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Original Research

The 6-week Effects of HIIT on Biomarkers of Tissue and Oxidative Damage in Wistar Rats Previously Supplemented with Pyridoxine

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ABSTRACT

International Journal of Exercise Science 14(7): 369-381, 2021. We aimed to analyze the effects of long high-intensity interval training (HIIT) associated with pyridoxin supplementation on tissue and oxidative injury markers in animals. Male Wistar rats were divided into three groups (n = 8): sedentary (GS), HIIT (GH), and HIIT + pyridoxine (GHP). The HIIT comprised 18 sessions of 7 repetitions of 2min × 2min rest, 3 times per week. Pyridoxine was administered to the GHP group 1h before the exercise. The Thiobarbituric acid reactive substances (TBARS) and sulfhydryl group (SH) were analyzed as markers of oxidative stress and CK, LDH, ALT and AST as tissue lesions. There was an increase in the correlation between CK and LDH of 172.86% and 268.83% in the GH group compared with the GS group, respectively. There was a reduction in CK (34.37%) and LDH (34.74%) compared with the GH group, which had an increase of 229.03% in ALT. Pyridoxine supplementation reduced ALT by 80.62% in the GHP group compared with no-supplementation GH group. In addition, there was a reduction in plasma MDA (52.92%), liver (20.30%) and cardiac (22.06%) tissues in GHP compared to GH. It was possible to conclude that administration of pyridoxine attenuated oxidative stress, and tissue injuries induced by HIIT.

KEY WORDS: Pyridoxine; HIIT, oxidative stress, antioxidant

INTRODUCTION

High-intensity interval training (HIIT) is defined by short intervals of high-intensity activity, >80%–95% of the maximum oxygen uptake (VO_{2max}) or >90% of the maximum heart rate (HRmax), interspersed with low-intensity passive or active rest periods, with the recovery period ≥2 times longer than the exercise duration (30, 37). HIIT has been investigated in recent decades for its capability to induce physiological and metabolic responses related to peripheral adaptations, such as increased skeletal muscle mitochondrial content, capillary density, and to vital factors, such as increased maximal systolic volume and maximum cardiac output and blood volume, in shorter exercise sessions than that of other types of training (14).

Few studies suggest that the increased metabolic rate promoted by HIIT results in increased production of reactive oxygen species (ROS) in various tissues and body fluids. This is due in part to the increased oxygen uptake or stimulation of anaerobic metabolism and muscle damage (21). Oxidative stress develops when the concentration of ROS exceeds the upper limit of the body's antioxidant capacity (4).

Oxidative stress can trigger damage to the cell structures, including lipids, protein membranes, and DNA (21). During high-intensity physical exercise (HIPE), redox imbalance is a main cause of fatigue, as it reduces the production of skeletal muscle strength and promotes greater susceptibility to muscle injuries (23). These damages to cellular and tissue structures cause the extravasation of cytosolic enzymes, such as creatine kinase (CK) and lactate dehydrogenase (LDH), into the plasma. High plasma levels of these enzymes indicate muscle damage. Additionally, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are used as markers of liver injury (1).

Thus, various forms of supplementation are used to enhance exercise performance (29). Pyridoxine, or vitamin B6, is a compound comprising a group of six chemically related compounds containing a pyridine ring as their core. These compounds are pyridoxal, pyridoxamine, pyridoxine, pyridoxal-5-phosphate, pyridoxamine-5-phosphate, and pyridoxine-5-phosphate (19). They are essential to synthesize neurotransmitters and proteins and regulate some of the neuronal activities (17). Pyridoxal-5-phosphate, a cofactor of pyridoxine, participates in enzymatic reactions of proteins, fats, and carbohydrates (10, 19). Recent studies have been investigating its antioxidant capacity (28, 38). However, its effects on stress conditions, such as in experimental HIIT training models, remain unknown.

This study aimed to analyze the effects of a long HIIT protocol on tissue biomarkers and oxidative lesions in rats supplemented with pyridoxine. Studies have demonstrated the effects of long HIIT on oxidative stress and tissue damage biomarkers and have elucidated pyridoxine as a supplementary alternative to improve performance.

