean} · A · 6 · 10\textsuperscript{-6}, L/min) was calculated by multiplying the weighted mean blood velocity (V\textsubscript{mean}, m/s) by the cross-sectional area of the femoral artery (A).\textsuperscript{30} To avoid contribution of blood from the lower leg in either study, an occlusion cuff placed just below the knee was inflated to 260 mm Hg. TBF values in both studies represent the average of two measurements made from 2 to 3 minutes of exercise and 1 to 2 minutes after the start of ATP infusion. Heart rate was obtained from the continuously recorded ECG signal. Arterial blood pressure was continuously monitored by a pressure transducer (Pressure Monitoring Kit, Baxter) inserted in the femoral artery at the level of inguinal region.

[ATP] in plasma and whole blood samples were determined using the luciferin-luciferase technique.\textsuperscript{31} Blood samples (2.7 mL) for determination of either plasma or whole blood [ATP] were obtained using vacuum syringes containing 4.3 mg EDTA (5-monovette, 2.7 mL KE; Sarstedt). For plasma ([ATP] analysis, blood samples in the collection tubes were centrifuged immediately for 30 seconds at 14 000 rpm (4°C; Sigma 1 to 15 K, Osterode am Harz). Plasma was then pipetted into prechilled tubes and frozen down in dry ice. The duration of the whole procedure from blood withdrawal to plasma separation was ~90 seconds. For whole blood [ATP] analysis, 25 μL
Skeletal Muscle Hemodynamics and Circulating ATP Under Normoxic Conditions

TBH, mean arterial pressure, and TVC increased progressively during incremental exercise under normoxic conditions and subsequently returned to values that were not significantly higher than rest after 10 minutes of recovery (Figure 1). With an unchanged ctO₂, during incremental exercise, the gradual elevation in O₂ delivery to the thigh was the sole result of the increase in TBF. Femoral arterial and venous plasma [ATP] did not change in the transitions from rest to 20 W or the transition from rest to passive exercise (n=4), which augmented blood flow by 0.9±0.1 L/min. However, they increased progressively during incremental exercise, being significantly higher at 67 W compared with rest ([ATP]ₐ=997±143 versus 654±110 nmol/L, respectively; [ATP]ₐ=1835±410 versus 642±82 nmol/L, respectively; both P<0.05), and remained elevated after 10 minutes of recovery (≈1500 nmol/L). Thigh arterial and venous plasma ATP, which accounts for the changes in thigh plasma flow, increased progressively from 119±22 to 6350±1567 nmol/min (P<0.05) but declined to ≈1100 (±355) nmol/min after 10 minutes of recovery (Figure 2). In the nonexercising limbs, venous plasma [ATP] also increased from 428±23 nmol/L at rest to 929±171 nmol/L at 67 W (P<0.05) and remained at 912±212 nmol/L after 10 minutes of recovery.

Skeletal Muscle Hemodynamics and Circulating ATP When Exposed to Systemic Hypoxia, Systemic Hyperoxia, and CO in Combination With Normoxia

The exposure to systemic hypoxia and CO+normoxia equally reduced ctO₂ by ≈22% compared with normoxia, whereas the exposure to systemic hyperoxia increased ctO₂ by ≈9% above normoxic levels. No significant changes in ctO₂ were observed during incremental exercise in any of the experimental conditions (Table). Despite the similar decline in arterial ctO₂, O₂Hb, and FO₂Hb in hypoxia and CO+normoxia, arterial O₂ saturation and FO₂Hb+COHb in CO+normoxia were similar to normoxia, but they were significantly reduced in hypoxia (99 versus ≈77%, respectively). In CO+normoxia, thigh O₂ extraction was significantly reduced compared with the other trials (eg, 20 W: 49% versus 54% to 59±6%, respectively; P<0.05), reflecting a high ctO₂ similar to that in normoxia. Concomitant to changes in ctO₂ and ctO₂, TBF and TVC were elevated with hypoxia (11% to 14%) and CO+normoxia (20% to 25%), but were reduced with hyperoxia (8% to 11%; Figure 1). The compensatory adjustments in TBF and a-VO₂diff with hypoxia, hyperoxia, and CO+normoxia afforded similar thigh O₂ delivery and VO₂ among the 4 experimental conditions (Figure 1).

