Two weeks of muscle immobilization impairs functional sympatholysis but increases exercise hyperemia and the vasodilatory responsiveness to infused ATP

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Mortensen SP, Mørkeberg J, Thaning P, Hellsten Y, Saltin B. Two weeks of muscle immobilization impairs functional sympatholysis but increases exercise hyperemia and the vasodilatory responsiveness to infused ATP and adenosine-induced vasodilation. Eight male subjects (22 ± 2 yr, V̇O₂max: 49 ± 2 ml O₂-min⁻¹·kg⁻¹) were studied before and after 5 wk of one-legged knee-extensor training (3–4 times/wk) and 2 wk of immobilization of the other leg. Leg hemodynamics were measured at rest, during exercise (24 ± 4 watts), and during arterial ATP (0.94 ± 0.03 μmol/min) and adenosine (5.61 ± 0.03 μmol/min) infusion with and without coinfusion of tyramine (11.11 μmol/min). During exercise, leg blood flow (LBF) was lower in the trained leg (2.5 ± 0.1 l/min) compared with the control leg (2.6 ± 0.2 l/min; P < 0.05), and it was higher in the immobilized leg (2.9 ± 0.2 l/min; P < 0.05). Tyramine infusion lowers LBF similarly at rest, but, when tyramine was infused during exercise, LBF was blunted in the immobilized leg (2.5 ± 0.2 l/min; P < 0.05), whereas it was unchanged in the control and trained leg. Mean arterial pressure was lower during exercise with the trained leg compared with the immobilized leg (P < 0.05), and leg vascular conductance was similar. During ATP infusion, the LBF response was higher after immobilization (3.9 ± 0.3 and 4.5 ± 0.6 l/min in the control and immobilized leg, respectively; P < 0.05), whereas it did not change after training. When tyramine was coinfused with ATP, LBF was reduced in the immobilized leg (P < 0.05) but remained similar in the control and trained leg. Training increased skeletal muscle P2Y2 receptor content (P < 0.05), whereas it did not change with immobilization. These results suggest that muscle inactivity impairs functional sympatholysis and that the magnitude of hyperemia and blood pressure response to exercise is dependent on the training status of the muscle. Immobilization also increases the vasodilatory response to infused ATP.

Adenosine 5′triphosphate; adenosine; blood flow; sympathetic nerve activity

BLOOD FLOW TO CONTRACTING muscle is regulated to match O₂ delivery to the metabolic demand and is thought to be determined by an interaction between locally formed vasoactive substances and the degree of sympathetic vasoconstriction (6). During exercise, the sympathetic nervous system is engaged (1, 11, 23, 44). Sympathetic nerve activity is increased in both resting and contracting skeletal muscle (10, 35, 41, 46). In inactive tissues, the increase in sympathetic nervous activity causes vasoconstriction as evidenced by reductions in limb vascular conductance (3, 5, 40). In contracting muscles of young, healthy individuals, the increased sympathetic vasoconstrictor activity can be attenuated or even abolished in part by local metabolic products of contraction (9, 37, 47, 49). This phenomenon, termed functional sympatholysis, is thought to allow adequate perfusion and O₂ delivery to exercising muscles. With ageing (16, 18) and in essential hypertension (50) functional sympatholysis is impaired. The degree of functional sympatholysis has been suggested to be altered in the arms, but not the legs, of trained cyclists compared with sedentary controls (51). Local adaptations in skeletal muscle with exercise training or inactivity could be important in determining the degree of functional sympatholysis, but this has not been investigated in humans.

ATP has been proposed to play a role in skeletal muscle blood flow regulation by inducing local vasodilation via P2 receptors (7) and attenuating sympathetic vasoconstrictor activity (15, 38). Intravascular adenosine does not appear to be sympatheticolytic (38) but has been suggested to play a role in the regulation of exercise hyperemia by stimulating nitric oxide and prostacyclin formation (26, 30, 33). Arterial infusion of ATP or adenosine in the human leg can induce vasodilation close to that observed during maximal exercise (8, 33, 38). Despite the proposed vascular effects of intraluminal ATP and adenosine, the role of muscle training status on ATP- and adenosine-induced vasodilation remains unexplored. ATP is likely to exert its vascular effect via endothelial P2Y2 receptors within the muscle tissue (24, 25, 39), but it is not known if the skeletal muscle content of P2Y2 receptors is affected by activity level of the muscle.

