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ORIGINAL ARTICLE

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Effect of a low-carbohydrate diet on plasma and sweat ammonia concentrations during prolonged nonexhausting exercise

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Abstract The purpose of this investigation was to examine the effect of low body glycogen stores on plasma ammonia concentration and sweat ammonia excretion during prolonged, nonexhausting exercise of moderate intensity. On two occasions seven healthy untrained men pedalled on a cycle ergometer for 60 min at 50% of their predetermined maximal O_2 uptakes ($\dot{V}O_{2max}$) firstly, following 3 days on a normal mixed diet (N-diet) (60% carbohydrates, 25% fat and 15% protein) and secondly, following 3 days on a low-carbohydrate diet (LC-diet) (less than 5% carbohydrates, 50% fat and 45% protein) of equal energy content. Blood was collected from the antecubital vein immediately before, at 30th and at 60th min of exercise. Sweat was collected from the hypogastric region using gauze pads. It was shown that plasma ammonia concentrations after the LC-diet were higher than after the N-diet at both the 30th and 60th min of exercise. Sweat ammonia concentration and total ammonia loss through the sweat were also higher after the LC-diet. The higher ammonia concentrations in plasma and sweat after the LC-diet would seem to indicate an increased ammonia production, which may be related to reduced initial carbohydrate stores.

Key words Ammonia · Exercise · Low-carbohydrate diet · Sweat

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Introduction

It has been known for many years that contracting muscles produce and release ammonia (Parnas 1929). One source of ammonia is adenosine 5′-monophosphate (AMP), which is deaminated to inosine 5′-monophosphate (IMP) in a reaction catalysed by AMP-deaminase. This reaction has been shown to be the first stage of the purine nucleotide cycle (Lowenstein 1972). It has been demonstrated that another important source of ammonia is the deamination of amino acids, mainly branched-chain amino acids (BCAA) (MacLean et al. 1991; Wagenmakers et al. 1991).

It has been shown that the role of AMP deamination is to maintain high energy potential [adenosine 5-triphosphate/adenosine 5-diphosphate (ATP/AMP) and ATP/ADP] (Lowenstein 1972). Formation of IMP and ammonia has been said to reflect a condition of imbalance between resynthesis and utilization of ATP (Sahlin and Katz 1988). During high-intensity exercise this process is known to be due to impaired regeneration of ATP by high concentrations of lactic acid and low concentration of phosphocreatine (Dudley and Terjung 1985 a,b; Sahlin et al. 1978, 1981). During prolonged, submaximal exercise to fatigue the increase in purine nucleotide degradation and muscle ammonia production has been found to be linked to muscle glycogen depletion (Broberg and Sahlin 1988, 1989). However, Graham et al. (1991) have pointed out that they found this only to be true when the muscles approach exhaustion and that during non exhausting exercise an increase in plasma ammonia concentration is unrelated to glycogen depletion. In addition, MacLean et al. (1991) have shown that muscle ammonia production is not affected by increased carbohydrate availability, modified by ingestion of a high-carbohydrate diet. On the other hand Greenhaff et al. (1991) have shown that plasma ammonia accumulation during intermittent incremental exercise after consumption of a low-carbohydrate diet tended to be greater than after a normal diet.

Thus, a relationship between carbohydrate stores in the body and ammonia production during exercise is not clear. In the work of Broberg and Sahlin (1988, 1989) they found muscle glycogen to be depleted by previous exercise and thus they have suggested subsequent ammonia production during the second bout of exercise, after a short recovery time, might be influenced by other factors than decreased carbohydrate availability in the body. The body glycogen stores may be modified not only by preceding exercise, but also by consumption of diets of variable carbohydrate content. Consumption of a low carbohydrate diet has been shown to reduce the body glycogen stores (Bergström et al. 1967; Galbo et al. 1979). Therefore, the aim of the present investigation was to study plasma ammonia concentration during prolonged non-exhausting exercise performed after the intake of a low carbohydrate diet to diminish glycogen stored both in muscle and liver. Moreover, it has been recently shown (Czarnowski and Górski 1991) that much ammonia produced during exercise is lost with sweat. This factor has not been taken in to account by the other authors and its investigation could shed some more light on ammonia metabolism during exercise. Therefore we also examined sweat ammonia excretion during exercise.

