Effects of gender on resting leg blood flow: implications for measurement of regional substrate oxidation

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Jensen, Michael D., Tu T. Nguyen, A. Hernández Mijares, C. Michael Johnson, and Michael J. Murray. Effects of gender on resting leg blood flow: implications for measurement of regional substrate oxidation. J. Appl. Physiol. 84(1): 141–145, 1998.—These studies were designed to examine whether the respiratory quotient (RQ) of leg tissue (primarily skeletal muscle) would increase to a greater degree in women than in men during meal ingestion. We found that mean leg and systemic RQ values were similar in men under both basal and fed conditions, whereas the agreement was poor in women. In women, leg RQ values tended to be greater than the systemic RQ, whereas splanchic RQ values tended to be lower than the systemic RQ. The possibility that measurement imprecision accounted for the different findings in women could not be excluded because the arteriovenous blood O2 differences were almost twice as great in men as in women (53.7 ± 5.4 vs. 28.6 ± 2.9 ml of O2/l, respectively; P < 0.01), as were venoarterial blood CO2 differences. The smaller arteriovenous differences in women appeared to limit our ability to accurately measure their leg RQ values. O2 uptake relative to leg fat-free mass (FFM) was not different between men and women, whereas leg blood flow relative to leg FFM was greater in women than in men (55 ± 3 vs. 39 ± 2 ml·kg FFM−1·min−1, respectively; P < 0.001). These findings were confirmed by examining data from other studies conducted in our laboratory to create a larger data set. We conclude that resting leg blood flow in women is greater (relative to FFM) than in men, making it more difficult to accurately measure leg RQ in women.

blood oxygen; blood carbon dioxide; indirect calorimetry; body composition

WE RECENTLY REPORTED significant gender differences in adipose tissue metabolism after meal ingestion (4). Postprandial free fatty acid (FFA) flux was suppressed to a greater degree in women than in men. Greater postprandial FFA availability in men than women would be expected to result in greater postprandial fatty acid oxidation by skeletal muscle. This question could potentially be addressed by using limb-balance studies; however, most previous studies that examined lipid glucose and fat oxidation by using indirect calorimetry have focused primarily (10, 11, 13) or exclusively (8, 9) on men.

To address this deficiency in the literature, experiments were undertaken to measure changes in leg respiratory quotient (RQ) in men and women undergoing studies of FFA metabolism (14). A continuous meal ingestion protocol was used to achieve a steady-state postprandial leg RQ. The average leg RQ values were different from systemic RQ values in women but not in men; however, substantial gender-related differences in resting leg blood flow were noted that may have reduced the accuracy and/or precision of the measurement in women.

METHODS

Subjects. Informed written consent was obtained from nine nonobese, premenopausal women and eight nonobese men. All volunteers were in good health, taking no medications, and had maintained a stable weight for >2 mo before the study. A summary of the subjects' characteristics is provided in Table 1. Most of these volunteers were studied as part of a FFA turnover research project (14).

Materials and assays. Indocyanine green (Cardio-Green; Becton Dickinson, Cockeysville, MD) was used for these studies. Arterial and venous blood gases were measured by an Instrument Laboratories (Lexington, MA) 1301 blood-gas analyzer and 282 CO-oximeter. Total plasma CO2 was measured by using a Corning 965 CO2 analyzer (Corning Medical and Scientific, Medfield, MA). Body fat and fat-free mass (FFM), as well as leg fat, leg FFM, and total leg mass, were measured by using dual-energy X-ray absorptiometry (Lunar Radiation, Madison, WI). Plasma and indocyanine green concentrations were measured on the day of the study by using a spectrophotometer.

Protocol. Each volunteer consumed all meals as provided by the Mayo Clinic General Clinical Research Center (GCRC) for 3 days before the study. The diet provided 40% of energy intake as fat, 40% as carbohydrate, and 20% as protein. The energy intake for weight maintenance for each volunteer was based on a measurement of resting energy expenditure by indirect calorimetry (DeltaTrac Metabolic Cart, SensorMedics, Yorba Linda, CA) and on our experience with long-term feeding studies in the GCRC (4). Each subject was admitted to the GCRC the evening before the study, and an 18-gauge infusion catheter was placed in a forearm vein and infused with 0.45% NaCl at 20 ml/h. The next morning before the indocyanine green infusion was started, blood was sampled to be used for the construction of the indocyanine green calibration curve.

