Sex and limb-specific ischemic reperfusion and vascular reactivity

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The incidence of cardiovascular disease differs significantly between men and women. This is thought to be predominantly due to sex-specific differences in risk factors and the hormonal milieu. Indeed, epidemiologic studies have revealed that atherosclerosis, hypertension, and peripheral vascular and coronary artery diseases occur with greater prevalence in men and in premenopausal women compared with premenopausal women (4, 23, 26), while clinical assessments of endothelium-dependent peripheral artery vasomotion, flow-mediated vasodilation (FMD), in healthy populations suggest that vascular function in the arms and legs will not be different between men and women. This is thought to be predominantly due to sex-specific differences in risk factors and the hormonal milieu. Indeed, epidemiologic studies have revealed that atherosclerosis, hypertension, and peripheral vascular and coronary artery diseases occur with greater prevalence in men and in premenopausal women compared with premenopausal women (4, 23, 26), while clinical assessments of endothelium-dependent peripheral artery vasomotion, flow-mediated vasodilation (FMD), in healthy populations suggest that vascular function is superior in premenopausal women compared with their postmenopausal counterparts and with men (27, 40, 52). En masse, these studies have implicated estrogen as both a promoter of vascular dysfunction and subsequent atherogenic disease states. However, in studies assessing FMD, when the initial value of arterial diameter is taken into consideration, vasodilation appears similar between males and females (18). This suggests that some of the documented sex differences in vascular reactivity could be the consequence of a mathematical bias rather than the beneficial effects of the female hormonal milieu.

There is an emerging belief that vascular function in the arms and legs of humans is not uniform, but to our knowledge there is a relative paucity of studies that examine limb-specific sex differences in vascular reactivity. Most studies of arterial FMD have been conducted in the brachial artery (BA), but the extent to which this can index systemic vascular function has recently been questioned (35, 54). Humans, as upright bipeds, are regularly subjected to large hydrostatic and transmural forces in the legs that appear to contribute to differential vasoreactivity (11, 28, 48) and arterial stiffening compared with upper extremities (3, 7). Additionally, in those with atherosclerosis the lower extremities appear to exhibit both a higher prevalence and a greater degree of impaired endothelial function compared with the upper extremities (1, 8). Thus, in light of the potential vascular function differences when females are compared with males (27, 40, 52), it is currently unknown whether the assumption of limb vascular heterogeneity, which may proceed the onset of disease, can be extended to a young, healthy, sedentary group of women.

Consequently, the purpose of this study was to extend previous findings of heterogeneous vascular reactivity and the limb-specific regulation of muscle blood flow, but with the added focus of determining the effect of sex. Specifically, we tested the following hypotheses. 1) After 5 min of ischemic cuff occlusion, postischemic cuff hyperemia and subsequent FMD will be greater in the BA of the arm than in the popliteal artery (PA) of the leg in both males and females, extending previous findings of limb vascular heterogeneity. 2) Given the purported beneficial antioxidant effects of estrogen on NO bioavailability, the impact of a reduced hemoglobin concentration ([Hb]), and smaller baseline vessel diameters in females, ischemic reperfusion (IR) and relative FMD will be more pronounced in the arms and legs of the female group. However, 3) when initial vessel diameter and shear rate after cuff occlusion are taken into account (i.e., absolute FMD/shear rate), vascular function in the arms and legs will not be different between males and females.

METHODS

Subjects and General Procedures

Subjects. Fifteen young (26 ± 1 yr) healthy men (height 179 ± 2 cm, weight 77 ± 4 kg) and fifteen young (23 ± 1 yr) healthy women
(height 165 ± 3 cm, weight 59 ± 2 kg) participated in this study. All subjects were nonsmokers, normotensive (<140/90 mmHg), of similar activity level, not partaking in any special diet, and free of overt cardiovascular disease as assessed by a prescreening interview. Subjects were excluded from participation if they were taking any medications that would alter vascular responsiveness. Female subjects were studied in the follicular phase (days 1–7) of their menstrual cycle, and 12 of the 15 female subjects were not taking oral contraceptives. The protocol was reviewed and approved by the University of California, San Diego, Human Subjects Protection Program, and informed consent was obtained according to this program’s requirements. After the completion of health histories and physical examinations, each subject reported to the laboratory in a fasted state (>4 h postprandial) and had refrained from caffeine and exercise before the studies (>12 h). All studies were performed in a thermonutral environment.