The purpose of the present study was to examine the influence of muscle training status on exercise hyperemia, functional sympatholysis, ATP- and adenosine-induced vasodilation, and P2Y2 receptor content. To accomplish these aims, we measured leg hemodynamics during exercise and arterial ATP and adenosine infusion with and without simultaneous infusion of tyramine, before and after 5 wk of exercise training with one leg and 2 wk of immobilization of the other leg in healthy, male subjects. We choose this approach to create a large difference in limb training status within the same individual. We hypothesized that sympatholysis, the vasodilatory response to ATP and adenosine, and P2Y2 receptor content, would be increased and reduced by exercise training and immobilization, respectively.
ROLE OF MUSCLE TRAINING STATUS ON VASCULAR FUNCTION

A total of eight recreationally active male subjects with a mean age of 22 ± 2 yr, body weight of 73 ± 3 kg, height of 180 ± 3 cm, and \( V_\text{O}_{2\text{max}} \) of 49 ± 1 ml·kg\(^{-1}\)·min\(^{-1}\) participated in the study. All subjects had normal electrocardiogram and blood pressure and were free of any medications. The subjects were informed of the 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 and was conducted in accordance with the guidelines of the Declaration of Helsinki.

**Experimental protocol.** On the first visit to the laboratory, the subjects completed a training session to become accustomed to the one-legged knee-extensor ergometer, and they completed an incremental test to exhaustion with each leg to determine maximal workload. On a separate day, the subjects performed an incremental bicycle ergometer exercise test (Excalibur Sport, Lode, The Netherlands) in which the maximal oxygen uptake was determined with a metabolic system (Quark b2 system; Cosmed, Rome, Italy). The subjects were examined before and after a training/immobilization period. Before the training/immobilization period, one leg was examined (randomized), and the legs were then again randomized into the training or immobilization protocol.

**Training and immobilization period.** The subjects completed 5 wk of one-legged knee-extensor exercise (3–4 times/wk). Three weeks into the training protocol, the other leg was immobilized for 2 wk. Training consisted of a 5-min warm up followed by 10 2-min intervals with 1-min rest periods between intervals. The workload was individually adjusted such that subjects were exhausted upon completion of each training session. During the first training session, the mean workload was 42 ± 5 watts, and, during the last training session, the mean workload was 80 ± 7 watts. After 3 wk of training, the other leg was immobilized. Immobilization was accomplished by whole leg casting using a lightweight fiber cast applied from just above the malleoli to just below the groin. The cast was positioned in 30–35° of knee joint flexion to circumvent walking ability of the casted limb. The subjects were carefully instructed to perform all activities on crutches and abstain from ground contact, as well as performing isometric contractions of quadriceps of the immobilized leg. The immobilization period was limited to 2 wk to avoid joint stiffness and severe muscle atrophy. The day before the experimental day, the cast was removed, and the leg was passively moved to confirm full range of motion. A knee brace (Don-Joy) was applied at a fixed position (30°) until the following day.

