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Effects of ATP-induced leg vasodilation on $\dot{V}O_{2\,peak}$ and leg O_2 extraction during maximal exercise in humans

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Calbet, J. A. L., C. Lundby, M. Sander, P. Robach, B. Saltin, and R. Boushel. Effects of ATP-induced leg vasodilation on $\dot{V}O_{2\,peak}$ and leg O_2 extraction during maximal exercise in humans. *Am J Physiol Regul Integr Comp Physiol* 291: R447–R453, 2006. First published February 16, 2006; doi:10.1152/ajpregu.00746.2005.— During maximal whole body exercise $\dot{V}O_{2\,peak}$ is limited by O_2 delivery. In turn, it is though that blood flow at near-maximal exercise must be restrained by the sympathetic nervous system to maintain mean arterial pressure. To determine whether enhancing vasodilation across the leg results in higher O_2 delivery and leg $\dot{V}O_2$ during near-maximal and maximal exercise in humans, seven men performed two maximal incremental exercise tests on the cycle ergometer. In random order, one test was performed with and one without (control exercise) infusion of ATP (8 mg in 1 ml of isotonic saline solution) into the right femoral artery at a rate of 80 $\mu g \cdot kg$ body $mass^{-1} \cdot min^{-1}$. During near-maximal exercise (92% of $\dot{V}O_{2\,peak}$), the infusion of ATP increased leg vascular conductance (+43%, $P < 0.05$), leg blood flow (+20%, 1.7 l/min, $P < 0.05$), and leg O_2 delivery (+20%, 0.3 l/min, $P < 0.05$). No effects were observed on leg or systemic $\dot{V}O_2$. Leg O_2 fractional extraction was decreased from 85 ± 3 (control) to 78 ± 4% (ATP) in the infused leg ($P < 0.05$), while it remained unchanged in the left leg (84 ± 2 and 83 ± 2%; control and ATP; $n = 3$). ATP infusion at maximal exercise increased leg vascular conductance by 17% ($P < 0.05$), while leg blood flow tended to be elevated by 0.8 l/min ($P = 0.08$). However, neither systemic nor leg peak $\dot{V}O_2$ values where enhanced due to a reduction of O_2 extraction from 84 ± 4 to 76 ± 4%, in the control and ATP conditions, respectively ($P < 0.05$). In summary, the $\dot{V}O_2$ of the skeletal muscles of the lower extremities is not enhanced by limb vasodilation at near-maximal or maximal exercise in humans. The fact that ATP infusion resulted in a reduction of O_2 extraction across the exercising leg suggests a vasodilating effect of ATP on less-active muscle fibers and other noncontracting tissues and that under normal conditions these regions are under high vasoconstrictor influence to ensure the most efficient flow distribution of the available cardiac output to the most active muscle fibers of the exercising limb.

muscle sympathetic nerve activity; fatigue; performance

WHEN O_2 DELIVERY IS ENHANCED by increasing arterial O_2 content (Ca_{O_2}) either by raising blood hemoglobin concentration (3, 8, 14, 16, 43) or with hyperoxia (15, 21, 26, 31), $\dot{V}O_{2\,peak}$ is enhanced. Conversely, when the Ca_{O_2} is reduced by isovolemic hemodilution (14, 22), hypoxia (5), or carbon monoxide administration (15), peak O_2 uptake ($\dot{V}O_{2\,peak}$) is lower. When submaximal exercise is performed at an intensity close to 80% of $\dot{V}O_{2\,max}$ or higher with increased Ca_{O_2}, as a result from human recombinant erythropoietin treatment (3) or hyperoxia (29, 32), $\dot{V}O_2$ and endurance time are enhanced. Consequently, the energy required and not supplied by aerobic metabolism at near-maximal exercise, i.e., at an intensity between 90 and 100% of $\dot{V}O_{2\,max}$, should be provided by anaerobic pathways, as reflected by the high lactate values observed at these exercise intensities. In fact, both the elevation of hematocrit (9, 13, 16, 38) and hyperoxia (29) lower blood lactate concentrations during near-maximal exercise at the same absolute intensity used before the intervention. This implies that during near-maximal exercise there is a mismatch between O_2 delivery and O_2 demand. The underlying cause of this apparent mismatch in O_2 demand and supply is intriguing, given that at ~90% of $\dot{V}O_{2\,max}$ cardiac output can be further elevated (7). Thus despite a functional pumping reserve to increase muscle perfusion and O_2 delivery, this reserve is not exploited. Perhaps the active muscle fibers are not receiving all the O_2 delivery they need due to insufficient vasodilation, since they may be restrained by the sympathetic nervous activity to maintain blood pressure and perfusion (6, 27, 34, 42). Alternatively, the active muscle fibers may be fully vasodilated and the mismatch between O_2 delivery and O_2 uptake may occur reflecting that the maximal vasodilatory response has been attained in the active muscle fibers and that any further increase in $\dot{V}O_2$ would require the recruitment of additional motor units and/or an enhancement of O_2 extraction.