METHODS

Sample

Twenty-four male Wistar rats, each weighing 250–300 g, were obtained from the Sectoral Vivarium for Creation and Experimentation of the Intracellular Signaling Research Center/Department of Morphology/CCBS of the Federal University of Sergipe. The animals were randomly maintained at a controlled temperature of 22 ± 3°C with a 12/12 h light/dark cycle and free access to rodent-specific feed (Labina®) and water ad libitum.

The rats were divided into three groups (n = 8): sedentary group (GS), which did not receive any type of intervention; HIIT group (GH), which performed the protocol of 18 HIIT training sessions; and pyridoxine-supplemented HIIT group (GHP), which performed the 18-session HIIT protocol and was supplemented with pyridoxine 60 min before each training session. The research was approved by the Animal Research Ethics Committee (CEPA) of the Federal University of Sergipe (protocol 03/2019). This research was carried out fully in accordance to the ethical standards of the International Journal of Exercise Science (18).

The animals were acclimatized to the liquid environment, following a protocol adapted from Manchado-Gobatto et al. (15), using 80-cm deep × 80-cm diameter cylinders, with the temperature of water being maintained at 31 ± 1°C. The GH and GHP groups were acclimatized for 15 uninterrupted days, the first 3 days in 10-cm deep shallow water for 15 min; from days 4 to 10 in water 60-cm deep water, increasing the time by 2 minutes each day; and from days 11 to 15, overload equivalent to 3% of the body weight was added for 5 min.

The protocol followed by the GS group was similar to the other groups until day 10, but from days 11 to 15, the animals were placed in water for 25 min without overload. This adaptation aims to reduce the stress in the animals (15). The experimental design of the study is provided in Figure 1.

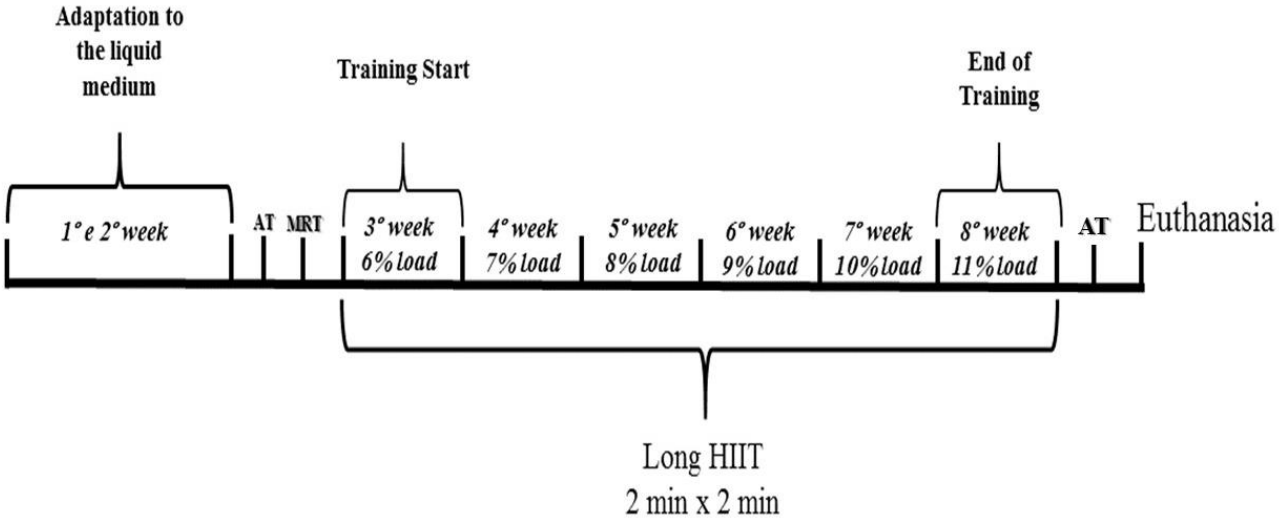

Figure 1. Experimental design of the intervention protocol. Anaerobic threshold (AT); Maximum Repeat Test (MRT); High Intensity Interval Training (HIIT).

To determine the overload used in the HIIT training, we adapted the anaerobic threshold protocol of Manchado-Gobatto (15), which consists of six swimming stages, followed by 5 min

of exercise, with loads equivalent to 3.5%; 4.0%; 4.5%; 5.0%, 5.5%; and 6.0% of the animal's body weight, tied to its back. At each of these moments, a drop of blood was drawn from the tail to measure blood glucose levels by using the Accu-Check Go Glucometer (Roche Diagnostics GmbH, D-68298 Mannheim, Germany). Individual graphs were drawn with the obtained glycemic values to identify the anaerobic threshold.