**Experimental days.** On the two experimental days, the subjects arrived at the laboratory after a light breakfast. Catheters were placed under local anesthesia (Lidocain) in the femoral artery of both legs, 2 cm below the inguinal ligament, and advanced 10 cm in the proximal direction. Catheters were also placed in the femoral vein and advanced 5 cm proximal to the inguinal ligament. A muscle biopsy was obtained from both vastus lateralis muscles under local anesthesia (Lidocain). Following 30 min of rest, subjects completed 6 min of: 1) one-legged knee-extensor exercise (24 ± 4 watts, i.e., 35% of maximal workload before the training/immobilization period), 2) arterial ATP infusion (0.94 ± 0.03 \( \mu \text{mol/min}\), i.e., 0.08 \( \mu \text{mol·min}^{-1} \cdot kg\) leg mass\(^{-1}\); A7699; Sigma Aldrich, St. Louis, MO), and 3) arterial adenosine infusion (5.61 ± 0.03 \( \mu \text{mol/min}\), i.e., 0.50 \( \mu \text{mol·min}^{-1} \cdot kg\) leg mass\(^{-1}\); ITEM Development). Tyramine (11.11 \( \mu \text{mol/min}\), i.e., 1.0 \( \mu \text{mol·min}^{-1} \cdot kg\) leg mass\(^{-1}\); Sigma) was infused during the last 3 min of exercise/ATP/adenosine infusion. This dose of tyramine has been found to result in the same vasoconstrictor response as 0.5 \( \mu \text{mol·min}^{-1} \cdot kg\) leg mass\(^{-1}\) (Mortensen, Nyberg, Thaning, Jensen & Hellsten, unpublished observations, \( n = 6 \)) and thus considered to induce a maximal vasoconstrictor response. Tyramine was also infused for 3 min in resting conditions. The order of the exercise/ATP/adenosine trials was randomized and separated by 30 min of supine rest.

**Measurements.** Arterial and venous blood samples (1–5 ml) were drawn simultaneously before each trial and during the 6 min of exercise/infusion (2.5 and 5.5 min). Arterial pressures were obtained from the catheter in the contralateral limb with the pressure transducers positioned at the level of the heart (Pressure Monitoring Kit; Baxter). Leg mass was calculated from whole body dual-energy X-ray absorptiometry scanning (Prodigy; GE Medical Systems). Blood gases and hemoglobin concentrations were measured using an ABL725 analyzer (Radiometer, Copenhagen, Denmark). Venous plasma norepinephrine concentrations were determined with a radioimmunoassay (LDN, Nordhorn, Germany).

**Femoral arterial blood flow.** Femoral arterial blood flow [leg blood flow (LBFl)] was measured with ultrasound Doppler (Philips Ice33; Philips Healthcare) equipped with a probe operating an imaging frequency of 9 MHz and Doppler frequency of 5.0 MHz. The site of blood velocity measurements in the common femoral artery was distal to the inguinal ligament but above the bifurcation into the superficial and profound femoral branch to avoid turbulence from the bifurcation. All recordings were obtained at the lowest possible insonation angle and always below 60°. The sample volume maximized according to the width of the vessel and kept clear of the vessel walls. A low-frequency filter (velocities <1.8 m/s) rejected noises caused by turbulence at the vascular wall. Doppler tracings and B-mode images were recorded continuously, and Doppler tracings were averaged over 16 heart cycles. The arterial diameter was determined after each Doppler recording and averaged over three cardiac cycles. Arterial diameter measures were assessed during the systole from arterial B-mode images with the transducer parallel to the vessel.

**Quantification of purinergic P2Y2 receptor expression.** Biopsies were freeze-dried and dissected free from fat, blood, and connective tissue. Approximately 5 mg dry wt of the biopsy were homogenized in homogenization buffer (10% glycerol, 20 mM sodium pyrophosphate, 150 mM NaCl, 50 mM HEPES, 1% Nonidet P-40, 20 mM β-glycerophosphate, 2 mM Na3VO4, 10 mM NaF, 2 mM phenylmethylsulfonyl fluoride, 1 mM EDTA and EGTA, aprotinin, leupeptin, and benzamidine) while kept on ice at all times. The protein concentration of the lysate samples was determined by a BCA protein assay using BSA as standard (Pierce, Rockford, IL). Lysate proteins (20 μg) were separated using 10% SDS gels (Bio-Rad Laboratories) and transferred to polyvinylidene difluoride membranes (Immobilion Transfer Membrane; Millipore). The membranes were incubated with primary polyclonal antibodies against the purinergic P2Y2 receptor (APR-010; Alomone Laboratories). Secondary antibody horseradish peroxidase-conjugated goat anti-rabbit (P-0448; Dako, Glostrup, Denmark) was used for detection. Subsequent to exposure (Kodak Image Station, 2000MM; Kodak, Glostrup, DK) and quantification (Kodak Molecular Imaging software; Kodak), the protein content was expressed in arbitrary units relative to standard samples run on each gel. The membranes were analyzed for GAPDH as loading control after inactivation of peroxidase activity (45) and reprobing with anti-GAPDH antibody (mAbcam 9484; Abcam).