The aim of this study was to test the hypothesis that insufficient vasodilation is limiting leg $\dot{V}O_2$ during near-maximal and maximal whole body exercise in healthy humans. For this purpose, ATP, a potent vasodilating agent and also able to completely abolish sympathetic vasoconstriction at rest as well as during submaximal exercise in the active skeletal muscles (33), was infused at maximal doses into the femoral artery of one leg during near-maximal and maximal exercise on the cycle ergometer in healthy humans. ATP may cause vasodilation by binding to P2Y purinergic receptors on vascular endothelial cells, triggering the release of nitric oxide, prostaglandins, and endothelium-derived hyperpolarizing factor (EDHF) (30). Additionally, ATP may evoke conducted vasodilation (11).

MATERIALS AND METHODS

Subjects. Ten healthy males, age 24 ± 2 yr, height 180 ± 2 cm, and weight 74 ± 2 kg, volunteered to participate in the study. The subjects

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had a $\dot{V}O_{2\,peak}$ of 4.3 ± 0.1 l/min or 55 ± 2 ml·kg^{-1}·min^{-1}, assessed during an incremental test to exhaustion on a cycle ergometer (model Monark 829E; Varberg, Sweden). All subjects were informed about the possible risks and discomfort involved before giving their written consent to participate. This study was carried out according to the Declaration of Helsinki and was approved by the Ethical Committee of Frederiksberg and Copenhagen Counties.

Experimental preparation. On the experimental day, the subjects reported to the laboratory at 8:00 AM, and catheters were placed under local anesthesia (2% lidocaine). A 20-gauge catheter (model ES-14150; Arrow, Reading, PA) was inserted percutaneously using the Seldinger technique into the right femoral artery, 2 cm below the inguinal ligament and advanced 5–10 cm in the proximal direction. This catheter was connected to a blood pressure transducer positioned at the height of the fourth intercostal space (model T100209A; Baxter, Unterschleissheim, Germany) and was also used to sample arterial blood. A similar catheter was inserted in the same femoral artery 5 cm below the inguinal ligament and advanced 5–10 cm in the proximal direction for intra-arterial infusion of ATP. In the right femoral vein, a venous catheter with side holes (Radiopack TFE; Cook, Bjaerverskov, Denmark) was inserted and advanced ~5 cm proximal to the inguinal ligament for the injection of iced physiological saline solution (1). A thin polyethylene-coated thermistor (model 94-030-2.5F T.D. Probe; Edwards/Edslab/Baxter, Irvine, CA) was inserted through the venous catheter for blood flow measurements by the constant infusion thermodilution technique (1). A flow-through chamber (model 93-505; Edwards/Edslab/Baxter) was connected to the entry of this catheter to measure infusate temperature during ice-cold saline infusion. In the same vein, an additional 20-gauge catheter (Hydrocath, Ohmeda, Wiltshire, UK) was also inserted 2–3 cm below the inguinal ligament and advanced 7–10 cm in the distal direction beyond the merger with the saphenous vein. This catheter was connected to another blood pressure transducer positioned at the height of the fourth intercostal space (model T100209A; Baxter, Unterschleissheim, Germany) and used to measure femoral vein pressure and to obtain femoral venous blood samples. Finally, in four subjects an additional 20-gauge catheter (model ES-14150; Arrow) was also inserted in the left femoral vein, 2–3 cm below the inguinal ligament, and advanced 7–10 cm in the distal direction, beyond the merger with the saphenous. This catheter was used exclusively to sample femoral venous blood from the left leg, thus no measurements of blood flow were carried out in the left leg. An additional venous catheter was inserted into an antecubital vein to inject indocyanine green (Akorn, IL) when cardiac output was measured, as explained below.

A three-lead ECG was displayed on a monitor during catheterization and the rest of the experimental procedures (Dialogue 2000; Danica, Copenhagen, Denmark). The ECG, blood pressure and the temperatures registered by the thermistor, as well as the infusate temperatures, were recorded simultaneously with the data acquisition system (MacLab 8/s; ADInstruments, Sydney, Australia).