The maximum HIIT test was performed to define the amount of repetitions during the training. A day after the anaerobic threshold, the animals performed the maximum repeat test, following the protocol of Pimenta et al. (20).

Protocol

The HIIT protocol of Terada et al., (36) was adapted for this study, and the swimming training was used. The long HIIT protocol comprises sets of 7 repetitions of 2-min individual swimming, interspersed with 2-min passive rest, 3 times a week for 6 weeks, totaling to 18 sessions, with 6% body weight overload tied to the back of the animal. With each week of training, 1% overload was added until the load equivalent was 11% at the end of the training sessions. Each training session lasted for 28 min for each animal. This protocol is presented in Table 1. Buchheit and Laursen (3) pointed out that periods of >60 s of intense activity interspersed with rest of >60 s characterize long HIIT.

Table 1. Long HIIT training protocol, adapted from Terada (35).

Weeks	Protocol	Load (g)
1°	2 min swimming X 2 min passive recovery	6%
2°		7%
3°		8%
4°		9%
5º		10%
6º		11%

The GHP group animals were intragastrically (gavage) administered 4 mg/kg pyridoxine (Purifarma with Internal Lot: PURI003567 and Manufacturer Lot PH17044081) diluted with distilled water 60 min before each HIIT session, 3 times a week (34).

After 24 h of the last training day, the animals were anesthetized by injecting a combination of ketamine (100 mg/kg) and xylazine (10 mg/kg) and then euthanized by cardiac puncture. The blood was immediately collected and centrifuged at 4000 × g for 15 min at ±4°C, and the supernatant was stored at ± −80°C to analyze enzyme markers and oxidative stress. Meanwhile, the gastrocnemius muscle, liver, and heart tissues were removed for further analysis of oxidative stress markers.

Lipid oxidation was determined by measuring thiobarbituric acid reactive substances (TBARS) according to the method described by Lapenna et al., (12). Antioxidant level of the tissues was

determined by quantifying the sulfhydryl groups (SH) according to the method described by Faure and Lafond (8) with some modifications.

The extent of muscle and liver damage caused by HIIT was evaluated by measuring enzyme activity of tissue injury such as CK, lactate dehydrogenase (LDH), alanine aminotransferase (ALT), and aspartate aminotransferase (AST). The analysis was performed using a commercial kit (Labtest®) according to the manufacturer's instructions.

Statistical Analysis

The data obtained were expressed as mean ± standard deviation, using the Shapiro-Wilk normality test, to test the homogeneity of the data. For comparison between groups, ANOVA one-way analysis of variance with Bonferroni's Post-Hoc was used, with a statistical difference of $p < 0.05$ being considered significant. The calculations were made using the Graph Pad Prism statistical software version 7.0 (Graph Pad Software, San Diego, CA, USA).

RESULTS

Data related to markers of muscle and liver damage in the GS, GH, and GHP groups are presented in Table 2. Regarding CK and LDH concentrations, there was an increase of 172.86% ($p < 0.0001$) and 268.83% ($p < 0.0001$) in the GH group compared with the GS group, respectively. However, pyridoxine (GHP) supplementation was able to reduce CK and LDH concentrations by 34.37% ($p < 0.001$) and 34.74% ($p < 0.0001$), respectively, when compared with exercise without supplementation (GH group).

Regarding liver enzyme concentrations (Table 2), the GH group showed a 229.03% increase in the ALT concentration when compared to the GS group ($p < 0.0001$). However, the pyridoxine supplementation (GHP group) was able to reduce ALT concentration by 80.62% ($p < 0.0001$) when compared to exercise without supplementation (GH group). The plasma AST concentrations were similar between the GS, GH, and GHP groups ($p > 0.05$) (Table 2).

Table 2. Muscle and liver damage biomarkers.