**Statistical analysis.** A two-way repeated-measures ANOVA was performed to test significance within and between the control, trained, and immobilized leg. A one-way repeated-measures ANOVA was performed to test significance before and after exercise training and immobilization.

**Table 1. Peak workload and leg mass before and after exercise training and immobilization**

<table>
<thead>
<tr>
<th></th>
<th>Before (Control)</th>
<th>Immobilized</th>
<th>Trained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak workload, watts</td>
<td>68 ± 7</td>
<td>60 ± 8*</td>
<td>88 ± 7*#</td>
</tr>
<tr>
<td>Leg mass, kg</td>
<td>11.3 ± 0.6</td>
<td>11.1 ± 0.7</td>
<td>11.5 ± 0.6</td>
</tr>
<tr>
<td>Fat-free mass, kg</td>
<td>9.6 ± 0.4</td>
<td>9.2 ± 0.5*</td>
<td>9.6 ± 0.4#</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Different from the control leg, \( P < 0.05 \). #Different from the immobilized leg, \( P < 0.05 \).
performed to test significance between the percent change in leg hemodynamics during tyramine infusion between the control, trained, and immobilized leg. After a significant $F$-test, pairwise differences were identified using Tukey’s honestly significant difference post hoc procedure. Presented data are $n = 8$, except immobilization data ($n = 7$) and plasma norepinephrine concentrations ($n = 6$). The significance level was set at $P < 0.05$, and data are means $\pm$ SE.

**RESULTS**

Exercise training increased peak power, whereas immobilization lowered peak power compared with control (Table 1). The relative workload during the experiment was $36 \pm 2$, $40 \pm 3$, and $26 \pm 1\%$ of peak power in the control, immobilized, and trained leg, respectively. There was no difference in leg mass between trials, but fat-free mass was lower after immobilization compared with the control and trained leg.

Leg hemodynamics during arterial tyramine infusion before and after training or immobilization. Arterial tyramine infusion lowered LBF from $0.41 \pm 0.07$ to $0.25 \pm 0.05$, $0.44 \pm 0.06$ to $0.22 \pm 0.03$, and $0.38 \pm 0.05$ to $0.21 \pm 0.03$ l/min in the control, immobilized, and trained leg, respectively ($P < 0.05$).

![Graphs showing leg hemodynamics](image.jpg)

**Fig. 1.** Leg blood flow, mean arterial pressure, leg vascular conductance, leg $O_2$ delivery, leg arteriovenous ($a$–$v$) $O_2$ difference, and leg $\dot{V}O_2$ at rest and during exercise (24 ± 4 watts) in the control, immobilized, and trained leg. *Different from baseline conditions, $P < 0.05$. †Different from the control leg, $P < 0.05$. ‡Different from the immobilized leg, $P < 0.05$. §Different from exercise without tyramine, $P < 0.05$. 

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Table 2. Blood variables and heart rate during resting conditions and one-legged knee-extensor exercise with the control, immobilized, and trained leg with and without infusion of tyramine

