MODERATE-INTENSITY EXERCISE ASSOCIATED WITH KETOGENIC DIET. A MODEL FOR THE STUDY OF AMMONIA METABOLISM?

Flavio Bachini¹,² f.bachini@gmail.com
João Pedro Saar Werneck-de-Castro¹,² joaopedrowerneck@pq.cnpq.br
Leonardo Cristiano Moretzsohn¹,⁴ leo.c.moretzsohn@bol.com.br
Adriana Bassini-Cameron¹ adriana.bassini@terra.com.br
Luiz-Cláudio Cameron¹,⁴,⁵ cameron@unirio.br

doi:10.3900/fpj.8.3.226.e


ABSTRACT

Introduction: Exercise has been used as a model to study the metabolism of ammonia (NH₃ + NH₄⁺), which is highly toxic to the central nervous system. A ketogenic diet leads to several metabolic adaptations to maintain the ATP/ADP ratio, including a lack of glycogen reservoirs, the use of amino acids as carbon skeleton donors and increased oxidation of fatty acids. The formation of ammonia during high-intensity exercise is well studied, but its role in moderate-intensity exercise remains unclear. Here we investigate ammonia metabolism during moderate-intensity exercise associated with a ketogenic diet as a model system. Materials and Methods: Athletes (n=7) were physically evaluated and had their maximum oxygen consumption (VO₂max) and heart rate (HRmax) determined. The subjects remained on a ketogenic diet 72h prior to exercise. They then exercised for 60min at a power output of 60% of that at VO₂max and at 70%-75% of HRmax. Results: Basal ammonemia increased by 35% due to dietetic modifications, while exercise caused a 250% increase in ammonemia in parallel with this effect. Uremia was increased by 60% due to the ketogenic diet without response to exercise. We measured a 10% increase in serum urate that did not change during the exercise protocol. No changes were found in glycemia or lactatemia. Discussion: Our data suggest that moderate-intensity exercise associated with a ketogenic diet can be used to study the increase in ammonemia and as a model to understand ammonia metabolism during metabolic stress.

KEYWORDS

Hiperammonemia, Lactate, Urate, Central Nervous System.

¹ University Federal of the State of Rio de Janeiro - UNIRIO - Laboratory of Protein Biochemistry - Rio de Janeiro - Brazil
² University of Trás-os-Montes e Alto Douro - UTAD - Post Graduate Program in Physical Education and Sport - Vila Real - Portugal
³ University Federal of Rio de Janeiro - UFRJ - Department of Bioscience and Physical Activity - Laboratory of Molecular Biology and Exercise - Rio de Janeiro - Brazil
⁴ University Castelo Branco - UCB - Post Graduate Program in Science of Human Motricity - Rio de Janeiro - Brazil
⁵ University Federal of Uberlândia - UFU - Institute of Genetics and Biochemistry - Uberlândia - Brazil
EXERCÍCIO DE INTENSIDADE MODERADA ASSOCIADO À DIETA CETOGÊNICA. UM MODELO DE ESTUDO PARA O METABOLISMO DE AMÔNIA?

RESUMO

Introdução: O exercício tem sido utilizado como modelo para compreender a produção de amônia (NH₃ + NH₄⁺) um metabólito tóxico para o sistema nervoso central. A dieta cetogênica é caracterizada pela redução no consumo de carboidratos levando modificações metabólicas para manutenção da relação ATP/ADP, que incluem a diminuição da reserva de glicogênio, o uso de aminoácidos como fornecedores de esqueletos de carbono e aumento da b-oxidação de ácidos graxos. A produção de amônia durante o exercício de alta intensidade é um evento bastante estudado, porém pouco se conhece deste metabolismo em exercício de intensidade moderada. Neste estudo investigamos o metabolismo de amônia durante o exercício de intensidade moderada e longa duração associado à dieta cetogênica como induitores de estresse metabólico.