On the morning of study, volunteers were transferred to the Vascular Radiology Laboratory where a 5-Fr Terumo sheath was introduced into the right femoral artery by using standard percutaneous technique. A 20-cm-long, 4-Fr straight catheter with six distally placed side holes (special-order Cook) was placed through the sheath with the catheter tip in the common iliac artery. This catheter was used for arterial blood sampling, and the sheath was used to infuse indocyanine green. The right femoral vein was then punctured in a similar manner, and a 6-Fr Terumo sheath was introduced. The distal tip of the sheath was placed in the external iliac vein a few centimeters above the inguinal ligament. A 5-Fr Simmons II catheter with four distal side holes was placed through the sheath, and the catheter tip was placed in the right hepatic vein. Catheter position was confirmed with the injection of ~5 ml iodinated contrast material. A solution of 0.45% NaCl was infused through the sheaths and the cath-
Table 1. Subject characteristics

<table>
<thead>
<tr>
<th></th>
<th>Men</th>
<th>Women</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>27 ± 3</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>Height, cm</td>
<td>180 ± 4</td>
<td>165 ± 3</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>78.2 ± 3.2</td>
<td>59.7 ± 3.2</td>
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<tr>
<td>%Body fat</td>
<td>19 ± 2</td>
<td>29 ± 2</td>
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<tr>
<td>Leg mass, kg</td>
<td>11.7 ± 0.5</td>
<td>9.9 ± 0.7</td>
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<tr>
<td>Leg FFM, kg</td>
<td>9.7 ± 0.4</td>
<td>6.5 ± 0.3</td>
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Values are means ± SE for 8 men and 9 women. FFM, fat-free mass. With exception of age, all variables are significantly (P < 0.05) different for men and women.

deters to maintain patency. The volunteers were then transferred back to the GCRC for completion of the study.

After returning to the GCRC, the volunteers rested in bed for 90 min before beginning to consume the experimental liquid meal, which consisted of Ensure Plus (Ross Laboratories). Each meal was measured to within 2 ml for each individual and was calculated to provide one-third of daily energy needs (980 ± 47 kcal for men, 709 ± 26 kcal for women). The meal contained 53% of energy as carbohydrate, 32% as fat, and 15% as protein. A primed, continuous infusion of indocyanine green (220 μg/min) was begun through the femoral artery sheath 30 min before the first blood samples. Arterial, femoral venous, and hepatic venous blood samples were taken at 15-min intervals over 45 min before the meal was begun. After the basal blood samples were obtained, the volunteers began consuming the meal, beginning with an initial priming (double) dose, followed by equal portions at 20-min intervals over 6 h. The indocyanine green infusion was discontinued after the basal blood samples were collected and was restarted 60 min before the second series of blood samples were begun, which were obtained at 20-min intervals during the last 80 min of the study. After completion of the study, the catheters were removed and local hemostasis was obtained. The volunteers remained hospitalized under observation until the next morning.

Systemic O2 consumption and CO2 production rates were measured continuously for the 30 min before the subjects began feeding and for the last 10 min of every 30 min for the last 2 h of the study.

Leg and splanchnic indirect calorimetry. During the 45-min basal period, simultaneous arterial, femoral venous, and hepatic venous blood samples were collected at 15-min intervals for measurement of plasma CO2 content in triplicate. The plasma CO2 content was adjusted to whole blood CO2 content by using a regression equation that predicts values of whole blood CO2 content (1). During the final 30 min of the basal period, arterial, femoral venous, and hepatic venous blood samples were collected in duplicate at 15-min intervals for measurement of blood O2 content. These blood-gas samples were collected in heparinized syringes, submerged in ice, and analyzed within 30 min of when they were drawn. Blood O2 content was calculated by using the hemoglobin concentration, percent hemoglobin saturation, and the PO2 (2). During the last 80 min of the 360-min meal ingestion time period, blood samples were obtained at 20-min intervals. Plasma CO2 content was measured in triplicate on each sample, and duplicate sets of blood gases were taken from each site during the last 60 min.

