Effect of carbohydrate ingestion on ammonia metabolism during exercise in humans

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Effect of carbohydrate ingestion on ammonia metabolism during exercise in humans. J Appl Physiol 88: 1576–1580, 2000.—The present study was undertaken to examine the effect of carbohydrate ingestion on plasma and muscle ammonia (NH3 denotes ammonia and ammonium) accumulation during prolonged exercise. Eleven trained men exercised for 2 h at 65% peak pulmonary oxygen consumption while ingesting either 250 ml of an 8% carbohydrate-electrolyte solution every 15 min (CHO) or an equal volume of a sweet placebo. Blood glucose and plasma insulin levels during exercise were higher in CHO, but plasma hypoxanthine was lower after 120 min (1.7 ± 0.3 vs. 2.6 ± 0.1 µmol/l; P < 0.05). Plasma NH3 levels were similar at rest and after 30 min of exercise in both trials but were lower after 60, 90, and 120 min of exercise in CHO (62 ± 9 vs. 76 ± 9 µmol/l; P < 0.05). Muscle NH3 levels were similar at rest and after 30 min of exercise but were lower after 120 min of exercise in CHO (1.51 ± 0.21 vs. 2.07 ± 0.23 mmol/kg dry muscle; P < 0.05; n = 5). These data are best explained by carbohydrate ingestion reducing muscle NH3 production from amino acid degradation, although a small reduction in net AMP catabolism within the contracting muscle may also make a minor contribution to the lower tissue NH3 levels.

MUSCLE AMMONIA (NH3 denotes ammonia and ammonium) production increases during prolonged exercise, and the source of this production is likely to be AMP and/or amino acid catabolism (19). Muscle NH3 production from AMP catabolism occurs when ATP utilization exceeds ATP synthesis and involves the action of AMP deaminase (19). Amino acid catabolism provides a fuel source to help meet the ATP requirements of the contracting muscle, and this source, although minor, may become quantitatively more important with increasing exercise duration and concomitant carbohydrate depletion (11, 22). The progressive increase in muscle NH3 production observed during prolonged exercise may therefore provide a marker of muscle metabolic stress because its production reflects the extent of the reliance of active muscle on amino acid catabolism in conjunction with its inability to match ATP demand with supply.

Several studies (1, 14, 15, 21, 22) have manipulated the supply of carbohydrate to contracting skeletal muscle in an attempt to alter NH3 production during prolonged exercise at moderate intensities. Experiments that have examined the effect of muscle glycogen content on NH3 metabolism indicate that muscle NH3 production during exercise is similar in muscle with normal compared with high glycogen contents (14, 15). The effect of low glycogen content on NH3 metabolism is currently unclear because one study (1) reported that muscle NH3 production was enhanced in this circumstance, whereas another (21) found no change. Wagensmackers et al. (22) studied the influence of carbohydrate ingestion combined with altered muscle glycogen content during 2 h of cycling exercise. Their data are difficult to interpret because the exercise intensity was higher throughout most of the carbohydrate-supplemented and glycogen-loaded trial compared with the non-carbohydrate-supplemented and glycogen-depleted trial. However, during the first 20 min of exercise when the exercise intensities were identical, plasma NH3 concentration was elevated in the subjects depleted of carbohydrates, and the higher plasma NH3 concentration was attributed to an increased muscle NH3 production derived from amino acid catabolism. Unfortunately, no measurements of muscle NH3 content or muscle NH3 efflux were obtained to ascertain the source of the elevated plasma NH3 concentration. Furthermore, it was not possible to determine the effect of carbohydrate ingestion alone on muscle NH3 metabolism because muscle glycogen content was also manipulated. The aim of the present study was to examine the effect of carbohydrate ingestion on muscle NH3 metabolism during prolonged submaximal exercise. We hypothesized that the consumption of carbohydrate during exercise would decrease NH3 levels in blood and muscle.

METHODS

Subjects. This study was conducted in two parts. Initially, eight endurance-trained men volunteered to exercise and allowed cardiorespiratory and blood samples to be obtained. Subsequently, two of these subjects and a further three subjects volunteered to undertake the same experimental protocol with muscle sampling. The common data obtained for the two subjects participating in both parts of the experi-
ment were averaged, resulting in n = 11. There were some plasma measurements made in the first part of the study (n = 8) but not in the second (n = 5) and vice versa. These slight changes in experimental procedures account for the variation in the number of subjects from which the mean data are presented. The mean, age, weight, and peak pulmonary oxygen consumption (V\textsubscript{O2peak}) of the subjects (n = 11) were 27.7 ± 1.6 yr, 69.8 ± 1.7 kg, and 4.40 ± 0.08 l/min, respectively. All subjects were fully informed of the experimental procedures and signed an informed consent statement. The experiments were approved by the Human Research Ethics Committee of Victoria University of Technology.