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Immobilized Leg</th>
<th>Trained Leg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest</td>
<td>Ex</td>
<td>Ex + Tyr</td>
</tr>
<tr>
<td>Po2, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>99 ± 2</td>
<td>96 ± 2</td>
<td>100 ± 2</td>
</tr>
<tr>
<td>v</td>
<td>29 ± 2</td>
<td>23 ± 0*</td>
<td>20 ± 1*</td>
</tr>
<tr>
<td>Hemoglobin, g/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>146 ± 2</td>
<td>149 ± 2</td>
<td>148 ± 2</td>
</tr>
<tr>
<td>v</td>
<td>146 ± 2</td>
<td>150 ± 3*</td>
<td>149 ± 2</td>
</tr>
<tr>
<td>PO2, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>98.0 ± 0.1</td>
<td>97.6 ± 0.2</td>
<td>97.9 ± 0.3</td>
</tr>
<tr>
<td>v</td>
<td>49.7 ± 3.9</td>
<td>31.1 ± 1.2*</td>
<td>25.8 ± 1.9*</td>
</tr>
<tr>
<td>O2 saturation, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>194 ± 3</td>
<td>198 ± 3</td>
<td>198 ± 3</td>
</tr>
<tr>
<td>v</td>
<td>95 ± 8</td>
<td>63 ± 3*</td>
<td>52 ± 4*</td>
</tr>
<tr>
<td>Lactate, mmol/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>42 ± 1</td>
<td>43 ± 1</td>
<td>43 ± 1</td>
</tr>
<tr>
<td>v</td>
<td>47 ± 1</td>
<td>59 ± 1*</td>
<td>62 ± 1*$</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>1.3 ± 0.1</td>
<td>2.2 ± 0.2</td>
<td>2.2 ± 0.2</td>
</tr>
<tr>
<td>v</td>
<td>1.2 ± 0.1</td>
<td>2.3 ± 0.2$</td>
<td>2.3 ± 0.2$</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>66 ± 4</td>
<td>88 ± 3*</td>
<td>84 ± 3*</td>
</tr>
</tbody>
</table>

Values are means ± SE. Ex, exercise; Tyr, tyramine; a, arterial; v, venous. *Different from baseline conditions, P < 0.05. §Different from immobilized leg, P < 0.05. #Different from exercise without tyramine, P < 0.05.

Tyramine infusion increased mean arterial pressure (MAP) in the immobilized leg (P < 0.05), whereas it tended to increase in the control (P = 0.063) and trained (P = 0.095) leg. Leg vascular conductance (LVC) was lowered by 42 ± 2, 51 ± 6, to 44 ± 7%, respectively. There was no difference in LBF, MAP, and LVC between trials.

**Leg hemodynamics during one-legged knee extensor exercise before and after training or immobilization.** Baseline LBF was similar in the control, immobilized, and trained leg (0.31–0.36 l/min). Exercise increased LBF to 2.61 ± 0.21 l/min in the control leg (Fig. 1). This increase in blood flow was lower (P < 0.05) than in the immobilized leg and higher (P < 0.05) than in the trained leg (2.93 ± 0.21 and 2.47 ± 0.12 l/min, respectively). In all three conditions, MAP increased during exercise, but it was lower during exercise with the trained leg compared with the immobilized leg (P < 0.05). There was no difference in LVC during exercise between the control, immobilized, and trained leg. The lower LBF during exercise with the trained leg was paralleled by an increase in leg arteriovenous (a-v) O2 difference (P < 0.05) such that leg VO2 was similar (Table 2).

In the immobilized leg, tyramine infusion during exercise resulted in a reduced LBF, LVC, and O2 delivery compared with during exercise alone (P < 0.05), whereas in the control leg and trained leg these variables were unaffected by tyramine infusion during exercise. Tyramine infusion did not alter MAP in any of the three conditions. The lower LBF in the immobilized leg after tyramine infusion was paralleled by an increase in leg a-vO2 difference (P < 0.05), such that leg VO2 remained similar in all three trials. The percent change in LBF with tyramine infusion was larger in the immobilized leg and smaller in the trained leg compared with the control leg (P <
0.05), whereas the change in LVC was larger in the immobilized leg compared with the trained leg ($P < 0.05$) (Fig. 2).

**Leg hemodynamics during arterial ATP infusion before and after training and immobilization.** Baseline LBF was similar in the control, immobilized, and trained legs (0.27–0.39 l/min).

Arterial ATP infusion increased LBF to $3.86 \pm 0.33$, $4.48 \pm 0.63$, and $3.53 \pm 0.36$ l/min in the control, immobilized, and trained leg, respectively ($P < 0.05$; Fig. 3), and LBF was higher in the immobilized leg compared with the control and trained leg ($P < 0.05$). ATP infusion lowered MAP in all three conditions.

