

# ACUTE AEROBIC EXERCISE RESTORES TRYPTOPHAN CONCENTRATION IN THE BRAIN OF RATS WITH HYPERPHENYLALANINEMIA

EXERCISE AND  
SPORTS SCIENCES



ORIGINAL ARTICLE

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## ABSTRACT

**Introduction:** Phenylketonuria (PKU) is characterized by deficiency of the enzyme phenylalanine hydroxylase, leading to accumulation of phenylalanine. Early diagnosis and subordination to low-phenylalanine diet are important to prevent the harmful effects of hyperphenylalaninemia. In case the diet is not strictly followed, some possible effects are imbalance in the neutral amino acids that use the same carrier of phenylalanine to cross the blood-brain barrier, causing hence reduction in tryptophan entry, the precursor of serotonin in the brain. This neurotransmitter has been implicated in the regulation of mood states, and its high production is linked to central fatigue in individuals subjected to prolonged exercise. Physical exercise increases free tryptophan levels in the blood, which facilitates its influx in the brain, and therefore, may be useful in hyperphenylalaninemia states. **Objective:** To assess whether aerobic exercise is able to normalize the concentrations of tryptophan in the brain of rats with hyperphenylalaninemia. **Methods:** 32 rats were randomly assigned to sedentary (Sed) and exercise (Exe) groups, and then divided into control (SAL) and hyperphenylalaninemia (PKU). Hyperphenylalaninemia was induced by administration of alpha-methylphenylalanine and phenylalanine for three days, while the SAL groups received saline. Exe groups held a session of aerobic exercise lasting 60 minutes and speed of 12 m.min<sup>-1</sup>. **Results:** The concentration of tryptophan in the brain of PKU groups was significantly lower than SAL groups (both in Sed and Exe groups), compatible with the condition of hyperphenylalaninemia. The exercise increased brain tryptophan levels comparing to sedentary animals. The most interesting finding was that the brain tryptophan levels of ExePKU group were not different from SedSAL group. **Conclusion:** The results indicate an important role of aerobic exercise to restore the concentration of tryptophan in the brain in hyperphenylalaninemic rats.

**Keywords:** phenylketonuria, phenylalanine, serotonin.

## INTRODUCTION

Phenylketonuria (PKU) is the most common innate error among the amino acids metabolism disorders and it occurs with the frequency of 1:14.000 births<sup>1</sup>. The classical form of this condition is caused by genetic mutations of the hepatic enzyme phenylalanine hydroxylase, located in the 12 chromosome<sup>2</sup>, making the action of this enzyme responsible for the transformation of the essential amino acid phenylalanine in tyrosine impossible. When this route is inactivated, the phenylalanine accumulates and takes alternative routes, causing disorders, mainly in the central nervous system. If not early treated, the child with phenylketonuria presents a clinical scenario characterized by important episodes to the central nervous system, presenting severe mental retardation, microcephalia, seizures, motor alterations, among other neurological symptoms<sup>3</sup>.

In order to avoid these consequences, the phenylketonuric patients adopt a hypoprotein diet especially poor in phenylalanine for maintenance of the blood levels of this amino acid within normality. However, the protein needs do not allow its total elimination from diet, causing oscillations in the plasma phenylalanine concentration even in those individuals early and continuously submitted to a special diet. Phenylalanine follows alternative metabolism

routes and the increase of phenylalanine and its metabolites causes the toxic effects to the body whose mechanisms are still not completely known<sup>3,4</sup>.

These substances inhibit important enzymes necessary for the synthesis of neurotransmitter, such as 5-hydroxytryptophane decarboxylase, which originates serotonin<sup>5</sup>. The high concentration of phenylalanine causes decrease in the concentration of other neutral amino acids, harming hence their passage through the hematoencephalic barrier<sup>3,6,7</sup>. Moreover, the carriers present there evidence more intimacy with phenylalanine<sup>8</sup>, causing therefore decrease of availability of tryptophan in the brain, and consequently, lower serotonin production. In phenylketonuric individuals early treated, but who do not strictly follow the diet, lower concentrations of tryptophan and serotonin in the plasma<sup>9</sup> and decreased serotonin excretion in the urine are found<sup>10</sup>. This situation is very common among these patients due to the great difficulty in strictly respecting the dietetical restriction, which may lead to cognitive harm<sup>10,11</sup>. The lower production of this neurotransmitter is even more pronounced in untreated patients, and reduction of 30-40% in serotonin concentration and of 40-50% in the tryptophan levels in the brain was observed *post mortem*<sup>12</sup>.