Exercise tests. At least 1 wk before the trials, each subject performed an incremental maximal exercise test on a Monark cycle ergometer to determine V\textsubscript{O2peak}. The subjects were subsequently studied during 2 h of cycle ergometer exercise, at a workload requiring 65% V\textsubscript{O2peak}, on two occasions at least 1 wk apart in randomized order. During one trial (CHO group), subjects ingested 250 ml of an 8% carbohydrate-electrolyte solution at the onset of exercise and every 15 min thereafter; in the other (Con group), they received an equal volume of a sweet placebo. Subjects were instructed to refrain from strenuous exercise, caffeine, and alcohol for 24 h before all exercise testing. They were also asked to record their food intake for the 24-h period before the first trial and to consume the same food the day before the second trial. Subjects presented to the laboratory on the morning of the trials after an overnight fast. Heart rate, respiratory exchange ratio (RER), and pulmonary oxygen consumption (V\textsubscript{O2}) were measured at 30-min intervals during each trial (n = 11). Heart rate was measured during submaximal exercise tests by use of a heart rate monitor (Sports Tester PE3000). Expired air samples were collected by using Douglas bags. Fractions of oxygen and carbon dioxide were determined by electronic analyzers (Applied Electrochemistry S-3A and CD-3A analyzers, respectively, Ametek, Pittsburgh, PA) while gas volumes were measured by using a Parkinson-Cowan gas meter that had been calibrated against a Tissot spirometer.

Blood sampling and analysis. Blood samples were obtained from an indwelling Teflon catheter inserted into a vein in the antecubital space. The catheter was kept patent by flushing with small amounts of heparinized saline (10 IU/ml). Blood was sampled at rest and after 30, 60, 90, and 120 min of exercise. Two milliliters of blood from each sample were placed in a fluoride-heparin tube and stored on ice for glucose analysis (n = 11). Immediately after blood glucose analysis, the remaining blood of the resting, 60-min, and 120-min samples was spun, and the plasma was stored at −80°C until being analyzed for insulin (n = 8). A further portion of the blood sample was immediately placed into ice-cold 3 M perchloric acid (PCA) and spun, and the extract was stored for later blood lactate analysis (n = 8). In addition, blood was immediately placed into lithium-heparin tubes, mixed, and spun. The resulting plasma was frozen in liquid nitrogen for later NH\textsubscript{3} (n = 11) and hypoxanthine (n = 5) analysis.

Muscle sampling and analysis. Muscle samples were obtained from vastus lateralis muscle in five subjects by percutaneous needle biopsy at rest and after 30 and 120 min of exercise. Each sample was obtained from a separate incision located ~3 cm apart along the belly of the muscle. Sampling always occurred from the distal to proximal incision. Leg selection was random, and in the second trial the contralateral leg was biopsied. Muscle samples were quickly frozen and stored in liquid nitrogen. The estimated time between cessation of exercise and freezing of muscle was <20 s. Muscle samples were divided into two portions that were weighed at −80°C. One portion (8–15 mg wet wt) was dissected into small pieces and stored at −80°C until analyzed for NH\textsubscript{3}. These pieces were extracted at −20°C by using 0.6 M PCA-10% methanol, neutralized with KOH, and analyzed for NH\textsubscript{3} by a flow-injection technique (10, 18). The second portion was weighed at −30°C, freeze-dried, weighed again, and subsequently powdered and extracted by using the methods described by Harris et al. (9). The extracts were then analyzed enzymatically for creatine (Cr), creatine phosphate (PCr), and lactate by using fluorometric detection (12). In addition, reverse-phase HPLC was used to quantify ATP, ADP, AMP, and IMP (23). Separation was achieved by using a Merck Hichar Lipchrosphere 100 CH-18/2 250-mm × 4-mm column using a Bio-Rad model 700 chromatography workstation. The total adenine nucleotide (TAN) pool was calculated by summing ATP, ADP, and AMP. Muscle metabolites, except for lactate and NH\textsubscript{3}, were adjusted to the peak total Cr for each subject. Muscle NH\textsubscript{3} values were expressed per dry mass by using the wet mass-to-dry mass ratio determined from the second portion of muscle.