Experimental protocol. The experimental protocol was divided into two consecutive phases. During the first phase, the vasodilatory effect of ATP was assessed in the 10 subjects while they were resting in the supine position. During the second phase, two incremental exercise tests to exhaustion were performed on a cycle ergometer (Monark 829E) with and without infusion of ATP into the right femoral artery. Exercise measurements were performed in only eight subjects.

During the resting part of the experiments, measurements were performed in the supine position with and without infusion of ATP (Sigma cat. no. A7699 dissolved in isotonic saline to 8 mg/ml) into the right femoral artery at a constant rate of 80 μg·kg body mass^{-1}·min^{-1} during 24 min using a Harvard infusion pump (Harvard Apparatus, Millis, MA). The measurements at rest included pulmonary $\dot{V}O_2$, leg blood flow, arterial blood pressure, femoral vein blood pressure, muscle sympathetic nerve activity (MSNA), and arterial and venous femoral blood gases.

After the resting measurements, subjects performed two maximal exercise tests to exhaustion separated by at least 1 h resting period. The tests were done with right femoral artery infusion of ATP (same dose as during rest) or without (control); in random order. Given the small amount of saline infused during ATP-infusion experiments, it was not considered necessary to infuse a similar amount of saline without ATP during the control experiments. To minimize the risk of hypotension or premature fatigue due to excessive cardiac work, the ATP infusion was started when the subjects had completed 140 or 180 W, depending on the maximal exercise intensity they were able to reach in previous exercise tests. For analysis, the first load after the start of the infusion was excluded, to ensure that the first set of measurements represented a condition for which the drug has filled the lines completely and actually reached the resistance vessels. The exercise protocol started with a warm-up of 15 min at 100 W, then the load was increased by 40 W every 1.5 min until exhaustion. At each exercise intensity, measurements started after 45 s with the assessment of blood flow, followed immediately by the withdrawal of blood samples from both femoral veins and from the femoral artery for the determination of blood gas status and acid-base balance. Pulmonary $\dot{V}O_2$, heart rate, and arterial and femoral vein pressures were measured continuously during the exercise tests. Heart rate and blood pressures were averaged during 15 s around the blood flow measurements. During the incremental exercise test $\dot{V}O_2$ was averaged every 15 s. The $\dot{V}O_2$ corresponding to each load was calculated as the mean $\dot{V}O_2$ of the last four consecutive 15-s $\dot{V}O_2$ averages. $\dot{V}O_{2\,peak}$ was defined as the maximal 15-s $\dot{V}O_2$ value recorded during the test. The exercise load reached at exhaustion was considered as the maximal exercise intensity (W_{max}). The number of valid submaximal blood flow measurements obtained for each subject during the ATP infusion ranged between 1 and 3. These values were averaged to obtain a single submaximal value per subject, which we called near-maximal exercise or submaximal. The averaged $\dot{V}O_2$ of these submaximal measurements represented 92% of the $\dot{V}O_{2\,peak}$. The same individual submaximal absolute loads were exactly used in each subject to obtain the "near-maximal value" in the control test.

Blood flow. Femoral venous blood flow was measured by constant-infusion thermodilution as described in detail elsewhere (1). Briefly, iced saline was infused (Harvard pump, Harvard Apparatus, Millis, MA) through the femoral vein at flow rates sufficient to decrease blood temperature at the thermistor by 0.5–1°C. At rest, saline infusions were continued for at least 60 s, while during exercise 15- to 20-s-long infusions were used until femoral vein temperature had stabilized at its new lower value. Blood flow was calculated on thermal balance principles, as detailed by Andersen and Saltin (1).

Respiratory variables. Pulmonary $\dot{V}O_2$, CO_2 production ($\dot{V}CO_2$), and expired minute ventilation were measured continuously using an automated metabolic cart (Quark b^2; Cosmed, Rome, Italy). Before each test, ambient conditions were measured, and then the gas analyzer and the flowmeter were calibrated with high-precision gases.

