Sex differences in leg vasodilation during graded knee extensor exercise in young adults

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Parker BA, Smithmyer SL, Pelberg JA, Mishkin AD, Herr MD, Proctor DN. Sex differences in leg vasodilation during graded knee extensor exercise in young adults. J Appl Physiol 103: 1583–1591, 2007. First published August 23, 2007; doi:10.1152/japplphysiol.00662.2007. — Limb vascular conductance responses to pharmacological and nonexercise vasodilator stimuli are generally augmented in women compared with men. In the present investigation, we tested the hypothesis that exercise-induced vasodilator responses are also greater in women than men. Sixteen women and 15 men (20–30 yr) with similar fitness and activity levels performed graded quadriceps exercise (supine, single-leg knee extensions, 40 contractions/min) to maximal exertion. Active limb hemodynamics (left common femoral artery diameter and volumetric blood flow), heart rate (ECG), and beat-to-beat mean arterial blood pressure (MAP; radial artery tonometry) were measured during each 3-min workload (4.8 and 8 W/stage for women and men, respectively). The hyperemic response to exercise (slope of femoral blood flow vs. workload) was greater (P < 0.01) in women as was femoral blood flow at workloads >15 W. The leg vasodilatory response to exercise (slope of calculated femoral vascular conductance vs. absolute workload) was also greater in women than in men (P < 0.01) because of the sex difference in hyperemia and the women’s lower MAP (~10–15 mmHg) at all workloads (P < 0.05). The femoral artery dilated to a significantly greater extent in the women (~0.5 mm) than in the men (~0.1 mm) across all submaximal workloads. At maximal exertion, femoral vascular conductance was lower in the men (men, 18.0 ± 0.6 ml·min⁻¹·mmHg⁻¹; women, 22.6 ± 1.4 ml·min⁻¹·mmHg⁻¹, P < 0.01). Collectively, these findings suggest that the vasodilatory response to dynamic leg exercise is greater in young women vs. men.

There is substantial evidence to suggest that limb vasodilator responsiveness is sex specific. For example, young women exhibit augmented brachial artery flow-mediated dilation (33, 51) and β-adrenergic-mediated forearm vasodilation (27) relative to young men. Moreover, the forearm vasodilatory response to acetylcholine (12) as well as peak calf reactive hyperemia (40, 46) tend to be higher in women. Collectively, these results suggest that women exhibit augmented dilatary responsiveness in the limbs. Rogers and Sheriff (48) recently reported that female rats exhibit greater hindlimb vascular conductance during incremental treadmill exercise than male rats, suggesting that limb vasodilator responses to dynamic exercise may also be sex specific. The effect of sex on blood flow to active muscles and its regulation has not been systematically investigated in human during dynamic exercise, although our laboratory recently observed evidence of greater leg blood flow responses in women compared with men during graded leg cycling (28). One difficulty of using treadmill or conventional cycle ergometer exercise to examine sex differences in exercising leg blood flow, however, is that vasodilation of two or more limbs can exceed maximal cardiac output, leading to sympathetic restraint of active muscle blood flow to preserve systemic blood pressure (3, 50). Such effects could confound the comparison of limb vasodilatory responses to exercise in women vs. men because of documented sex differences in baroreflex responsiveness (8, 26), α-adrenergic responsiveness (19, 27), and the modulatory interaction between vasodilators and vasoconstrictors (37).

Therefore, the purpose of this study was to investigate blood flow and vascular conductance responses in the femoral artery (the conduit inflow to the leg vasculature) during dynamic knee extensor exercise, a mode of exercise involving a limited mass of active muscle (2, 3), in healthy young men and women. Based on the work of Rogers and Sheriff (48) as well as the precedent of greater limb vasodilator responsiveness in young women, we hypothesized that young women would exhibit augmented leg vascular conductance relative to young men during graded knee-kick exercise to maximal exertion.

