Influence of vascular dimension on gender difference in flow-dependent dilatation of peripheral conduit arteries

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Influence of vascular dimension on gender difference in flow-dependent dilatation of peripheral conduit arteries. Am J Physiol Heart Circ Physiol 282: H1262–H1269, 2002; 10.1152/ajpheart.00209.2001.—To assess the influence of initial diameter on the gender difference in flow-dependent dilatation (FDD) of the conduit artery, we measured radial artery internal diameter (echotriangulation), flow (Doppler) and total blood viscosity in 24 healthy (25 ± 0.8 yr) men and women during reactive hyperemia (RH) and during a gradual hand skin heating (SH). At baseline, mean diameter (men, 2.76 ± 0.09 mm vs. women, 2.32 ± 0.07 mm, P < 0.05), flow (men, 21 ± 4 vs. women, 10 ± 1 ml/min, P < 0.05), and blood viscosity (men, 4.13 ± 0.07 vs. women, 3.92 ± 0.13 cP, P < 0.05) were higher in men but mean shear stress (MSS) was not different between groups. During RH, the percent increase in diameter was lower in men (men, 9 ± 1% vs. women, 13 ± 1%, P < 0.05). This difference was suppressed after correction for baseline diameter. During SH, the increase in diameter with flow was higher in women (P < 0.01). However, the increase in MSS was higher in women because of their smaller diameter at each level of flow (P < 0.01) and there was no difference between groups for the increase in diameter at each level of MSS. These results demonstrate in a direct manner that initial diameter influences the magnitude of FDD of conduit arteries in humans by modifying the value of the arterial wall shear stress at each level of flow and support the interest of the heating method in presence of heterogeneous groups.

endothelium; shear stress; skin heating; vasodilatation

CONDUIT ARTERY FLOW-DEPENDENT vasodilatation is a fundamental mechanism that regulates vascular conductance at rest and during exercise and maintains wall shear stress within physiological values (3, 22, 28). This vasodilatation is mainly mediated by the endothelium release of nitric oxide (NO) in response to shear stress (17, 22). Postischemic hyperemia, by increase of arterial flow, is currently used in clinical studies (1, 6, 11, 17, 20, 23) for noninvasive evaluation of flow-dependent dilatation and is considered (i.e., by comparison with the vasodilating effect of exogenous NO) an index of endothelium-derived NO bioavailability. Gender differences in flow-dependent dilatation have been reported with a higher magnitude of conduit artery flow response in nonmenopausal women when compared with age-matched men, an effect that was considered as a consequence of the positive effect of endogenous estrogens on the bioavailability of NO (11, 21). However, despite the fact that numerous studies (2, 7, 12–14, 24, 26) support this positive effect of estrogens on NO bioavailability, when the initial value of arterial diameter is taken into consideration in the statistical analysis, the increase in diameter appears similar between men and women (21). This suggests that gender difference could be the consequence of the larger baseline diameter observed in the men (11, 20, 21). Indeed, for the same increase in flow, because of higher diameter in men, the increase in shear stress during hyperemia could have been smaller in men than in women and thus may explain these different results. Because of technical limitations, it was not possible to compare the flow-dependent dilatation between men and women at the same level of stimulus, i.e., at the same level of shear stress (6, 7, 21).

The present study was thus designed to compare in men and women the flow-dependent vasodilatation of a peripheral conduit artery at the same level of stimulus and to directly assess the influence of the initial vascular diameter on the gender differences observed in flow-dependent response. For this purpose, we studied by use of a high-resolution echo-tracking device coupled to a Doppler system, the flow-dependent vasodilatation of the radial artery in men and age-matched nonmenopausal women characterized by the presence of physiological differences in arterial diameter, blood flow and blood viscosity. This comparison was performed in response to postischemic hyperemia and by use of a diameter-wall shear stress relationship obtained during gradual distal hand skin heating, a
method developed to gradually increase forearm blood flow with no changes in systemic hemodynamics.

METHODS

Subjects

Twenty-four healthy volunteers (12 men and 12 women, 25 ± 0.8 (SE) yr) participated in the study. All subjects were normotensive nonsmokers with no regular medication. They were deemed healthy on the basis of a medical history and a complete medical examination, including a normal electrocardiogram and recent routine laboratory tests. Women had regular menstrual cycles (28–31 days) with no history of pregnancy. Four subjects used low-dose oral contraceptives. The protocol was approved by the hospital ethical committee and written informed consent was obtained from all participants.