**Arm FMD protocol.** Subjects lay supine, and a pneumatic cuff was positioned on the upper right arm proximal to the elbow, distal to the placement of the ultrasound Doppler probe on the BA (35, 43). After a 20-min rest period, baseline measurements were made, and the arm cuff was then inflated to suprasystolic pressure (>250 mmHg) for 5 min. Full occlusion was documented by the loss of ultrasound spectra in vessels at the wrist (radial artery), distal to the cuff.

**Leg FMD protocol.** Subjects lay supine on a gurney modified to allow dorsal ultrasound Doppler access to the PA. A pneumatic cuff was positioned on the lower right leg below the knee, distal to the placement of the ultrasound Doppler probe on the PA. As with the arm, after a 20-min rest period and baseline measurements, the leg cuff was inflated to suprasystolic pressure (>250 mmHg) for 5 min. Again, full occlusion was documented by the loss of ultrasound spectra in vessels at the ankle (peroneal artery), distal to the cuff.

**Measurements**

Ultrasound Doppler and FMD measurement. The ultrasound system (Logiq 7, GE Medical Systems, Milwaukee, WI) was equipped with a linear array transducer operating at an imaging frequency of 10 MHz. Vessel lumen diameter was measured at a perpendicular angle along the central axis of the scanned area, where the best spatial resolution was achieved.

The blood velocity profile was obtained with the same transducer and a Doppler frequency of 4.0–5.0 MHz, an insonation angle of ≤60°, a sample volume maximized according to vessel size, and a sample depth of 1.0–3.5 cm. Real-time ultrasound imaging and the pulse-wave velocity profile were assessed in duplex mode, allowing them to be viewed simultaneously. From artery diameter and mean blood velocity ($V_{\text{mean}}$), blood flow was calculated as blood flow (ml/min) = $V_{\text{mean}}\pi (\text{diameter}/2)^2/60$.

Ultrasound images and Doppler velocity waveforms were measured at rest for a 20-s period, a 20-s period during the last minute of occlusion, and again at 4–16, 25–45, 55–75, and 85–105 s after cuff release. At all sample points, arterial diameter [near-to-far intima-media interface, multiple measurements obtained within close proximity (~0.05 cm) and averaged] and angle-corrected, time-averaged, and intensity-weighted $V_{\text{mean}}$ values were calculated with commercially available software (Logiq 7). Data processing was performed as previously documented (35). All ultrasound vessel diameter measurements were evaluated during end diastole (corresponding to an R wave documented by the simultaneous ECG signal) (Logiq 7).

Total blood flow was quantified by using the AUC for blood flow over time (ml or ml/100 g) integrated with the use of commercially available software (SigmaPlot 8.0, Systat Software, Point Richmond, CA). Cumulative blood flow AUCs were integrated with the trapezoidal rule and calculated as $\sum [y_i(x_{i+1} - x_i) + (1/2)(y_{i+1} - y_i)(x_{i+1} - x_i)]$.

**Shear rate.** Shear rate was calculated with the equation shear rate ($s^{-1}$) = $4 \times V_{\text{mean}}$ (cm/s)/vessel diameter (cm) (7, 35, 53, 54). Cumulative shear rate was expressed with the AUC ($s^{-1}$) for shear rate over time (35, 38, 41), integrated as for cumulative blood flow described above, with the use of commercially available software (SigmaPlot 8.0). To mathematically normalize vasodilatation for shear rate, relative FMD (%) and absolute FMD (Δ cm) were divided by cumulative shear rate up to the point of maximal dilation (% diameter change/s$^{-1}$ and Δ cm/s$^{-1}$, respectively) (41).

