Adipose tissue extracts plasma ammonia after sprint exercise in women and men

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Esbjörnsson, Mona, Jens Bülow, Barbara Norman, Lene Simonsen, Jacek Nowak, Olav Rooyackers, Lennart Kajiser, and Eva Jansson. Adipose tissue extracts plasma ammonia after sprint exercise in women and men. J Appl Physiol 101: 1576–1580, 2006. First published November 10, 2005; doi:10.1152/japplphysiol.01119.2004.—This study evaluates a possible contribution of adipose tissue to the elimination of plasma ammonia (NH3) after high-intensity sprint exercise. In 14 healthy men and women, repeated blood samples for plasma NH3 analyses were obtained from brachial artery and from a subcutaneous abdominal vein before and after three repeated 30-s cycle sprints separated by 20 min of recovery. Biopsies from subcutaneous abdominal adipose tissue were obtained and analyzed for glutamine and glutamate content. After exercise, both arterial and abdominal venous plasma NH3 concentrations were lower in women than in men (P < 0.01 and P < 0.001, respectively). All postexercise measurements showed sex-independent positive arterio-subcutaneous abdominal venous plasma NH3 concentration differences (a-vabd), indicating a net uptake of NH3 from blood to adipose tissue. However, the fractional extraction (a-vabd/a) of NH3 was higher in women than in men (P < 0.05). The glutamine-to-glutamate ratio in adipose tissue was increased after the second and third bout of sprint exercise (2.2 ± 0.7 and 1.6 ± 0.8, respectively) compared with the value at rest (1.2 ± 0.6), suggesting a reaction of the extracted NH3 with glutamate resulting in its conversion to glutamine. Adipose tissue may thus play an important physiological role in eliminating plasma NH3 and thereby reducing the risk of NH3 intoxication after high-intensity exercise.

glutamine; glutamate; biopsy; sex; Wingate test

HIGH-INTENSITY SPRINT EXERCISE induces a pronounced breakdown of ATP in skeletal muscle and a corresponding increase of inosine monophosphate (IMP) as a result of subsequent deamination of AMP. A portion of the ammonia/ammonium (NH3) produced in this deamination process is released into the blood. The removal of NH3 from the blood is of significant physiological importance (2, 22) because high plasma NH3 concentration causes negative effects like vomiting and impairment of the central nervous system and motor functioning.

We recently showed that the degree of muscle ATP net breakdown in skeletal muscle per unit muscle mass and the concomitant increase in IMP generation during high-intensity sprint exercise does not differ between women and men either in type I (slow) or in type II (fast) fibers (9–11). However, in this situation, plasma NH3 concentrations reach much lower levels in women than in men, despite the fact that IMP and NH3 are formed in equal quantities per unit muscle mass during deamination of AMP. To some extent, however, the sex difference in plasma NH3 may depend on the smaller skeletal muscle mass (relative to body size) in women than in men. However, as estimated earlier, the difference in muscle mass can account for only 15–25% of the observed sex difference in plasma NH3 after sprint exercise (10). This indicates the existence of an additional mechanism that would generate the observed sex difference. A greater clearance of NH3 from blood in women may possibly constitute a pivot of such a mechanism and thus explain the existing sex difference.

NH3 is eliminated from blood by the liver, lungs, and kidney as well as through sweat. Redistribution of NH3 from blood to other organs, including adipose tissue, may, however, take place as well (15, 24). In this context, it should be kept in mind that there is an active metabolism that is taking place in adipose tissue, in which the enzyme glutamine synthase transforms glutamate and NH3 to glutamine (6, 13, 19). Studies in animals and humans have demonstrated accordingly that adipose tissue takes up glutamate and releases glutamine into blood and that a majority of the produced glutamine originates from NH3 and glutamate (12, 19). Released from adipose tissue, glutamine acts as a carrier for NH3 and is retransformed into glutamate and NH3 in the kidney, NH3 being finally excreted in the urine (6). Inasmuch as women have about twice as much fat (relative to body mass) as men (23), adipose tissue may in fact be instrumental in the increased plasma NH3 clearance after sprint exercise observed in women.

Against this background, the aim of the present study was to evaluate a possible physiological role of adipose tissue in the elimination of plasma NH3 after sprint exercise in men and women. It is hypothesized that an uptake of NH3 may occur from plasma to adipose tissue and that this uptake may increase after sprint exercise concurrently with an increasing arterial plasma NH3 concentration.