MSNA. At rest, recordings of multiunit MSNA (n = 8) were obtained with microelectrodes inserted into the peroneal nerve (44). MSNA was characterized by pulse-synchronous bursts, and the minimum requirement for signal-to-noise ratio was 3:1. The neural signals were amplified (95.5 × 103), filtered (bandwidth, 700–2,000 Hz), rectified, and integrated (time constant, 0.1 s) to obtain a mean voltage neurogram. After stabilization, the MSNA recordings were obtained in blocks of 6 min. The MSNA activity registered during the first 6 min of infusion was similar to that obtained between the 18th and the 24th min of ATP infusion, suggesting that a steady MSNA response to the ATP infusion was achieved during the resting experiments. Analysis of the neurograms was performed blinded (i.e., digital records were coded and scored without knowledge of infusion). MSNA was expressed as the number of bursts per minute (burst frequency), number of bursts per hundred heart beats (100 RR) (burst incidence), and as mean burst amplitude (setting the noise level to 1

unit, the burst amplitude was expressed as a signal/noise unit) × bursts per minute (total activity).

Vascular conductances. Leg vascular conductance was calculated as the quotient between leg blood flow and the pressure difference between the femoral artery and the femoral vein.

Cardiac output. Cardiac output was measured with the dye-dilution method using indocyanine green as previously reported (4, 17).

Blood samples and analytical procedures. Blood was sampled anaerobically in heparinized syringes and immediately analyzed for hemoglobin (Hb), O_2 saturation (OSM3 hemoxymeter; Radiometer, Copenhagen, Denmark), blood pH, CO_2, and O_2 tension (model ABL700; Radiometer). Femoral venous blood was also measured with the Radiometer ABL7000. Blood gases were corrected for measured femoral vein blood temperature. Blood O_2 content (Ca_{O_2} and Cv_{O_2}) was computed from the saturation and Hb concentration ([Hb]), i.e., $(1.34 \times [Hb] \times So_2) + (0.003 \times Po_2)$.

Statistical analysis. The effect of ATP was examined by using the paired Student's *t*-test tests. To reduce the likelihood of a type II error, no corrections for multiple comparisons were performed (28). The significance level was set at $P < 0.05$. Data is expressed as means ± SE, unless otherwise stated.

RESULTS

ATP infusion at rest. ATP increased leg blood flow up to 6.2 ± 0.4 l/min and leg vascular conductance from 11.2 ± 1.3 to 98.5 ml·min^{-1}·mmHg^{-1}. In addition, femoral mean venous pressure was 9 mmHg higher during the ATP infusion than during the control condition (16.5 ± 1.0 and 7.1 ± 0.7 mmHg, respectively). Mean arterial blood pressure decreased by 9 mmHg during the ATP infusion (from 90.8 ± 2.0 to 80.2 ± 2.2 mmHg). Heart rate increased by 24 beats/min (from 58.3 ± 2.9 to 81.9 ± 2.9 beats/min) and MSNA incidence and total activity increased from 43 ± 8 to 53 ± 8 bursts/100 RR and from 116 ± 22 to 206 ± 37 signal-to-noise units/min, respectively.

Near-maximal exercise. Near-maximal exercise intensity represented 92 ± 2% of $\dot{V}O_{2\,peak}$. During near-maximal exercise, the infusion of ATP increased leg vascular conductance by 43% without changing significantly mean arterial pressure (Fig. 1, *B* and *D*) nor cardiac output (24.5 ± 1.2 and 23.9 ± 0.7 l/min, ATP and control, respectively, $n = 5$). Systemic O_2 delivery was similar in both conditions (4.9 ± 0.1 and 4.9 ± 0.2 l/min). Leg blood flow and O_2 delivery were both increased by ~20% with ATP (1.7 and 0.3 l/min, $n = 6$) (Fig. 1*A* and Fig. 2*B*), but there was no significant effect on leg $\dot{V}O_2$ (Fig. 2*D*) or pulmonary $\dot{V}O_2$ (Table 1). Leg O_2 fractional extraction was decreased from 85 ± 3 (control) to 78 ± 4% (ATP) in the infused leg (Fig. 2*C*), while it remained unchanged in the left leg when the condition with ATP infusion was compared with the condition without ATP (83 ± 2% and 84 ± 2, respectively). Femoral venous lactate concentration was similar in both conditions (9.7 ± 1.3 and 9.6 ± 1.1 mmol/l, control and ATP, respectively).