**Blood pressure and heart rate.** Arterial blood pressure was measured with an automated radial tonometry (Medwave Vasotrac APM205A; BioPac Systems, Goleta, CA), with one measurement every 8–10 s. Heart rate was recorded from a standard three-lead ECG, an integral part of the Doppler system (Logiq 7).

**Tissue volume measurements.** Forearm and lower leg circumferences (distal, proximal end, and one-third distal to the proximal end) and length (joint to joint) were measured to calculate limb volume (19). Additionally, ventral (forearm) and dorsal (lower leg) skinfold measurements were taken to assess subcutaneous fat and allow the calculation of muscle volume for the lower leg and forearm (10, 19, 42). Muscle mass of the complete forearm and lower leg was then calculated from the anthropometric assessment of muscle volume by multiplying by the density of muscle (1.06 g/cm$^3$), an approach that has good validity based on an absolute agreement with X-ray absorptiometry (10).

**Statistics.** Statistics were performed with commercially available software (SigmaStat 3.10, Systat Software). Repeated-measures ANOVA, ANOVA, and Student’s $t$-tests were used to identify significant changes in variables within and between age groups and limbs, with the Bonferroni test used for post hoc analysis when a significant main effect was found. All group data are expressed as means ± SE. Statistical significance was established at $P < 0.05$.

**RESULTS**

**Subject Characteristics**

Subject characteristics of both male and female groups are presented in Table 1.

**Diameters, Shear Rates, and Limb Blood Flows**

Arterial diameters, shear rates, and blood flows in the BA and PA of the male and female groups before, during, and after 5 min of suprasystolic cuff occlusion are presented in Table 2.

**Table 1. Subject characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr†</td>
<td>26±2</td>
<td>23±1</td>
</tr>
<tr>
<td>Height, cm†</td>
<td>179±2</td>
<td>165±3</td>
</tr>
<tr>
<td>Weight, kg†</td>
<td>77±4</td>
<td>59±2</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>64±2</td>
<td>61±2</td>
</tr>
<tr>
<td>Mean arterial pressure, mmHg</td>
<td>92±2</td>
<td>84±1</td>
</tr>
<tr>
<td>Forearm muscle mass, kg*†</td>
<td>0.93±0.04</td>
<td>0.42±0.03</td>
</tr>
<tr>
<td>Lower leg muscle mass, kg†</td>
<td>2.60±0.10</td>
<td>1.77±0.13</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n = 15$ men, 15 women. *Significant difference between arm and leg ($P < 0.05$); †significant difference between male and female ($P < 0.05$).
Table 2. Brachial and popliteal artery diameter, shear rate, blood flow, and blood flow normalized for muscle mass

<table>
<thead>
<tr>
<th>Diameter, cm</th>
<th>Male (BA)</th>
<th>Female (PA)</th>
<th>Male-Female P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>0.41±0.01</td>
<td>0.31±0.01†</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cuff on</td>
<td>0.46±0.02</td>
<td>0.30±0.01‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Peak</td>
<td>0.62±0.02</td>
<td>0.46±0.02‡</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Area under curve, cm</td>
<td>1.83±164</td>
<td>1.925±157</td>
<td>0.64±0.82</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Shear rate, s⁻¹</th>
<th>Male (BA)</th>
<th>Female (PA)</th>
<th>Male-Female P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>90±14</td>
<td>82±14</td>
<td>0.58 ±0.81</td>
</tr>
<tr>
<td>Cuff on</td>
<td>12±12</td>
<td>10±22</td>
<td>0.99 ±0.53</td>
</tr>
<tr>
<td>Peak</td>
<td>305±24‡</td>
<td>432±33‡</td>
<td>&lt;0.05 ±0.11</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blood flow, ml/min</th>
<th>Male (BA)</th>
<th>Female (PA)</th>
<th>Male-Female P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>63±8</td>
<td>69±6</td>
<td>&lt;0.001 ±0.001</td>
</tr>
<tr>
<td>Cuff on</td>
<td>25±6‡</td>
<td>3±1‡</td>
<td>&lt;0.001 ±0.001</td>
</tr>
<tr>
<td>Peak</td>
<td>330±23†</td>
<td>453±52‡</td>
<td>&lt;0.001 ±0.001</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Normalized blood flow, ml·100 g⁻¹·min⁻¹</th>
<th>Male (BA)</th>
<th>Female (PA)</th>
<th>Male-Female P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rest</td>
<td>6.9±0.9</td>
<td>6.9±1.3</td>
<td>0.52 ±0.79</td>
</tr>
<tr>
<td>Cuff on</td>
<td>1.0±0.2‡</td>
<td>0.9±0.2‡</td>
<td>0.87 ±0.66</td>
</tr>
<tr>
<td>Peak</td>
<td>28±2.7‡</td>
<td>29±3.0‡</td>
<td>&lt;0.05 ±0.82</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 15 men, 15 women. *Significant difference between brachial artery (BA) and popliteal artery (PA) (P < 0.05); †significant difference between male and female (P < 0.05); ‡significant difference from rest (P < 0.05).

