Reduced submaximal leg blood flow after high-intensity aerobic training

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Proctor, David N., Jordan D. Miller, Niki M. Dietz, Christopher T. Minson, and Michael J. Joyner. Reduced submaximal leg blood flow after high-intensity aerobic training. J Appl Physiol 91: 2619–2627, 2001.—This study evaluated the hypothesis that active muscle blood flow is lower during exercise at a given submaximal power output after aerobic conditioning as a result of unchanged cardiac output and blunted splanchnic vasoconstriction. Eight untrained subjects (4 men, 4 women, 23–31 yr) performed high-intensity aerobic training for 9–12 wk. Leg blood flow (femoral vein thermodilution), splanchnic blood flow (indocyanine green clearance), cardiac output (acetylene rebreathing), whole body O₂ uptake (V̇O₂), and arterial-venous blood gases were measured before and after training at identical submaximal power outputs (70 and 140 W; upright 2-leg cycling). Training increased (P < 0.05) peak V̇O₂ (12–36%) but did not significantly change submaximal V̇O₂ or cardiac output. Leg blood flow during both submaximal power outputs averaged 18% lower after training (P = 0.001; n = 7), but these reductions were not correlated with changes in splanchnic vasoconstriction. Submaximal leg V̇O₂ was also lower after training. These findings support the hypothesis that aerobic training reduces active muscle blood flow at a given submaximal power output. However, changes in leg and splanchnic blood flow resulting from high-intensity training may not be causally linked.

exercise; gender; splanchnic blood flow; cardiac output; oxygen uptake

IT IS WELL KNOWN THAT AEROBIC exercise training of sufficient intensity, frequency, and duration augments maximal skeletal muscle blood flow capacity in humans. Cardiac output and active limb blood flow during maximal exercise are higher after training (25), and the maximal vasodilatory capacity of the trained muscles is elevated (i.e., reactive hyperemic flow; Refs. 17, 29).

Less is known about training-induced adaptations in active muscle blood flow during submaximal exercise. It is generally assumed that blood flow to the working muscles at a given submaximal power output is the same or slightly less after aerobic training because of unchanged cardiac output and increased perfusion of nonactive tissues (e.g., reduced splanchnic vasoconstriction; Refs. 6, 19). The few longitudinal studies that have directly compared leg blood flow before and after exercise training suggest reduced (15), no change (2) (23), or even increased (3) muscle blood flow at a given submaximal power output. However, several of these studies used the xenon clearance technique (6, 32), which is confounded by nonmuscle (e.g., lipid) uptake of the tracer (6). Other exercise training studies have used indicator dilution blood flow techniques (e.g., thermodilution, dye dilution), but these studies focused on maximal leg blood flow (25) or involved training of only one leg (15, 27) or knee extensor (23). Consequently, the question of whether blood flow to the working muscles at submaximal power outputs is less after conventional whole body physical conditioning is unresolved and has not been addressed in a direct or comprehensive way.

Therefore, the primary purpose of the present study was to test the hypothesis that leg blood flow during submaximal power outputs is less after aerobic training as a result of unchanged cardiac output and blunted splanchnic vasoconstriction. To do this, we measured leg and splanchnic blood flow and cardiac output before and after training at identical submaximal power outputs. We used a high-intensity interval-based exercise program that has been shown to result in significant central (7) and peripheral (10) circulatory adaptations. A secondary purpose was to determine whether aerobic training alters leg O₂ uptake (V̇O₂) during submaximal exercise.

METHODS

Subjects

Healthy young men and women were recruited from Rochester, Minnesota and surrounding communities. All volunteers were informed of potential risks and discomforts and signed an informed consent form approved by the Institutional Review Board of Mayo Clinic. Subjects were recruited who had prior experience with formal exercise training but who had not participated in aerobic exercise training during

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the previous 1–2 yr. This was done to increase the likelihood that subjects would tolerate the physical demands of high-intensity training. Subjects were nonsmokers, nonobese (% fat <35%), not taking medications, and had normal hemoglobin levels (15.4 ± 0.3 ml/dl for men; 13.6 ± 0.6 ml/dl for women). A graded treadmill test to maximum exertion was used to characterize each subject’s maximal (i.e., whole body) VO\textsubscript{2} (VO\textsubscript{2 max}). The women’s average VO\textsubscript{2 max} was 39.4 ± 2.5 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} (75th percentile) and the men’s was 43.6 ± 0.5 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} (55th percentile). Eight subjects (4 women, 4 men; 23–31 yr) completed the training program and all pre- and posttraining tests.