Calculations. The mean systemic O2 consumption and CO2 production rates over the 30-min basal period and over the last 120 min of the meal ingestion period were used to calculate the RQ. The basal and meal arterial and venous CO2 and O2 content were taken as the mean of the respective determinations for each subject. The coefficient of variation (CV) for the repeated measures of basal and meal blood CO2 and O2 content was calculated for each individual and subsequently used to determine group means. Arteriovenous (a-v) differences in blood O2 content and blood CO2 content were used to calculate the regional RQ. Leg and splanchnic O2 uptake were measured by multiplying the a-v difference in blood O2 content by leg or splanchnic blood flow. Regional CO2 production was also measured by multiplying the venoarterial (v-a) differences in blood CO2 content by leg or splanchnic blood flow. Leg (7) and splanchnic (3) plasma flow was calculated as previously described and converted to blood flow by using each individual's hematocrit value.

All data are presented as means ± SE. Statistical comparisons between mean steady-state basal and meal values were made by using a non-paired t-test. Comparisons between mean steady-state basal and meal values were made by using a two-tailed paired Student's t-test. Linear regression analysis was performed to test the association between leg size and blood flow or O2 uptake. To compare these relationships between men and women, multiple linear regression analysis was used that included gender as an additional independent variable.

RESULTS

Subjects. Men and women participating in this study were matched for age (Table 1). Men were taller and heavier with less body fat, greater leg mass, and greater leg FFM.

Systemic and regional RQ. The basal systemic RQ was 0.79 ± 0.01 and 0.79 ± 0.02 in men and women, respectively. Over the last 1 h of the meal the systemic RQ increased (P < 0.001) to 0.86 ± 0.01 in both men and women.

The individual values for the systemic vs. leg RQ for the basal and meal time intervals for women and men are provided in Fig. 1. Systemic RQ values clustered in a narrow range for both groups at both time intervals; however, the leg RQ values were substantially more variable, especially in women. The basal leg RQ in men was 0.79 ± 0.03 and over the last 1 h of the meal increased (P < 0.01) to 0.87 ± 0.04. In women, the basal leg RQ was 0.92 ± 0.05 and was not different during the last 1 h of the meal (0.96 ± 0.04; P = 0.56). Neither basal nor meal leg RQ values were significantly different between men and women.

The individual splanchnic RQ values for the basal and meal time intervals for women and men are provided in Fig. 2. An extremely wide range of values was observed, and although overlap was observed between the two groups, basal splanchnic RQ in men was greater (P < 0.05) than in women (0.97 ± 0.11 vs. 0.68 ± 0.04, respectively). Over the last hour of the meal, these values were 0.87 ± 0.08 and 0.79 ± 0.06 [P = not significant (NS) vs. basal and men vs. women].

Reproducibility of blood O2 and CO2 content measurements. The mean CVs of the basal arterial, femoral venous, and hepatic venous blood O2 content were 0.4 ± 0.4, 3.2 ± 1.8, and 1.7 ± 1.1%, respectively. The CVs of the blood CO2 content for arterial, femoral venous, and hepatic venous blood were 2.3 ± 1.6, 1.7 ± 1.1, and 1.7 ± 1.2%, respectively. The reproducibility of the
measurements was not different in women and men or between the basal and the meal ingestion samples.

Although the precision of the individual arterial and venous blood O₂ and CO₂ content measurements was acceptable and consistent with previous reports (10), the precision of the a-v difference values was substantially worse. The CV of the leg and splanchnic a-v O₂ differences was 15 ± 9 (SD) and 6 ± 4%. The CV of the v-a blood CO₂ content differences for the leg and the splanchnic bed was 33 ± 14 (SD) and 38 ± 20%.

Regional (leg and splanchnic) gas exchange. The regional O₂ uptake and CO₂ release in men and women were examined (Table 2). The leg a-v O₂ and v-a CO₂ differences were almost twice as great in men as in women (P < 0.01). Men also had larger (P < 0.05) splanchnic a-v O₂ and v-a CO₂ blood content differences than women; however, these gender-related differences were not as great as those observed across the leg tissues.