Tissue damage (U/L)	GS	GH	GHP
CK	445,3 ± 41,83[a]	1215±163,8[b]	797,4±134,5[c]
LDH	186,1±23,65[a]	686,4±52,9[b]	447,9±55,49[c]
ALT	79,02±18,23[a]	260,6±71,37[b]	50,49±11,52[ac]
AST	116,3±26,47[a]	135±21,87[a]	114,5±17,7[a]

a, b, c - The letters represent the statistical differences between groups, with $p < 0.05$. Equal letters represent that there were no statistical differences with $p > 0.05$

Group of sedentary animals (GS), group training for six weeks of high intensity interval training (HIIT) (GH) and the group exercised and supplemented with 4mg / kg of pyridoxine (GHP). The values represent the mean ± standard deviation (n = 8). The statistical differences were determined by ANOVA one way, with a Bonferroni post test.

To investigate oxidative stress, concentrations of TBARS and the production and determination of sulfhydryl groups (SH) were analyzed. Figure 3 shows the plasma, hepatic, muscular, and cardiac MDA values. The GH group showed a 39.56% ($p < 0.001$) increase in plasma MDA concentrations when compared with the GS group. However, the training group previously supplemented with pyridoxine (GHP) showed a reduction in plasma MDA concentrations of 52.92% ($p < 0.0001$) when compared to the GH group (Figure 2A). The hepatic fraction of MDA was increased by 123.34% ($p < 0.0001$) in the GH group when compared to the GS group. Prior supplementation with pyridoxine (GHP group) promoted a decrease in hepatic MDA of 20.30% ($p < 0.01$) when compared with exercising without pyridoxine supplementation (GH group) (Figure 2B).

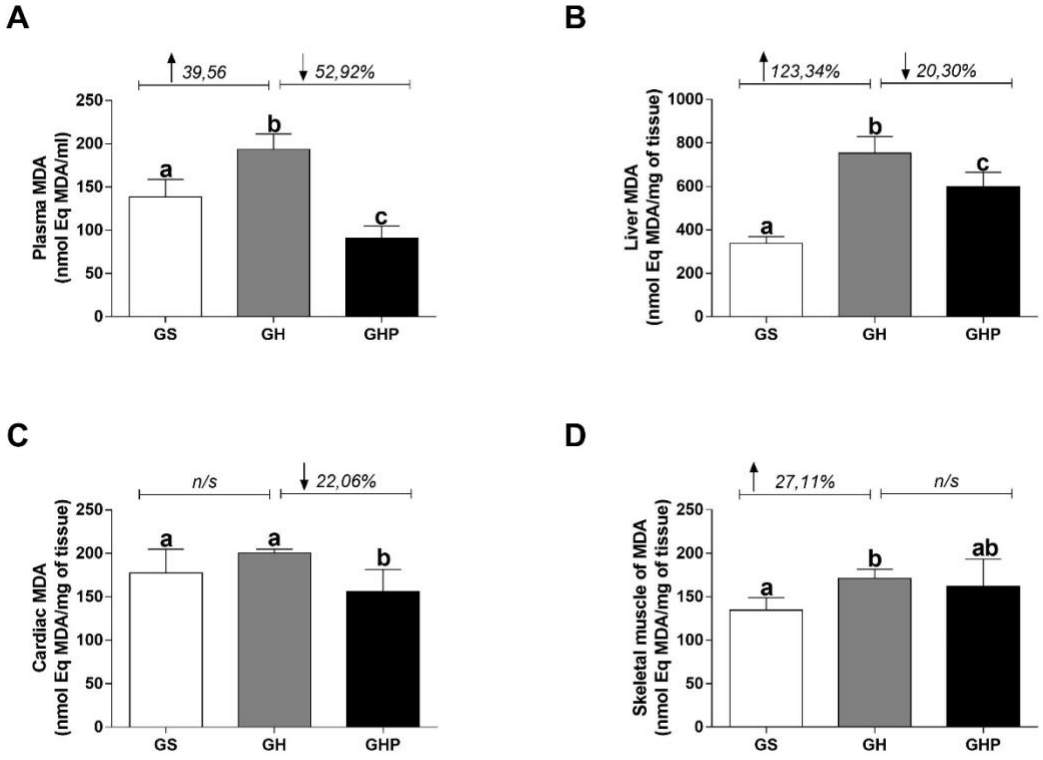

Figure 2. Analysis of Malonaldehyde (MDA) in plasma, heart, liver and gastrocnemic muscle. a, b, c - The letters represent the statistical differences between groups, with $p < 0.05$. Equal letters represent that there were no statistical differences with $p > 0.05$