METHODS

Subjects. Six men and seven women, most of them students at a college for sports and recreation instructors, volunteered for the study. All the subjects were well trained but no one was at an elite or competitive athletic level. A special questionnaire was used to estimate the physical activity level during leisure time. The subjects answered nine different questions from which an activity index (minimum value 5.5 and maximum value 20.5) was calculated (17).

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Fat-free body mass was estimated from skinfold measurements (triceps, biceps, and subscapula; Ref. 8). Smokers, individuals on medication, and women in menstrual phase were excluded from the study. The subjects were fully informed about the procedures and potential risks of the experiment before giving their consent to participate. The study was approved by the Ethics Committee of Karolinska Institutet.

Experimental protocol. The subjects were asked to refrain from any heavy exercise during the 24-h period preceding the experiment. After subjects were familiarized at least 24 h before the experiment, they performed three cycle sprints of 30-s duration [Wingate test (4)] on a mechanically braked cycle ergometer (Cardionics, Sweden) with 20 min rest between the sprints (Fig. 1). The subjects were instructed to pedal as fast as possible at an individual braking load set at 0.075 kilopound/kg body wt. A sensor-microprocessor assembly counted flywheel revolutions, and the flywheel progression per pedal revolution was 6 m. The average power output was automatically printed immediately after collection. The supernatant was frozen in liquid nitrogen and stored at −70°C until analysis by flow injection method (26). Blood lactate was determined in neutralized perchloric acid extract of whole blood by a fluorometric enzymatic method (20).

Statistics. Values in the text are means ± SD unless otherwise stated. The P values were accepted as statistically significant at P < 0.05. Student’s t-tests for independent groups were applied for comparison of subject characteristics. For the fat biopsy variable, glutamine-to-glutamate ratio, a one-factor ANOVA (repeated-measures design) was applied to test the response to time. For the plasma variables, a two-factor ANOVA (repeated-measures design; sex and time) was applied to test the sex difference in response to time. The statistical analysis of the relationship between arterio-subcutaneous abdominal venous NH3 plasma concentration difference (a-vabd) and the arterial plasma NH3 concentration was performed by a regression analysis that fits parallel lines through each subject’s data (3). The common slope for men was compared with the common slope for women by applying Student’s t-test for independent groups.

RESULTS

Anthropometrical data as well as physical activity level and power output during Wingate cycling are presented in Table 1. As can be seen from Table 1, the activity index did not differ between men and women. However, peak and mean power were significantly higher in men compared with women.

Table 1. Subject characteristic and power output in 7 women and 6 men

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>26±7 (21–43)</td>
<td>28±6 (21–37)</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>64±8</td>
<td>77±11*</td>
</tr>
<tr>
<td>Fat free mass, kg</td>
<td>47±8</td>
<td>62±9†</td>
</tr>
<tr>
<td>Fat mass, kg</td>
<td>17±6</td>
<td>15±6</td>
</tr>
<tr>
<td>Height, cm</td>
<td>165±5</td>
<td>178±6†</td>
</tr>
<tr>
<td>Activity index</td>
<td>16.8±3.0</td>
<td>15.2±1.6</td>
</tr>
<tr>
<td>Peak power, W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sprint 1</td>
<td>602±94</td>
<td>862±124‡</td>
</tr>
<tr>
<td>Sprint 2</td>
<td>564±96</td>
<td>844±126‡</td>
</tr>
<tr>
<td>Sprint 3</td>
<td>553±108</td>
<td>814±133‡</td>
</tr>
<tr>
<td>Mean power, W</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sprint 1</td>
<td>471±63</td>
<td>665±78‡</td>
</tr>
<tr>
<td>Sprint 2</td>
<td>453±56</td>
<td>648±66‡</td>
</tr>
<tr>
<td>Sprint 3</td>
<td>450±53</td>
<td>640±74‡</td>
</tr>
<tr>
<td>Peak power, W/kg body mass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sprint 1</td>
<td>9.4±1</td>
<td>11.1±0.8*</td>
</tr>
<tr>
<td>Sprint 2</td>
<td>8.8±1</td>
<td>10.9±0.9†</td>
</tr>
<tr>
<td>Sprint 3</td>
<td>8.7±1</td>
<td>10.5±1.0</td>
</tr>
<tr>
<td>Mean power, W/kg body mass</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sprint 1</td>
<td>7.4±1</td>
<td>8.6±0.5*</td>
</tr>
<tr>
<td>Sprint 2</td>
<td>7.1±1</td>
<td>8.4±0.9*</td>
</tr>
<tr>
<td>Sprint 3</td>
<td>7.1±1</td>
<td>8.3±0.9*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05; †P < 0.01; ‡P < 0.001.