### Body Composition and Blood Volumes

Total body fat and lower extremity fat-free mass were estimated both before and after training using dual-energy X-ray absorptiometry (Lunar, Madison, WI) as described previously (22). The instrument was calibrated monthly by using a series of meat blocks of known composition. Total plasma estradiol levels (15.4 ± 0.3 ml/dl for men; 13.6 ± 0.6 ml/dl for women). A graded treadmill test to maximum exertion was used to characterize each subject’s maximal (i.e., whole body) VO\textsubscript{2} (VO\textsubscript{2 max}). The women’s average VO\textsubscript{2 max} was 39.4 ± 2.5 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} (75th percentile) and the men’s was 43.6 ± 0.5 ml·kg\textsuperscript{-1}·min\textsuperscript{-1} (55th percentile). Eight subjects (4 women, 4 men; 23–31 yr) completed the training program and all pre- and posttraining tests.

#### Exercise Testing

All exercise testing was performed in the upright posture using a Lode electronically braked cycle ergometer with toe clips. Subjects were not permitted to lean forward or stand during testing. Pulmonary gas exchange (VO\textsubscript{2}, CO\textsubscript{2} production, and minute ventilation) was measured by using an automated breath-by-breath gas exchange system (Med Graphics). Heart rate (HR) was monitored (electrocardiogram) and ratings of perceived exertion were assessed using the Borg 20-point scale (18).

#### Peak VO\textsubscript{2} Testing

Our primary indicator of training effectiveness was peak VO\textsubscript{2} measured during cycle ergometer exercise to exhaustion. Two exercise protocols were used to measure peak VO\textsubscript{2}. For the first test, exercise started at a power output between 20 and 40 W and increased by 20–40 W each minute until exhaustion. After 20–30 min of recovery, peak VO\textsubscript{2} was measured via a protocol that more closely simulated the interval training workouts (i.e., constant load). For this test, subjects pedaled (60–90 rpm) at 75% of their peak incremental power output (i.e., 75% of peak power for test 1) for 2 min, followed by pedaling to exhaustion at 85–95% of their peak incremental power output (total time ranged from 3 to 4.5 min). Peak VO\textsubscript{2} measured using these two protocols did not differ (P > 0.05) during either pre- or posttraining tests. Therefore, peak VO\textsubscript{2} was calculated as the average for the two tests. Peak VO\textsubscript{2} was measured in this way before training, after the first 3–4 wk of training, and every 2–3 wk thereafter.

#### Cardiac Output Testing

On a subsequent visit, cardiac output was estimated at rest and during cycle ergometer exercise by using the acetylene (C\textsubscript{2}H\textsubscript{2}) rebreathing technique as described previously (21). Briefly, subjects rebreathed 8–10 breaths from a rubber bag initially containing a mixture of 0.7% C\textsubscript{2}H\textsubscript{2}, 40% O\textsubscript{2}, 10% He, and balance N\textsubscript{2}. Cardiac output was computed by using the equations outlined by Triebwasser et al. (31).

Cardiac output was measured while the subject rested on the bike seat and during steady-state, submaximal work rates (typically 70 and 140 W for women; 70, 140, and 210 W for men). Resting trials were conducted three to four times, with the two closest values used for averaging. During each submaximal power output, HR and VO\textsubscript{2} were monitored for 2–4 min until steady state was achieved, followed by cardiac output measurements at ~4 and 7 min of exercise. When two cardiac output values differed by >10%, the bout was repeated a second time after a short rest period. In most cases, this approach provided at least three values for averaging at each submaximal power output.

During preliminary testing, we determined that it was logistically difficult to have the subjects perform the C\textsubscript{2}H\textsubscript{2} rebreathing maneuver for cardiac output measurement during the invasive leg/splanchnic blood flow session. Consequently, cardiac output and leg blood flow/splanchnic blood flow were not measured simultaneously on the same study day. However, identical power outputs on the same cycle ergometer were used for both studies, and steady-state HR and whole body VO\textsubscript{2} responses at each power output were similar (± 2–3%) during the two visits.

#### Leg Blood Flow Testing

**Subject preparation.** Subjects had a venous blood sample drawn 1–2 days before leg blood flow testing to measure hemoglobin concentration. Female subjects also had a urine pregnancy test at that time. Subjects arrived at the General Clinical Research Center (GCRC) on the evening before the leg blood flow study and consumed a standard meal (55% carbohydrate, 35% fat, 15% protein; 20 kcal/kg). Fluid intake was encouraged, and no caffeine was allowed after 10 PM. Subjects remained in the GCRC overnight.

On the morning of testing, subjects consumed a light breakfast and were instructed on how to shave their right groin region and apply a topical anesthetic (Emla cream). Preparation for catheter placement began at 7–8 AM. Catheters were placed using aseptic procedures and local anesthetic (1% lidocaine) in the right femoral vein (for leg blood flow and venous blood sampling), the right radial artery (for arterial pressure and blood sampling), and the left antecubital vein [for iodocyanine green dye (ICG) infusion]. A thermister wire (IT-18, Physitemp Instruments, Clifton, NJ) and an 18-gauge infusion catheter with 10 side hole ports (Cook royal flush II 4.0 Fr) were placed ~15 cm apart in the femoral vein (anterograde and retrograde, respectively) as previously described by our group (20). A 20-gauge Teflon catheter (Arrow arterial catheterization set FA-04020) was inserted in the radial artery.