The cardiac MDA contents were similar between the GS and GH groups ($p > 0.05$). However, prior supplementation with pyridoxine (GHP group) promoted a decrease in cardiac MDA of 22.06% ($p < 0.05$) when compared with exercising without supplementation (GH group) (Figure 2C). Gastrocnemius muscle MDA levels were similar between the GH and GHP groups ($p > 0.05$). However, there was an increase in skeletal muscle MDA of 27.11% ($p < 0.05$) in the GH group when compared to the GS group (Figure 2D).

The quantification of SH groups showed the tissue antioxidant levels (Figure 3). Plasma and hepatic and gastrocnemius muscle SH concentrations were similar between the GS, GH, and GHP groups ($p > 0.05$) (Figure 3 A, B, D).

The cardiac sulfhydryl concentration was increased by 80.66% ($p < 0.05$) in the GH group when compared to that in the GS group. However, the cardiac sulfhydryl concentration was similar in both the GHP and GH groups ($p > 0.05$) (Figure 3C).

Group of sedentary animals (GS), group training for 6 weeks of high-intensity interval training (HIIT) (GH) and the group exercised and supplemented with 4 mg/kg pyridoxine (GHP). Figure 2A: Represents the plasma; Figure 2B: represents liver tissue; Figure 2C: represents cardiac tissue; Figure 2D: represents muscle tissue. The values are represented as mean ± standard deviation (n = 8). The statistical differences were determined by ANOVA one way, with a Bonferroni post test.

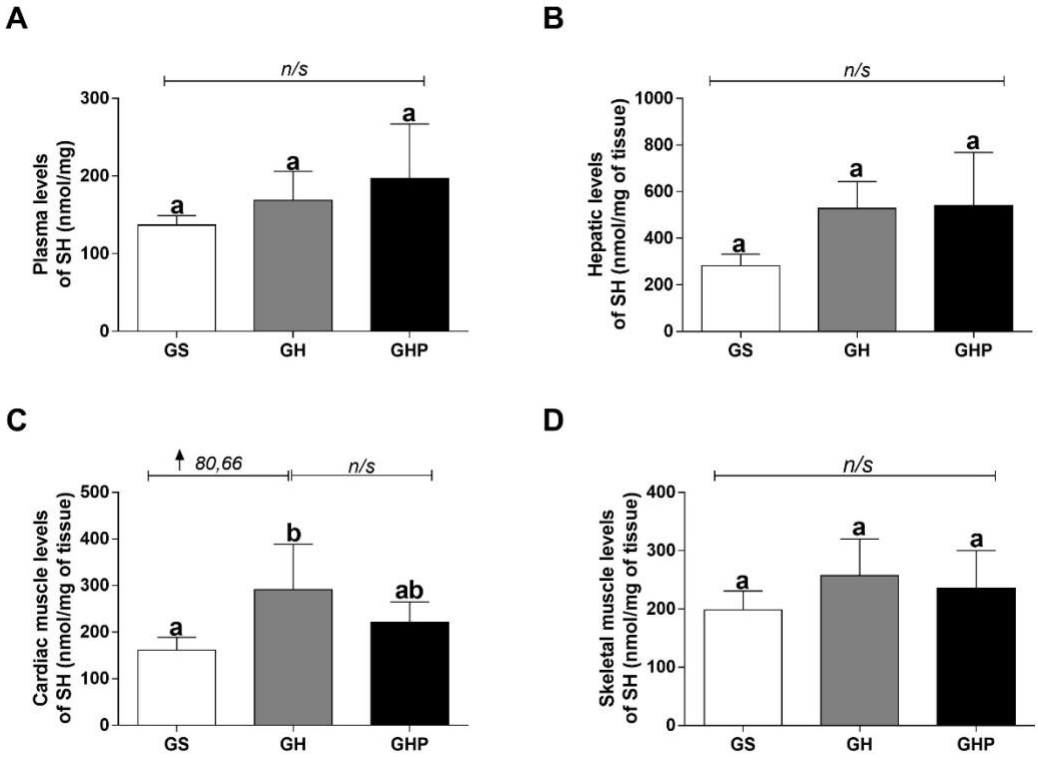

Figure 3. Analysis of sulfhydryl groups (SH) in plasma, heart, liver and gastrocnemic muscle. a, b, c - The letters represent the statistical differences between groups, with $p < 0.05$. Equal letters represent that there were no statistical differences with $p > 0.05$