Physiological Measurements

Measurements of radial artery and systemic hemodynamics. Radial artery internal diameter was continuously measured by use of a high-precision A-mode echo-tracking device (NIUS 02, Asulab; Neufchâtel, Switzerland) (35). Briefly, a 10-MHz focused transducer was positioned ~7 cm distal to the antecubital fossa over the radial artery of the right arm. The probe was set perpendicular to the artery so that its focal zone was in the center of the arterial lumen using a stereotaxic arm with micrometric screws while proper positioning was adjusted using a stereo Doppler mode. After switching to A-mode, the echoes from both anterior and posterior walls of the artery were visualized on a screen and tagged by electronic trackers, allowing continuous recording of the artery internal diameter. Radial artery internal cross-sectional area was then calculated from the measurement of internal diameter. Radial artery blood flow velocity was measured by continuous-wave Doppler (8-MHz transducer at a 60° angle with a width of beam of 1 mm at the focal point of 15 mm), distal to the echo-tracking probe, at the site of diameter measurement. After the 10-MHz probe was positioned, the Doppler transducer (Doptek 2000, Deltex; Chichester, UK) was rotated around the axis of the echotracking probe to provide the highest velocity. When this was achieved, the Doppler beam was positioned in the center of the arterial lumen at an angle of 60° with the longitudinal axis of the artery that was used for the calculation of the arterial flow velocity. Radial artery blood flow was calculated as the product of time-averaged mean velocity and arterial lumen cross section. Systolic, diastolic, mean arterial pressure, and heart rate were measured with the use of an oscillometric blood pressure recorder (Sentron 500, Bard Cie; Lombard, IL), and the cuff was placed around the left arm. In addition, arterial pressure recorder (Sentron 500, Bard Cie; Lombard, IL), and diameter were recorded for 5 min at ambient temperature. The device was then filled with water to maintain the hand skin temperature at 20°C for at least 20 min. Recordings were repeated during the last 3 min and then the water temperature was gradually changed to increase the skin temperature from 20°C to 25°, 30°, 34°, 36°, 37°, 39°, 40°, and 42°C, respectively. Each level of temperature was maintained for 10 min. At each level of temperature, 3 min before the end of each stage, systemic hemodynamics measurements were repeated and followed by 3 min of continuous vascular parameter recording.

The reproducibility of the skin heating test and the comparison with the reference method, i.e., postischemic hyperemia, was assessed in the 12 first volunteers by using the results obtained on the first day and by repeating the skin heating maneuver at the same hour after a 1-day interval.

From the individual values of radial artery internal diameter (d), blood flow (Q), and total blood viscosity (μ) and assuming a Poiseuillean model, the mean arterial wall shear stress (τ) was calculated at each level of flow as τ = 4μQ/πr³, where r is the internal artery radius (r = d/2) (25). Finally, from the individual values, the diameter flow, the wall shear stress flow, and the diameter wall shear stress curves were constructed.

Forearm flow clamp. In a separate set of experiments, an occlusion cuff was located on the wrist. The skin-heating procedure was started at the temperature of thermoneutral-
Table 1. Characteristics of study population

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Men</th>
<th>Women</th>
<th>ANOVA F Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>25 ± 1</td>
<td>25 ± 1</td>
<td>0.267</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.81 ± 0.02</td>
<td>1.69 ± 0.01</td>
<td>17.556*</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>71.2 ± 2.8</td>
<td>61.2 ± 1.2</td>
<td>11.006*</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>21.7 ± 0.6</td>
<td>21.3 ± 0.4</td>
<td>0.348</td>
</tr>
<tr>
<td>Body area, m²</td>
<td>1.90 ± 0.05</td>
<td>1.70 ± 0.02</td>
<td>14.374*</td>
</tr>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>119 ± 2</td>
<td>117 ± 1</td>
<td>0.508</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>71 ± 2</td>
<td>66 ± 1</td>
<td>3.389</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>87 ± 2</td>
<td>83 ± 1</td>
<td>2.245</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>56 ± 2</td>
<td>63 ± 3</td>
<td>3.472</td>
</tr>
<tr>
<td>Radial artery diameter, mm</td>
<td>2.76 ± 0.09</td>
<td>2.32 ± 0.07</td>
<td>16.360*</td>
</tr>
<tr>
<td>Radial artery flow, ml/min</td>
<td>21 ± 4</td>
<td>10 ± 1</td>
<td>7.037*</td>
</tr>
<tr>
<td>Total blood viscosity, cP</td>
<td>4.13 ± 0.07</td>
<td>3.92 ± 0.13</td>
<td>7.938*</td>
</tr>
<tr>
<td>Arterial wall shear stress, dyn/cm²</td>
<td>6.85 ± 1.11</td>
<td>5.65 ± 0.73</td>
<td>0.845</td>
</tr>
</tbody>
</table>

Values are means ± SE. Systemic and radial artery parameters were obtained in the healthy volunteers at baseline. *P < 0.05, men vs. women.