METHODS

Subject Characteristics and Initial Screening

Fifteen young men and 16 young women (ages 20–30 yr) completed the study. All subjects were nonobese (body mass index ≤30 kg/m²), were nonsmokers, had clinically normal blood chemistry (i.e., hemoglobin concentrations ranged from 11.1 to 16.2 g/dl, total cholesterol ≤240 mg/dl, low-density lipoprotein cholesterol ≤150 mg/dl), and had normal resting supine ankle-brachial index ratings (between 0.90 and 1.30; model VP2000, Colin Medical). All subjects were normotensive (resting blood pressure ≤140/90 mmHg) and were neither extremely sedentary nor extremely fit (i.e., had treadmill maximal oxygen uptake (VO₂max) values between 20 and 80% of age-predicted norms (11)). Subjects were free of overt chronic diseases as evaluated by medical history questionnaire, a physical examination, and resting ECG. Additionally, no subjects were taking medications having significant hemodynamic effects (including oral contraceptives) for at least the last 12 mo. Young female subjects were studied in days 1–7 of their menstrual cycle to standardize the influence of female hormones. On the study day, subjects were asked to refrain from alcohol, exercise, caffeine, aspirin, ibuprofen, or herbal supple-
ments for at least 12 h before testing. All subjects gave their written, informed consent to participate. This study was approved by the Office for Research Protections and the Institutional Review Board at The Pennsylvania State University. Subject characteristics are presented in Table 1.

Subjects also completed a physical activity questionnaire to assess routine physical activity [Baecke Questionnaire of Habitual Physical Activity (4)]. None of the subjects participated in moderate- to high-intensity aerobic exercise >3 days/wk or regular lower body resistance training >2 days/wk during the past 12 mo. To objectively quantify aerobic fitness status, all of the subjects performed a continuous incremental treadmill test (SensorMedics, Yorba Linda, CA) to maximal exertion to determine \( V_{\text{O2,max}} \).

Total and regional body composition was estimated using dual-energy X-ray absorptiometry (DXA; model QDR 4500W, Hologic, Waltham, MA) with subjects in the supine position as described previously (40). In addition, thigh volume was estimated by the anthropometric method described by Jones and Pearson (23) from thigh patellar-pubic length, skinfold thicknesses, and circumference. Quadriceps femoris muscle mass was then estimated from thigh volume as originally described and Andersen and Saltin (3).

### Study Procedures

**Exercise modality.** Single-leg knee-extensor exercise, designed to isolate the quadriceps muscle group, was performed as described previously (3, 45). Briefly, subjects were reclined in a seat in the supine position [to minimize cardiopulmonary baroreceptor-mediated decreases in muscle sympathetic nerve activity during knee-kick exercise (44)] as well as sex-related differences in stroke volume responses to exercise (14)] with knees flexed at an angle of 90°. The subject’s torso and both upper legs were fixed by straps attached to the chair to reduce extraneous movement and straining, and the left leg was strapped into a boot attached by lever arm to the pedal of a cycle ergometer. The right leg was allowed to hang free, although subjects were instructed not to swing or move this leg. One extension of the quadriceps muscle moved the subject’s lower leg 90–170° and the ensuing flexion was a passive return pulled by the flywheel of the ergometer. Subjects kicked at a constant cadence of 40 kicks/min (0.67 Hz), because our initial trials demonstrated that this cadence (rather than 60 kicks/min) was easier for subjects to maintain proficiently with minimum motion artifact and consistent duty cycles, without straining the upper body or recruiting the active hamstring. Resistance was increased by increasing the weight attached to a belt surrounding the flywheel such that friction on the flywheel increased proportionately. Subjects participated in two familiarization visits totaling ~1 h of kicking such that they could learn to avoid accessory muscle recruitment (i.e., of the hamstring, inactive leg, and upper body) and maintain cadence. The first familiarization visit consisted of just knee extension exercise, while the second visit involved instrumentation and data acquisition identical to that described below for the actual study visit.