BA diameter was significantly smaller than PA diameter in both male and female groups (P < 0.05). Female BA and PA diameters were statistically smaller than male BA and PA diameters (P < 0.05). A statistically significant vasodilation from rest was observed after cuff release in the BA and PA of both sex groups (P < 0.05). Consistent with the literature, peak dilations occurred, on average, between 50 and 70 s (6) and were not different within and between limb and sex groups (Table 2).

Resting, post-cuff occlusion peak, and AUC shear rates were significantly larger in the BA than in the PA in both male and female groups (P < 0.05). Between groups, female shear rate AUC was larger than male shear rate AUC in the BA (P < 0.05) but was not significantly different in the PA (P = 0.09) (Table 2).

Resting absolute blood flow was not different between forearm and lower leg within the sexes; however, there were significant differences between sex groups for both limbs (P < 0.05). Total blood flow from cuff release to the final time period examined (95–105 s after cuff release) was not different between the forearm and the lower leg in the men. Conversely, forearm total blood flow in the women was significantly attenuated compared with the lower leg (P < 0.05). Between sex groups, there was a significant difference in total blood flow in the forearm (P < 0.05); however, although attenuated, female lower leg total blood flow did not reach statistical significance compared with male lower leg total blood flow (P = 0.08) (Table 2).

Resting blood flow normalized for muscle mass was significantly higher (~3-fold) for both sexes in the forearm than in the lower leg (P < 0.05). In contrast to absolute blood flow, there was no sex difference in resting blood flow normalized for muscle mass with either forearm or lower leg. Total blood flow per 100 g of muscle mass from the cuff release to the final time period examined (95–105 s after cuff release) was significantly greater (~3-fold) in the forearm than in the lower leg for both male and female groups (P < 0.05). Again, unlike absolute blood flow, there was no statistical difference in total blood flow per 100 g of muscle mass between sex groups for both limbs.

Flow-Mediated Dilation

Expressed in traditional terms (% diameter change), a larger FMD in the BA than in the PA (P < 0.05) was revealed in both male and female groups (Fig. 1A). Between the sex groups, relative FMD (% diameter change) was significantly larger in the female group for the BA (male 6.7 ± 0.6%, female 8.7 ± 1.1%; P < 0.05) and was not statistically different in the PA (male 4.4 ± 0.6%, female 5.4 ± 0.8%) (Fig. 1A). With the statistically smaller resting diameters in the female group for both BA and PA (Table 2), FMD was also assessed as an absolute change in diameter (absolute diameter change). When assessed in this manner, there was no statistical difference within and between limbs and sex groups (male: BA 0.027 ± 0.002 and PA 0.027 ± 0.003 cm; female: BA 0.027 ± 0.003 and PA 0.024 ± 0.003 cm) (Fig. 1B).