RESULTS

Subjects

The characteristics of the men and women who participated in the study are presented in Table 1. There were no differences between groups for the mean age and the mean body mass index. However, the mean height, weight, and body area were significantly higher in men than in women (all P < 0.05).

There was no significant difference between groups for baseline systolic, diastolic, mean blood pressure and heart rate. Radial artery blood flow, diameter, and total blood viscosity were significantly higher in men (all P < 0.05), but there was no significant difference between men and women for the mean shear stress at baseline.

Table 2. Distal hand skin heating test

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>20°C</th>
<th>42°C</th>
<th>ANOVA F Values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mmHg</td>
<td>117 ± 3</td>
<td>115 ± 3</td>
<td>118 ± 4</td>
<td>0.684</td>
</tr>
<tr>
<td>Diastolic blood pressure, mmHg</td>
<td>68 ± 2</td>
<td>68 ± 2</td>
<td>69 ± 3</td>
<td>0.305</td>
</tr>
<tr>
<td>Mean blood pressure, mmHg</td>
<td>85 ± 2</td>
<td>84 ± 2</td>
<td>86 ± 3</td>
<td>0.525</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>60 ± 2</td>
<td>59 ± 2</td>
<td>63 ± 2</td>
<td>2.750</td>
</tr>
<tr>
<td>Plasma epinephrine, pg/ml</td>
<td>31 ± 4</td>
<td>35 ± 4</td>
<td>34 ± 3</td>
<td>0.366</td>
</tr>
<tr>
<td>Plasma norepinephrine, pg/ml</td>
<td>230 ± 56</td>
<td>224 ± 32</td>
<td>203 ± 25</td>
<td>0.299</td>
</tr>
<tr>
<td>Total blood viscosity, cP</td>
<td>3.99 ± 0.08</td>
<td>4.05 ± 0.08</td>
<td>4.05 ± 0.08</td>
<td>0.256</td>
</tr>
</tbody>
</table>

Values are means ± SE. Systemic hemodynamics and biological parameters were measured in healthy volunteers before and during hand skin heating from 20 to 42°C.
Postischemic hyperemia. Radial artery peak flow was higher in men than in women (P < 0.05) but the percent increase in blood flow was not significantly different between groups (524 ± 99 vs. 540 ± 82%, respectively) (Fig. 3). Thus the absolute value of flow increase was higher in men (85 ± 12 ml/min) versus women (50 ± 6 ml/min) (P < 0.01).

Radial artery peak diameter was higher in men than in women (P < 0.05) but the percent increase in diameter was higher in women compared with men (men 9 ± 1 vs. women 13 ± 1%, P < 0.05). Thus the absolute value of diameter increase was higher in women (men 0.24 ± 0.03 vs. women 0.30 ± 0.02 mm, P < 0.05). The AUC (men 1,030 ± 168 vs. women 773 ± 70, NS) and t½ of flow during hyperemia (men 35 ± 5 s vs. women 32 ± 6 s, NS) were not significantly different between groups.

Multiple regression analysis. When multiple analysis was carried out with the arterial diameter at baseline, the arterial flow at baseline and at peak, the AUC, and the t½ of flow as cofactors, there was no significant residual correlation between gender and diameter at peak during hyperemia. The optimal fit model after stepwise analysis included only the diameter at baseline as a significant predictor of diameter at peak (r² = 0.981, baseline diameter F = 449.20, P < 0.0001; gender, F = 3.468, P < 0.081; and t½, F = 2.747, P < 0.117).

Distal Skin Heating

Diameter-flow curve. Figure 4A shows the diameter-flow curve in men and women during distal hand skin heating. The diameter increased with arterial blood flow in the two groups (P < 0.01). There was a significant downward shift of the curve toward the lower}

**Fig. 1.** Means ± SE of radial artery diameter (A) and flow (B) measured the first day at baseline before water addition (○, skin temperature: 33°C) and after water addition the first (○) and second day (●) from the skin temperature of 20–42°C, enabling the construction of the diameter-flow relationship (C). Increase in skin temperature was associated with an increase in radial artery flow and diameter that was reproducible during day-to-day experiments. There was thus no significant difference between the diameter-flow curves obtained the first and second day. P < 0.01, time effect; *P < 0.05 vs. 33°C.

Postischemic hyperemia was associated with an increase in radial artery flow to 99 ± 13 ml/min (P < 0.05). The AUC and t½ of flow during hyperemia were 973 ± 94 arbitrary units (AU) and 34 ± 5 s, respectively. The increase in flow was associated with an increase in radial artery diameter to 2.87 ± 0.11 mm (P < 0.05).