**Exercise protocol.** On the study day, the subjects began the protocol with 3 min of quiet rest, followed by 3 min of unloaded passive exercise (a research technician moved the subject’s leg at 40 kicks/min). The purpose of the passive bout was to investigate the increase in flow due to mechanical influences and tachycardia separate from metabolic stimuli (55). The subject was then instructed to begin kicking against no resistance (0 W) for 3 min, after which resistance increased incrementally every 3 min until the subject could no longer maintain cadence. The workload increases were 8 W in men and 4.8 W in women. These increases were designed to produce similar time to exhaustion in both sexes, taking into account the reduced quadriceps muscle mass of women. Responses of men and women were compared at the same absolute workloads. Additionally, to take into account the different peak power outputs between men and women, each subject’s maximal workload was then used to estimate the relative intensity of each workload at which measurements were collected.

### Data Acquisition and Measurements

All variables were collected online at a sampling frequency of 400 Hz and stored using a Powerlab system (AD Instruments, Castle Hill, Australia). Heart rate and beat-to-beat systolic and diastolic blood pressure (radial tonometry of the right hand; Colin, Medical Instruments) were measured continuously throughout the study. In addition, manual auscultation was used every 3 min throughout the study to check the accuracy of the Colin during exercise. Electromyogram (EMG) signals of the active (left) biceps femoris and inactive (right) rectus femoris (to ensure that contraction was limited to the active quadriceps of the active leg, respectively) were collected with bipolar silver chloride surface electrodes (Bio-Tac, Tyco Healthcare Group LP, Mansfield, MA) fixed lengthwise over the middle of the muscle belly placed 10–20 cm apart. Reference electrodes were placed on the knee of each leg. Electrode signals were amplified (Gould Universal Amplifier model 13 4615 55, Cleveland, OH, and Powerlab bioamplifier) with a bandwidth frequency ranging from 1.5 Hz to 2 kHz and simultaneously digitized using Powerlab. Knee kick cadence was captured using a Cateye Astrale 8 (Cateye, Boulder, CO) cycle computer attached to the flywheel. Knee-kick force tracings were obtained using a load cell attached to the boot arm of the knee kick ergometer.

A Doppler ultrasound machine (model HDI 5000, Philips, Bothell, WA) equipped with a high-resolution 7- to 4-MHz linear-array transducer was used to measure mean blood velocity and vessel diameter of the left common femoral artery, distal to the inguinal ligament but above the bifurcation into the superficial and profunda femoral branch. For velocity measurements, the artery was insonated at a constant angle of 60° with the sample volume adjusted to cover the width of the artery, while diameter measurements were obtained with the artery insonated perpendicularly. Velocity measurements were taken continuously during minutes 1 and 3 of rest, passive exercise, and each workload, while high-resolution diameter measurements (taken in two-dimensional mode to optimize imaging) were taken during minute 2 of every workload (except the peak workload, during which diameter measurements were not taken). A custom-designed interface unit processed the angle-corrected, intensity-weighted Doppler audio information from the ultrasound system into a flow velocity signal that was sampled in real time by Powerlab. Postprocessing using PowerLab’s Chart application package yielded mean blood velocities.

### Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Men (n = 15)</th>
<th>Women (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yrs</td>
<td>24±1</td>
<td>22±1*</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>128±3</td>
<td>110±2*</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>62±2</td>
<td>55±2*</td>
</tr>
<tr>
<td>Height, cm</td>
<td>177±2</td>
<td>165±2*</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>77±2</td>
<td>63±3*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25±1</td>
<td>23±1</td>
</tr>
<tr>
<td>( V_{\text{O2,max}} ), %</td>
<td>54±2.6</td>
<td>43±2.5</td>
</tr>
<tr>
<td>Baecke Questionnaire score</td>
<td>7.4±0.3</td>
<td>7.4±0.3</td>
</tr>
<tr>
<td>Quadriceps muscle mass, kg</td>
<td>2.5±0.1</td>
<td>2.1±0.1*</td>
</tr>
<tr>
<td>Common femoral diameter, mm</td>
<td>8.4±0.2</td>
<td>7.2±0.2*</td>
</tr>
<tr>
<td>Resting FBF, ml/min</td>
<td>346±35</td>
<td>303±29</td>
</tr>
<tr>
<td>Resting FVC, ml·min(^{-1})·mmHg(^{-1})</td>
<td>4.1±0.3</td>
<td>4.2±0.4</td>
</tr>
<tr>
<td>Hemoglobin, gm/dl</td>
<td>15.0±0.2</td>
<td>12.8±0.2*</td>
</tr>
</tbody>
</table>