Statistical analyses. Repeated-measures ANOVA were used to compare data between trials and over time. Simple main effects analysis and Newman-Keuls post hoc tests were used to locate differences when ANOVA revealed a significant interaction. The level of probability to reject the null hypothesis was set at P < 0.05. All data are reported as means ± SE.

RESULTS

Cardiorespiratory data. There were no differences in mean V\textsubscript{O2} (Con: 2.92 ± 0.08, CHO: 2.86 ± 0.09 l/min) and heart rate (Con: 150 ± 3, CHO: 149 ± 1 beats/min) during exercise between the trials. RER was not different at any time point during exercise (data not shown); however, mean RER was higher in CHO (0.87 ± 0.01) than in Con (0.84 ± 0.01; P < 0.05), as was the mean rate of carbohydrate oxidation during exercise (1.96 ± 0.07 vs. 1.69 ± 0.10 g/min; P < 0.05).

Blood and plasma metabolites. Blood glucose concentrations were similar at rest but were elevated (P < 0.05) at all sampling time points during exercise in CHO compared with Con (Table 1). Similarly, plasma insulin concentrations were not different at rest but were higher (P < 0.05) after 60 and 120 min of exercise in CHO vs. Con (Table 1). Blood lactate concentrations were not different at rest or during exercise between the trials (Table 1). Plasma NH\textsubscript{3} levels increased (P < 0.05) during exercise in both trials from similar resting levels (Fig. 1). Plasma NH\textsubscript{3} concentration was higher (P < 0.05) in Con compared with CHO after 60, 90, and 120 min of exercise (Fig. 1). Plasma hypoxanthine concentrations were similar at rest and during most of the exercise period (Table 1). An exercise-induced increase (P < 0.05) in plasma hypoxanthine was observed in both trials; however, after 120 min of exercise, the hypoxanthine concentration was lower (P < 0.05) in CHO compared with Con (Table 1).

Muscle metabolites. Muscle ATP, ADP, AMP, and TAN contents were not different between the trials (Table 2). Resting muscle PCR was similar in the two treatments, and, in both, the PCR levels were reduced (P < 0.05) as a result of exercise. The PCR concentration after 30 min of exercise was higher (P < 0.05) in CHO than in Con; however, the small difference after 120 min was not significant (P = 0.1; Table 2). There was no significant
interaction between treatment and time for muscle IMP (Table 2). There was, however, a significant main

effect for time, with IMP concentration at 120 min being higher (P < 0.05) than the resting and 30-min

time points. Muscle NH₃ at rest was not different between treatments but was increased (P < 0.05) at each

subsequent measurement point in Con. In CHO, only at 120 min was muscle NH₃ concentration higher (P <

0.05) than the resting and 30-min levels. The latter two means were not different. Muscle NH₃ concentration

after 120 min of exercise was higher (P < 0.05) in Con compared with CHO (Fig. 2).

**DISCUSSION**

The results of the present study indicate that carbohydrate ingestion attenuates muscle and plasma NH₃

accumulation during the latter stages of prolonged submaximal exercise. These data are best explained by

carbohydrate ingestion reducing muscle NH₃ production from amino acid degradation. Elevated carbohydrate

availability may have also led to a small reduction in net AMP catabolism within the contracting

muscle, as reflected by the lower plasma hypoxanthine after 120 min of exercise, suggesting that this source of

NH₃ may also make a minor contribution to the lower tissue NH₃ levels.

The major potential sources of NH₃ during submaximal exercise include AMP deamination and amino acid
catabolism (19). In the present study, muscle IMP levels were not different between the two trials at any

time point, although plasma hypoxanthine after 120 min of exercise was lower with carbohydrate ingestion.

This finding suggests that carbohydrate ingestion may have resulted in a better balance between ATP degrada-
tion and resynthesis during the latter stages of prolonged exercise. However, the absolute plasma hypoxan-
thine levels and the magnitude of difference between the trials are relatively small, and thus differences in

net AMP deamination are unlikely to account for the lower NH₃ accumulation. Similar conclusions on the

role of net AMP deamination during prolonged exercise have been made by other authors (13–15), suggesting

that amino acid catabolism is the major source of NH₃ production. Carbohydrate ingestion also appeared to

result in a better maintenance of PCr levels during exercise (Table 2), suggesting a small shift from PCr
degradation to carbohydrate oxidation for ATP generation.