Group of sedentary animals (GS), group training for 6 weeks of high-intensity interval training (HIIT) (GH) and the group exercised and supplemented with 4 mg/kg pyridoxine (GHP). Figure 3A: represents the plasma; Figure 3B: represents liver tissue; Figure 3C: represents cardiac tissue; Figure 3D: represents muscle tissue. The values are represented as mean ± standard deviation

(n = 8). The statistical differences were determined by ANOVA one way, with a Bonferroni post test.

DISCUSSION

The study demonstrated that, six weeks of long HIIT with swimming, was able to increase the concentrations of some indicators of oxidative stress, muscle damage and liver damage in Wistar rats. In addition, animals previously supplemented with pyridoxine showed attenuation of oxidative and tissue damage in some of these biomarkers.

The biochemical parameters of CK, LDH, ALT and AST concentrations in Wistar rats differ considerably in some studies, few describe the reference values for laboratory rodents, mainly in sedentary groups (5). Several factors can influence these values, however the location of the sample and the methodologies used must be kept constant and adequate, avoiding possible variables (48). Boehm et al. (5) highlighted some baseline values of biochemical parameters for drafts. In addition, studies by dos Santos et al (10), Altinoz et al (3), Mas et al (20); Zang et al (47), Salimi et al (34) also present in the enzymatic concentrations of CK, LDH, ALT and AST values approximate to those found in this study.

The increased expressions of CK and LDH enzymes in the GH group compared with those in the GS group suggest that high-intensity exercise could trigger skeletal muscle damage. Muscle injury is due in part to the identification of sarcolemma rupture, changes in the contractile components of the myofibrils and extracellular matrix, and cytoskeleton damage (6). In addition to these factors, extravasation of plasma cytosolic enzymes such as CK and LDH are considered important markers of muscle damage induced by high-intensity exercise (1, 6).

A study by dos Santos et al. (7) identified an increase in the concentration of CK and LDH enzymes being associated with morphological changes in the gastrocnemius skeletal muscle after a high-intensity training protocol for 4 weeks in rats. In another human study, sprint training resulted in greater muscle damage than longer submaximal intervals, demonstrating that HIIT protocols of different duration and intensity result in different physiological changes (40). Thus, strenuous exercise can induce morphological changes such as rupture of myofibrillar structures and cause reduction in performance.

In addition, it was shown that rats submitted to HIIT and supplemented with pyridoxine showed a reduction in CK and LDH levels. These results suggest that pyridoxine may contribute by attenuating or preventing muscle damage induced by high-intensity exercise.

Pyridoxine has cofactors that play important roles in energy metabolism, improving enzymatic activities (13). Pyridoxal-5-phosphate (PLP) is a cofactor of biologically active pyridoxine, which is linked to glycogen phosphorylase, present in skeletal muscle and brain. Among its functions, active PLP hydrolyzes α-1,4-glycosidic bonds to generate glucose-1-phosphate (31) and also participates in transamination reactions for synthesis and degradation of amino acids (18, 32).

Virk et al., (39) identified that exercise-associated vitamin B6 supplementation improved plasma concentrations of energy substrates, such as glucose, fatty acids, and amino acid content, during strenuous resistance exercise without affecting performance.

Suidassari et al., (33) pointed out that pyridoxine supplementation could increase endurance in skeletal muscle, prolonging muscle contraction. Thus, these findings suggest that pyridoxine may improve skeletal muscle performance by attenuating muscle damage, as shown by decreased extravasation of CK and LDH enzymes after 18 HIIT sessions.

It is demonstrated that the PLP present in the supplementation of pyridoxine, would act in increasing the availability of glycogen to the muscle, providing a greater energy demand for exercise, maintaining the integrity of the cell, thus preserving the muscle tissue, reducing the leakage cytosolic enzymes CK and LDH.