At rest, femoral arterial and venous plasma [ATP] with normoxia, hypoxia, hyperoxia, and CO+normoxia ranged from 495 to 838 nmol/L (P=NS). During incremental exercise, plasma [ATP] and thigh plasma ATP increased significantly with increasing exercise intensity in all conditions (all P<0.001; Figures 2 and 3). Moreover, venous plasma [ATP] at a given work rate tended (P=0.14) to be higher with...
### Femoral Blood Variables at Rest During Incremental Knee-Extensor Exercise and After 10 Minutes of Recovery When Exposed to Normoxia, Hypoxia, Hyperoxia, and CO Inhalation Combined With Normoxia

<table>
<thead>
<tr>
<th>Power Output, W</th>
<th>Normoxia</th>
<th></th>
<th></th>
<th>Hypoxia</th>
<th></th>
<th></th>
<th>CO + Normoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Hb], mmol/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>8.3 ± 0.2</td>
<td>8.4 ± 0.2</td>
<td>8.3 ± 0.2</td>
<td>8.3 ± 0.2</td>
<td>8.5 ± 0.2</td>
<td>8.2 ± 0.2</td>
<td>8.3 ± 0.2</td>
</tr>
<tr>
<td>v</td>
<td>8.3 ± 0.2</td>
<td>8.4 ± 0.2</td>
<td>8.3 ± 0.2</td>
<td>8.4 ± 0.2</td>
<td>8.6 ± 0.2</td>
<td>8.1 ± 0.2</td>
<td>8.3 ± 0.2</td>
</tr>
<tr>
<td>Hct, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>41.1 ± 1.3</td>
<td>41.2 ± 1.3</td>
<td>41.3 ± 0.9</td>
<td>41.6 ± 0.9</td>
<td>42.3 ± 0.9</td>
<td>40.7 ± 0.9</td>
<td>41.4 ± 1.1</td>
</tr>
<tr>
<td>v</td>
<td>41.3 ± 1.0</td>
<td>41.6 ± 1.0</td>
<td>41.6 ± 1.0</td>
<td>41.5 ± 1.0</td>
<td>42.3 ± 1.0</td>
<td>40.7 ± 1.0</td>
<td>41.3 ± 1.0</td>
</tr>
<tr>
<td>COHb, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>1.8 ± 0.1</td>
<td>1.8 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>1.7 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.6 ± 0.1</td>
<td>1.7 ± 0.1</td>
</tr>
<tr>
<td>v</td>
<td>1.5 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>1.1 ± 0.2</td>
<td>1.0 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>O2Hb, mL/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>180 ± 5</td>
<td>181 ± 4</td>
<td>181 ± 5</td>
<td>185 ± 4</td>
<td>179 ± 4</td>
<td>152 ± 7</td>
<td>147 ± 7</td>
</tr>
<tr>
<td>v</td>
<td>125 ± 9</td>
<td>70 ± 7*</td>
<td>66 ± 7*</td>
<td>60 ± 6*</td>
<td>54 ± 6*</td>
<td>110 ± 6</td>
<td>52 ± 5*</td>
</tr>
<tr>
<td>F02Hb, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>96.8 ± 0.3</td>
<td>96.8 ± 0.2</td>
<td>97.0 ± 0.2</td>
<td>97.0 ± 0.2</td>
<td>96.9 ± 0.2</td>