Previous studies have observed a link between carbohydrate availability and amino acid catabolism during

exercise. Davies et al. (4) demonstrated that glucose ingestion attenuated leucine oxidation, as measured by

¹³CO₂ production from infused [¹³C]leucine. Sweat urea nitrogen excretion, an indirect marker of protein catabo-
lism, is lower during exercise commenced in a glycogen-loaded state compared with depleted-muscle-glycogen

levels (11). Similarly, prior muscle glycogen depletion has been shown to increase net protein degradation
during single-leg, knee extension exercise (20). Greater increases in the activity of skeletal muscle branched-
chain oxoacid dehydrogenase, the rate-limiting step in branched-chain amino acid oxidation, have been ob-
served during exercise under conditions of reduced carbohydrate availability (20, 22). These results sug-
gest that amino acid catabolism is reduced by increased carbohydrate availability and provide the most plau-

**Table 1. Plasma glucose, lactate, hypoxanthine, and insulin during 120 min of cycling at ~65% peak pulmonary

oxygen consumption with and without carbohydrate ingestion**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>0</th>
<th>30</th>
<th>60</th>
<th>90</th>
<th>120</th>
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<tr>
<td>Glucose, mmol/l</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Con</td>
<td>11</td>
<td>4.5±0.1</td>
<td>4.9±0.1</td>
<td>4.6±0.2</td>
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<td>3.8±0.1</td>
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<td>CHO</td>
<td></td>
<td>4.4±0.1</td>
<td>6.2±0.2*</td>
<td>5.1±0.2*</td>
<td>4.9±0.1*</td>
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<td>Lactate, mmol/l</td>
<td></td>
<td>0.7±0.1</td>
<td>1.7±0.5</td>
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<tr>
<td>CHO</td>
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<td>0.7±0.1</td>
<td>1.7±0.3</td>
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<td>Hypoxanthine, µmol/l</td>
<td></td>
<td>0.9±0.2</td>
<td>1.9±0.2</td>
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<td>1.8±0.2</td>
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<tr>
<td>CHO</td>
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<td>0.9±0.2</td>
<td>1.8±0.3</td>
<td>1.6±0.1</td>
<td>1.4±0.1</td>
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<td>Insulin, pmol/l</td>
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<td>40±4</td>
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<td>23±2</td>
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<tr>
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<td>CHO</td>
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<td>38±4</td>
<td></td>
<td>66±12*</td>
<td></td>
<td>54±12*</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects. Con, without carbohydrate ingestion; CHO, with carbohydrate ingestion. *Different from Con, P < 0.05.

**Fig. 1. Plasma ammonia and ammonium (NH₃) during 120 min of cycling at ~65% peak pulmonary oxygen consumption with (CHO) and without (Con) carbohydrate ingestion. Values are means ± SE; n = 11 subjects. *Different from Con, P < 0.05.**
Table 2. Muscle metabolites at rest and after 30 and 120 min of cycling at \( \sim 65\% \) peak pulmonary oxygen consumption in CHO and Con trials

<table>
<thead>
<tr>
<th></th>
<th>Con</th>
<th>CHO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rest 30 min 120 min</td>
<td>Rest 30 min 120 min</td>
</tr>
<tr>
<td>ATP</td>
<td>25.7 ± 1.3 27.3 ± 0.9 24.9 ± 1.0</td>
<td>25.6 ± 1.5 27.7 ± 2.3 25.9 ± 1.6</td>
</tr>
<tr>
<td>ADP</td>
<td>1.93 ± 0.13 2.26 ± 0.19 2.22 ± 0.19</td>
<td>1.95 ± 0.15 2.10 ± 0.23 2.06 ± 0.17</td>
</tr>
<tr>
<td>AMP</td>
<td>0.08 ± 0.01 0.08 ± 0.01 0.09 ± 0.01</td>
<td>0.09 ± 0.01 0.07 ± 0.01 0.08 ± 0.01</td>
</tr>
<tr>
<td>TAN</td>
<td>27.7 ± 1.4 29.7 ± 1.0 27.3 ± 1.2</td>
<td>27.7 ± 1.5 29.9 ± 2.5 28.0 ± 1.7</td>
</tr>
<tr>
<td>IMP</td>
<td>0.05 ± 0.01 0.06 ± 0.01 0.14 ± 0.01</td>
<td>0.06 ± 0.01 0.07 ± 0.02 0.12 ± 0.04</td>
</tr>
<tr>
<td>PCR</td>
<td>88.0 ± 2.4 67.5 ± 3.4 58.5 ± 2.6</td>
<td>86.3 ± 5.2 79.2 ± 3.0* 68.3 ± 4.2</td>
</tr>
<tr>
<td>Cr</td>
<td>43.6 ± 0.6 62.2 ± 1.4 73.2 ± 4.0</td>
<td>45.4 ± 3.6 52.4 ± 2.3 62.5 ± 4.1</td>
</tr>
<tr>
<td>Lactate</td>
<td>6.9 ± 0.7 9.7 ± 2.2 7.7 ± 1.5</td>
<td>5.7 ± 1.3 7.0 ± 1.2 9.4 ± 1.8</td>
</tr>
</tbody>
</table>

Values are means ± SE expressed in mmol/kg dry mass; \( n = 5 \) subjects. TAN, total adenine nucleotide; PCR, creatine phosphate; Cr, creatine. *Different from Con, \( P < 0.05 \).