Maximal exercise. Vascular conductance at maximal exercise without ATP was 36% higher than during maximally ATP-induced vasodilation at rest. ATP infusion at maximal exercise increased leg vascular conductance by 17% (Fig. 1*D*). Leg blood flow showed a trend to a higher value with ATP (9.6 ± 1.0 and 10.4 ± 0.9 l/min, $P = 0.08$) (Fig. 1*A*). With ATP, maximal cardiac output was increased from 24.6 ± 0.8 to 26.1 ± 0.7 l/min ($n = 5$, same subjects as during submaximal exercise, $P < 0.05$), without a significant repercussion on mean

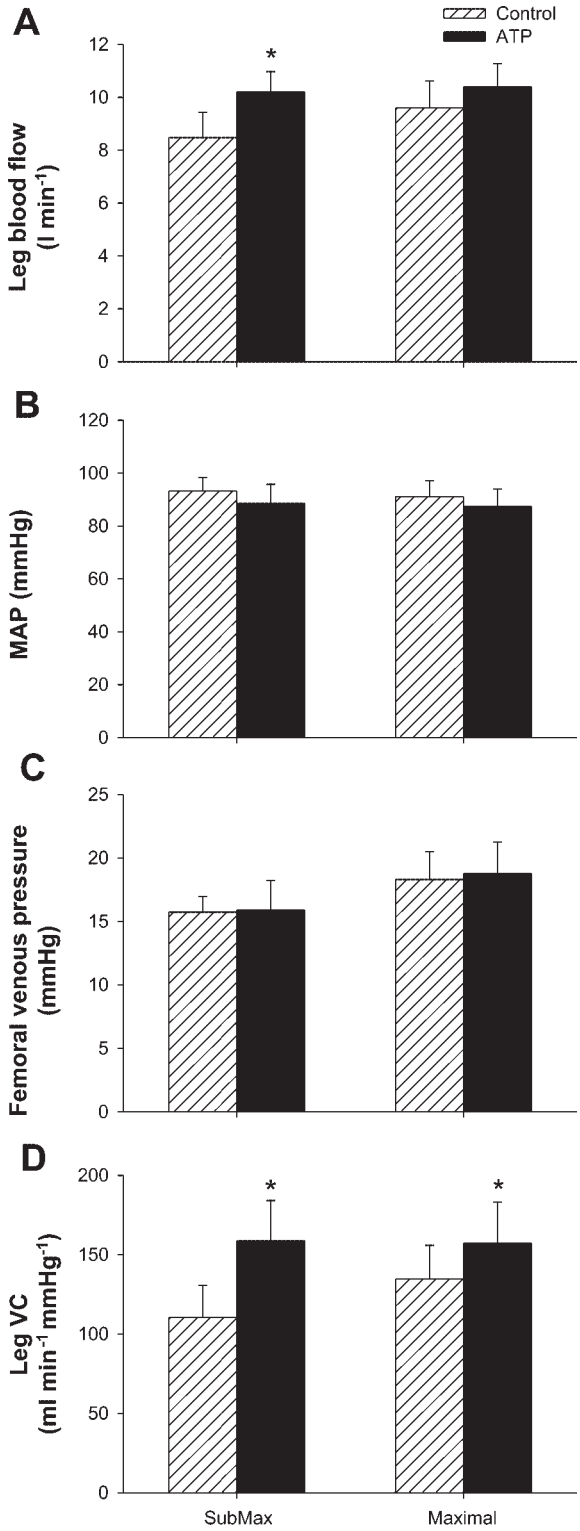

Fig. 1. Hemodynamics. Leg blood flow, mean arterial pressure (MAP), femoral venous pressure, and leg vascular conductance (VC) during near-maximal (SubMax) ($n = 7$) and maximal exercise ($n = 6$) on the cycle ergometer with or without intra-arterial infusion of ATP into the right femoral artery. *$P <$ 0.05 when compared with control.