<td>91.4 ± 2.7</td>
<td>77.6 ± 2.5*</td>
</tr>
<tr>
<td>v</td>
<td>66.9 ± 3.7</td>
<td>37.0 ± 3.2*</td>
<td>35.1 ± 2.9*</td>
<td>38.1 ± 2.7*</td>
<td>27.9 ± 1*</td>
<td>74.7 ± 2.8</td>
<td>59.3 ± 2.7</td>
</tr>
<tr>
<td>F(O2 + CO)Hb, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>98.6 ± 0.2</td>
<td>98.5 ± 0.1</td>
<td>98.7 ± 0.2</td>
<td>98.7 ± 0.1</td>
<td>98.5 ± 0.1</td>
<td>91.4 ± 2.7</td>
<td>78.3 ± 2.4</td>
</tr>
<tr>
<td>v</td>
<td>66.8 ± 3.7</td>
<td>38.2 ± 3.3*</td>
<td>36.3 ± 3.0*</td>
<td>38.2 ± 2.8</td>
<td>28.9 ± 2.5*</td>
<td>60.9 ± 2.7</td>
<td>28.8 ± 2.6*</td>
</tr>
<tr>
<td>Pao2, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>101 ± 2</td>
<td>101 ± 2</td>
<td>106 ± 2</td>
<td>102 ± 2*</td>
<td>109 ± 2*</td>
<td>104 ± 2*</td>
<td>102 ± 2*</td>
</tr>
<tr>
<td>v</td>
<td>37 ± 3</td>
<td>23 ± 1*</td>
<td>23 ± 1*</td>
<td>21 ± 1*</td>
<td>22 ± 1*</td>
<td>44 ± 4*</td>
<td>37 ± 1*</td>
</tr>
<tr>
<td>PtcO2, mL/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>183 ± 5</td>
<td>184 ± 4</td>
<td>184 ± 5</td>
<td>185 ± 5</td>
<td>188 ± 4</td>
<td>182 ± 4</td>
<td>175 ± 7</td>
</tr>
<tr>
<td>v</td>
<td>126 ± 9</td>
<td>70 ± 7*</td>
<td>67 ± 7*</td>
<td>61 ± 6*</td>
<td>55 ± 6*</td>
<td>137 ± 6*</td>
<td>111 ± 6</td>
</tr>
<tr>
<td>SaO2, %</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>98.6 ± 0.2</td>
<td>98.5 ± 0.1</td>
<td>98.7 ± 0.2</td>
<td>98.7 ± 0.1</td>
<td>98.5 ± 0.1</td>
<td>82.8 ± 2.8</td>
<td>78.0 ± 2.5*</td>
</tr>
<tr>
<td>v</td>
<td>68.0 ± 3.8</td>
<td>37.5 ± 3.3*</td>
<td>35.6 ± 2.9*</td>
<td>32.1 ± 2.7*</td>
<td>28.2 ± 2.5*</td>
<td>75.9 ± 2.9</td>
<td>60.3 ± 2.7</td>
</tr>
<tr>
<td>Pco2, mm Hg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>39 ± 1</td>
<td>40 ± 1</td>
<td>39 ± 1</td>
<td>39 ± 1</td>
<td>39 ± 1</td>
<td>36 ± 1</td>
<td>36 ± 1</td>
</tr>
<tr>
<td>v</td>
<td>46 ± 1</td>
<td>55 ± 1*</td>
<td>59 ± 1*</td>
<td>63 ± 1*</td>
<td>70 ± 2*</td>
<td>44 ± 2</td>
<td>42 ± 1</td>
</tr>
<tr>
<td>pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>7.41 ± 0.01</td>
<td>7.40 ± 0.01</td>
<td>7.39 ± 0.01</td>
<td>7.37 ± 0.01*</td>
<td>7.37 ± 0.01*</td>
<td>7.43 ± 0.01*</td>
<td>7.40 ± 0.01*</td>
</tr>
<tr>
<td>v</td>
<td>7.37 ± 0.01</td>
<td>7.33 ± 0.01*</td>
<td>7.30 ± 0.01*</td>
<td>7.27 ± 0.01*</td>
<td>7.21 ± 0.01*</td>
<td>7.34 ± 0.01</td>
<td>7.40 ± 0.01</td>
</tr>
</tbody>
</table>