**Measurement of leg blood flow and arterial pressure.** Leg blood flow was measured during exercise by using the constant infusion thermodilution technique as described previously (20). Ice-chilled sterile saline (2–5°C) was infused for 10–15 s until femoral vein temperature decreased to a stable level. Before training, the rate of saline infusion was adjusted to achieve ~1.0–1.5°C drop in femoral vein temperature at each power output. After training, saline infusion rates were matched with those used pretraining to minimize leg blood flow measurement variability. Thermistor signals and saline bag weight changes were displayed and analyzed using Windaq software. Leg blood flow (l/min) was calculated by using the thermal balance equation described by Andersen and Saltin (1) and was doubled to give two-leg blood flow.
Within-day reproducibility of thermodilution measurements in our laboratory is consistent with other that in laboratories (i.e., within-trial coefficient of variation = 5–7%). In the absence of a “gold standard” for measuring muscle blood flow, we computed leg VO₂ as a percentage of whole body VO₂. When this was done, seven of our eight subjects had leg blood flow measurements at 70 and 140 W that yielded physiologically valid leg VO₂ values (i.e., 50–90% of whole body VO₂). However, one woman had leg VO₂ values that were all significantly below the normal physiological range (i.e., <35% of whole body VO₂). Because her blood gas data were within normal limits, we determined that her leg blood flow recordings were in error, likely as a result of positioning of the proximal thermister wire in an adjacent femoral branch. Therefore, we had to exclude her leg blood flow data from all analyses.

Simultaneous recordings from the radial artery pressure transducer (Baxter PX-MK099) were also displayed, recorded, and analyzed by using WinDaq software as described previously (20). The arterial pressure transducer was zeroed at the artery level for each test. Arterial pressure recordings from two studies (two women) were unintentionally deleted. Consequently, we did not have a sufficient number of subjects to determine the effects of gender or training on mean arterial pressure responses or calculated leg conductance during exercise.

**Measurement of leg VO₂.** The arterial and venous blood-gas samples (3 ml each) were collected anaerobically in heparinized syringes, placed on ice, and analyzed within 15 min. All blood gas variables (total hemoglobin, %oxyhemoglobin saturation, PO₂, PCO₂, and pH) were analyzed by using an Instrumentation Laboratories blood-gas analyzer. All blood-gas measurements were made at 37°C and corrected to the femoral vein blood temperature obtained immediately before blood sampling. Blood O₂ content was calculated as (1.39 × corrected hemoglobin concentration × %O₂ saturation) + (0.003 × blood PO₂). Leg arteriovenous O₂ content difference [(a-v)O₂] was calculated as the difference between arterial and venous blood O₂ content. Leg (a-v)O₂ and leg blood flow (2 legs) were then multiplied to give leg VO₂ (l/min).

**Measurement of splanchnic blood flow.** Splanchnic blood flow was estimated by using the indocyanine green dye (ICG) clearance technique described by Kenney and co-workers (11, 13). ICG infusion began after placement of the thermodilution arterial and pressure catheters while the subject rested in the supine position. A priming dose of ICG (0.5 mg/kg) was infused into the antecubital venous catheter followed by a continuous infusion (0.5 mg/kg/hr) that continued for the remainder of the leg blood flow study. Blood samples were collected after 30, 35, and 40 min of supine rest to confirm that the blood concentration of ICG had reached steady state. Splanchnic blood flow at rest and during exercise was calculated from ICG clearance (CICG) and hematocrit (Hct) as CICG/(1 – Hct). We assumed that training did not alter the ICG extraction ratio at rest or during exercise in either men or women. However, there was considerable variation in resting splanchnic blood flow pre- to postraining. Therefore, changes in splanchnic blood flow during exercise were expressed relative to baseline resting levels (i.e., delta reduction in l/min) to give an indication of splanchnic vasoconstriction.

**Catecholamines and lactate.** Blood samples for arterial and venous plasma catecholamine concentrations (5 ml each) were measured by using high-performance liquid chromatography with electrochemical detection (5). Rate of norepinephrine (NE) spillover (net overflow) from the leg was calculated as described by Savard et al. (28). Arterial and venous lactate concentrations (2 ml each) were also measured at each power output by using a standard enzymatic assay (catalog no. 735-10, Sigma Chemical). Approximately 250 ml of blood were collected from each subject over the course each leg blood flow study.