The maximal value of blood flow was higher after RH than after distal skin heating (P < 0.05) but the maximal value of radial artery diameter was higher after distal skin heating than after hyperemia (P < 0.05). In these conditions, by taking the values obtained at 34°C as baseline values during heating, the maximal increase in flow was greater after hyperemia (percent change from baseline: RH, 508 ± 46% vs. skin heating, 311 ± 50%; P < 0.05) but the maximal increase in diameter was greater after hand skin heating (percent change from baseline: RH, 10 ± 1% vs. skin heating, 17 ± 2%; P < 0.05).
values of diameter and flow in the women as expected from the smaller vascular dimension observed in this group ($P < 0.01$). However, the slope of the diameter-flow curve was slightly but significantly higher in women than in men, suggesting a higher magnitude of flow-dependent dilatation in the women ($P < 0.01$).

**Diameter-shear stress curve.** Figure 4 shows the diameter-shear stress curve in men and women during distal hand skin heating. The diameter increased with arterial shear stress in the two groups ($P < 0.01$). There was a significant downward shift of the curve toward the lower values of diameter in the women ($P < 0.01$). However, in contrast to the previous relationships, there was no significant difference for the slope of the diameter-shear stress curves between men and women, suggesting the same radial artery flow reactivity between groups when compared at the same level of stimulus.

**Shear stress-flow curve.** Figure 5 shows the shear stress-flow curve in men and women during distal hand skin heating. The shear stress increased with the blood flow in the two groups ($P < 0.01$). However, the increase in shear stress was higher in women than in men because of the smaller arterial diameter at each level of flow ($P < 0.01$).

**DISCUSSION**

The present study demonstrates by direct evaluation that arterial diameter explains the gender difference reported in conduit artery flow-dependent dilatation when studied at each level of flow by modifying the mean arterial wall shear stress. This result was obtained by use of a distal skin heating method that allows the comparison of the flow-dependent dilatation of peripheral conduit arteries at a known level of stimulus.

As previously reported (5, 31, 36), local skin heating provided reproducible increases in regional blood flow. Because the vasodilating effect of local heating is limited to the skin with no dilatation of the underlying muscular vascular bed and no effect on the central thermoregulation, we observed no significant modification in systemic hemodynamics (5, 9, 18, 31, 36, 38). Moreover, in the present study, no modification was observed in plasma catecholamines or total blood viscosity during the test. By gradually increasing the distal hand skin temperature, it was possible to obtain, in contrast to postischemic hyperemia, a successive and sustained increase in radial artery flow and diameter (16). Furthermore, by means of the flow-clamp procedure, we have demonstrated that under these experimental conditions, the radial artery dilatation was an exclusive flow-dependent process. Therefore, it
was possible to study the flow-dependent vasodilation, not only at instantaneous and maximal flow increase, but also at different levels of flow. As a consequence, the shear stress at each level of flow can be assessed to compare the flow responses between groups at known levels of stimulus and establish a reproducible response curve at these different stimuli.

In the present study, we characterized the flow-dependent vasodilation in men and women both by skin heating and posts ischemic hyperemia. During posts ischemic hyperemia, the radial artery flow-dependent vasodilation was significantly higher in women despite the fact that the increase in flow was significantly higher in men. This response is currently accepted and has previously been reported in the brachial artery of women (11, 21). However, by use of multiple regression analysis, we observed in our study population, in accordance with the literature, that this difference is entirely explained by the difference in baseline diameter (20, 21). Thus, in the present study, as in other reported studies (6, 7, 20), the diameter at peak appears as a direct function of the base diameter. In addition, it has been previously stressed that the increase in large artery diameter after hyperemia is inversely related to the basal value of the diameter when expressed in percent change from baseline (21, 27).

In this context, the distal hand skin heating method used in the present study permitted us to obtain the shear stress-diameter relationship by gradually increasing the flow and by simultaneously measuring arterial diameter to directly compare the arterial vasodilation between groups at the same level of stimulus. At baseline, we first compared the wall shear stress obtained in the two groups. However, no significant difference between wall shear stress calculated in men and women was observed. This result has been previously demonstrated in the carotid artery (30).

This result is because in men the higher flow and higher blood viscosity are associated with a higher diameter and that the ratio between these parameters, which determines the wall shear stress value remains not significantly different between groups (25). Thus the variations of flow-dependent increases in diameter obtained during heating cannot be explained by different basal values of shear stress in men and women.