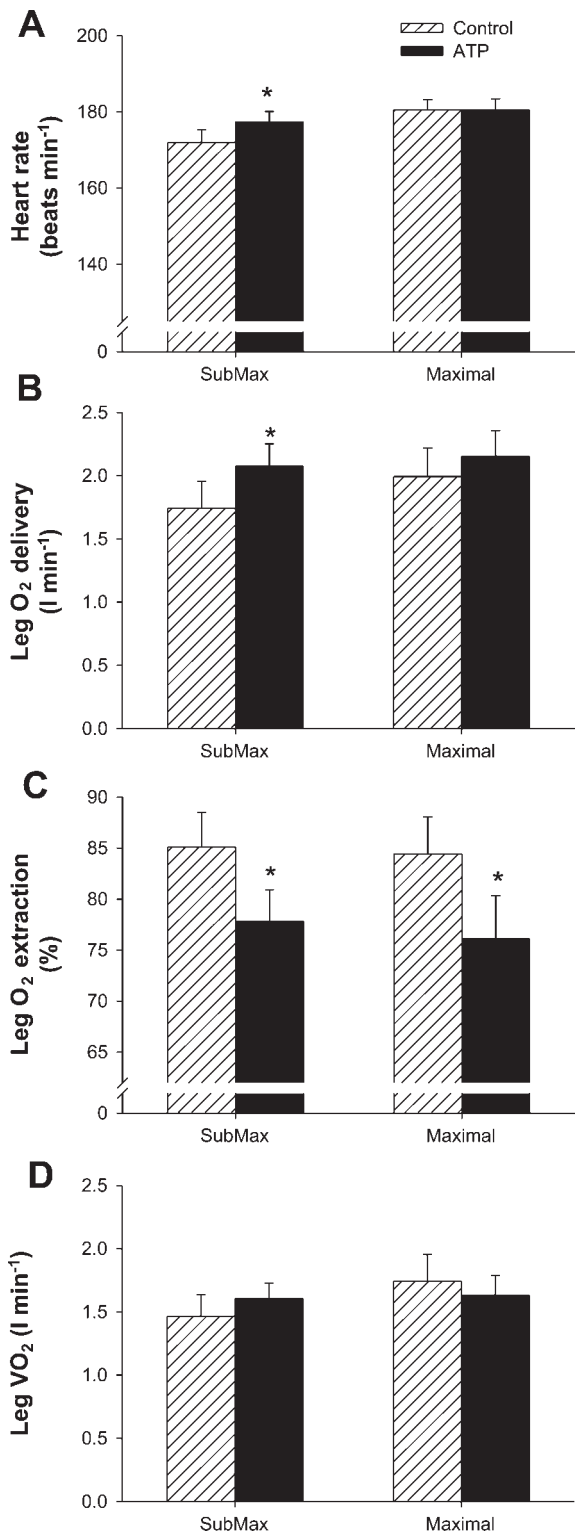

Fig. 2. O_2 delivery and O_2 extraction. Effects of ATP infused into the right femoral artery on heart rate, leg O_2 delivery, leg fractional extraction of O_2, and leg $\dot{V}O_2$ during near-maximal and maximal exercise on the cycle ergometer ($n = 7$). *$P < 0.05$ when compared with control.

Table 1. *Effects of ATP infusion on blood gas exchange during near-maximal (submaximal) and maximal exercise*

		Submaximal	Maximal
Systemic V_{O_2}, ml/min	C	3.825±0.132	4.105±0.098
	ATP	3.799±0.134	4.146±0.117
Alveolar P_{O_2}, mmHg	C	112.3±01.0	115.0±1.4
	ATP	114.0±1.9	117.6±1.3
A-a gradient, mmHg	C	14.9±1.2	18.2±1.9
	ATP	15.6±2.3	18.4±2.0
Ca_{O_2}, ml/l	C	205±2	205±2
	ATP	203±3	205±2
Cv_{O_2}, ml/l	C	31±7	32±8
	ATP	45±7*	49±9*
Sa_{O_2}, %	C	96.2±0.2	95.6±0.4
	ATP	96.4±0.4	95.9±0.4
Sv_{O_2}, %	C	14.3±3.3	14.8±3.5
	ATP	21.4±3.1*	22.9±4.2*

Data are mean ± SE. Ca_{O_2}, arterial-oxygen content. *$P < 0.05$ when compared with control.

arterial pressure (Fig. 1*B*). Consequently, systemic O_2 delivery was 0.3 l/min higher with ATP (5.1 ± 0.2 and 5.4 ± 0.1 l/min, $P < 0.05$). Since ATP reduced O_2 extraction (Fig. 2*C*), the maximal values of leg and systemic $\dot{V}O_2$ were not altered by the ATP infusion (Fig. 2*D* and Table 1). No significant differences were observed in femoral venous lactate concentration between the control and ATP condition (11.3 ± 1.0 and 12.6 ± 1.4 mmol/l, respectively).

DISCUSSION

In contrast to our hypothesis, this study shows that the $\dot{V}O_2$ of the skeletal muscles of the lower extremities is not elevated by enhanced blood flow induced by vasodilator infusion at near-maximal exercise in humans. Infusion of ATP enhanced the degree of leg vasodilation at near-maximal and maximal exercise, but reduced O_2 extraction without any net effect on leg or pulmonary $\dot{V}O_2$. The fact that during the infusion of ATP the fractional extraction of O_2 across the exercising leg was reduced at near-maximal exercise, whereas it was maintained in the contralateral leg, suggests that a great part of the extra-flow gained with the ATP infusion during near-maximal exercise is directed to leg tissues other than the active muscle fibers. In addition, this study shows that during maximal exercise on the cycle ergometer the intra-arterial infusion of a maximal vasodilating dose of ATP into one femoral artery has only a marginal effect on peak leg blood flow, despite inducing a small elevation of maximal cardiac output. At most, the intra-arterial infusion of ATP results in an elevation of peak convective O_2 transport in the infused leg, but since it also reduces the fractional O_2 extraction, leg and systemic $\dot{V}O_2$ remain unchanged. The latter suggests either a stealing effect, deviating part of the flow perfusing the active muscle fibers to other vascular beds of the limb, or the larger amount of O_2 available is not extracted due to a too short mean transit time, or the combination of both effects.