Values are group averages ± SE; n, no. of subjects. BMI, body mass index; FBF, femoral blood flow; FVC, femoral vascular conductance; \( V_{\text{O2,max}} \), maximal oxygen uptake. *Significant difference between men and women, \( P < 0.05 \).
Diameter measurements were stored on VHS tape and digitized at 4 frames/second using brachial imager software (Medical Imaging Applications, Iowa City, IA). Post test analysis of diameters was performed using edge-detection software (Brachial Analyzer Software, Medical Imaging Applications); briefly, the technician (always the same and blind to any subject information) selected a region of interest along the arterial wall and the edge of the wall was detected by pixel density and represented by a line of best fit. Each sequence of images was reviewed by the technician and adjusted to ensure that diameter measurements were always calculated from the intimal-lumen interface at both the distal and proximal vessel wall. Images affected by motion artifact were excluded based on visual inspection by the technician as well as a calculation by the software program that the confidence limit for the best-fit line was <80%. Diameter measurements for each workload comprised an average of ~80 frames.

Data Analysis and Computations

For all study variables, values were calculated as the average over the last minute of rest, passive exercise, and each workload [to allow hemodynamic parameters to reach steady-state conditions (20, 36)], with the exception of peak measurements, which were calculated with first and/or second minute data if the subject did not complete the peak workload. Mean arterial blood pressure (MAP; in mmHg) was calculated as (1/3 systolic pressure) + (2/3 diastolic pressure). The EMG signals were full-wave rectified, squared, and median filtered, from which the root-mean-square (RMS) value was derived. A spike-triggered average of EMG over the last minute was then derived from the cadence and calculated as a percentage of the RMS value obtained from maximal isometric contraction (EMG averaged from three, 5-s isometric contractions performed at the beginning of the study). Femoral artery blood flow (FBF) for each condition or workload was calculated by multiplying the cross-sectional area of the femoral artery (the diameter taken in minute 2 of each condition or workload) by mean blood velocity, according to the formula:

\[
\text{FBF} = \text{mean blood velocity} \times \pi \times \left(\frac{\text{femoral diameter}}{2}\right)^2 \times 60
\]

where the FBF is in milliliters per minute, the mean blood velocity is in centimeters per second, the femoral diameter (averaged across the cardiac cycle) is in millimeters, and 60 is used to convert from milliliters per second to milliliters per minute. To validate the assumption that the diameter taken in minute 2 was representative of the diameter underlying the blood flow measured in minute 3 of each condition or workload, four subjects performed knee extensor exercise at various 3-min workloads with high-resolution diameter imaged constantly in two-dimensional mode. The within-subject correlation between minute 2 and minute 3 diameters was 0.99. In addition, because peak diameter measurements were not obtained, peak FBF measurements were calculated with the diameter from the previous workload. Femoral vascular conductance (FVC) was calculated as FBF/MAP and, in the case of normalized FVC, divided by each workload. Femoral vascular conductance (FVC) was calculated as a measure of each condition or workload

Statistical Analysis

Statistical analyses were performed using SAS (SAS 9.1, Cary, NC) software. All data are reported as means ± SE with significance set at \( P < 0.05 \). A Student’s \( t \)-test for independent groups and Tukey post hoc analysis were used to compare baseline differences between male and female groups. An autoregressive, random-coefficients model (PROC MIXED) using a continuous predictor (either absolute workload or workload as a percentage of maximal workload attained), fitting a random intercept and slope with workload as the within-individual factor and sex as the between-individual factor, was used to determine differences between subject groups in outcome variables (FVC, FBF, MAP, heart rate, EMG). This repeated-measures model fits the linear or curvilinear trend in response variables based on the measured outcome variables at the absolute workload or calculated percentage of maximal workload in all subjects, and then it estimates and compares the outcome variable at designated common values between subjects and groups (34). A Bonferroni post hoc adjustment was performed when significant sex \( \times \) workload differences were detected.