Materiais e Métodos: Os atletas (n=7) foram avaliados segundo diversos parâmetros clínicos e tiveram seu consumo máximo de oxigênio (VO₂max) e frequência cardíaca máxima (FCmax) individualmente estimados. Os sujeitos permaneceram em dieta cetogênica nas 72h prévias ao experimento que aconteceu com intensidade de 60% da potência desenvolvida no VO₂max e 70%-75% da FCmáx durante 60min. Resultados: Houve aumento de 35% da amonemia basal em resposta à dieta cetogênica. O exercício causou elevação de 250% na amonemia, coerentemente a uremia basal se elevou 60% devido à dieta cetogênica, sem mudanças em resposta ao exercício. O urato sérico basal se elevou 10% sem ser modificado pelo exercício. Não detectamos mudanças na glicemia ou lactatemia durante qualquer fase do estudo. Discussão: Nossos achados parecem indicar que o exercício de intensidade moderada associado à dieta cetogênica pode ser usado como modelo para elevação da amonemia, possibilitando o seu uso como indutor de estresse metabólico para o estudo do metabolismo de amônia.

PALAVRAS-CHAVE
Hiperamonemia, Lactato, Urato, Sistema Nervoso Central.

INTRODUCTION

When ATP hydrolysis associated with the increased muscle contraction exceeds the speed of ATP resynthesis, the intracellular AMP concentration increases. AMP is deaminated by AMP deaminase, resulting in inosine monophosphate (IMP) and ammonia (here considered as NH₃ + NH₄⁺). Moreover, the increase in intracellular ADP concentration stimulates the entry of amino acids into the tricarboxylic acid cycle through oxidative deamination, producing ammonia and keto acids that are used to produce ATP and to preserve glycemia. The liver quickly responds to changes in levels of systemic ammonia, activating the urea cycle as well as glutamate and glutamine synthesis.

A ketogenic diet is characterized by reduced carbohydrate ingestion, which may decrease muscular glycogen storage, resulting in reduced glycolytic flux and subsequent lactate production. Consequently, a ketogenic diet...
increases the oxidation of amino acids, fatty acid and keto bodies for ATP production. When a ketogenic diet is associated with exercise, it increases the amount of amino acids used as skeleton carbon donors for ATP production or gluconeogenesis, thereby increasing the levels of free ammonia. This pathway is of vital importance in the interaction of metabolic pathways because the increased activity of tricarboxylic acid results in more ATP production\(^9,10\). In hepatocytes, ammonia activates glutamate hydrolysis via glutamate dehydrogenase, resulting in the production of 2-oxoglutarate and more ammonia.

It has been suggested that high levels of ammonia are associated with or responsible for the fatigue, loss of motor coordination, ataxia, and torpor that appear during or following exercise\(^11\). These symptoms resemble those in many hepatic diseases. Muscular ammonia moves into the blood and can cross the blood-barrier\(^12\), leading to alterations in the specific central nervous system (CNS) sensitivity to glutamate.

**Figure 1** - Glycemic response to diet and exercise. The ketogenic diet and exercise did not modify blood glucose concentrations.

![Figure 1](image1)

(\(\downarrow\)) blood collection.

**Figure 2** - Ammonemia increased in response to diet and exercise. The ketogenic diet and exercise acted synergistically for induction of ammonia production and did not modify lactate production.

![Figure 2](image2)

(\(^\ast\)) D0 different from Pre; (#) Pre different from Post; (●) ammonia; (▼) lactate; (\(\downarrow\)) blood collection

**Figure 3** - Ureagenesis increased in response to diet.

![Figure 3](image3)

(\(^\ast\)) D0 different from Pre; (\(\downarrow\)) blood collection

**Figure 4** - Creatinine as control for renal function. The concentration of blood creatinine remains constant during diet and post exercise.

![Figure 4](image4)

(\(\downarrow\)) blood collection
This may explain the variability of factors involved in CNS dysfunction.

In this study, we investigated ammonia metabolism using a model of low-intensity exercise associated with a ketogenic diet in order to increase metabolic stress.

**MATERIAL AND METHODS**

**Approval of the study**

The experimental protocol met the requirements for research in human subjects (resolution 196/1996 of Health National Council, Brazil) in accordance with the Helsinki Declaration (1975, updated 2000). The study was approved by the Ethical Committee in Research of Universidade Federal do Estado do Rio de Janeiro (CEP 117/2007).