Methods

Subjects

Seven healthy untrained men volunteered for the study. None of them had been actively engaged in any sport activity 1 week before of during the study. Their age range was 19–24 years, height 170–188 cm and body mass 67.8–88.8 kg; they had maximal oxygen uptakes ($VO_{2\,max}$) of 37.9–58.0 $ml \cdot kg^{-1} \cdot min^{-1}$. The $VO_{2\,max}$ was determined before the experiment (on a separate day) during a graded exercise test at 60 rpm in the upright position on an electrically braked cycle ergometer (Monark-Crescent, AB, Varberg, Sweden) to fatigue. The peak oxygen uptake was considered to be $VO_{2\,max}$. The subjects were well informed about the protocol of the study and written consent was obtained from each of them before the tests. The project was accepted by the Ethics Committee at the Medical Research Centre of the Polish Academy of Sciences in Warsaw.

Procedures

The subjects performed two identical cycle ergometer exercise tests (60 rpm) for 60 min at 50% of the predetermined $VO_{2\,max}$, at an ambient temperature 20°C, separated by a 3-day period. The first exercise test was performed 12 h after 3 days of eating a normal (controlled) mixed diet (N-diet) (60% carbohydrates, 25% fat and 15% protein). Afterwards, the same subjects remained for 3 days eating or low-carbohydrate (LC) diet less than 5% carbohydrates, 50% fat and 45% protein) and the exercise test was performed again 12 h after the last meal. Both diets had the same energy content (2,200 $kcal \cdot 75\ kg^{-1}$ body mass daily). During the exercise tests the subjects were dressed in shorts, socks and shoes. Their heart rates were continuously monitored on an electrocardiograph. A catheter was inserted percutaneously in to the antecubital vein of each subject 0.5 h before each exercise Blood samples were taken immediately before, at the 30th and 60th min of exercise. A 5% solution of ethylenediaminetetraacetic acid was used as an anticoagulant. Sweat was collected using gauze pads. The skin of the hypogastric region was cleaned with soap and water and then washed with distilled water and 70% ethyl alcohol. A sterile 10-ply gauze pad (10 × 5 cm) was applied to the skin of this region with forceps immediately before the exercise and covered with alcohol-cleansed plastic. The sides of the plastic were taped to the skin. After 30 min of exercise the gauze pad was removed, the skin was washed with 70% ethyl alcohol and a new pad was applied to the same place and covered with fresh plastic. It was removed immediately after exercise. both gauze pads were placed in separate centrifuge tubes on a plastic diaphragm and centrifuged for 10 min at 3,000 rpm at 4°C. The diaphragms were fixed 3-cm above the bottom of the tube and had many holes, which enabled separation of sweat from the gauze and thus its collection. Both the sweat and plasma were immediately analysed for ammonia (DaFonseca-Wolleim 1973) by means of Monotest Ammoniak (Boehringer Mannheim GmbH). Lactic acid was also determined (Noll 1974) using Test-Combination Lactat Vollenzymatisch (Boehringer Mannheim GmbH). The plasma concentrations of lactic acid were determined only at rest and at the end of exercise. The volumes of the sweat samples obtained after 30 and 60 min of exercise were determined separately. The differences between these volumes were negligible. The concentrations of examined compounds in the sweat were determined in the following way:

$$C = \frac{C_{30} \times V_{30} + C_{60} \times V_{60}}{V_{30} + V_{60}}$$

where C was the concentration of examined compound in the sweat C_{30}, C_{60} the concentrations of the compound examined after 30 and 60 min of exercise, respectively and V_{30}, V_{60} the volumes of the sweat sample after 30 and 60 min of exercise, respectively.

The gauze itself had previously been found to contain ammonia (Czarnowski and Górski 1991). Therefore, the gauze pad was applied to the skin of six resting subjects. It was removed after 30 min, soaked in ammonia-free water and centrifuged as above. The mean amount of ammonia eluted from the gauze was then calculated to be 2.84 μmol. The intragauze differences were negligible. Therefore this value was considered as a "blank" value and was subtracted from each individual value obtained. The concentration of ammonia in each sweat sample was then recalculated.

The subjects were weighed naked to the nearest 50 g before each exercise and afterwards when the sweat had been wiped off. The amount of sweat lost was calculated according to Lemon and Mullin (1980).

Statistics

The results were evaluated statistically using the Wilcoxon's rank sum test. A P value less than 0.05 was considered to be statistically significant.