When relative FMD (% diameter change) was normalized for the shear rate AUC, the PAs of both male and female groups revealed a significantly greater FMD for a given shear rate than their respective BAs (male group: PA 8.3 ± 10⁻⁴ ± 0.1 × 10⁻⁴ and BA 3.8 × 10⁻⁷ ± 0.3 × 10⁻⁴Δs⁻¹, P < 0.05; female group: PA 7.5 × 10⁻⁴ ± 1.6 × 10⁻⁴ and BA 3.5 × 10⁻⁴ ± 0.5 × 10⁻⁴Δs⁻¹, P < 0.05) (Fig. 1C). Between the male and female groups, FMD normalized for shear rate in both limbs revealed no sex-related differences (Fig. 1C). Consistent with the relative change in diameter,
when absolute FMD was normalized for the shear rate AUC, the PAs of both the male and female groups revealed a significantly greater FMD for a given shear rate than their respective BAs (male group: PA 5.0 ± 0.8 × 10^{-6} and BA 1.6 ± 0.1 × 10^{-6} cm/s^{−1}·s, P < 0.05; female group: PA 3.3 ± 0.6 × 10^{-6} and BA 1.2 ± 0.2 × 10^{-6} cm/s^{−1}·s, P < 0.05) (Fig. 1D). Therefore, in contrast to the relative FMD/shear rate AUC, there was a sex-related difference in PA absolute FMD/shear rate AUC (P < 0.05) but no sex-related difference in BA (Fig. 1D).

Another aspect of the vascular sex differences explored in this study was the relationship between baseline artery diameter and both relative FMD and absolute FMD. Indeed, there was a significant, negative, linear relationship between the baseline diameter and the relative FMD in either the BA (r = 0.85; Fig. 2B) and the PA (r = 0.98; Fig. 2A) (male group: PA 5.0 ± 0.8 × 10^{-6} and BA 1.6 ± 0.1 × 10^{-6} cm/s^{−1}·s, P < 0.05; female group: PA 3.3 ± 0.6 × 10^{-6} and BA 1.2 ± 0.2 × 10^{-6} cm/s^{−1}·s, P < 0.05). Therefore, in contrast to the female group, when absolute FMD was normalized for shear rate (Fig. 3A), there was a significant, negative, linear relationship between the baseline diameter and absolute FMD for both the BA (r = 0.004, P = 0.02; Fig. 3C) and the PA (r = 0.03, P = 0.1; Fig. 3D). Interestingly, when these data were normalized for shear rate the results were quite different (Fig. 3). Specifically, there was no longer a relationship between the baseline diameter and the relative FMD in either the BA or the PA when normalized for shear (Fig. 3A and B). Additionally, there was now a significant relationship between baseline artery diameter and absolute FMD for the BA (r = 0.004, P = 0.02; Fig. 3C), but this was not the case for the PA (Fig. 3D).

**Additional Analyses**

On examination of the complete data set there was no evidence that the three women who were taking oral contraceptives were “outliers” in the female data set, and thus they were not excluded from the data interpretation.

**DISCUSSION**

The purpose of this study was to extend previous findings of limb vascular heterogeneity and the limb-specific regulation of muscle blood flow, but with the additional goal of determining differences between males and females. Vascular reactivity, as assessed by FMD, revealed a greater relative FMD (% diameter change) in the BA than in the PA of both male and female groups. In addition, relative FMD in the female BA was larger than in the male BA, a finding that is consistent with the literature in suggesting augmented vascular endothelium-dependent vasodilation in females. However, because of significant differences in starting diameter, limb FMD responses were also assessed in absolute terms and revealed no difference within or between groups. When FMD (relative and absolute) was normalized for the shear stimulus, both sexes revealed a greater normalized FMD response in the PA than in the BA. Together, these findings extend the recognition of limb-specific sensitivity to a shear stimulus that favors the legs in males to a female group (35) but fail to support the concept of enhanced vascular function in females. In fact, these findings reveal an attenuated vascular function in the legs of women (Fig. 1D). In addition, these data emphasize the importance of taking into account starting vessel diameter in subject groups such as males and females, because this can introduce a powerful mathematical and shear-related bias in which smaller vessels with greater shear rates (females) reveal a much greater dilatory response that may skew the interpretation of sex-specific vascular reactivity.