There was an increase in plasma ALT expression, identified in the GH compared with the GS; the increase in this enzyme is usually associated with liver tissue damage (13). The enzyme ALT catalyzes the reversible transamination reaction of alanine and α-ketoglutarate in pyruvate and glutamate using PLP as a cofactor (19). The increase of tissue injuries after exhaustive exercises depends on the intensity, density, and type of exercise that are performed (5, 7).

In HIPE, there is an increased blood flow to muscle tissues, which in turn decreases blood flow to other tissues, such as the liver, which can cause damage. These effects are due in part to hypoxia in hepatocytes, ALT enzyme release, and necrosis (26). In a study by Ramos et al., (27), an increase in the concentration of ALT was observed after a high-intensity exercise protocol compared with that observed after a low-intensity exercise protocol in rats. In another study, Shin et al., (31) found that strenuous exercise in runners increased the ALT plasma level, demonstrating liver injury after running, in line with the results of this study.

By contrast, there was a significant reduction in ALT in the GHP group when compared with the GH group. It can be said that pyridoxine administration may have attenuated the liver damage probably induced by HIIT, demonstrated by the reduction of ALT, when associated with supplementation. Roh et al., (28) analyzed acetaminophen-induced hepatocyte damage in vitro in pyridoxine-supplemented rats. The results showed reduced ALT levels, implying the protective action of pyridoxine against liver cell damage.

In addition, a reduction in oxidative damage to liver tissue in exercised rats supplemented with pyridoxine was shown in the results of the present study, showing the antioxidant action of this vitamin in relation to exhaustive exercise. Studies by Wen et al (45) and Anand (1) with rats and cell cultures indicated a decrease in oxidative stress induced by drugs, when associated with pyridoxine supplementation. However, it has been shown that pyridoxine would act to reduce oxidative damage in the liver cell, as well as, in tissue integrity, indicated by the reduction of the ALT enzyme.

These effects are due in part to the effects of pyridoxine on attenuating exhaustive exercise-induced oxidative injuries and improving physical performance and recovery after fatigue (34).

In addition to tissue injury markers, the present study analyzed the biomarkers of oxidative stress in the plasma, liver, heart, and muscle tissues. After 6 weeks of HIIT, the MDA concentrations in the plasma and liver and muscle fractions increased significantly in the GH group compared with the GS group. Some authors report that MDA is the end product of a series of chemical reactions that occur during lipid peroxidation, characterized by oxidation of cell membrane phospholipids and other biomolecules, such as thiol compounds, enzymatic cofactors, proteins, nucleotides, and DNA, and resulting in changes in homeostasis, which leads to fatigue (22, 25).

It was observed an increase in muscle MDA in the GH group compared with the GS group. This could be due to the generation of ROS in the skeletal muscle, predominantly by contraction during physical exercise (9). Increased generation of ROS during HIPE causes a reduction in sarcolemmal repolarization capacity, disturbances in sarcoplasmic reticulum Ca^{2+} release, interaction between damaged actin and myosin, blood flow restriction, and reduction of enzymatic activities, resulting in contractile dysfunction and fatigue (1, 2, 7).

However, Powers and Hogan (24) pointed out that the generation of ROS induced by moderate intensity exercises plays an important role in the physiological functions (hormesis), mainly in the regulation of muscle strength production and increase in muscle antioxidant production, because the induction of oxidative stress depends on the exercise intensity, duration, and volume (11, 25).

Therefore, it can be noted that in the results of the present study that, previous supplementation of pyridoxine in rats trained with HIIT, can act in the generation of energy for the muscle fiber, being its cofactor responsible for the glycogen catalysis by the enzyme glycogen phosphorylase supplying energy to the fiber muscular (16). In addition, oxidative stress markers were reduced, highlighting the antioxidant action of the vitamin, which shows that the action of pyridoxine, in addition to providing an energy source for the muscle fiber, prevented oxidative damage from ROS, maintaining the integrity of the muscle fiber and reducing the tissue damage.

It was possible to concluded that long HIIT protocol in rats induced an increase in muscle and liver damage, as demonstrated by the elevated plasma concentrations of the enzymes CK, LDH, and ALT. On the other hand, supplementation with pyridoxine, administered 60 min before each training session, reduced the oxidative stress markers concentrations e tissue lesion. Therefore, this vitamin can act as an antioxidant, in pro-oxidant situations caused by physical training.

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