Results

Sweat and plasma concentrations of compounds examined are given in Table 1. The LC-diet did not affect the plasma ammonia concentration at rest, compared to the N-diet. Exercise increased the plasma ammonia concentration in each group but the values after the LC-diet were significantly higher than after the N-diet, both at 30th and 60th min of exercise. The plasma lactic acid concentration after the LC-diet was lower than

Table 1. Effect of N- and LC-diets on sweat and plasma concentrations of ammonia and lactic acid at rest and after exercise

Compound	Diet	Plasma						Sweat	
		Rest	Exercise						
			30 min			60 min			
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ammonia	N	30.4	6.4	38.1	6.9	58.9	8.1**	3290.5	676.5
(μmol·l^{-1})	LC	30.0	5.4	58.9	7.4**	77.9	8.3**,[a]	4885.4	940.3[a]
Lactic acid	N	1.0	0.1	–	–	1.4	0.3*	18.2	2.6
(mmol·l^{-1})	LC	0.8	0.2[a]	–	–	1.1	0.2*,[a]	17.9	2.4

**$P < 0.001$ *$P < 0.05$ vs the resulting value; [a]$P < 0.05$ vs the value before LC-diet

after the N-diet, both at rest and after exercise. Exercise caused a small, but significant, increase in the plasma concentration of this compound in each group. Sweat lactic acid concentration was not influenced by diet. The average volume of sweat lost during exercise in both the groups examined was similar [700 (SD 130) ml and 679 (SD 110) ml for the N- and the LC-diet, respectively]. Total loss of ammonia in the sweat was 2.30 (SD 0.62) mmol·l^{-1} and 3.24 (SD 0.60) mmol·l^{-1} ($P < 0.05$) for the N- and the LC-diet, respectively.

Discussion

The present study demonstrated that prolonged, non-exhausting exercise of moderate intensity resulted in an increase in the plasma ammonia concentration and that the magnitude of this increase depended on the preceding diet. Eriksson et al. (1985) have shown that splanchnic ammonia uptake did not change in response to exercise and that the working muscles released ammonia. It is therefore clear that the increase in the plasma ammonia during exercise is due to its continuous production in working muscles.

In the present study plasma ammonia concentration increased faster and reached higher values during exercise after the LC-diet than after the N-diet. It has been established that carbohydrate restriction after exercise markedly delays glycogen resynthesis in the muscles. As a result glycogen concentrations in the muscles has been shown to remain low (Bergström et al. 1967; Galbo et al. 1979). Although we did not examine muscle glycogen content in this study, it can be assumed, that glycogen stores were substantially different after both the diets used. This assumption was also partly supported by lower concentrations of blood lactic acid after the LC-diet, both at rest and during exercise. More pronounced hyperammonaemia during exercise after the LC- than after the N-diet in our study indicated that muscle ammonia production depended on carbohydrate availability in the body. This confirms data obtained by Broberg and Sahlin (1988, 1989). However their exercise protocol for depletion of glycogen was quite different. They employed two exhausting tests at 70% $VO_{2\,max}$ separated by only 75 min of rest. The short and incomplete recovery might have influenced ammonia production by factors other than glycogen depletion. In our study the exercise tests at lower intensity were separated by 3 days of rest. Therefore, it may be concluded that ammonia production depended on glycogen stores not only at fatigue, but also throughout the exercise. Our results on the relationship between carbohydrate availability and plasma ammonia concentration during prolonged exercise of moderate intensity were similar to those obtained by Greenhaff et al. (1991) during intermittent incremental exercise. However, these authors have not also investigated ammonia loss with the sweat.

In humans there is no protein that is a "storage protein" It has been shown that when a high-protein diet is fed, a minimal amount is used in the normal turnover (degradation and resynthesis) of all proteins, and the remainder is metabolized with a following increase in urea production (see Dohm 1986). It would seem that this leads to dramatic elevation on plasma urea concentrations (e.g. Greenhaff et al. 1988). Our subjects were examined 12 h after the last meal and had normal values for the concentration of ammonia in the plasma at rest. This would seem to suggest that excessive dietary nitrogen had been detoxified to urea. There is no reason to presume that exercise increased specifically the degradation of the dietary proteins and that those proteins were the source of extra ammonia produced in the LC-group. However, differences in dietary composition can alter amino acid metabolism during exercise and might therefore affect plasma and sweat ammonia concentrations during exercise.