**Fig. 3.** Leg blood flow, mean arterial pressure, and leg vascular conductance during baseline conditions and arterial ATP or adenosine (ADO) infusion in the control, immobilized, and trained leg. *Different from baseline conditions, $P < 0.05$. €Different from control leg, $P < 0.05$. #Different from immobilized leg, $P < 0.05$. §Different from ATP/ADO infusion without tyramine, $P < 0.05$. 

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conditions ($P < 0.05$), and there was no difference between trials. Consequently, LVC was higher in the immobilized leg (53 ± 4 ml min$^{-1}$ mmHg$^{-1}$) compared with the control (46 ± 4 ml min$^{-1}$ mmHg$^{-1}$) and trained (42 ± 4 ml min$^{-1}$ mmHg$^{-1}$) leg. When tyramine was coinfused with ATP, LBF was reduced in the immobilized leg ($P < 0.05$), whereas it did not change in the control or trained leg. Coinfusion of tyramine with ATP increased MAP ($P < 0.05$) to baseline levels in all three conditions. LVC was lower during combined ATP and tyramine infusion in the control and immobilized leg compared with ATP infusion alone ($P < 0.05$), whereas it remained unchanged in the trained leg. Leg VO$_2$ remained similar to baseline conditions during ATP infusion with and without coinfusion of tyramine, and there was no difference between trials (Table 3). The change in LBF with tyramine infusion tended to be higher in the immobilized leg compared with the control ($P = 0.088$) and trained ($P = 0.055$) leg, whereas the change in LVC tended ($P = 0.063$) to be larger in the immobilized leg compared with the trained leg.

### Table 3. Blood gas variables and heart rate during baseline conditions and arterial ATP infusion in the control, immobilized, and trained leg with and without coinfusion of tyramine

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Immobilized Leg</th>
<th>Trained Leg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>ATP</td>
<td>ATP + Tyr</td>
</tr>
<tr>
<td>PO$_2$, mmHg</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>99 ± 2</td>
<td>100 ± 2</td>
<td>102 ± 2</td>
</tr>
<tr>
<td>v</td>
<td>35 ± 1</td>
<td>77 ± 1*</td>
<td>73 ± 2*</td>
</tr>
<tr>
<td>Hemoglobin, g/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>144 ± 3</td>
<td>145 ± 3</td>
<td>147 ± 2</td>
</tr>
<tr>
<td>v</td>
<td>144 ± 3</td>
<td>145 ± 3</td>
<td>147 ± 2</td>
</tr>
<tr>
<td>O$_2$ saturation, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>97.9 ± 0.2</td>
<td>97.8 ± 0.1</td>
<td>98.0 ± 0.2</td>
</tr>
<tr>
<td>v</td>
<td>61.9 ± 3.0</td>
<td>94.8 ± 0.5*</td>
<td>94.5 ± 0.4*</td>
</tr>
<tr>
<td>O$_2$ content, ml/l</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a</td>
<td>191 ± 4</td>
<td>193 ± 3</td>
<td>196 ± 3</td>
</tr>
<tr>
<td>v</td>
<td>120 ± 7</td>
<td>187 ± 3*</td>
<td>188 ± 3*</td>
</tr>
<tr>
<td>Leg VO$_2$, ml/min</td>
<td>23 ± 2</td>
<td>26 ± 4</td>
<td>26 ± 4</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>63 ± 4</td>
<td>74 ± 4*</td>
<td>65 ± 2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *Different from baseline conditions, $P < 0.05$. €Different from control leg, $P < 0.05$. #Different from immobilized leg, $P < 0.05$. §Different from ATP infusion without tyramine, $P < 0.05$.
conditions. The change in LBF and vascular conductance with tyramine infusion tended to be higher in the immobilized leg compared with the trained leg \( (P = 0.052 \) and \( 0.055 \), respectively).