Serotonin (5-hydroxytryptamine or 5-HT) is an important

monoaminergic neurotransmitter of the central nervous system, and is derived from the essential aromatic amino acid tryptophan which is carried by the albumin protein in the blood stream. The free tryptophan is picked by the encephal, after having crossed the hematoencephalic barrier and transformed in 5-hydroxytryptophan, being subsequently decarboxylated into active neurotransmitter, serotonin<sup>13</sup>. Due to its activity in many receptors, serotonin has been implied in the mood regulation, including depression, anxiety, food intake and compulsive violence<sup>4</sup>. While low production of serotonin causes disturbance in the mental health of an individual, its high production is related to central fatigue, as demonstrated by many studies with aerobic exercise in animal<sup>15,16</sup> and human models<sup>17-19</sup>.

Physical exercise is able to promote increase in the production of serotonin due to higher availability of its precursor amino acid. In aerobic conditions of low intensity and long duration, the adipose tissue is recruited by the hormone system, releasing fatty acids for energy production. These lipids will be carried away by albumin in the blood stream until they reach the active musculature<sup>13</sup>. The need to make free fatty acids available for oxidation ends up releasing tryptophan, since both use the same carrier protein. Therefore, the free tryptophan concentration in the hematoencephalic barrier becomes increased<sup>20,21</sup>.

Thus, the aim of this study was to evaluate the effect of one session of aerobic exercise in the tryptophan concentration in the brain of rats with hyperphenylalaninemia.

## METHODS

### Animals

All the animal experiments were performed according to the Brazilian resolutions under the Bioethics in Animal Experimentation (Law # 6.638, from May 8, 1979 and legal opinion # 24.645, from July 10, 1934). In this study, 32 male Wistar rats, approximate age of 30 days were used. The animals were kept in polyethylene collective cages and kept in rooms with controlled temperature and exposed to light/dark cycles of 12:12 hours, with free water access and food *ad libitum*.

### Experimental groups

At 28 days of life, the animals were randomly sorted in four groups: 1) sedentary control (SedSAL) – healthy rats; 2) sedentary PKU (SedPKU) – rats with hyperphenylalaninemia; 3) exercise control (ExeSAL) – healthy rats which exercised; and 4) exercise PKU (ExePKU) – rats with hyperphenylalaninemia which exercised.

### PKU model

The PKU groups received an injection with  $\alpha$ -methyl-phenylalanine (phenylalanine hydroxylase inhibitor) during three days at 9h and with phenylalanine in the  $1.6\mu\text{mol}\cdot\text{g}^{-1}$  and  $2.1\mu\text{mol}\cdot\text{g}^{-1}$  concentrations, respectively and at 18h they received the same dose of phenylalanine<sup>22</sup>. The SAL groups received saline solution at the same conditions.

### Exercise and tryptophan measurement

On the fourth day, the Sed groups remained in their cages, while the Exe groups performed run on treadmill specific for rodents (Insight EP 131) during 60 minutes and velocity of  $12\text{m}\cdot\text{min}^{-1}$ <sup>23</sup>.

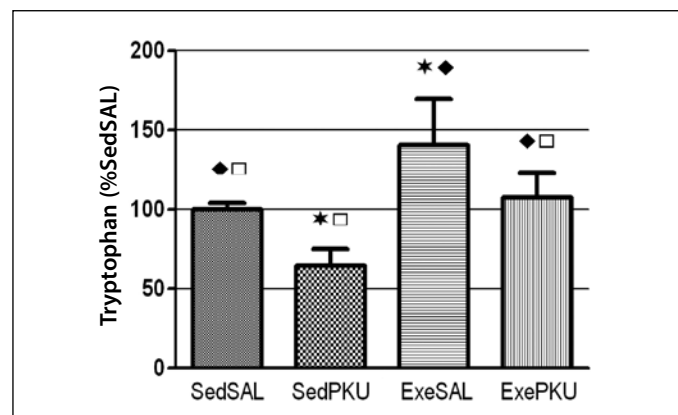
Immediately after, the animals were sacrificed by decapitation without anesthesia and the brains were quickly removed, cleaned (cerebellum, olfactory bulb, superficial bridge and blood vessels removal) and kept in ice. The brain mass was measured and they were cleaned with buffering solution of sodium phosphate ( $\text{Na}_3\text{PO}_4$  – 20mM) with potassium chloride (KCl – 140mM), in 1:5 dilution. The product was placed in *eppendorfs* for centrifuging during 10min at velocity of 1,000g and temperature of 4°C (Eppendorf Centrifuge 5417R). The supernatant was used for measurement of the tryptophan by spectrophotofluorimetry (Molecular Devices – Espectra Max Gemini XPS), with excitation of 280nm and emission of 360nm<sup>24</sup>. The total proteins of the samples were also measured by Lowry et al.<sup>25</sup> technique, using bovine albumin as standard. The results were normalized ( $\mu\text{M}\cdot\text{mg}_{\text{protein}}^{-1}$ ) and calculated in % of the control (SedSAL group) and expressed as mean and standard deviation. Data normality was verified through the Shapiro-Wilk test, and the differences between groups were examined by two-way ANOVA, followed by Bonferroni test, using the statistical package SPSS, version 12.0 for Windows. The significance level adopted was of 0.05.