**Sample**

The participants in this study included seven male professional cyclists between the ages of 25 and 35 (27.4±3.5 years old). In order to ensure the homogeneity of the group, the subjects were subjected to anthropometric, ergospirometric (VO$_{2 \text{max}}$=61.7±5.9ml.kg$^{-1}$.min$^{-1}$; HR$_{\text{max}}$=193±9 bpm; W$_{\text{max}}$=390±96W), clinical and hematologic assessment one week prior to exercise (D0). The cyclists had a mean height of 170.0±7.9cm; a mean corporal mass of 64.4±11.5kg and a corporal mean of 7.5±2.5%. The group did not train or use any medications or supplements during the study.

**Model of study**

The athletes were hematological and biochemical evaluated before experiment (D0). They began their ke-

| Table 1 - Biochemical and metabolic analyses of cyclists in D0. The athletes arrived at 12h for blood collection. All values were found to be in the normal range. The values are expressed as mean ± standard error. |
|-------------------------|------------------|
| **Hemogram + Platelet** | **D0**          |
| Erythrocytes (x 10$^{12}$.L$^{-1}$) | 5.2 ± 0.2        |
| Hemoglobin (mmol.L$^{-1}$)               | 2.3 ± 0.1        |
| Hematocrit (%)                        | 44.5 ± 0.9       |
| MCV (fL)                         | 85.7 ± 1.6       |
| MCH (pg)                          | 29.1 ± 0.7       |
| MCHC (g.L$^{-1}$)                 | 33.9 ± 0.2       |
| RDW (%)                           | 13.7 ± 0.2       |
| Leucocytes (10$^{9}$.L$^{-1}$)       | 5.1 ± 4.2        |
| Basophils                          | 0.1 ± 8.4        |
| Eosinophils                        | 0.2 ± 0.1        |
| Lymphocytes                        | 2.1 ± 0.1        |
| Monocytes                          | 0.5 ± 0.1        |
| Neutrophils                        | 2.8 ± 0.3        |
| Platelet (x 10$^{9}$.L$^{-1}$)      | 244.6 ± 14.9     |
| **Macronutrients**                | **D0**          |
| Total cholesterol (mmol.L$^{-1}$)   | 4.5 ± 0.3        |
| LDL                               | 2.6 ± 0.3        |
| HDL                               | 1.3 ± 0.0        |
| VLDL                              | 0.5 ± 0.1        |
| Triacylglycerol (mmol.L$^{-1}$)    | 1.2 ± 0.1        |
| Total Protein (g.L$^{-1}$)         | 78.3 ± 1.9       |
| Albumin (g.L$^{-1}$)               | 46.8 ± 1.3       |
| Globulin (g.L$^{-1}$)              | 3.4 ± 1.6        |
| A/G Ratio (g.L$^{-1}$)             | 15.1 ± 0.9       |
| **Hormones**                      | **D0**          |
| T3 (mmol.L$^{-1}$)                 | 1.7 ± 0.1        |
| T3 livre (mmol.L$^{-1}$)           | 6.3 ± 0.1        |
| T4 (pmol.L$^{-1}$)                 | 95.7 ± 13.8      |
| T4 free (pmol.L$^{-1}$)            | 13.8 ± 0.6       |
| TSH (mUI.L$^{-1}$)                 | 1.9 ± 0.3        |
| GH (µg.L$^{-1}$)                   | 0.1 ± 0.01       |
| IGF-1 (ng.mL$^{-1}$)               | 183.7 ± 16.8     |
| Insulin (µU.mL$^{-1}$)             | 5.4 ± 0.3        |
| Testosterone (nmol.L$^{-1}$)       | 14.5 ± 0.4       |
| Testosterone free (pg.mL$^{-1}$)   | 23.2 ± 1.7       |
| Leptin (ng.mL$^{-1}$)              | 10.5 ± 1.9       |