**Note:** Values are mean ± SD, except where otherwise indicated.*P < 0.05 vs. rest; †P < 0.05 vs. hypoxia; ‡P < 0.05 vs. hyperoxia; §P < 0.05 vs. CO inhalation.
hypoxia compared with the other conditions (Figure 2). The increases in venous plasma [ATP] with incremental exercise in all conditions were inversely related to declines in femoral venous FO2Hb and O2 saturation (Figure 3). Plasma [ATP] in exercising limb at rest, during peak exercise, and after 10 minutes of recovery tended to be higher than that in the nonexercising limbs (P=0.068; main effect; Figure 4). During peak exercise, venous plasma [ATP] in the exercising and nonexercising limbs was closely related to alterations in venous F(O2+CO2)Hb (r²=0.93 and r²=0.96, respectively; P<0.01; Figure 5). Red blood cell [ATP] in the femoral artery and femoral vein did not change significantly with either increasing exercise intensity or altered blood oxygenation [1.7 to 1.8 (±0.1) mmol/L]. In all experimental conditions, arterial and femoral venous creatine kinase concentrations were not different, and remained unaltered with increasing exercise intensity and during recovery from exercise [≈100 (±13) U/L].

**Skeletal Muscle Hemodynamics With Infracrural Artery ATP Infusion**

Intraarterial infusion of ATP in resting normoxic conditions resulted in significant increases in TBF, TVC, and heart rate, without altering mean arterial pressure or blood flow in the control thigh (Figure 6). The constant infusion of ATP did not produce a significant increase in plasma [ATP] after 2 minutes of the start of infusion. However, when accounting for the increase in thigh plasma flow, thigh venous plasma ATP increased from 0.4±0.1 to 4.6±1.7 μmol/min (P<0.05). The increase in TBF paralleled a proportional drop in thigh O2 extraction (from 27±1% to 2±1%; P<0.05), allowing the maintenance of thigh VO2 in the face of a 10-fold increase in O2 delivery. The bolus infusion of 2000 mmol/L of ATP, which augmented arterial plasma [ATP] from 1304±45 to 2652±101 mmol/L, increased TBF in less than 10 seconds reaching a value of 2.3±0.1 L/min after ≈30 seconds (baseline TBF 0.4±0.1 L/min).

**Discussion**

There are several novel findings in this study that, on one hand, indicate a tight coupling between alterations in circulating plasma ATP and changes in the oxygenation and carboxylation state of hemoglobin and, on the other hand, demonstrate the physiological relevance of such variations in circulating ATP: (1) the progressive increases in femoral venous plasma [ATP] during incremental exercise with normoxia, hypoxia, hyperoxia, and CO+normoxia closely mirrored the declines in femoral venous O2Hb; (2) differences in
venous plasma [ATP] among conditions were strongly related to variations in venous $F(O_2+CO)Hb$ ($r^2=0.93$); (3) at peak exercise, venous plasma [ATP] in the nonexercising limbs tended to be lower than in the exercising limbs, being strongly correlated to venous $F(O_2+CO)Hb$ ($r^2=0.96$); (4) CO+normoxia, which produced the same decline in $ctO_2$ than hypoxia but increased femoral venous $O_2Hb$ and $(O_2+CO)Hb$, resulted in an abrupt lowering in plasma [ATP]; and (5) intrafemoral artery infusion of ATP at rest in normoxia evoked similar increases in TBF and TVC than those observed during dynamic exercise. Together, our results support the hypothesis that the erythrocyte functions as an $O_2$ sensor, contributing to the regulation of local skeletal muscle blood flow and $O_2$ delivery by releasing ATP depending on the number of unoccupied $O_2$ binding sites in the hemoglobin molecule.