During hand skin heating, the evaluation of the relationship between radial artery diameter and flow demonstrates that the flow-dependent increase in diameter was slightly but significantly higher in women than in men. This result suggested, according with previous experiments performed by use of hyperemia and without any correction for the brachial artery diameter at baseline, a higher magnitude of radial artery flow-dependent dilatation in women (11, 21). However, when we compared the shear stress-diameter relationship, no significant difference between groups for the shear stress-dependent increase in diameter was observed. The curve obtained in men was shifted upward toward the higher values of diameters according to their higher body area but the increase in diameter in response to the increase in flow was similar in men and women and the curves were parallel. These results can be explained by the shear stress-flow relationship. When we compared the shear stress-flow relationship, it appears that the shear stress is higher in women than in men at each level of flow and that the difference between men and women was found to increase with flow, thus resulting in a significantly different relationship between these two parameters in the two groups. Under our experimental conditions it appears that with similar increases in flow, increases in shear stress and in diameter are more marked in women than in men. Because for identical basal values of diameter and viscosity, identical increases in flow induce identical increases in shear stress; the role of the initial diameter seemed to be the determinant in our results to explain these discrepancies between men and women. Thus, when basal diameter is smaller, an identical increase in flow is associated with a higher increase in shear stress. This was observed in the case of women and was clearly evident from the flow-shear stress relationship. To eliminate the influence of the basal diameter, we evaluated the diameter-shear stress relationship, which shows a nonstatistically different evolution between increases in diameter and shear stress during increasing flows by heating and thus demonstrates similar flow-dependent vasodilating responses in men and women. It thus appears that with this methodological approach of the study of flow-dependent dilatation, endothelial function could be directly evaluated independently of the arterial diameter.

With regard the absence of significant difference between men and women, the mechanism that could explain the presence of the same arterial response to increase in flow, despite the fact that estrogens increase the magnitude of arterial flow-dependent dilatation and increase the bioavailability of endothelial
NO, remains unknown and could not be determined from the present study (2, 7, 11–13, 24). One explanation could be the fact that estrogens appear to primarily increase basal release than the stimulated NO bioavailability and that this increase could be sufficient to explain the vascular gender differences (19, 29, 33). Indeed, it was demonstrated in healthy subjects that the arterial vasoconstrictive effect of NO synthase inhibitors was higher in women than in men despite the same vasodilating effect of acetylcholine when doses were adjusted to the forearm volume (8, 19, 34). In addition, the estrogen substitution of perimenopausal women increased the basal rather than the acetylcholine-stimulated NO bioavailability in the forearm volume (33). However, the positive effect of estrogens on stimulated release of NO could be unmasked in nonmenopausal women in pathological states. Thus, in hypertension and hypercholesterolemia, the endothelium-mediated vasodilation in response to acetylcholine appears lower in men in contrast to women (8, 34). This effect could be explained by the stimulating effect of estrogens on NO synthase activity or moreover, as previously suggested by the estrogen-mediated decrease in NO inactivation, by oxygen-derived free radicals (2, 4, 12, 37). Nevertheless, despite the fact that the role of NO could be implicated, it cannot be excluded that the plasma levels of estrogens could not reflect local concentration and/or activity of the hormones within the blood vessel wall, thus leading to underestimate the estrogens activity in men (10, 29).

Finally, it could be pointed out that the absence of characterization of the peak wall shear stress could be a limitation because it has been shown that, at the level of elastic conduit arteries, the peak shear stress can be higher in men than in women at similar mean wall shear stresses (30). However, in our study, if the peak shear stress would have been the main determinant of the flow-mediated response, a higher peak shear stress in men in presence of a similar flow-dependent response at the same mean shear stress level would have been associated with a lower flow-dependent dilatation in this group when compared at peak shear stress. This is not the case with postischemic hyperemia after statistical correction for differences in baseline diameter. This type of absence in the difference between groups for flow-dependent dilatation in these experimental conditions has been previously reported (21). In addition, recent experiments demonstrated that the mean shear stress was a better determinant of the endothelium flow-dependent dilatation in the iliac artery of the dog than the peak shear stress (32).

In conclusion, the present study performed in healthy human volunteers on the radial artery clearly demonstrates that the initial diameter influences the magnitude of flow-dependent vasodilatation of conduit arteries by modifying the value of the arterial wall shear stress at each flow stage and that the gender difference previously reported in flow-dependent vasodilation could be the consequence of the difference in diameter at baseline. These results were obtained by use of a distal hand skin heating method that allows the direct comparison of flow-dependent dilatation of peripheral conduit arteries at the same stimulus between groups, characterized by differences in arterial diameter, flow, and blood viscosity.

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REFERENCES