VCM: mean corpuscular volume; HCM: mean corpuscular hemoglobin; CHCM: mean corpuscular hemoglobin concentration; RDW: red distribution width; LDL: low density lipoproteins; HDL: high density lipoproteins; VLDL: very low density lipoproteins; PCT: procalcitonin; T3: triiodothyronine; T4: tetraiodothyronine; TSH: thyroid hormone stimulant; GH: growth hormone; IGF-1: insulin-like growth factor-1.
Ketogenic diet

The athletes were placed on a ketogenic diet for 72h before the experiment to minimize the chance of altered enzyme expression in response to the change in diet. The diet was isoenergetic, with a minimum of 1.5g.kg⁻¹ (actual weight) of proteins; it contained a maximum of 50g of carbohydrates per day. Water was consumed ad libitum.

Blood collection

Blood samples were collected from the antecubital vein at D0; Pre- and Post- time points. Immediately after collection, the blood was centrifuged and the plasma was separated, frozen and stored at -70°C to avoid the loss of volatile material and/or metabolites. The blood and plasma were analyzed by Bittar Laboratory Ltda (Bittar, Brazil).

In this study, “ammonia” is understood to be NH₃ + NH₄⁺.

Statistical analyses

Results were analyzed by Student’s “t” test and significance was accepted for p<0.05. The data are expressed as mean ± standard error.

RESULTS

To eliminate the possibility of sub-clinical diseases, the metabolisms of macronutrients and hormones linked to anabolism were analyzed. The capacity for gas transport was checked by hemograms and ergospirometry (Table 1). Infections and tissue injuries were evaluated by the analysis of leukocytes and thrombocytes associated with classical enzymatic markers of cellular injury (Tables 1 and 2).

Hepatic lesions and skeletal and cardiac muscles were assessed through the plasma activities of enzymes (γGT, AST, ALT, ALP, LDH, CK and CKMB) expressed by these tissues (Table 2). There were no significant differences in the levels of these enzymes between the athletes as all of them were in training, and the levels were similar to those previously described for athletes³,¹³,¹⁴,¹⁵.

The ketogenic diet did not alter basal glycemia for subjects when compared with D₀, and lactatemia was also not affected by this diet. Exercise did not modify glycemia or lactatemia (Figures 1 and 2).

Basal ammonemia increased by 35% due to the ketogenic diet, while the addition of exercise to this diet increased ammonemia by 250% (Figure 2). In agreement with this finding, basal uremia increased by 60% due to the ketogenic diet but did not change with the addition of exercise (Figure 3).

Levels of serum creatinine were unaffected by the ketogenic diet or exercise (Figure 4). There was an increase in basal serum urate of about 10% that was not modified by exercise (Figure 5).

DISCUSSION

The metabolic homogeneity of the group of athletes was first confirmed using hematological and biochemical assessments as previously described¹⁶.

Reductions in available glucose provoked by dietary manipulation induce catabolism of amino and fatty acids in order to maintain the ATP/ADP ratio and to enable continuous long-term exercise. A ketogenic diet reduces the storage of glycogen/glucose, decreasing glycolytic flux in skeletal muscle⁴,⁵,⁶ and inducing gluconeogenesis. The inhibition of the glycolytic pathway promotes an increase in the IMP concentration and in the intermediates of the tricarboxylic acid cycle¹⁷,¹⁸,¹⁹. In this study, we combined an increased exercise load and a glycogen deficit in order to increase the plasma ammonia²⁰,²¹,²²,²³,²⁴.

Table 2 - Marker enzymes of hepatic and muscular injuries. There was no difference between enzymes levels at different time points. The values are expressed as mean ± standard error.