**Exercising Skeletal Muscle Blood Flow Responds to Changes in the Affinity and Amount of $O_2$ Bound to Hemoglobin, Rather Than to Changes in the Amount of $O_2$ Dissolved in Blood**

The present elevations in exercising TBF and TVC with hypoxia and CO+normoxia, and reductions with hyperoxia, are generally consistent with previous reports producing large changes in $ctO_2$. The main difference compared with our recent studies was that with greater herein COHb levels in the CO+normoxia trial (22% versus 18%) thigh $O_2$ delivery did not increase significantly, and that with lower herein $FIO_2$ levels in the hypoxia trial (10% versus 12%) thigh $O_2$ delivery tended to decline somewhat at high work rates. In every study, however, thigh $V_{O_2}$ was remarkably similar among conditions owing to compensatory adjustments in TBF and $a-vO_2$diff. These hemodynamic data illustrate the ability of the circulatory system in adjusting skeletal muscle blood flow so that total $O_2$ flux from muscle capillaries to mitochondria is maintained in conditions of vastly different $O_2$ gradients. Such precise circulatory regulation is thought to be controlled by extracellular and cellular $O_2$ sensors, which are capable of changing vascular responses in contracting and resting muscle in accordance with $O_2$ availability.

The present results shed more light into the quest for the primary extracellular site of $O_2$ sensing. Collectively, our present and previous reports using normoxia, hypoxia, anemia, hyperoxia, and CO+normoxia as interventions provide compelling evidence that elevations in TBF and TVC during steady state exercise are independent of pronounced alterations in $Pao_2$ (40 to 540 mm Hg), but are closely linked to reductions in arterial $O_2Hb$ and altered venous $O_2Hb$. New evidence from the comparison of present and previous results also argues against the idea that skeletal muscle hemodynamics carefully senses changes in $O_2$ dissolved in arterial blood. In the present study, we observed that hyperoxia that elevated $Pao_2$ to
\[ H_1 \text{ mm Hg}, \] lowered TBF by \( 0.4 \text{ L/min (9\%)} \) compared with normoxia. This contrasts sharply to our previous observation that CO hyperoxia increased TBF by \( 1.0 \text{ L/min (33\%)} \) compared with normoxia despite the similarly elevated \( \text{PaO}_2 \). Therefore, it seems that the main vascular O2 sensor locus lies in the erythrocyte itself, rather than in the \( \text{PO}_2 \)-sensitive regions of the endothelium or vascular smooth muscle. It is worth noting that intracellular O2 markers such as \( \text{PO}_2 \) and \( \text{MbO}_2 \) saturation cannot explain the profound increases in TBF with CO inhalation because quadriceps muscle \( \text{PO}_2 \) and \( \text{MbO}_2 \) saturation have been shown to be similar in normoxia, \( \text{CO} \)-normoxia, and \( \text{CO} \)-hyperoxia. A central question then relates to how the red blood cell signals the vascular endothelium to increase or decrease skeletal muscle blood flow in direct proportion to \( \text{O}_2 \text{Hb} \). According to the model proposed by Ellsworth et al., the erythrocyte would release ATP in proportion to the offloading of O2 from hemoglobin. Free plasma ATP would in turn bind to purinergic receptors (\( P_2y \)) in the vascular endothelium, resulting in a vasodilatory response mediated by NO- and/or endothelium-derived hyperpolarization factor. Hence, treatments that modify the amount of O2 bound to Hb, such as those used in this study, would be hypothesized to alter plasma [ATP] and muscle blood flow.

Changes in Circulating Plasma ATP Are Closely Coupled to Changes in Oxygenation and Carboxylation State of Hemoglobin
Venous plasma ATP has previously been shown to increase significantly during forearm exercise compared with rest in humans. A major novel finding in this study was that the magnitude of increase in femoral venous plasma [ATP] during incremental knee-extensor exercise varied profoundly with alterations in blood oxygenation, being more tightly coupled to alterations in \( \text{O}_2 \text{Hb} \) and \( \text{(O}_2 \text{CO)} \text{Hb} \) fractions than \( \text{PO}_2 \). This conclusion is based on three observations: (1) the greater venous plasma [ATP] at a given work rate with hypoxia was associated with lower venous \( \text{O}_2 \text{Hb} \), whereas the opposite occurred with \( \text{CO} \)-normoxia, which resulted in higher venous \( \text{O}_2 \text{Hb} \) than hypoxia (Figure 3); (2) the drop in femoral \( \text{PvO}_2 \) cannot explain the higher venous plasma [ATP] in hypoxia compared with \( \text{CO} \)-normoxia as \( \text{PvO}_2 \) values were essentially the same in both trials; and (3) the greater plasma [ATP] in the exercising compared with nonexercising limbs was intimately related to the lower \( \text{(O}_2 \text{CO)} \text{Hb} \) fraction, but weakly related to \( \text{PvO}_2 \) (Figure 5). Thus, the present alterations in plasma [ATP] with vast variations in blood oxygenation and metabolic O2 demands are intimately linked to the offloading of O2 from Hb as well as the binding of CO to Hb, but are poorly related to changes in circulating free O2.