RESULTS

Subject Characteristics

Subject characteristics are shown in Table 1. In addition to data presented in Table 1, there were no significant between-group differences in resting blood lipid parameters such as total cholesterol (men: 159 ± 6 mg/dl; women: 155 ± 7 mg/dl), low-density lipoprotein cholesterol (men: 93 ± 7 mg/dl; women: 82 ± 5 mg/dl), and triglycerides (men: 90 ± 10 mg/dl; women: 71 ± 6 mg/dl), although high-density lipoprotein cholesterol was significantly \(( P = 0.03)\) higher in women (men: 49 ± 3 mg/dl; women: 60 ± 4 mg/dl).

Peak Power Output

Men achieved a significantly higher peak knee extensor workload than women (men: 38 ± 2 W; women: 25 ± 2 W; \( P < 0.01 \)), although there was no between-group difference in the number of 3-min workloads taken to reach peak (men: 5.8 ± 0.2 workloads; women: 6.2 ± 0.3 workloads) such that the exercise protocols were of similar duration. Ensuing data are represented at either absolute workloads or as each subject’s workloads normalized to his or her peak workload (% of maximal workload) to account for the different workload increases used in men vs. women.

Blood Flow Responses to Incremental Exercise

FBF, estimated leg oxygen delivery, FBF normalized to estimated quadriceps muscle mass, and FBF partitioned by duty cycle into contraction and relaxation flows at rest, passive exercise, and incremental knee extensor exercise to exhaustion are shown in Fig. 1, A–D, respectively. FBF was significantly \(( P < 0.05)\) higher in women at workloads greater than or equal to \( \geq \) 15 W. In addition, the slope of the response was greater in women (47.1 ± 3.7 vs. 29.1 ± 1.8 ml·min\(^{-1}\)·W\(^{-1}\); \( P < 0.01 \)). Although there was no sex difference in estimated oxygen delivery at workloads \( > \) 5 W, the slope of the response was greater in women (0.0073 ± 0.0006 vs. 0.0056 ± 0.0003 ml·min\(^{-1}\)·W\(^{-1}\); \( P = 0.02 \)). Normalizing FBF to estimated quadriceps muscle increased the sex difference such that women exhibited significantly \(( P < 0.05)\) greater normalized blood flow at workloads \( \geq \) 5 W. Relaxation flows were greater \(( P < 0.05)\) in women than men at all workloads \( \geq \) 10 W, and contraction flows were greater \(( P < 0.05)\) in women than men at all workloads \( \geq \) 20 W.

FVC Response to Incremental Exercise

FVC at rest, passive exercise, and incremental knee extensor exercise to exhaustion are shown in Fig. 2. FVC was signifi-
Hemodynamic Responses to Incremental Exercise Expressed Relative to Maximal Workload

MAP, FVC, and FVC normalized to estimated quadriceps muscle mass responses at rest, passive exercise, and incremental knee extensor exercise to exhaustion are shown in Fig. 3. MAP was significantly (P < 0.01) lower (10–15 mmHg) in women at every workload, and FVC was significantly (P < 0.05) higher at workloads >40% of maximal workload in women. In addition, the slope of the conductance response was greater in women than men (0.12 ± 0.01 vs. 0.08 ± 0.01 ml·min⁻¹·mmHg⁻¹·%maximal workload⁻¹; P = 0.02). Normalizing to estimated quadriceps muscle exaggerated the sex difference such that with the exception of passive exercise, women had significantly (P < 0.05) higher normalized FVC at rest and every ensuing workload, and the slope of the normalized FVC response was again greater in women (0.05 ± 0.01 vs. 0.03 ± 0.002 ml·min⁻¹·mmHg⁻¹·%maximal workload⁻¹ greater in women than men; P = 0.01).