## RESULTS

In the analysis of the tryptophan in the brain, significant differences were found between the treatments saline and hyperphenylalaninemia [ $F_{(1,28)} = 83.53$   $p < 0.001$ ], and also between the sedentary and exercise conditions [ $F_{(1,28)} = 102.06$   $p < 0.001$ ], figure 1. No interaction effect between the studies factors was observed [ $F_{(1,28)} = 3.58$   $p = 0.069$ ].

In the sedentary groups, the hyperphenylalaninemia model used (SedPKU group) significantly decreased the tryptophan concentration in the brain compared with the control group (SedSAL) ( $p < 0.001$ ). The same has occurred with the groups which performed exercise, in which the tryptophan concentration was lower in the ExePKU group when compared with the ExeSAL group ( $p < 0.001$ ).

Concerning the exercise effect, the tryptophan in the brain of exercised animals was significantly higher when compared with their controls, with higher values for the ExeSAL group compared with the SedSAL ( $p < 0.001$ ), and higher for the ExePKU group compared with SedPKU ( $p < 0.001$ ). Significant differences were not found between the trained hyperphenylalaninemic animals (ExePKU) and the controls which did not perform physical exercise (SedSAL) ( $p = 0.903$ ).



**Figure 1.** Relative tryptophan concentration in the brain (%SedSAL). Results expressed in mean  $\pm$  standard deviation. \* indicates difference SedSAL; ♦ indicates difference of SedPKU; □ indicates difference of ExeSAL, for  $p < 0.05$ .

## DISCUSSION

The hyperphenylalaninemic model used in this study<sup>22</sup> was effective in significantly decreasing the tryptophan concentration in the brain derived from increase in phenylalanine. The majority of the phenylalanine concentration in the plasma, and consequently in the hematoencephalic barrier, harms the passage to the brain of other amino acids which use the same transportation system, decreasing the tryptophan influx in an important way<sup>3,6,7,26,27</sup>. Phenylalanine is able to saturate the membrane proteins, which guarantee the permeability of the hematoencephalic barrier, making the entrance of the tryptophan and of other amino acids in the brain even more difficult<sup>9</sup>, probably due to its higher affinity with this transportation system<sup>8</sup>.

Tryptophan decrease means lower contribution of substrate for production of serotonin by the hypothalamic system, making the individual more sensitive to mood disorders<sup>14</sup>. Ardis et al.<sup>28</sup> found negative alterations in the behavior and cognition of rats, derived from the diet poor in tryptophan to which they were submitted, to lower tryptophan concentration in the plasma and lower serotonin concentration in the brain, evidencing the importance of the serotonergic system in cognitive variables. Negative influence in the synthesis of amines in the brain was observed in animal model of chronic hyperphenylalaninemia, which caused cognitive deficit in adult rats<sup>29</sup>.

The proposed physical exercise<sup>23</sup> was significant in the increase of the tryptophan concentration in the brain, both in control and hyperphenylalaninemic animals. Our results agree with many studies with healthy animals<sup>15,16,23,30</sup>, which found increase of free tryptophan concentration in the plasma and in the brain and consequently, higher serotonin production. Increase in serotonin production is caused by the increase of the activity of the sympathoadrenergic system, characteristic of physical stress. Lypolysis stimulation

causes higher availability of fatty acids released in the blood stream and increases the albumin need to lead them to the musculature under exercise, causing the tryptophan ligated to albumin to be released. The Free tryptophan in increased concentration reaches the hematoencephalic barrier in advantage compared to other amino acids, resulting in its higher influx in the brain, and consequently, in higher serotonin production.

The most interesting finding of this study was that the tryptophan value in the brain of the ExePKU group did not present statistical difference from the SedSAL group. Therefore, aerobic exercise was able to revert decrease of tryptophan found in the hyperphenylalaninemic rats which did not exercise, reaching values similar to the control animals. It is expected that serotonin production increases with the higher contribution of substrate, since the tryptophan supplementation presents this effect in hyperphenylalaninemic patients<sup>3,4</sup>.

The results obtained in this investigation showed that aerobic physical exercise may be an interesting strategy for restoration of the tryptophan concentration in the brain in hyperphenylalaninemic status. No studies which could relate aerobic exercise with hyperphenylalaninemic individuals were found in the scientific literature. Thus, further investigation should be carried out to support the use of aerobic physical exercise under hyperphenylalaninemia, a characteristic of individuals with PKU, as strategy of increase in the tryptophan levels in the brain. It is extremely important to know the acute responses to physical exercise as the ones presented in this investigation for the conduction of future research which assesses the effects of physical training in hyperphenylalaninemia.

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All authors have declared there is not any potential conflict of interests concerning this article.

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