**Ischemic Reperfusion**

It has recently become evident that muscle blood flow in the arm and leg differ in response to a variety of stimuli (10, 33, 34). To better understand the potential sex-associated and limb-specific regulation of muscle blood flow, we assessed IR...
responses following 5 min of suprasystolic cuff occlusion in the arm and the leg. Absolute blood flow at rest, post-cuff release peak, and post-cuff release AUC were similar between arm and leg in men (Table 2). In contrast, although similar at rest and after cuff release peak, post-cuff release AUC absolute blood flow of the female group was significantly less in the arm than in the leg (Table 2). Nevertheless, when assessing blood flow in limbs of differing size, especially at rest, where metabolic demand and therefore muscle mass dictate oxygen delivery and blood flow, it is important to normalize for the mass of perfused muscle. With such an approach, there was a clear blood flow difference between limbs in both sex groups, with the larger IR in the arm (Table 2). This difference is primarily driven by the significant differences in muscle mass between limbs for both sexes (Table 1): compared with the arm, females have a lower leg muscle mass relatively more similar to that of

Fig. 2. Baseline artery diameter and its influence on relative flow-mediated dilation (% diameter change) (brachial, A; popliteal, B) and absolute flow-mediated dilation (brachial, C; popliteal, D).

Fig. 3. Baseline artery diameter and its influence on relative flow-mediated dilation (% diameter change) (brachial, A; popliteal, B) and absolute flow-mediated dilation (brachial, C; popliteal, D) when normalized for shear rate (AUC).
males. Interestingly, because of the significantly smaller muscle masses in both limbs of the female group, there was no statistical difference in IR between men and women when normalized for muscle mass in both the arm and the leg (Table 2). This finding of a similar IR response between sexes after 5 min of cuff occlusion is consistent with data previously documented in the arm (20), but now extends these findings to both the upper and lower extremities.

Blood flow dynamically responds to arterial oxygen content (22, 44), and it is well known that females have a reduced red blood cell count and plasma [Hb] compared with males (9, 18, 46). Thus, with the expected lower [Hb] in women vs. men, it was hypothesized that women would reveal a greater blood flow both at rest and during IR than men. However, although on average IR per unit of muscle mass was greater in the women, this response was not statistically different between the sexes.

A Hormonal Milieu Effect on Flow-Mediated Dilation?

Sex differences in FMD have been reported concomitantly with a greater post cuff hyperemia in premenopausal women compared with age-matched men (27, 51). This effect has been explained by the positive influence of endogenous estrogens on the availability of NO (16), the principal vasodilator in shear-mediated vasodilation (31). Indeed, numerous studies have implicated estrogen as both a prostaglandin promoter and an antioxidant (2), protecting NO from degradation and facilitating increased vasomotion (17, 29, 39). 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 most similar to concentrations measured in men (16).

Recognizing the acute effects of estrogen on blood flow regulation (45), it is unknown whether the results of Hashimoto et al. (16), who demonstrated that the greatest endothelium-dependent vasodilation, assessed with the cuff occlusion model, coincided with the end of the follicular phase of the menstrual cycle, when serum estradiol levels were highest, may have been affected by an altered reactive hyperemia response. In light of this and the present data, it would be interesting to investigate FMD normalized for shear in women throughout the menstrual cycle to comprehensively elucidate the acute effects of estrogen on human vascular function. Despite the hypothesis of an increased vasodilation in the BA and/or PA of the female group, there appears to be no female-specific augmentation of peripheral artery vasodilation. In fact, there is evidence to the contrary, with decreased vascular function in the PA of women when absolute FMD is normalized for shear rate AUC (Fig. 1D).

Sex and Limb-Specific Vascular Function

Parker et al. (37) recently published findings in young and older women that identified no limb difference in response to a FMD test in the BA and PA. Although the authors did not study men, it was suggested that the failure to identify the hypothesized limb difference in vascular function could be attributable to sex. This conclusion was based in part on recently published findings from our group (35) of a limb-specific, shear-mediated vasodilation favoring the PA compared with the BA in young, healthy, sedentary men. The present study confirms this limb difference in males when the shear stimulus is taken into account while, in contrast to Parker et al. (37), also extending this limb specificity to females. In a clinical context, recent studies have revealed some vascular adaptations with both disease and aging to be limb dependent, with evidence that vascular dysfunction may occur in the lower extremities without significant changes in vessels of the upper extremities (1, 30, 50). Although lengthy clinical speculations are beyond the scope of this study, it will be interesting to evaluate whether aging and the hormonally based physiological effects of menopause result in a sex-related, limb-specific alteration in vascular function.