The question then arises as to whether the release of ATP from the red blood cell could account for the present changes in plasma ATP during exercise. Theoretically, the release of
very small amounts of the ATP contained in red blood cells can cause large increases in plasma ATP because the [ATP] in red blood cells is almost 3000-fold higher than in plasma (1.8 mmol/L versus 0.5 to 2 μmol/L, respectively). Several in vitro studies have clearly documented an enhanced ATP release from red blood cells with exposure to hypoxia in the presence of hypercapnia,27 hypoxia, and mechanical deformation.28 Furthermore, a recent in vitro study has elegantly shown that CO, which displaces O₂ from the heme subunits of the Hb molecule and increases the affinity of the remaining subunits for O₂,14 drastically inhibits ATP release from red blood cells.8 In congruence with this in vitro finding, the present correlation at peak exercise is stronger between plasma [ATP] and F(0₂+CO)Hb (r²=0.93 to 0.96) than plasma [ATP] and O₂ saturation (r²=0.68 to 0.86) or plasma [ATP] and F0₂Hb (r²=0.03 to 0.45) (Figure 5). This suggests that not only the lower O₂ offloading, but also the persistent binding of CO to Hb explains the lower venous plasma ATP in CO+/H normoxia compared with the other trials. In this context, the erythrocyte might be seen as the major source accounting for the observed differences in plasma ATP.

However, there are other potential sources of plasma ATP. Although the effects of hypoxia, hyperoxia, and CO+/normoxia on interstitial ATP are unknown, studies with microdialysis in human skeletal muscle in normoxia have shown that adenosine, AMP, ADP, and ATP increase in the muscle interstitium in proportion to the intensity of contraction.25 The augmented interstitial [ATP], which possibly reflects increases in sympathetic activity and/or intracellular ATP turnover, could be another potential source for plasma ATP during exercise. It has long been known that sympathetic nerves corelease ATP with noradrenaline depending on the frequency of stimulation.21 In this light, results from a parallel study showed that hypoxia and CO+/normoxia caused remarkably similar significant increases in muscle sympathetic nerve activity (MSNA) during dynamic handgrip exercise.36 A similar increase in plasma ATP would be expected in CO+/normoxia and hypoxia if sympathetic nerves were major contributors to plasma ATP. However, the observations that venous plasma [ATP] was lower with CO+/normoxia despite MSNA was equally high in CO+/normoxia and hypoxia in our parallel study, suggest that sympathetic nerves were not the main source of plasma ATP.

Muscle cells do not appear to serve as a source for extracellular ATP as intracellular ATP content decreases with incremental exercise in all the present conditions.20 Flow- or shear stress–induced increase in ATP from endothelial cells cannot explain our results either, because CO+/normoxia resulted in lower [ATP] than hypoxia, despite the somewhat higher TBF than in hypoxia. Lastly, the strikingly constant creatine kinase concentration throughout the entire protocol clearly argues against a noticeable leakage of ATP from interstitium. Together results from the present and parallel studies seem to indicate that the erythrocyte is the source accounting for the majority of the changes in plasma ATP.