ATP-induced vasodilation reduces fractional O_2 extraction. Despite an ATP-induced 20% increase in blood flow (+1.7 l/min) in one leg at near-maximal exercise, $\dot{V}O_2$ in this leg did not change significantly. In agreement, pulmonary $\dot{V}O_2$ was also not affected by the ATP infusion. These findings can be interpreted in two different ways: *1*) assuming that the ATP-

induced vasodilation occurred around fully activated muscle fibers or *2*) that most of the increase in leg blood flow was due to vasodilation of the less active muscle fibers and/or nonmuscular tissues. The increase in femoral venous O_2 levels during the infusion of ATP is consistent with the second explanation. In fact, if all of the 1.7 l/min of extra blood had been irrigating inactive tissue, the resulting O_2 content in femoral vein would have been around 66 ml/l (assuming that the additional O_2 delivery to the less active muscle fibers or nonmuscular tissues is not used). However, the measured value during the ATP infusion was 46 ml/l, which suggests that ~50% of the extra blood flow was directed to the active muscle fibers, whereas the remaining blood was distributed elsewhere.

The degree of venous admixture during the infusion of ATP was similar at near-maximal and maximal exercise, but leg blood flow was only marginally increased at maximal exercise. This implies that part of the flow that normally would be perfusing the active muscle fibers at maximal exercise was deviated to less active muscle fibers or nonmuscular tissues with the ATP infusion. This stealing effect was likely present and resulted in a marginally lower leg $\dot{V}O_2$ (that 6% less is a type II error is assumed since this difference did not reach statistical significance, $P = 0.3$).

Regulation of skeletal muscle vascular conductance during exercise. Is some degree of vasoconstriction needed? The reason why ATP may cause redistribution of blood toward inactive tissue may be related to ATP sympatholytic properties. During the sympathoexcitation accompanying intense exercise, sympathetic vasoconstriction is prominent in inactive tissue, but is less efficient in active skeletal muscle due to metabolic inhibition. Thus any sympatholytic agent would exert a more pronounced effect in inactive tissue. Our resting data are consistent with an ATP-related sympatholytic effect. Despite the fact that MSNA almost doubled during the resting ATP-infusion, the degree of leg vasodilation was remarkable, as reflected by the very high levels of leg vascular conductance achieved. In contrast, the high MSNA elicited by the ATP infusion resulted in an increase of leg O_2 extraction (data not shown) in the contralateral leg (which did not receive ATP) suggesting vasoconstriction in this territory, which is in agreement with previous observations (19). Our data suggest that the ATP-induced vasodilation of inactive tissue and partially active muscle fibers during maximal exercise may become detrimental to O_2 uptake, and muscle metabolism, as previously reported in rats (25).

The finding that ATP infusion caused an increase in leg vascular conductance at near-maximal and maximal exercise may provide interesting insight into the balancing influences of sympatholytic and vasoconstrictor mechanisms in a large muscle mass during exercise. Previous studies have provided evidence that sympathetic vasoconstriction is present in exercising human forearm muscle (18, 39, 41). Conversely, there is strong evidence that in both the human forearm and thigh muscle sympathetic vasoconstriction is counteracted in exercising muscle (18, 36, 45). Moreover, it has been shown that this sympathetic vasoconstricting activity is counteracted more efficiently as exercise intensity increases (20, 42). Likely, part of the ATP-induced increase in flow at near-maximal exercise is directed toward active muscle, which suggests incomplete vasodilation or residual sympathetic vasoconstriction in the active muscle even at this high intensity. This vasoconstriction is likely matching O_2 demand with O_2 delivery in some less-active muscle fibers, i.e., it is necessary to avoid heterogeneity in the perfusion/$\dot{V}O_2$ relationship (25). The fact that fractional O_2 extraction was not altered in the contralateral active leg (the left leg in our experiments) suggests that blood flow was not reduced in the left leg when ATP was infused in the right leg.