Vessel Size and Flow-Mediated Dilation

In general, caution should be exercised when comparing vessels of different sizes because there may be a mathematical bias in favor of smaller arteries yielding a larger relative difference (if a similar absolute change is achieved in a smaller vessel, although this could be argued to be unlikely). However, in the present limb comparison for both sexes, because the PA has a larger starting diameter than the BA (handicapping % change in the PA), this mathematical bias cannot explain the improved vascular responsiveness of the PA compared with the BA, but would, in fact, reduce the chance of this observation. Between sexes, however, because there are significant differences in starting diameters between males and females for the BA and the PA and a potential for this mathematical bias, caution should be adopted when conclusions are based on relative diameter change data. In the present study, as in other studies (5, 18, 24, 32), the flow-induced change in artery diameter is inversely related to the basal value of the diameter when expressed in percent change from baseline (relative FMD) in both the BA and the PA (Fig. 2, A and B). This supports the idea that larger arteries show a less convincing relative vasodilation compared with smaller arteries. Correlation analysis of the flow-induced change in artery diameter expressed as an absolute change from basal diameter reveals no such relationship (Fig. 2, C and D). This supports the latter analytical approach (avoiding the potential mathematical bias) as the most unbiased interpretation when considering vessels of different size.

However, following the present theme of recognizing the important modulating role of shear as a stimulus in FMD, when these data were normalized for shear rate the results were quite different (Fig. 3). At least in terms of the BA, this approach appears to have reduced the variance in the FMD response, supporting the concept that shear can explain some of the variance in FMD (Fig. 3A). Additionally, the inclusion of shear in the assessment removes the relationship between initial vessel diameter and relative FMD, suggesting that the relationships noted above and illustrated in Fig. 2, A and B, are more the consequence of fluid dynamics (greater shear forces in smaller vessels) than the simple mathematical bias of comparing the relative change of vessels with differing internal diameters. Finally, this approach potentially illustrates, yet again, a difference between the vessels of the arms and legs in that there was a significant relationship between baseline artery diameter for the BA (Fig. 3C) but this was not the case for the PA (Fig. 3D). Thus definitive statements concerning differences in endothelial function between vessels of different size and there-
fore differing shear stimuli, such as between the sexes, must be carefully interpreted.

**Vascular Function and Vascular Disease Progression**

Several studies have now demonstrated that impaired endothelial function in both the coronary and the peripheral circulation precedes the development of pathologies such as atherosclerosis (14, 15, 36, 47). Additionally, in those with atherosclerosis the PA appears to exhibit both a higher prevalence and a greater degree of impaired endothelial function than the BA (1, 8). Therefore, in healthy subjects, the study of endothelial function in the upper and lower extremities may reveal a greater predisposition toward limb-specific vascular dysfunction and therefore vascular disease in these anatomically distinct locations. Indeed, the study of Angerer et al. (1), which investigated the effects of coronary artery disease on FMD in the BA and PA, did find an attenuated PA FMD compared with that in the BA in both patients and age-matched control subjects (≈50 yr), with the greatest reduction in vascular function in the diseased patients. Unfortunately, they failed to account for the shear stimulus, perhaps an essential component in the true interpretation of FMD. The present data reveal a potential paradox in that, regardless of sex, when FMD is appropriately normalized for the shear stimulus the PA reveals a greater endothelium-derived vasodilation than the BA. This is especially interesting, because it could be argued that although the BA and PA are in different limbs their relative positions in the arterial tree have some similarities; both supply the majority of blood flow to a muscle mass distal to the second joint of a limb. However, it should be noted that that this investigation is limited to young, healthy subjects and it is likely that the limb-specific progression of vascular disease is the consequence of differing stresses experienced by the limbs over the life span, such as larger hydrostatic and transmural forces in the leg as well as the continued stresses associated with daily lower limb-powered locomotion (11, 28, 48, 49). Thus it will be very interesting to determine whether the greater shear stress reactivity exhibited in the legs of young healthy men and women is apparent in a much older, but healthy, cohort. It is also interesting to note that it has previously been recognized that young healthy women may exhibit stiffer conduit vessels than their male counterparts (12). Although vessel stiffness per se was not measured in the present study, there is a physiological link between vascular function and vessel stiffness, mediated by NO bioavailability, and therefore the work of Ferreira et al. (12) and others may not be at odds with the present data and inferences regarding sex and vascular health. This, in combination with the present findings of no difference or potentially impaired vascular function in the legs of women certainly set the stage for some interesting sex-specific differences with age when women ultimately lose the potential vascular protection afforded by estrogen.