**Exercise protocol.** All seven subjects completed at least two common power outputs both before and after training (70 and 140 W). In general, each submaximal power output lasted for ~7 min and was repeated twice according to the following protocol (70→140 W, 30–45 min rest, 70→140→peak watts). HR and VO₂ were monitored during minutes 3–7 of each submaximal power output. Leg blood flow and arterial pressure measurements were obtained at least three times during each submaximal workload (at ~3, 5, and 7 min), and arterial and femoral venous blood samples were obtained at ~6 min. For all subjects, this protocol provided between three and six leg blood flow values for averaging at each of the two common workloads (70 and 140 W). A fan was placed in front of the subjects during exercise testing, and they were encouraged to drink water between bouts to remain well hydrated.

**Exercise Training Program.** Most (i.e., >90%) of the training sessions took place in the employee fitness center at Mayo Clinic and were supervised by certified fitness instructors. Training consisted of high-intensity aerobic intervals on a stationary cycle ergometer (3–4 days/wk) and continuous running on a treadmill (1 day/wk). A 2-wk aerobic build-up program (6 sessions) preceded the high-intensity training to orient the subjects to the training program and to provide time for subjects to fully replenish the blood that was removed during the pretraining leg blood flow study (i.e., ~250 ml). The basic design of the bike interval-training program was similar to that developed by Hickson and colleagues (10) and consisted of six 5-min cycling bouts with recovery periods of 2–5 min (i.e., recovery HR of ~120 beats/min). The first three intervals of each session were gradually increased from light to moderate intensity (i.e., 50–75% of peak HR), and the last three intervals were designed to elicit maximal exertion (95–100% of peak HR) during the final 1–2 min. Pedal rates were maintained between 60 and 90 rpm for each interval. Treadmill training consisted of continuous jogging for 15 min/day during the first week, increasing to 30 min/day after 2–3 wk. The pace and/or grade were increased every 5–10 min during these sessions such that subjects reached near maximal effort (rating of perceived exertion (RPE) ≥ 17) during the final 5–10 min. Training intensity was monitored with Polar HR watches and recorded in a training log along with RPE data and subjective comments. Throughout the training program, subjects were given encouragement and counseling by the fitness instructors and study investigators to enhance compliance to this physically demanding training program. Subjects were also continually encouraged to drink fluids to avoid the effects of underhydration. Body weight was also measured once per week. If subjects lost (or gained) more than 4% of their body weight, they were referred to a dietician.

Training continued until improvements in peak VO₂ (l/min) had reached a clear plateau, which ranged from 9 to 12 wk (including the 2-wk build-up period) in these subjects. Postraining laboratory measurements were conducted over a 2- to 3-wk period, during which high-intensity training continued, but at a reduced frequency (2–3 days/wk). One female subject had to discontinue training for a 6-wk period because of uncontrollable scheduling conflicts. This subject resumed high-intensity training (primarily bike intervals)
for an additional 6 wk before completing posttraining measurements.

Statistical Analyses

All statistics were performed by using Minitab (version 13). A two-way ANOVA was used initially to determine if the pre- to posttraining difference for each variable was significantly different between the two power outputs (i.e., 70 vs. 140 W) or between men and women. This analysis indicated that only two sets of variables (blood lactate concentrations, leg NE spillover) responded differently to training at the two power outputs. For these two sets of variables, the effect of training was assessed for each power output separately by using a paired t-test. For all other variables, the effect of training was assessed across the two submaximal power outputs (i.e., data for both power outputs combined) by using a paired t-test. For those variables that exhibited a gender effect by the initial two-way ANOVA (i.e., variables that responded to training differently in men vs. women), paired t-tests were conducted to determine the effect of training for each gender separately. For body composition and peak exercise variables, gender and training effects were analyzed by using one-way ANOVA and subsequent paired t-tests. Statistical significance was accepted for $P \leq 0.05$, except where more than one paired t-test was performed on the same variable, i.e., variables for which there was a power output or gender effect. In these cases, the level of significance was conservatively adjusted to 0.05/2 (i.e., $P \leq 0.025$).

RESULTS

Subject Characteristics

Both before and after the training program, the men were heavier, leaner, and had more leg fat-free mass than the women (Table 1). Training did not alter body weight, total body fat, or leg composition (i.e., leg fat mass or fat-free mass) in either sex. However, there was a larger ($P = 0.03$) training-induced increase in total blood volume in the women (+15%) compared with the men (+2%). This was primarily due to a larger plasma volume expansion in the women (+20 vs. +3%).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men Pretraining</th>
<th>Men Posttraining</th>
<th>Women Pretraining</th>
<th>Women Posttraining</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight, kg</td>
<td>95.3 ± 5.6</td>
<td>96.0 ± 7.0</td>
<td>66.8 ± 7.5</td>
<td>68.2 ± 7.3</td>
<td>0.276</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>27.2 ± 1.2</td>
<td>27.3 ± 2.4</td>
<td>32.4 ± 3.5</td>
<td>32.4 ± 3.2</td>
<td>0.979</td>
</tr>
<tr>
<td>Leg fat-free mass, kg</td>
<td>23.0 ± 1.2</td>
<td>23.0 ± 0.8</td>
<td>14.3 ± 1.2</td>
<td>14.4 ± 0.9</td>
<td>0.939</td>
</tr>
<tr>
<td>Blood volume, ml</td>
<td>6,170 ± 359</td>
<td>6,291 ± 211</td>
<td>3,982 ± 404</td>
<td>4,590 ± 466</td>
<td>0.068</td>
</tr>
<tr>
<td>Plasma volume, ml</td>
<td>3,795 ± 179</td>
<td>3,919 ± 91</td>
<td>2,562 ± 271</td>
<td>3,062 ± 301</td>
<td>0.024*</td>
</tr>
<tr>
<td>Red blood cell volume, ml</td>
<td>2,376 ± 184</td>
<td>2,372 ± 128</td>
<td>1,420 ± 147</td>
<td>1,529 ± 169</td>
<td>0.224</td>
</tr>
<tr>
<td>Arterial O$_2$ content, ml/dl</td>
<td>20.3 ± 0.3</td>
<td>19.9 ± 0.6</td>
<td>18.0 ± 0.8</td>
<td>18.4 ± 0.7</td>
<td>0.924</td>
</tr>
</tbody>
</table>