The question should then be a asked as to whether the more pronounced hyperammonaemia during exercise after the LC-diet than after the N-diet could have been a consequence of an altered distribution of ammonia between plasma and other tissues. The relative distribution of ammonia between tissue compartments and the direction of its movement across membranes have been thought to be determined by distribution of hydrogen ion (Stabenau et al. 1959). Ammonia is a weak base (pK = 9.3) and thus at the physiological

pH it has been shown to exist mainly in the form of ammonium ion and less than 5% in the form of ammonia (Mutch and Banister 1983). The permeability of cell membranes for ammonia has been found to be similar to water, whereas the permeability for ammonium ion has been found to be small (Visek 1968). In consequence, ammonia has been demonstrated to accumulate in compartments with lower pH (Stabenau et al. 1959). Greenhaff et al. (1988) have shown that consumption of a low-carbohydrate diet for 4 days caused only mild reduction in blood pH and did not change muscle pH compared to a high-carbohydrate diet. After exercise (at 100% $VO_{2\,max}$) the plasma pH did not differ between these two groups, whereas muscle pH was much lower on a low-carbohydrate diet. Our subjects exercised only at 50% $VO_{2\,max}$ and thus no essential difference in the plasma and muscle pH between the N- and the LC-group would have been expected. As a result, it seems likely that the muscles of both groups retained a similar percentage of ammonia. Therefore, more pronounced hyperammonaemia after exercise in subjects on the LC- than on the N-diet reflected increased ammonia production in subjects fed the LC-diet.

Muscle ammonia may derive from degradation of purine nucleotides and oxidation of amino acids. A decreased availability of carbohydrates in the muscle has been found to impair energy metabolism and to induce an increase in purine nucleotide degradation resulting in enhancement of muscle ammonia production (Broberg and Sahlin 1988, 1989). In contrast, MacLean et al (1991), using a similar exercise protocol, have shown that purine nucleotides were not degraded during exercise. They have suggested that the principal source of ammonia was oxidation of amino acids, mainly BCAA. The amino group from metabolized BCAA has been shown to be removed by transdeamination catalyzed by aminotransferase and glutamate dehydrogenase (MacLean et al. 1991). The restriction of carbohydrates in the diet leads to several changes in hormone milieu and may promote protein catabolism. The plasma concentration of insulin has been found to decrease, whilst concentrations of catecholamines, glucagon and glucocorticoids to increase (Galbo et al. 1979; Jansson et al. 1982; Langfort et al. 1993) Lemon and Mullin (1980) have shown that protein was utilized to a greater extent, when initial muscle glycogen was depleted.

The mechanism whereby a deficiency of carbohydrates increases muscle ammonia production may be explained by a reduction of tricarboxylic acid (TCA) intermediates. Sahlin et al. (1990) have suggested that low muscle glycogen content leads to decreased production of pyruvate and phosphoenolpruvate, and secondarily, to reduced availability of TCA intermediates. The decreased availability of TCA intermediates has been found to reduce the flux through the TCA cycle, the rate of oxidative phosphorylation and, in consequence, enhances activation of AMP deamination to IMP and ammonia during the latter period of submaximal exercise to fatigue (Sahlin et al. 1990). Carbohydrate supplementation during prolonged exercise has been found to reduce muscle glycogen depletion and attenuates IMP accumulating in muscle (Spencer et al. 1991). Carbohydrate deprived diet has been shown to increase the relative contribution of fat to oxidative metabolism (Jansson et al. 1982). The first stage of fatty acids metabolism is β-oxidation with the formation of acetyl-coenzyme A, which is metabolized further in the TCA cycle. A reduced concentration of TCA intermediates could impair complete oxidation of fatty acids. One of the more important ways for the replenishment of the TCA intermediates has been shown to be the purine nucleotide cycle (PNC) (Sahlin et al. 1990). It has been demonstrated that the net effect of this series of reactions is the deamination of aspartate and the formation of fumarate (for the TCA cycle) and ammonia (Lowenstein 1972). Broberg and Sahlin (1989) have shown that PNC was active during prolonged, submaximal exercise. The second way for the replenishment of TCA intermediates in deamination of glutamate to 2-oxoglutarate in the reaction catalysed by glutamate dehydrogenase. A higher concentration of plasma ammonia during exercise after the LC-diet than after the N-diet in our study may reflect altered muscle metabolism for maintaining TCA intermediate concentration to ensure complete oxidation of fat.

We have also shown that the higher ammonia concentration after the LC-diet was accompanied by increased ammonia excretion in the sweat. This confirms our previous findings that plasma ammonia is the principal source of ammonia in sweat (Czarnowski and Górski 1991, 1992). This factor has not been taken into consideration by others and it appears that ammonia production during exercise may have been underestimated.

In summary, the present study demonstrated that plasma ammonia concentration during exercise depends on carbohydrate availability in the body. It has also confirmed that part of the ammonia formed during exercise would seem to be lost with the sweat.

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