To test whether the greater a-v gradients in men than women were unique to this group of subjects or a more generalized phenomenon, we combined data from previous studies (5, 6) together with data from this study for a total of 25 men and 25 women (splanchnic data available for 23 men and 22 women). The leg a-v O₂ differences in the combined groups were greater (P < 0.001) in men than in women (49.5 ± 2.6 vs. 29.7 ± 2.1 ml/l, respectively). The splanchnic a-v blood O₂ difference was also greater in men than in women (39.6 ± 1.5 vs. 34.4 ± 1.1 ml/l, respectively; P < 0.005).

Gender-based differences in leg a-v blood O₂ gradients could be related to differences in the O₂ consumption of FFM, differences in blood flow, or a combination of factors. To test the former possibility, we examined the relationship of leg O₂ uptake (ml/min) and leg FFM in this larger group of men and women. Consistent with previous observations (5, 6), a strong correlation between leg O₂ uptake (ml/min) and leg FFM was present (Fig. 3), but the slope of the line was not different between men and women.

Regional blood flow. Basal leg blood flow in this larger group of men and women was 391 ± 25 and 382 ± 23 ml/min, respectively, (P = NS). Splanchnic blood flow in the same groups was 1,566 ± 78 vs. 1,346 ± 74 ml/min (P < 0.05, men vs. women, respectively).

A significant (P < 0.05) but weak (r = 0.36) relationship between blood flow and total leg mass was present (Fig. 4). Blood flow per kilogram leg mass was not significantly different in men (32 ± 2 ml·kg⁻¹·min⁻¹) and women (37 ± 2 ml·kg⁻¹·min⁻¹). Leg blood flow relative to leg FFM was greater (P < 0.001) in men than in women (55 ± 3 vs. 39 ± 2 ml·kg FFM⁻¹·min⁻¹, respectively). There was no apparent relationship between leg FFM and resting leg blood flow (Fig. 5).

Table 2. Basal regional blood O₂ and CO₂ differences

<table>
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<th>Men</th>
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<tbody>
<tr>
<td>Leg</td>
<td></td>
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<tr>
<td>a-v O₂ difference</td>
<td>53.7 ± 5.4</td>
<td>28.6 ± 2.9*</td>
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<tr>
<td>v-a CO₂ difference</td>
<td>42.5 ± 4.0</td>
<td>25.9 ± 2.8*</td>
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<tr>
<td>Splanchnic</td>
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<tr>
<td>a-v O₂ difference</td>
<td>40.5 ± 2.4</td>
<td>37.4 ± 1.1*</td>
</tr>
<tr>
<td>v-a CO₂ difference</td>
<td>37.6 ± 2.6</td>
<td>25.6 ± 1.8*</td>
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</tbody>
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Values are means ± SE in ml/l. Leg values are for 8 men, and 9 women. Splanchnic values are for 7 men and 8 women. a-v Difference, arteriovenous difference; v-a difference, venoarterial difference. *P < 0.05 vs. men.

Fig. 1. Systemic respiratory quotient (RQ) values vs. leg RQ values are plotted for both basal and meal conditions for men and women participating in this study.

Fig. 2. Individual splanchnic RQ values under basal and meal conditions for men and women participating in this study are plotted.
These studies were originally designed to assess whether leg RQ would increase more in women than in men during meal ingestion. This expectation was based on the greater postprandial suppression of FFA availability in women than in men (4). We found that the overnight postabsorptive systemic RQ was similar in men and in women. In men, the mean basal leg RQ was identical to the mean systemic RQ, and both values increased during meal ingestion. In contrast, the leg RQ values in women were greater than the systemic RQ, whereas splanchnic RQ values were less. Although this could be due to regional differences in substrate oxidation between men and women, the lesser a-v O2 and v-a CO2 gradients across leg tissues in women limit our confidence in the accuracy of these numbers because of the reduced measurement precision of the a-v differences in women compared with men. The smaller a-v gradients in women were not due to differences in the O2 uptake relative to leg FFM but rather to a greater resting leg blood flow relative to FFM in women. We conclude that resting leg blood flow (relative to FFM) is significantly greater in women than in men. This makes it more difficult to accurately and precisely measure leg uptake and release of O2 and CO2, respectively, in women.