Change in Femoral Diameter During Passive and Graded Exercise

Femoral diameter changes (relative to rest) during incremental exercise are shown in Fig. 4. Diameter measurements were not attained at each individual’s peak workload. The change in diameter was greater in women at workloads ≥0% of maximal workload.

Ipsilateral Hamstring Muscle and Contralateral Quadriceps Muscle Recruitment

There were no sex differences in hamstring EMG, expressed as a percentage of each subject’s maximal isometric contraction, at any workload. In addition, inactive quadriceps EMG
was significantly higher \((P < 0.05)\) in women at workloads >40% of maximal recruitment, although the magnitude of this difference was small (quadriceps EMG at rest and at maximal workload in women vs. men: 2.7 ± 0.5 to 6.6 ± 1.0% vs. 2.2 ± 0.5 to 4.2 ± 0.6%) (Fig. 5, A and B).

### DISCUSSION

The purpose of the present investigation was to test the hypothesis that vascular responses during dynamic exercise are sex specific in healthy young humans. We utilized single-leg knee extension exercise, thereby minimizing possible confounding effects of sex differences in cardiac output reserve and counterregulatory reflexes, to measure leg hemodynamics during incremental exercise to maximal exertion. Particular care was also taken to ensure that all subjects maintained the 40 contractions/min kick rate, and effective isolation of the quadriceps (up to 80% of maximum workload) was confirmed by ipsilateral hamstring EMG activity. Accordingly, the major new findings of the present study were 1) the hyperemic response to graded small muscle leg exercise was greater in young women compared with men, 2) exercise-induced femoral artery dilation was greater in women than men, and 3) young women exhibited lower blood pressure and augmented femoral vascular conductance during graded single-knee extensor exercise. These results suggest that the mechanisms by which leg vasodilation occurs during incremental exercise may be sex dependent in healthy young humans.

#### Hyperemic Responses to Dynamic Leg Exercise in Young Men and Women

When compared as a function of absolute work intensity, the absolute leg blood flow responses during moderate- and higher
intensity knee kicking, as well as the overall slope of the blood flow response to this mode of exercise, were greater in women vs. men (Fig. 1A). These results are in general agreement with our previous studies (28) showing a steeper rise in cycle ergometer leg blood flow (via femoral thermodilution) per unit increase in leg oxygen uptake in women compared with men. These findings could be explained by lower hemoglobin concentrations in women vs. men, as leg blood flow responses to exercise can be augmented when arterial oxygen content is reduced (29, 47). In support of this possibility, estimated leg oxygen delivery (calculated with the assumptions that venous and arterial hemoglobin were similar, oxygen saturation was consistently 97%, and arterial oxygen content thus did not change during incremental exercise) was closely matched in men vs. women at most workloads (i.e., >5 W; Fig. 1B). However, given that 1) the overall slope of the estimated leg oxygen delivery response was greater in women, 2) there was no relationship between venous hemoglobin and the change in FBF from 0 to 24 W (data not shown) in men and women, and 3) the flow response (slope of blood flow vs. absolute workload) was still greater (P < 0.01) in four men and four women matched for hemoglobin (13.8–14.2 g/dl), we have concluded that hemoglobin is not the only factor contributing to the sex differences in exercising leg hyperemia. This conclusion is consistent with a previous study showing that moderate reductions in hemoglobin (from 14.7 to 13.1 g/dl) do not significantly alter leg blood flow during single-knee extensor exercise (15). Other potential mechanisms contributing to the sex difference in exercising leg hyperemia include an enhanced oxidative capacity representing greater oxygen demand in the leg muscles of women (17, 49, 52), as well as greater mechanical compression of the muscle bed during contraction in men (18). This latter possibility is addressed below.