Values are means ± SE for 4 men and 4 women. All physical characteristics responded to training similarly between men and women. Therefore, $P$ values indicate the effects of training collapsed across both genders (n = 8) evaluated using a 2-sided paired t-test ($*P < 0.05$).

Systemic Responses During Submaximal Power Outputs

Training did not alter steady-state levels of whole body VO$_2$ during submaximal cycling at 70 or 140 W (Table 3). However, the relative intensity of these workloads (% of peak VO$_2$) was substantially less after training because of the increases in peak VO$_2$ and power output. Training also reduced HR, blood lactate, and NE concentrations at these power outputs. Reductions in femoral venous lactate were greater at 140 than at 70 W. Each of these metabolic adaptions to training was similar between men and women.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men Pretraining</th>
<th>Men Posttraining</th>
<th>Women Pretraining</th>
<th>Women Posttraining</th>
<th>P Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak VO$_2$, l/min†</td>
<td>3.44 ± 0.30</td>
<td>4.21 ± 0.25</td>
<td>2.18 ± 0.17</td>
<td>2.69 ± 0.12</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Peak power output, W†</td>
<td>315 ± 27</td>
<td>388 ± 21</td>
<td>192 ± 19</td>
<td>254 ± 13</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Peak heart rate, beats/min†</td>
<td>192 ± 2</td>
<td>187 ± 4</td>
<td>192 ± 2</td>
<td>188 ± 1</td>
<td>0.021*</td>
</tr>
<tr>
<td>Peak leg O$_2$ extraction, %†</td>
<td>72 ± 2</td>
<td>82 ± 1</td>
<td>72 ± 1</td>
<td>82 ± 2</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Values are means ± SE for 4 men and 4 women. Peak exercise variables responded to training similarly between men and women. Therefore, $P$ values indicate the effects of training collapsed across both genders (n = 8) evaluated using a 2-sided paired t-test ($*P < 0.05$).
Table 3. Systemic responses during submaximal power outputs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>Pretraining</th>
<th>Posttraining</th>
<th>Gender effect</th>
<th>Training effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole body VO₂, l/min</td>
<td></td>
<td></td>
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<tr>
<td>70</td>
<td>1.29 ± 0.03</td>
<td>1.30 ± 0.05</td>
<td>1.12 ± 0.05</td>
<td>1.10 ± 0.02</td>
<td>0.94</td>
<td>0.84</td>
</tr>
<tr>
<td>140</td>
<td>2.06 ± 0.04</td>
<td>2.05 ± 0.05</td>
<td>1.73 ± 0.16</td>
<td>1.74 ± 0.14</td>
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<tr>
<td>Percent of peak VO₂</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>70</td>
<td>38 ± 2</td>
<td>31 ± 1</td>
<td>52 ± 4</td>
<td>41 ± 1</td>
<td>0.22</td>
<td>0.10</td>
</tr>
<tr>
<td>140</td>
<td>61 ± 4</td>
<td>49 ± 2</td>
<td>80 ± 4</td>
<td>64 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cardiac output, l/min</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>70</td>
<td>11.8 ± 0.3</td>
<td>12.3 ± 0.3</td>
<td>10.7 ± 0.6</td>
<td>9.8 ± 0.6</td>
<td>0.005*</td>
<td>0.19 (men)</td>
</tr>
<tr>
<td>140</td>
<td>15.3 ± 0.4</td>
<td>15.4 ± 0.4</td>
<td>14.0 ± 1.1</td>
<td>13.2 ± 1.2</td>
<td>0.13 (women)</td>
<td></td>
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<tr>
<td>Splanchnic blood flow, Δl/min</td>
<td></td>
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<tr>
<td>70</td>
<td>0.65 ± 0.12</td>
<td>0.50 ± 0.07</td>
<td>0.48 ± 0.08</td>
<td>0.67 ± 0.18</td>
<td>0.003*</td>
<td>0.03* (men)</td>
</tr>
<tr>
<td>140</td>
<td>0.94 ± 0.13</td>
<td>0.69 ± 0.10</td>
<td>0.69 ± 0.10</td>
<td>0.94 ± 0.16</td>
<td></td>
<td>0.53 (women)</td>
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<tr>
<td>Arterial O₂ content, ml/dl</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>70</td>
<td>21.4 ± 0.5</td>
<td>20.4 ± 0.6</td>
<td>18.4 ± 1.1</td>
<td>18.6 ± 0.8</td>
<td>0.001*</td>
<td>0.01* (men)</td>
</tr>
<tr>
<td>140</td>
<td>21.3 ± 0.5</td>
<td>20.4 ± 0.6</td>
<td>18.7 ± 1.0</td>
<td>18.8 ± 0.8</td>
<td></td>
<td>0.53 (women)</td>
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<tr>
<td>Heart rate, beats/min</td>
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<td></td>
<td></td>
<td>&lt;0.01*</td>
</tr>
<tr>
<td>70</td>
<td>110 ± 10</td>
<td>108 ± 3</td>
<td>137 ± 4</td>
<td>121 ± 2</td>
<td>0.36</td>
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</tr>
<tr>
<td>140</td>
<td>135 ± 11</td>
<td>129 ± 6</td>
<td>174 ± 2</td>
<td>165 ± 2</td>
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<td></td>
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<tr>
<td>Venous lactate, mmol/l‡</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>2.4 ± 0.6</td>
<td>1.1 ± 0.2</td>
<td>3.0 ± 0.8</td>
<td>1.5 ± 0.4</td>
<td>0.80</td>
<td>0.02* (70W)</td>
</tr>
<tr>
<td>140</td>
<td>6.1 ± 1.2</td>
<td>2.7 ± 0.4</td>
<td>7.6 ± 1.4</td>
<td>3.9 ± 0.6</td>
<td>&lt;0.01*</td>
<td>&lt;140W)</td>
</tr>
</tbody>
</table>