It is possible that men and women, although displaying similar systemic RQ values, differ in the localization of substrate oxidation. The basal splanchnic RQ values <0.70 in women and not in men may indicate greater postabsorptive gluconeogenesis occurs in women than in men. Similarly, the greater basal leg RQ values in women may reflect lesser postabsorptive fatty acid oxidation in the skeletal muscle of women than of men. Because of the difficulties in measuring leg CO2 exchange in women, however, additional studies should be undertaken to test this hypothesis. Care in planning such studies, especially with regard to adequate sample collection and the number of subjects studied (see below) will be necessary.

The reason for the greater blood flow relative to FFM in women compared with men is uncertain. Blood flow relative to total leg mass was not substantially different in women than in men. Women, however, have significantly greater adipose tissue in their legs than do men. It is possible that the blood flow to adipose tissue could contribute to the differences in leg blood flow relative to FFM between men and women. Because adipose tissue is less metabolically active than skeletal muscle, the measurement of O2 uptake across the combined tissues may make it more difficult to accurately assess O2 uptake and CO2 release across the more metabolically active portion of leg tissue, namely, skeletal muscle. Differences in leg fat mass may not be the full explanation for the differences in blood flow.
between men and women, however. Women had, on average, 1.3 kg more leg fat (~1.6 kg more adipose tissue) than did men. At an average blood flow of 2.6 ml/100 g adipose tissue (12, 15), the greater leg adipose tissue mass in women would be expected to account for only ~42 ml/min (~10%) of additional leg blood flow. The possibility that blood flow to leg skeletal muscle is greater in women than in men must still be considered.

Our findings highlight a problem in the use of a-v difference measurements to assess the uptake and release of substances, including O2 and CO2. A value derived from the subtraction of one large number from another large number (e.g., venous CO2 minus arterial CO2 content) can be imprecise despite the use of highly precise individual measurement techniques. To overcome this problem, more samples could be collected to improve the accuracy of the measurement. The practicality of this approach seems limited, however. The SD of repeated measures of arterial and femoral venous blood CO2 content measurement was ~1.0 ml/100 ml blood for both men and women. Thus the 95% confidence interval for arterial and venous blood CO2 content was ~1.8 ml/100 ml (n = 4–6 samples each run in triplicate). Increasing the number of replicate samples to 16 could be expected to decrease the 95% confidence interval to ~1.0 ml of CO2/100 ml blood for both men and women. Whereas this may provide adequate accuracy for measuring leg CO2 release in men (average a-v CO2 difference of 4.3 ml/100 ml blood), it would still be insufficiently accurate for women (average a-v CO2 difference of 2.6 ml/100 ml blood). Because the narrowing of the confidence interval is related to the square root of the number of samples, it would require almost three times as many (n = 44) replicates to achieve comparable accuracy of leg CO2 release in women as in men.

It is not known whether the variations we observed in blood CO2 content are due to measurement error alone or some combination of measurement error and physiological variability in blood CO2 content. A significant degree of physiological variability in venous blood O2 content appears to occur; the reproducibility of arterial blood O2 content is substantially better than that of femoral venous blood O2 content. The former likely represents the true measurement error, whereas the latter represents both measurement error and physiological variations in leg O2 uptake or blood flow. A similar situation almost surely exists with regard to leg CO2 release.

In summary, in the course of attempting to study leg substrate oxidation in men and women, we found that gender-related differences in leg blood flow decrease the a-v blood O2 and CO2 differences in women compared with men. Therefore, despite relatively precise assays, it was difficult to accurately measure leg RQ in women. Increasing the number of samples obtained could theoretically improve the accuracy of the measurements, but significantly more women than men will likely need to be included in future studies to assess whether true gender difference in regional substrate oxidation are present between women and men. Further improvements in the measurement of blood CO2 content could make such studies more feasible.

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