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

Mona Esbjörnsson,1 Jens Bülow,2 Barbara Norman,1 Lene Simonsen,3 Jacek Nowak,1 Olav Rooyackers,2 Lennart Kaijser,1 and Eva Jansson1

1Department of Laboratory Medicine, Division of Clinical Physiology, 2Department of Anaesthesiology and Intensive Care at the Centre for Surgical Sciences, Karolinska Institutet, Stockholm, Sweden; and 3Department of Clinical Physiology, Bispebjerg Hospital, Copenhagen, Denmark

Submitted 5 October 2004; accepted in final form 7 November 2005

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 major fraction 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).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
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 (Cardioworx, 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 by 10.220.33.2 on May 13, 2017 http://jap.physiology.org/ Downloaded from

Three biopsies from the subcutaneous abdominal adipose tissue were obtained under local anesthesia by using a Hepafix needle (Braun Medical, Melsungen, Germany) normally used to obtain percutaneous liver biopsies: one before sprint 1, a second one 15 min after sprint 2, and the third one 15 min after sprint 3 (Fig. 1). The biopsies were frozen within 5 s in liquid nitrogen and stored at −70°C until analysis by flow injection method (26). Blood lactate was determined in neutralized perchoric 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 (Δv,Δa) 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.
Glutamine and glutamate in subcutaneous abdominal adipose tissue. The glutamine concentration in the abdominal adipose tissue increased significantly from 117/1006 60 at rest to 180/1006 70 mol/kg wet tissue after the second bout of exercise (sprint 2). In contrast, the glutamate concentration decreased significantly from 100/1006 34 at rest to 69/1006 26 mol/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 1.0/1006 0.5 in women and 1.0/1006 0.3 mmol/l in men, and the respective average values of the five postexercise samples were 10.8/1006 1.5 and 12.6/1006 1.5 mmol/l (sex × time; P < 0.05).

Plasma NH₃. The exercise-induced accumulation of plasma NH₃ 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-vabd differences in the plasma NH₃ concentrations at rest and during recovery after the three respective bicycle sprints is presented in Table 2. At rest, the a-vabd differences tended to be positive but increased and became significantly positive after sprint exercise and recovery, thus implying exercise-induced uptake of NH₃ in the subcutaneous abdominal adipose tissue. The greatest a-vabd differences were found after the first sprint. No sex differences were observed for the a-vabd differences either at rest or during recovery.

The fractional extraction of NH₃ (a-vabd/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-vabd difference of plasma NH₃ was related to the arterial plasma concentration of NH₃, the slope of the respective regression lines in men and women (Fig. 5) being an indicator of NH₃ extraction in subcutaneous adipose tissue at given arterial plasma NH₃ concentration. However, the slope of the regression line for women was significantly steeper than that for men (Fig. 5), thus implying a more efficient NH₃ uptake in subcutaneous adipose tissue in women than in men for any given arterial NH₃ 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 NH₃ from blood to the subcutaneous

Table 2. Arterio-abdominal venous (a-vabd) differences in plasma NH₃ content at rest and after sprint exercise in 6 men and 7 women

<table>
<thead>
<tr>
<th>a-vabd Difference, μmol/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>At rest</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1±1</td>
<td>89±32</td>
<td>34±26</td>
<td>36±18</td>
</tr>
<tr>
<td>Women</td>
<td>3±3</td>
<td>74±33</td>
<td>25±15</td>
<td>33±18</td>
</tr>
</tbody>
</table>

Values are means ± SE. ANOVA, P < 0.001 for time; nonsignificant difference for sex and for interaction time × 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 $\sim 150 \mu\text{mol/min}$ during a 10-min recovery period after intense exercise at the arterial NH3 concentration of $\sim 200 \mu\text{mol/l}$. 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 $\sim 4 \text{ ml} \cdot \text{100 g tissue}^{-1} \cdot \text{min}^{-1}$ (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 $\mu\text{mol} \cdot \text{100 g tissue}^{-1} \cdot \text{min}^{-1}$) 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 $\mu\text{mol/min}$ for the women and 53 $\mu\text{mol/min}$ 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 $\mu\text{mol/min}$ (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.

ACKNOWLEDGMENTS

We greatly appreciate the excellent statistical support of Staffan Ljungfeldt.

GRANTS

This study was supported by grants from the Swedish National Centre for Research in Sports, Foundation of Åke Wiberg, Foundation of Magnus Bergvall, Centre of Gender Related Medicine, and The Swedish Society of Medicine.
REFERENCES


J Appl Physiol • VOL 101 • DECEMBER 2006 • www.jap.org