**Skeletal muscle purinergic P2Y2 receptor content before and after training and immobilization.** Skeletal muscle purinergic P2Y2 receptor content was higher in the trained leg compared with the control and immobilized leg, whereas there was no difference between the control and immobilized leg (Fig. 4).

**Plasma catecholamine concentrations.** Resting venous plasma norepinephrine levels were similar in the three conditions \( (1.2–1.3 \text{ nmol/l}) \). Exercise increased plasma norepinephrine levels during exercise with the immobilized leg \( (2.0 \pm 0.3 \text{ nmol/l}; \ P < 0.05) \), whereas norepinephrine levels remained similar to resting levels during exercise with the control \( (1.7 \pm 0.2 \text{ nmol/l}) \) and trained \( (1.6 \pm 0.2 \text{ nmol/l}) \) leg. Plasma norepinephrine levels tended \( (P = 0.063) \) to be higher during exercise with the immobilized leg compared with the trained leg. Tyramine increased venous plasma norepinephrine concentrations in all three conditions \( (4.4 \pm 0.5, 4.2 \pm 0.3, \text{ and } 4.2 \pm 0.5 \text{ nmol/l in control, trained, and immobilized leg, respectively}) \), and there was no difference between conditions.

**DISCUSSION**

This study investigated the effects of training and immobilization on functional sympatholysis and ATP- and adenosine-induced vasodilation. The main findings were: 1) infusion of tyramine lowered exercise hyperemia in the immobilized leg, whereas exercise hyperemia was maintained during tyramine infusion in the control and trained leg. 2) exercise hyperemia was higher in the immobilized leg and lower in the trained leg compared with the control leg. 3) immobilization increased the vasodilatory response to infused ATP, whereas the level of vasodilation was unchanged after training. 4) training and immobilization did not alter the vasodilatory response to adenosine, and 5) P2Y2 receptor content was increased after training. These results demonstrate that a period of immobilization reduces the degree of functional sympatholysis in muscle. Moreover, the level of exercise hyperemia is associated with the training status of the muscle. Furthermore, the hyperemic response to infused ATP, but not adenosine, is increased in a muscle that has been immobilized for 2 wk.

One of the aims of the present study was to elucidate the influence of inactivity and exercise training on functional sympatholysis in leg muscle of young healthy subjects. In agreement with previous studies in moderately trained men, we found that exercise as well as arterially infused ATP, but not adenosine, could modulate sympathetic vasoconstriction \((15, 38, 39)\). An important observation was that the modulating effect of exercise and ATP on sympathetic vasoconstriction was impaired when the leg had been immobilized for 2 wk. The precise mechanism by which exercise and ATP can override sympathetic vasoconstrictor activity remains unknown, but the mechanism is thought to involve interference at the level of the postjunctional vascular \( \alpha \)-adrenoreceptors and/or their underlying intracellular signaling pathways in smooth muscle cells \((48)\).

The similar increase in plasma norepinephrine levels during tyramine infusion suggests that tyramine stimulated a similar norepinephrine release in the three conditions. An increased sympathetic nerve activity during exercise with the immobilized leg \((36)\), as suggested by the tendency toward higher venous plasma norepinephrine levels, may also be a mechanism contributing to the impaired sympatholysis during exercise with muscle that has been inactive. Moreover, the higher relative workload during exercise with the previously immobilized leg could have altered the degree of functional sympatholysis, since functional sympatholysis has previously been suggested to be influenced by the exercise intensity \((49)\). However, we observed a similar impairment in functional sympatholysis during ATP infusion, suggesting that other mechanisms are also involved. The endothelial function of the immobilized leg could be affected by the exercise training of the contralateral leg \((20, 31)\), but such changes would have reduced rather than enhanced the effect of immobilization. In both the control and the trained leg, tyramine administration did not lower exercise and ATP-induced vasodilation, suggesting the functional sympatholysis was intact in both conditions. However, we did observe a difference in the relative effect of tyramine on exercise hyperemia, indicating that exercise training did have a positive effect on functional sympatholysis. Although immobilization is a more drastic intervention than detraining and inactivity, the results suggest that a certain level of physical activity is required to maintain an intact functional sympatholysis.