Values are means ± SE for 4 men and 4 women during both 70- and 140-W power outputs. Gender effect P values (1-way ANOVA) indicate whether the effect of training was significantly different between men and women. Training effect P values (2-sided paired t-test) indicate whether the effect of training was significant (**P < 0.05). †Reduction in splanchnic blood flow from baseline rest condition. ‡Reductions in femoral venous lactate concentration after training were larger for 140 than for 70 W.

There was a significant effect of gender on the cardiac output response to training (P = 0.05, two-way ANOVA). In the men, cardiac output at rest (5.2 ± 0.1 vs. 5.6 ± 0.3 l/min, P = 0.26) and during submaximal exercise (Table 3) did not change with training. This differed from the response seen in the women, who showed a trend toward reduced cardiac outputs both at rest (4.6 ± 0.3 vs. 4.0 ± 0.1 l/min, P = 0.11) and during exercise (Table 3, P = 0.13). Gender also influenced the effect of training on the arterial O₂ content measured during exercise. In the men, arterial O₂ content was ~1 ml/dl lower after training. This differed from the responses seen in the women, who maintained their arterial O₂ content at both submaximal power outputs with training.

**Leg Responses**

**Reproducibility and validity of leg blood flows.** Seven of the eight subjects who completed the training had leg blood flow measurements at 70 and 140 W that yielded physiologically valid leg O₂ uptakes as described in METHODS. Leg blood flows and femoral venous blood gases during submaximal cycle ergometry were also obtained ~3 mo apart in two laboratory personnel (1 man, 1 woman) who did not change their physical activity habits between tests. Test-retest values for leg blood flow and leg VO₂ at 140 W for these “time control” subjects were within 5–10%.

**Training effects.** Steady-state leg blood flows at 70 and 140 W were significantly lower after training (P = 0.001; Fig. 1A). At 140 W, decreases of at least 0.6 l·min⁻¹·leg⁻¹ were seen in six of the seven subjects. Training-induced changes in leg blood flow at 70 W were smaller and more variable between subjects, but the mean reduction in leg blood flow was 18% for both power outputs. There was a trend toward larger training-induced reductions in leg blood flow in the women vs. men (~22% vs. ~12%), but the effect of gender was not statistically significant (P = 0.33; ANOVA). Leg NE spillover, the product of leg blood flow and estimated NE extraction, was almost 100% lower at the 140-W level after training (P = 0.03; Fig. 1B). No difference in leg NE spillover was seen at 70 W.