A possible explanation for the reduction in muscle and plasma NH\(_3\) accumulation we observed in the present study. It should be noted, however, that the reduction in tissue NH\(_3\) accumulation observed in the present study when subjects were fed carbohydrate may not be due to the substance fed (i.e., carbohydrate) but rather to the simple addition of energy. Indeed, Graham et al. (7) demonstrated that infusion of Intralipid attenuated the release of NH\(_3\) from contracting muscle.

The principal reactions believed to produce NH\(_3\) from the degradation of amino acids are purine nucleotide cycle and the glutamate dehydrogenase (GDH) reaction (19). The relative contribution to NH\(_3\) production from these pathways is currently unclear, and the present study is unable to resolve this problem. Although speculative, the lower muscle PCR level during Con suggests that the free ADP and free AMP were higher and that this would tend to activate AMP deaminase to a greater extent in the Con trial. Because there were no differences in muscle IMP content between treatments, the IMP produced may have been reaminated in the contracting muscle to form AMP via the other enzymes of the PNC at a faster rate in Con compared with CHO. The net effect of a more rapid operation of the PNC is a faster rate of NH\(_3\) production from the amino acid aspartate. It should be noted that previous research has questioned that the deamination and reamination legs of the cycle operate concurrently in contracting skeletal muscle (19), thereby casting doubt over aspartate deamination, via the PNC, as a source of NH\(_3\) production.

The ingestion of carbohydrate during prolonged cycling exercise increases muscle glucose uptake (16), which may account for an increased intramuscular glucose concentration late in exercise when fed carbohydrate (17). Glucose and pyruvate have been shown to inhibit branched-chain oxoacid oxidation in incubated rat diaphragm (2). However, because muscle glucose uptake does not appear to be increased by carbohydrate ingestion until after 20–30 min of exercise (16) and because net muscle glycogen use is unaltered (3, 8), an increase in intramuscular carbohydrate supply may not have been evident until later in the exercise bout. This could account for the observation that plasma and muscle NH\(_3\) levels were attenuated after 60 and 120 min of exercise, respectively, but were similar after 30 min of exercise. It is also possible that an increase in plasma insulin plays a role in the attenuation of ammonia accumulation because it is known to inhibit protein breakdown (6).

The attenuation of muscle NH\(_3\) content observed toward the latter stages of exercise in the carbohydrate ingestion trial may also be partly explained by enhanced alanine production. Alanine is one of the principal nitrogen carriers released from active skeletal muscle (5). In order for alanine to remove free NH\(_3\), the amino group from glutamate must have also originated from free NH\(_3\). The major reaction involving the fixation of NH\(_3\) to glutamate is catalyzed by GDH. Although the direction in which the GDH reaction proceeds is unknown, it has been argued that in contracting muscle the reaction favors NH\(_3\) production rather than removal (13). If this is the case, muscle alanine production is not likely to enhance free NH\(_3\) removal. Alternatively, there is the possibility that de novo alanine production from pyruvate and glutamate in the alanine aminotransferase reaction reduces the availability of glutamate for NH\(_3\) production. Carbohydrate ingestion has been shown to elevate muscle alanine content during prolonged submaximal exercise (17), suggesting that muscle alanine production is enhanced with carbo-

![Fig. 2. Muscle NH\(_3\) during 120 min of cycling at \( \sim 65\% \) peak pulmonary oxygen consumption in CHO and Con trials. Values are means ± SE; \( n = 5 \) subjects. dm, Dry mass. *Different from Con, \( P < 0.05 \).](http://jap.physiology.org/10.1210/jap.28.9.1579)
hydrate supplementation. In the present study we have no data to support or refute such a mechanism.

In summary, the results of the present study suggest that carbohydrate ingestion attenuates muscle and plasma NH3 accumulation during the latter stages of prolonged submaximal exercise by reducing NH3 production from amino acid degradation.

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