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


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 splanchnic 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. 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 limb 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 quotients (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 using 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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<tr>
<td>Age, yr</td>
<td>27 ± 3</td>
<td>29 ± 2</td>
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<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.

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
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 consumption 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 women than in men (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>
<thead>
<tr>
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<th>Men</th>
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<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>
</tr>
<tr>
<td>v-a CO₂ difference</td>
<td>42.5 ± 4.0</td>
<td>25.3 ± 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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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.
DISCUSSION

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 tissues, namely, skeletal muscle. Differences in leg fat mass may not be the full explanation for the differences in blood flow.

Fig. 3. Leg fat-free mass (FFM) as measured by dual-energy X-ray absorptiometry vs. basal leg O₂ consumption (V̇O₂) is plotted for men (○) and women (●) participating in this study. Data from previously conducted studies (5, 6) are included to create a larger data set.

Fig. 4. Leg mass as measured by dual-energy X-ray absorptiometry vs. basal leg blood flow is plotted for men (○) and women (●) participating in this study. Data from previously conducted studies (5, 6) are included to create a larger data set.

Fig. 5. Leg FFM as measured by dual-energy X-ray absorptiometry vs. basal leg blood flow is plotted for men (○) and women (●) participating in this study. Data from previously conducted studies (5, 6) are included to create a larger data set.
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 over-
come this problem, more samples could be collected to
improve the accuracy of the measurement. The practi-
cality 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% confi-
dence interval for arterial and venous blood CO₂ con-
tent 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 accu-
rate 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 narrow-
ing of the confidence interval is related to the square
root of the number of samples, it would require almost
time 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 signifi-
cant 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 com-
pared 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 measure-
ments, 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. Fur-
ther improvements in the measurement of blood CO₂
content could make such studies more feasible.

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