Fig. 1. Scheme for the experimental design.
Glutamine and glutamate in subcutaneous abdominal adipose tissue. The glutamine concentration in the abdominal adipose tissue increased significantly from 117 \( \pm \) 60 at rest to 180 \( \pm \) 70 mmol/kg wet tissue after the second bout of exercise (sprint 2). In contrast, the glutamate concentration decreased significantly from 100 \( \pm \) 34 at rest to 69 \( \pm \) 26 mmol/kg wet tissue after the third bout of exercise (sprint 3). Consequently, the ratio of glutamine to glutamate was significantly increased after both sprint 2 and sprint 3 compared with baseline (Fig. 2).

Blood lactate. The lactate concentrations in the arterial blood at rest were 1.0 \( \pm \) 0.5 mmol/l in women and 1.0 \( \pm \) 0.3 mmol/l in men, and the respective average values of the five postexercise samples were 10.8 \( \pm \) 1.5 and 12.6 \( \pm \) 1.5 mmol/l (sex \( \times \) time; \( P = 0.07 \)).

Plasma \( \text{NH}_3 \). The exercise-induced accumulation of plasma \( \text{NH}_3 \) in both the arterial and the subcutaneous abdominal venous blood was less pronounced in women than in men at all sampling points after exercise (Fig. 3).

The value of a-\( \text{V}_{\text{abd}} \) differences in the plasma \( \text{NH}_3 \) concentrations at rest and during recovery after the three respective bicycle sprints is presented in Table 2. At rest, the a-\( \text{V}_{\text{abd}} \) differences tended to be positive but increased and became significantly positive after sprint exercise and recovery, thus implying exercise-induced uptake of \( \text{NH}_3 \) in the subcutaneous abdominal adipose tissue. The greatest a-\( \text{V}_{\text{abd}} \) differences were found after the first sprint. No sex differences were observed for the a-\( \text{V}_{\text{abd}} \) differences either at rest or during recovery.

The fractional extraction of \( \text{NH}_3 \) (a-\( \text{V}_{\text{abd}}/a \)) was, however, significantly higher in women than in men at all sampling points during recovery after the exercise (Fig. 4). In fact, the performed regression analysis demonstrated that the a-\( \text{V}_{\text{abd}} \) difference of plasma \( \text{NH}_3 \) was related to the arterial plasma concentration of \( \text{NH}_3 \), the slope of the respective regression lines in men and women (Fig. 5) being an indicator of \( \text{NH}_3 \) extraction in subcutaneous adipose tissue at given arterial \( \text{NH}_3 \) concentration. However, the slope of the regression line for women was significantly steeper than that for men (Fig. 5), thus implying a more efficient \( \text{NH}_3 \) uptake in subcutaneous adipose tissue in women than in men for any given arterial \( \text{NH}_3 \) concentration.

The slope was found to be negatively related to the arterial blood lactate level after sprint exercise \(( r = -0.6, P < 0.02 \)) , i.e., the lower the blood lactate concentrations the steeper the slope.

DISCUSSION

The principal finding in the present study is the demonstration of a net uptake of \( \text{NH}_3 \) from blood to the subcutaneous

<table>
<thead>
<tr>
<th>a-( \text{V}_{\text{abd}} ) Difference, ( \mu \text{mol/l} )</th>
<th>At rest</th>
<th>After sprint 1</th>
<th>After sprint 2</th>
<th>After sprint 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 min</td>
<td>9 min 18 min</td>
<td>9 min 18 min</td>
<td>9 min 18 min</td>
</tr>
<tr>
<td>Men</td>
<td>3 \pm 3</td>
<td>74 \pm 33</td>
<td>25 \pm 15</td>
<td>33 \pm 18</td>
</tr>
<tr>
<td>Women</td>
<td>37 \pm 4</td>
<td>33 \pm 25</td>
<td>15 \pm 33</td>
<td>18 \pm 9</td>
</tr>
</tbody>
</table>

Values are means \( \pm \) SE. ANOVA, \( P < 0.001 \) for time; nonsignificant difference for sex and for interaction time \( \times \) sex.
Abdominal adipose tissue after high-intensity sprint exercise. This phenomenon may play a significant physiological role, because after high-intensity exercise the arterial plasma NH₃ concentrations can reach levels approaching the limit for brain toxicity (2, 22) and a rapid elimination of NH₃ would be of crucial importance for optimal physical performance. The exact fate of NH₃ in adipose tissue is not known, but there is a possibility that NH₃ and glutamate are converted to glutamine by the enzyme glutamine synthase. Indeed, glutamine synthase has been identified in adipose tissue (7), and the present data from fat biopsies support this concept by revealing an increased glutamine-to-glutamate ratio during recovery periods after exercise.