<table>
<thead>
<tr>
<th>Enzymes (U.L⁻¹)</th>
<th>D0</th>
<th>Pre</th>
<th>Post</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT</td>
<td>25.4 ± 2.4</td>
<td>24.1 ± 2.0</td>
<td>25.0 ± 1.6</td>
</tr>
<tr>
<td>AST</td>
<td>26.4 ± 3.8</td>
<td>29.6 ± 3.0</td>
<td>29.1 ± 2.5</td>
</tr>
<tr>
<td>γGT</td>
<td>18.6 ± 2.6</td>
<td>17.8 ± 1.8</td>
<td>18.1 ± 2.0</td>
</tr>
<tr>
<td>AP</td>
<td>57.0 ± 3.8</td>
<td>54.4 ± 3.6</td>
<td>56.1 ± 3.2</td>
</tr>
<tr>
<td>LDH</td>
<td>367.6 ± 41.4</td>
<td>395.0 ± 44.0</td>
<td>382.8 ± 34.4</td>
</tr>
<tr>
<td>CK</td>
<td>295.8 ± 86.9</td>
<td>283.0 ± 66.4</td>
<td>291.3 ± 67.9</td>
</tr>
<tr>
<td>CKMB</td>
<td>17.7 ± 3.1</td>
<td>22.6 ± 3.4</td>
<td>20.3 ± 2.2</td>
</tr>
</tbody>
</table>

AST: aspartate aminotransferase; ALT: alanine aminotransferase; γGT: glutamyltransferase gamma; AP: alkaline phosphatase; LDH: lactate dehydrogenase; CK: creatine kinase total; CKMB: creatine kinase Muscle Brain
It has been reported that the anaplerotic process increases the ammonia pool that is exported to the blood\textsuperscript{19}. In our study, we observed increased ammonemia between D\textsubscript{0} and the sample taken before exercise, probably due to the higher consumption of proteins in the diet. The effect of this diet was also reflected in the elevated uremia observed.

In this study we measure a 250\% increase in ammonemia in response to exercise and diet. In intensely contracted skeletal muscle, the speed of glycolysis increases, increasing the concentration of pyruvate, which is reduced to lactate or acetyl-S-CoA to enter the Krebs cycle. In addition, alanine and glutamine are deaminated concomitantly to maintain the production of ATP\textsuperscript{25}. Because mitochondrial activity is insufficient to rephosphorylate the generated ADP, there is a progressive increase in generation of ammonia that goes to the blood and can be taken up by many tissues\textsuperscript{1}. In our study, uremia increased in response to diet but was stable during exercise, even with the enhancement of ammonemia. These data can be explained by the lack of time available for urea to be generated by and to exit hepatocytes into the blood.

We did not measure lactatemia increase in response to exercise. Lactate formation is low in subjects depleted of carbohydrate ingestion. Prior studies in our laboratory have shown that kinetics of urea and urate appearance in the blood differs from those of ammonia, explaining their later appearance (Bessa \textit{et al.}, manuscript in preparation). Our findings indicate that the increase in serum urate before exercise could be caused by ingestion of foods rich in purine bases, the main source of IMP formation and urate\textsuperscript{30}.

Our data support the use of moderate exercise associated with a ketogenic diet as a model for enhancement of ammonemia, and we suggest that this could be used as a model of metabolic stress for the study of ammonia metabolism.

\textbf{REFERENCES}

23. Felig P, Marliss EB, Cahill Jr GF. Metabolic response to human growth
24. Smith DJ, Norris SR. Changes in glutamine and glutamate concentrations
25. Thomas C, Perrey S, Lambert K, Hugon G, Mornet D, Mercier J. Monocar-
boxylate transporters, blood lactate removal after supramaximal exercise,
26. Schulz H, Heck H. Glicogen depletion as indication for determination in
27. Chicharro JL, Vaquero AF, Tello R, Pérs M, Lúcia A. Relationship between
lactate and ammonia thresholds in Heart transplant patients. Chest.
28. Ament W, Huizenga JR, Mook GA, Gips CH, Verkerke GJ. Lactate and am-
monia concentration in blood and sweat during incremental cycle ergometer
29. Lowenstein JM, Goodman MN. The purine nucleotide cycle in skeletal
30. Thompson WJ, Appleman MM. Characterization of cyclic nucleotide phos-
phodiesterases of rat tissues. The J of Biol Chemistry. 1971;246(10):3145-
50.
31. Brule D, Sarwar G, Savoie L, Campbell J, Van Zeggelaar M. Differences in
uricogenic effects of dietary purine bases, nucleosides and nucleotides in

Submitted: 03/02/09 - Accepted: 04/15/09