**Experimental Considerations**

It is well documented both in our laboratory and in the literature that on average men and women typically differ in terms of [Hb] by 2 g/dl, and this has the effect of lowering blood viscosity from ≈3.7 mPa·s in men to ≈3.3 mPa·s in women (18, 25, 46). Because the present study was noninvasive, a limitation is that individual [Hb] was not measured to determine blood viscosity and therefore the quantification of the true physiological shear stimulus (shear stress) was not possible on a case-by-case basis. However, although the application of the above sex-specific blood viscosities (derived from typical [Hb] and confirmed in similar subjects in our laboratory of ≈12.5 and 14.5 g/dl for women and men, respectively) and the subsequent calculation of shear stress instead of shear rate does bring the male and female shear data closer together, female shear stress remains 15–18% greater than male. Indeed, the substitution of the literature-based blood viscosity for women into the calculation of shear stress (and not shear rate as used in this investigation) [the product of blood viscosity (η) multiplied by blood velocity (V), divided by vessel diameter (D) (13)] did not qualitatively alter the results of this study. Utilization of the same shear rate equation for both men and women serves to overestimate the true shear stimulus in the women. However, when considered in light of the 25% smaller vessels of the women (Table 2) and the large impact of this on the shear stress calculation, the relative contribution and accurate determination of the small sex-related difference in viscosity becomes relatively less significant.

Experimental evidence has revealed that oral contraceptives exert a beneficial effect on endothelial function by increasing NO availability and inhibiting superoxide anion generation (21). Despite this potential confounding effect of oral contraceptives on FMD, the three female subjects taking oral contraceptives were not statistical outliers, and their inclusion or exclusion from the analyses did not alter the conclusions from this study.

During the process of this research we considered the implications of including the hand and foot in the IR response of the limbs studied. Therefore, in an additional group of similar subjects (n = 8) we assessed hand/forearm and foot/leg volume (water displacement) and compared limb IR with and without the hand/foot occluded with an additional cuff. Interestingly, there was a very consistent ratio between hand and forearm (0.25 ± 0.02) and foot and lower leg (0.26 ± 0.02) volume. Additionally, IR assessed without the inclusion of the hand or foot (cuffed) was altered in proportion to the volume that was removed by cuffing. Specifically, the hand, when included in the hyperemic response of the arm accounted for 24% of the arm IR, while the foot when included in the hyperemic response of the lower leg accounted for 27% of the lower leg IR. Therefore, the inclusion or exclusion of the hand or foot in these studies quantitatively affects the results (lower IR), but the between-limb implications remain unaltered.

**Conclusions**

In conclusion, the present study extends previous findings in young, healthy men of greater endothelium-dependent vascular reactivity in the lower compared with the upper extremities to young healthy women. It is also concluded that basal vessel diameter and shear rates influence the magnitude of relative flow-mediated vasodilation and that the sex differences previously reported could be the consequence of a mathematical bias and differences in the shear stimulus rather than a sex-related physiological difference. These vascular characteristics are essential to appropriate interpretation and thus should be taken into account when comparing the vascular responsiveness of men and women. Therefore, when perhaps the most...
rigorous analysis for these conditions, absolute FMD normalized for shear rate, is applied, the BA does not exhibit a difference between the sexes, whereas the PA displays attenuated function in women.

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