Similar Increase in Skeletal Muscle Hemodynamics With Intraarterial Infusion of ATP Than During Incremental Exercise

Regardless of the mechanism or the source, the present study demonstrates that elevations in human skeletal muscle blood flow during incremental exercise are, in part, related to progressive increases in plasma ATP. A crucial question is whether the observed increases in plasma ATP during incremental exercise (0.5 to 2 μmol/L or 0.1 to 8 μmol/min) would be sufficient to increase skeletal muscle blood flow, independently of many other vasoactive compounds that also rise during exercise. Underpinning the physiological relevance of circulating ATP, we found that the infusion of ATP in the femoral artery at rest in normoxia evoked a dose-dependent increase in TBF and TVC comparable to that observed during exercise. This finding agrees with the well-characterized vasodilator effect of ATP infusion in the human forearm25,26 and isolated arterioles23,24 and extends previous results by directly comparing the hemodynamic effects of intraarterial infusion of ATP to those associated with endogenous ATP during exercise. The present observation that neither mean blood pressure nor blood flow in the control thigh were altered suggest that the infused ATP primarily acted locally.

Notably, the constant infusion of ATP up to 25 μmol/min in the femoral artery did not result in a corresponding elevation in femoral venous plasma [ATP] (Figure 6). There are several reasons for this finding: (1) ATP rapidly binds to surface receptors21,22; (2) ATP degrades to other nucleotides by the action of actonucleotidas21,22; and (3) changes in plasma flow should be taken into account to quantify the “true” increase in plasma ATP during incremental exercise. The effect of rapid decreases in thigh plasma flow is clearly demonstrated during recovery from exercise, where plasma [ATP] remains high even after 10 minutes, but the rate of thigh ATP outflow declines sharply. Furthermore, plasma [ATP] decreases somewhat when TBF increases with passive exercise or with adenosine infusion. Therefore, these data demonstrate that ATP induces a potent dose-dependent vasodilator response in resting human muscle that is comparable to that observed during incremental exercise.

In conclusion, this study provides evidence in resting and exercising human limbs that variations in plasma [ATP] are intimately coupled to the offloading of O₂ from the hemoglobin molecule to the muscle cells, the alterations in the affinity of O₂ for Hb and the binding of CO to Hb. Moreover, our finding that infusion of ATP at rest caused similar increases in skeletal muscle blood flow than incremental exercise demonstrate the physiological significance of the presently observed elevations in plasma ATP. Taken collectively, the herein results support the theory that the erythrocyte functions as an O₂ sensor (see Figure 7), contributing to the regulation of local skeletal muscle blood flow and O₂ delivery by releasing ATP depending on the number of unoccupied O₂ binding sites in the hemoglobin molecule. Our results together with those by McMahon et al.,37 which indicate a varied release of NO/S-nitrosothiol from human red blood cells as a function of O₂/Hb, clearly underpin the pivotal role of the erythrocyte in the control of vascular tone.
Acknowledgments
This study was supported by a grant from The Danish National Research Foundation (504-14). Special thanks are given to Dr Arne Lundin from Bio Thema AB, Sweden, for his insightful advice in the optimization of the ATP analysis. The excellent technical assistance of Mads Dalsgaard, Carsten Nielsen, Karin Hansen, Birgitte Jessen, Heidi Hansen, and Kristina Møller is acknowledged.

References
Erythrocyte and the Regulation of Human Skeletal Muscle Blood Flow and Oxygen Delivery: Role of Circulating ATP
José González-Alonso, David B. Olsen and Bengt Saltin

Circ Res. 2002;91:1046-1055; originally published online October 31, 2002;
doi: 10.1161/01.RES.000004939.73286.E2

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2002 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/91/11/1046

An erratum has been published regarding this article. Please see the attached page for:
/content/92/6/e61.full.pdf

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research 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 Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/
In an article by González-Alonso et al (Circ Res. 2002;91:1046–1055), “Erythrocyte and the Regulation of Human Skeletal Muscle Blood Flow and Oxygen Delivery: Role of Circulating ATP,” an error appeared in the table. The column headings appearing on page 1050 in the continuation of the table are incorrectly listed as Normoxia and Hypoxia. The correct column headings are Hyperoxia and CO+Normoxia, respectively.