Augmented Vasodilator Responses to Dynamic Leg Exercise in Young Women

Comparisons of FVC were evaluated at both absolute workloads (Fig. 2) as well as relative workloads (Fig. 3B), given that arterial blood pressure responses to dynamic exercise are determined by relative exercise intensity (16, 32). The results were similar: given that women achieved similar or greater (dependent on workload) leg blood flow with lower MAP during graded knee extensor exercise, FVC was augmented in women at absolute workloads >5 W and relative workloads >40% of maximal workload. This finding was in line with the previous observation in female rats of heightened hindlimb vascular conductance and lower MAP during treadmill exer-

Fig. 4. Change (Δ) in diameter relative to rest expressed as group means ± SE at percentage of maximal workload (% MW). Diameter measurements were not taken at each individual’s peak workload. For men, sample size was n = 15 until 62% MW, after which n = 10 at 76% MW, and n = 2 at 77% MW. For women, sample size was n = 16 until 60% MW, after which n = 11 at 72% MW; n = 6 at 78% MW, and n = 2 at 80% MW. Regarding sample size, please see the note in the legend of Fig. 3. *Significant difference between men and women at 0, 20, 40, 60, 80, and 100% of predicted responses, P < 0.05. A significant sex × workload interaction indicates a between-sex slope difference.

Fig. 5. Ipsilateral hamstring recruitment (A) and contralateral quadriceps recruitment (B), as represented by electromyographical (EMG) activity normalized to each individual’s maximal isometric contraction, during graded knee extensor exercise, expressed as group means ± SE at percentage of maximal workloads (% MW). For men, sample size was n = 15 until 83% MW, after which n = 10 at 95% MW, n = 2 at 93% MW, and n = 1 at 100% MW. For women, sample size was n = 16 until 80% MW, after which n = 11 at 88% MW, n = 6 at 93% MW, n = 2 at 94% MW, and n = 1 at 100% MW. Regarding sample size, please see the note in the legend of Fig. 3. *Significant difference between men and women at 0, 20, 40, 60, 80, and 100% of predicted responses, P < 0.05. A significant sex × workload interaction indicates a between-sex slope difference.
Exercise (48). Leg vasodilator responsiveness (i.e., the slope of the conductance response) was also greater in women vs. men in the present study; normalizing to estimated quadriceps muscle enhanced these sex differences (Fig. 3C). In addition, although it has been reported that femoral artery diameter does not change during knee extensor exercise (35, 41), an unexpected finding was the statistically significant conduit dilation with increasing workload in both men and women, with the magnitude of the increase being almost five times as great in women (−0.5 mm over the course of the exercise trial in women vs. the −0.1 mm in men). This finding underscores the importance of measuring arterial diameter at every workload (54) because the observation that conduit diameters remain uniform during exercise may be unique to the ergonomics of various knee-kick devices, femoral measurement site, and/or given populations. With respect to women in the present study, the augmented femoral dilation may be a mechanism through which the higher shear forces generated during exercise (i.e., greater blood velocity and smaller femoral diameter) are dissipated. We estimated the contribution of this diameter increase to femoral artery blood flow by comparing the observed blood flows with calculations of projected blood flow in the absence of any increase in femoral diameter; by this method, the observed dilation could have contributed up to 14% of the increase in blood flow achieved at the highest workloads in women but a rather insignificant 2% of the increase in blood flow in men under the same conditions. It should be noted that the extent to which conduit diameter changes influence downstream vascular control and blood supply to the working muscle may be minimal if the resistance vasculature is maximally dilated (22, 43); however, should the cross-sectional area of the quadriceps resistance vasculature be a limiting factor to vasodilation in women, then the conduit dilation may become more significant as exercise intensity increases.