Exercise training increased the purinergic P2Y2 receptor content, but, in contrast to our hypothesis, the vasodilatory response to ATP was lower in the trained leg compared with the immobilized leg because of an increased vasodilatory responsiveness in the immobilized leg. Immobilization did not

![Fig. 4. P2Y2 receptor content in vastus lateralis muscle before and after 14 days of immobilization of one leg and 5 wk of exercise training of the other leg. C, control; TR, trained; IM, immobilized. #Different from the control leg, \( P < 0.05 \). †Different from the immobilized leg, \( P < 0.05 \). Representative Western blots for P2Y2 and GAPDH are shown on right.](http://ajpheart.physiology.org/ by 10.220.33.1 on April 14, 2017)
alter adenosine-induced vasodilation, suggesting that the change in responsiveness to ATP was associated with changes in the signaling pathway of ATP rather than structural changes within the vasculature in response to immobilization. We investigated the skeletal muscle P2Y2 receptor content, since these receptors appear to be important mediators of ATP-induced vasodilatation (34, 39). We have previously shown that, in human skeletal muscle, P2Y2 receptors are present in microvascular endothelial and smooth muscle cells with no presence in skeletal muscle cells (4, 24), suggesting that the change in skeletal muscle P2Y2 receptor content occurred in the vasculature. However, it is not possible to determine whether the increase in P2Y2 protein occurred specifically in endothelial and/or smooth muscle cells. A possibility is that endothelial-bound nucleoside triphosphate diphosphohydrolase (21) is increased with training, resulting in a more rapid degradation of the infused ATP in the trained leg (27) and/or that P2Y2 receptor sensitivity is downregulated with training. A slightly larger variation in the individual response to infused adenosine may also have contributed to the lack of difference between conditions, although no nonresponders were observed (22). In contrast to the effect of immobilization on ATP-induced vasodilation, exercise training did not alter ATP- and adenosine-induced vasodilation. The lack of effect of exercise training on vasodilator responsiveness may be attributed to a decrease in regular daily physical activity during the immobilization period. Also, a limitation to the experimental model is that one-legged knee-extensor training targets mainly the quadriceps muscle group, whereas infused vasodilators are likely to induce vasodilation across the entire leg, which could mask small differences in vasodilator responsiveness within the quadriceps muscle group.

The finding that exercise training reduced limb blood flow at the same absolute workload is in agreement with some (13, 17, 19, 29, 32) but not all training studies (28). The underlying mechanisms appear to be confined to the exercising muscles because the trained and immobilized muscle were examined on the same experimental day. The lowering of LBF was associated with a lowering of perfusion pressure (17, 28), rather than a change in vascular conductance (19). The lower blood pressure and heart rate during exercise with a trained muscle within the same circulation suggests that a lower activation of the exercise pressor reflex arising in the muscle (2, 32, 36) may be a mechanism contributing to the lowering of blood flow after a period of training. The similar LVC in the control, trained, and immobilized leg is in contrast to the finding that functional sympatholysys is impaired with immobilization but in agreement with the observation that the vasodilatory response to infused ATP is higher in the immobilized muscle. Training adaptations within the skeletal muscle that result in an optimized blood flow distribution and improved conditions for oxygen diffusion could explain the lower blood flow in the trained leg (12, 29, 42, 43) despite an improved functional sympatholysis.

In conclusion, immobilization impairs functional sympatholysis but also increases exercise hyperemia and the vasodilatory responsiveness to infused ATP. The higher exercise hyperemia is linked to a higher blood pressure response during exercise with a limb that has been immobilized. The impaired functional sympatholysis despite increased responsiveness to infused ATP may be associated with differences in the activation of sympathetic nerve activity and relative workload. The increased vasodilatory responsiveness to ATP, but not to adenosine, after immobilization appears to be related to changes in receptor sensitivity to ATP and not P2Y2 receptor content, but further studies are needed to determine the precise mechanism.

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REFERENCES
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ROLE OF MUSCLE TRAINING STATUS ON VASCULAR FUNCTION