Leg VO₂ was also significantly lower after the training program (Fig. 1C). This was primarily due to reductions in leg blood flow because leg (a-v)O₂ difference was either unchanged (men; pre 14.1 ± 1.4 vs. post 13.7 ± 0.8 ml/dl) or significantly higher (women; pre 13.3 ± 1.5 vs. post 14.5 ± 0.9 ml/dl) after training. Figure 2 shows the linear relationship between training-induced reductions in leg blood flow and leg VO₂ at both power outputs. Fractional O₂ extraction by the legs averaged 3–4% higher after training at both submaximal power outputs in both men and women (P = 0.03).

**Splanchnic Blood Flow**

Supine resting levels of splanchnic blood flow before training averaged 2.0 ± 0.2 l/min in the men and 1.5 ± 0.1 l/min in the women. During upright cycling exercise, splanchnic blood flow decreased in an intensity-dependent fashion in all subjects, both before and after training. When splanchnic blood flow was expressed relative to baseline (i.e., supine rest), there was a statistically significant effect of gender on the splanchnic vasoconstriction adaptation to training (Table 3). In
the men, splanchnic blood flow reductions during exercise at 70 and 140 W averaged 0.65 and 0.9 l/min (−32 and −47% below resting), respectively, before training. After training, exercise-induced reductions in splanchnic blood flow were significantly less (−26% and −36% below resting; \( P = 0.03 \)). By contrast, the women did not experience a significant change in splanchnic blood flow after (−37 and −52% below resting) compared with before (−32 and −46% below resting) training at 70 or 140 W (\( P = 0.53 \)). The correlations between training-induced changes in leg blood flow and splanchnic vasoconstriction were not statistically significant at either power output (both \( P > 0.2 \)). The relationship between training-induced changes in leg blood flow and splanchnic vasoconstriction at 140 W is shown in Fig. 3.

**DISCUSSION**

Whether or not aerobic exercise training reduces active muscle blood flow at a given submaximal power output in humans has been debated since the early 1970s (6). It has generally been assumed that blood flow to the working muscles at a given submaximal power output is the same or slightly less after aerobic training as a result of unchanged cardiac output and reduced splanchnic vasoconstriction. To our knowledge, the present investigation is the first to directly address this hypothesis using simultaneous invasive leg and splanchnic blood flow techniques and a conventional mode of aerobic exercise training (i.e., 2-leg upright cycling). Our findings indicate that high-intensity aerobic training can decrease total leg blood flow at low- and moderate-intensity power outputs in young men.
men and women. However, there was no clear evidence that this training adaptation was associated with changes in splanchnic vasoconstriction. An additional new finding was that leg \( \text{VO}_2 \) during submaximal cycling was lower after high-intensity aerobic training.

**Leg Blood Flow and Training**

To our knowledge, only four previous studies involving leg cycle ergometry directly measured total leg blood flow at identical power outputs before and after aerobic exercise training in healthy humans (2, 3, 15) (23). Three studies (2) (15) (23) reported no effect of training on submaximal leg blood flow, whereas the study by Bergman et al. (3) reported a 10% increase. Thus our finding that submaximal leg blood flow was significantly reduced after training differs from these previous studies. The larger training-induced changes in leg blood flow we observed could reflect the higher training stimulus we used (bike interval training at 85–100% maximal HR) compared with previous studies (continuous training at 60–85% maximal HR). Divergent findings might also be due to differences in the procedures used to measure leg blood flow. For example, Beere et al. (2) and Bergman et al. (3) measured iliac vein blood flow, which includes blood flow from the skin draining the leg (i.e., greater saphenous vein), the gluteal muscles, and possibly other pelvic tissues. Finally, none of the previous studies included female subjects. Although we did note a trend toward greater training-induced reductions in leg blood flow in our female subjects (−22% vs. −12%), this gender difference was not statistically significant.

Femoral vein thermodilution estimates of leg blood flow represent the sum of blood flow to several tissues including skeletal muscle, bone, subcutaneous fat, and skin. We believe that most of the training-induced reductions in total leg blood flow during exercise were the result of reduced blood flow to muscle tissue. This is likely because skeletal muscle represents the largest component of the legs and has the highest requirement for \( \text{O}_2 \) delivery during exercise. Large changes in blood flow to skin are also possible during large muscle exercise, but skin blood flow at a given absolute work intensity is thought to be higher, rather than lower, after endurance training in humans (12).

**Splanchnic Blood Flow and Training**

In contrast to our original hypothesis, training-induced reductions in leg blood flow were not correlated with changes in splanchnic vasoconstriction during submaximal exercise (e.g., Fig. 3). However, there are several physiological and methodological factors unique to the present study that might explain this apparent lack of association. The most compelling physiological explanation is that not all of our subjects displayed the expected adaptation in splanchnic vasoconstriction with training (i.e., less vasoconstriction posttraining). It is generally assumed that blood flow to the splanchnic organs (i.e., liver, intestines, pancreas, and spleen) is increased at any given absolute work intensity after endurance training, because of the well-established relationship between \( C_{ICG} \) and relative (% of \( \text{VO}_2 \text{max} \)) intensity (13, 26). In the present study, splanchnic blood flow did decrease during exercise in all subjects in an intensity-dependent fashion, both before and after training. However, smaller reductions in splanchnic blood flow during exercise were not observed after training in the women (Table 3). This potential gender difference is difficult to explain, but maintained splanchnic vasoconstriction after training in women could compensate for small reductions in total cardiac output and/or mean arterial pressure at a given absolute power output (14). Additional research involving larger numbers of female subjects is needed to clarify these hemodynamic adaptations to training.