Possible Determinants of Augmented Leg Vasodilator Responses in Young Women

Mechanical and metabolic influences. Certainly, given the numerous anatomic, metabolic, and strength differences between men and women (9, 25, 39), there is the possibility that between-sex differences in the mechanics and/or metabolic demands of knee-kick exercise underlie the observed vasodilatory differences. For example, it is possible that the reduced quadriceps muscle mass in women necessitated an increased hamstring recruitment during return to maintain cadence, resulting in a greater metabolic demand throughout the thigh. However, there were no differences in hamstring recruitment expressed relative to maximal isometric contraction (Fig. 5A), which would support this theory. Moreover, greater inactive quadriceps recruitment by men could have evoked augmented counterregulatory and/or pressor responses; however, inactive quadriceps recruitment was only slightly (~2% of maximal isometric contraction) greater in women at higher workloads (Fig. 5B). We also investigated the possibility that men, generating more absolute knee extensor power during each contraction, exhibited a greater retrograde blood flow because of the increasing impedance of the muscle pump at higher workloads (18, 35). There were no statistical differences between men and women in either the retrograde flows at any workload or the slope of the retrograde flow response (data not shown). In addition, flows partitioned by duty cycle closely mimicked the overall sex differences in flow responses (Fig. 1D), and the length of the contraction duty cycle was also similar (P = 0.85) in men and women (0.83 ± 0.01 and 0.83 ± 0.02 s, respectively), providing no overt evidence of a contraction-dependent impedance to flow that was specific to this group of men.

Influence of fatigue. During isometric knee extension to exhaustion, women exhibit less muscle fatigue because they generate less absolute force than men during contraction (9). Under these conditions, fatigue appears coupled to blood flow as sex differences in muscle fatigue are eliminated under ischemia (25, 49). However, there is no evidence that greater fatigue is a cause of reduced blood flow during dynamic exercise (21, 25, 49). This is supported in the present study, where imposing greater fatigue on the women by doubling the length of the knee-kick protocol (incremental workload increases were reduced to 2.4 W so that time to exhaustion was effectively doubled; data were collected on the second familiarization visit) did not alter observed sex-specific differences in vasodilatory responsiveness (Fig. 6). Also, hamstring recruitment increased to a similar extent in men and women as maximal workload was approached, suggesting that the use of the hamstring to counteract quadriceps fatigue and maintain cadence at the highest workloads was occurring similarly between sexes.

Potential estrogenic factors. Through a series of inhibitory blockades and comparisons of ovariectomized and estrogen-replaced rats, Rogers and Sheriff (48) determined that the nature of their sex-specific findings was attributable to estrogenic modulation of vascular responses mediated through nitric
oxide- and prostaglandin-dependent pathways. Besides its influence on endothelial-derived vasodilators, estrogen can also act as a direct smooth muscle vasorelaxant (53), modulate reactive oxygen species generated through exercise (5–7), and mitigate blood pressure, myogenic contraction, and other smooth muscle cell contractile pathways (10, 11, 13, 24), actions that may also serve to augment femoral vascular conductance. However, it must be noted that in the present study, we standardized study visits for women to coincide with days 1–7 of the menstrual cycle, when circulating estrogen is most likely to be lowest and similar to concentrations measured in men. Thus any potential estrogenic mechanisms underlying the present observations in women would have to be exerted through the chronic, rather than acute, effects of estrogen on the exercising vasculature. While there are estrogen receptors located in both the vascular endothelium and smooth muscle cells that may alter genetic transcription of many vasoactive metabolites, such as prostacyclin synthase, prostacyclin, cyclooxygenase, endothelin-1, and endothelial nitric oxide synthase (38), additional research is necessary to determine whether chronic estrogen exposure alters dilator and constrictor pathways during exercise.

Experimental Considerations

It must be noted that our estimates of quadriceps muscle mass are based on previous work (3, 23) that has not been validated in large populations and specifically women. However, our estimates of quadriceps muscle mass in young men are similar to other published estimates in young men (30, 31, 42), there was a significant relation between DXA estimates of regional thigh volume and anthropometric estimates of quadriceps muscle mass in both men ($r^2 = 0.69$) and women ($r^2 = 0.84$) with no significant between-sex difference in these relations, and normalizing flow and conductance to DXA estimations of thigh muscle mass did not change the nature of our findings (data not shown). These comparisons lead us to believe that there was no sex-specific error associated with normalizing hemodynamic data to anthropometric estimates of quadriceps muscle mass.

Conclusions

The present study provides novel evidence for sex-specific leg blood flow and leg vascular conductance responses during single knee extensor exercise in healthy young men and women, consistent with previous findings of augmented limb vasodilatory responses in women to other physiological and pharmacological stimuli.

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