The small effect of ATP on peak leg vascular conductance at maximal intensity suggests that almost maximal leg vasodilation has been already achieved. The fact that maximal vascular conductance was one-third higher during maximal exercise than during maximal ATP-induced vasodilation at rest may be explained through the effect of the muscle pump, which contributes to increased blood flow by a mechanism independent of vasodilation (23).

Collectively, our results suggest that sympatholysis is already maximal in the fully activated muscle fibers of human lower extremities, whereas some degree of sympathetic vasoconstriction remains even at maximal exercise in partially activated muscle fibers and nonskeletal muscle tissues of the legs to optimize the perfusion/$\dot{V}O_2$ relationship. Disrupting this mechanism in one leg was well tolerated. However, if this mechanism was to be disrupted in the four extremities, an intolerable increase of vascular conductance and pressure drop should have been expected (7).

Limitations. In this study, we determined $\dot{V}O_{2\,peak}$ using an continuous unsteady-state protocol (4, 6, 24). The exercise intensity was increased by 40-W steps every 90 s, and $\dot{V}O_2$ was computed during the last 60 s, clearly during the transient. With this protocol, it may be more difficult to observe a plateau in the relationship between steady-state $\dot{V}O_2$ and power than with a steady-state protocol. However, supramaximal exercise intensities, higher than the minimal intensity eliciting $\dot{V}O_{2\,peak}$, are attained at the end of the protocol. Since we retained as $\dot{V}O_{2\,peak}$ the highest 15-s $\dot{V}O_2$ observed during the final step, it is very likely that, in fact, we succeeded in determining the actual $\dot{V}O_{2\,peak}$. Subsidiary criteria of maximality, such as lack of heart rate increase, R values >1.1, lactate levels >10 mM were accomplished in all subjects during the tests with or without ATP infusion. Moreover, $\dot{V}O_{2\,peak}$ can be equally achieved using very different exercise protocols, either incremental or of constant intensity (10). For example, it has been shown that $\dot{V}O_{2\,peak}$ is independent of the slope of the ramp function (in the range of 6–100 W/min), although the peak exercise intensity achieved is inversely related to the slope of the ramp test (12, 37, 40). Likewise, $\dot{V}O_{2\,peak}$ has been found to be also independent from the duration of the steps (2, 46). Therefore, it is very likely that during both tests, i.e., with or without ATP infusion, our subjects achieved their actual $\dot{V}O_{2\,peak}$.

Submaximal comparisons were performed at the same absolute workloads. Because we did not determine the minimal exercise intensity eliciting $\dot{V}O_{2\,peak}$, we cannot exclude the fact that this intensity differed in the two investigated conditions and, hence, the relative mechanical intensity of the submaximal exercise bout. However, the fact that in both tests, subjects achieved a similar $\dot{V}O_{2\,peak}$, maximal heart rate, and blood lactate concentration at the same peak power output implies that the workloads used during the submaximal tests were not only alike in absolute but also in relative terms. In agreement, similar pulmonary $\dot{V}O_2$ and leg $\dot{V}O_2$ values were observed at

submaximal intensity with or without ATP. Because we used an "unsteady-state" protocol, the aerobic demand of the "near-maximal exercise intensities" should have been higher than the actual $\dot{V}O_2$ observed during the transient, and likely greater than the minimal exercise intensity eliciting $\dot{V}O_{2\,max}$. Thus despite the aerobic demand had been higher than the measured $\dot{V}O_2$, increasing leg blood flow was not accompanied by an increase of leg $\dot{V}O_2$ at near-maximal exercise. This finding does not contradict the paradigm that holds that $\dot{V}O_{2\,max}$ is mainly determined by O_2 delivery in healthy humans (35), because the extra flow (and O_2 delivery) gained with the infusion of ATP was, at least in part, deviated away from the active muscle fibers.

In summary, the present investigation shows that the $\dot{V}O_2$ of the skeletal muscles of the lower extremities is not enhanced by limb vasodilation at near-maximal or maximal exercise in humans. That ATP infusion resulted in a reduction of O_2 extraction across the exercising leg suggests a sympatholytic effect of ATP on less active muscle fibers and other noncontracting tissues and that under normal conditions these regions are under high vasoconstrictor influence to ensure the most efficient flow distribution of the available cardiac output to the most active muscle fibers of the exercising limb. In healthy humans, it is not possible to increase peak leg $\dot{V}O_2$ by increasing vasodilation, likely due to an alteration in the perfusion/$\dot{V}O_2$ relationship.

GRANTS

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