Methodological factors, independent of possible gender differences, could also explain the lack of association between training-induced changes in leg blood flow and splanchnic vasoconstriction observed in the present study. First, training-induced changes in splanchnic blood flow during exercise are relatively small (<200–300 ml/min) compared with potential changes in leg blood flow and cardiac output (6). Second, we assumed that the hepatic extraction ratio for ICG was unchanged with training, both at rest and during exercise. Systematic changes in this ratio with training are not expected (13), but individual variation in this ratio could have contributed to variability in estimating splanchnic blood flow in the present study. Another methodological factor unique to the present study was our use of high-intensity interval training. It is possible that such training may not elicit the same changes in blood flow to the splanchnic region (or other “inactive” regions) that prolonged, lower intensity training does (16). Finally, it should be recognized that the small sample size limited our statistical power to detect a significant association between variables, particularly where substantial variation between subjects exists (e.g., Fig. 3).

Our findings also do not rule out potential training-induced changes in blood flow to other regional circulations. Renal blood flow, for example, decreases during exercise in an intensity-dependent manner and could undergo adaptations to exercise training similar to those observed in the splanchnic circulation (26). We did not measure renal blood flow because of concerns about excessive blood withdrawal and the technical complexity of simultaneous measurements of blood flow to several regions. Blood flow to the arms or other stabilizing muscles (postural) could also be altered by aerobic exercise training. There is a significant decrease in blood flow to the forearm muscles during high-intensity leg cycling (4), but the effects of training are unknown. Therefore, less vasoconstriction within regional circulations that we did not measure could account for changes in the distribution of cardiac output after high-intensity aerobic conditioning.
Potential Mechanisms of Reduced Leg Perfusion

An important new finding in the present study was that leg VO₂ was reduced during submaximal exercise in direct proportion to the decline in leg blood flow (i.e., Fig. 2). This is consistent with the concept that blood flow is tightly regulated to O₂ demand during dynamic exercise. This also suggests that the total metabolic vasodilator signal during submaximal exercise was reduced after training. There are several potential mechanisms by which aerobic training might reduce vasodilator signals in active muscle at a given absolute workload leading to reduced leg blood flow. First, aerobic training causes a marked increase in mitochondrial oxidative capacity, and a given absolute level of exercise is typically associated with less metabolic stress within the active muscles (30). If this were the case in the present study, then the efflux of vasodilator metabolites might have been less after training. Second, repeated bouts of high-intensity leg cycling could result in a more efficient pattern of leg muscle recruitment during submaximal cycling (i.e., improved leg economy). A third possibility is that the training enhanced leg muscle contractile efficiency per se. This could result from increases in the percentage of leg muscle fibers expressing myosin heavy chain (MHC) isoforms with low tension costs (i.e., MHCslow and MHC2A fibers) (9). Finally, it is possible that additional muscles not drained by the femoral vein (i.e., gluteals, hip flexors/extendors) were recruited to a greater extent during submaximal exercise after training. Such an increase in accessory muscle recruitment could reduce the apparent O₂ demand of the leg muscles (i.e., thigh and lower leg) during cycling.

An additional novel finding in the present study was that leg NE spillover during moderate intensity exercise (i.e., 140 W) was lower after training. This suggests that there was less sympathetic restraint of active muscle blood flow after training, possibly associated with a reduced metabolic vasodilator signal. The lower level of sympathetic outflow during moderate intensity exercise after training could reflect the subjects’ higher aerobic capacity in the trained state and/or improved leg economy (24). The lack of training-induced adaptation in leg NE spillover during low-intensity exercise (i.e., 70 W) could be due to large intersubject variability and/or the low absolute level of NE spillover at this workload.

Experimental Limitations

Sample size, particularly within each gender subgroup, was limited because of the complexity, invasiveness, and time demands associated with this study. Inclusion of a conventional, nonexercise control group was also logistically prohibitive. Despite these limitations, statistically and physiologically significant changes were observed for our primary outcome variables (i.e., leg blood flow, splanchnic blood flow, and leg VO₂).

In summary, the findings of this study demonstrate that high-intensity aerobic training can decrease leg blood flow and leg VO₂ during low- and moderate-intensity workloads in young men and women. The mechanisms underlying altered blood flow distribution to active and nonactive tissues after aerobic training and potential gender differences require further investigation. A more complete understanding of muscle blood flow regulation during submaximal exercise also has practical significance because most daily activities are carried out at submaximal intensities.

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