It has been demonstrated previously that inactive muscle also possesses capacity to take up NH₃ during and after exercise (1). This implies that both adipose tissue and skeletal muscle tissue may be involved in the removal of NH₃ from blood. Due to the considerable contribution of both tissues to the body mass, their involvement in the process of plasma ammonia clearance after high-intensity exercise may be quantitatively highly important.

The results of the present study clearly demonstrate that there exists a sex-dependent difference in the exchange of ammonia over adipose tissue. Accordingly, the fractional extraction of ammonia in the subcutaneous abdominal adipose tissue was 0.15 units higher at all the six sampling time points in women than in men. The mechanism of the observed sex difference is not known. It can be speculated, however, that the lower blood lactate levels after sprint exercise observed in women (9, 11) most likely result in somewhat higher blood pH value. Consequently, this would cause in women a more pronounced shift of the equilibrium between ammonia and the ammonium ion toward more lipophilic ammonia.

Katz et al. (18) estimated the total net clearance of NH₃ from blood to be ~150 μmol/min during a 10-min recovery period after intense exercise at the arterial NH₃ concentration of ~200 μmol/l. In the present study, only an estimation of the uptake of NH₃ over adipose tissue could be done due to the lack of blood flow determinations. However, the subcutaneous adipose tissue blood flow does not seem to differ between women and men, either before and during exercise or during postexercise recovery, and is ~4 ml·100 g tissue⁻¹·min⁻¹ (5, 16, 21). Thus, since the a-vabd concentration difference of NH₃ in the present study did not differ between the two sex groups, the absolute clearance of NH₃ in the subcutaneous abdominal adipose tissue (expressed as μmol·100 g tissue⁻¹·min⁻¹) ought to be of similar magnitude in women and men (0.30 and 0.36 for women and men, respectively). With the use of these clearance data in combination with the total fat mass (17 and 15 kg for women and men, respectively) from the present study and assuming that the ammonia uptake in the subcutaneous adipose tissue is representative for the uptake in the total fat mass, this calculation gives a whole body adipose tissue NH₃ uptake of 50 μmol/min for the women and 53 μmol/min for the men, 9 min after the first exercise bout. Hence our estimated uptake of NH₃ from blood to adipose tissue indicates that the adipose tissue significantly contributes to the removal of NH₃ from blood during a postexercise period. The estimated elimination rate of NH₃ by adipose tissue in the present study may also be compared with measured removal rates of NH₃ by the nonexercised leg 2–10 min after high-intensity exercise in men, which were on the order of 100–200 μmol/min (1, 16). These figures further support the conception of adipose tissue playing a significant role in balancing removal and production of NH₃ during and after exercise.

The results of the present experiments may help to explain the original observation of the lower plasma NH₃ concentration after sprint exercise in women because the currently observed clearance of NH₃ by adipose tissue in relation to the NH₃ production seemed to be more efficient in women than in men. Certainly, in absolute terms, the NH₃ clearance by adipose tissue was of similar magnitude in women and men (see calculations above), but the clearance relative to body mass was greater in women (data not shown). As estimated earlier, the smaller muscle mass relative to body mass in the female group and thereby NH₃ production can account for 15–25% of the observed sex difference in plasma NH₃ after sprint exercise (10). Thus both greater clearance and smaller production of NH₃ relative to the body mass may contribute to the lower plasma NH₃ levels after sprint exercise in women compared with men.

In conclusion, the present study demonstrates in accordance with the hypothesis that there occurs a net uptake of NH₃ from the blood to adipose tissue after high-intensity exercise. This uptake is of such magnitude that it may significantly contribute to the removal of NH₃ from blood. The removed NH₃ may then react with glutamate in the adipose tissue, being thus converted to glutamine. The present findings suggest an important physiological role of adipose tissue in reducing the risk of NH₃ intoxication after high-intensity exercise.

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GRANTS

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