Original Article

The anti-inflammatory effect of resistance training in hypertensive women: the role of purinergic signaling

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Background and methods: Essential arterial hypertension triggers a chronic inflammatory process that seems to be linked to purinergic signaling. Physical exercise exhibit anti-inflammatory properties and is able to modulates purinergic system. The aim of this study was to evaluate the effect of 6 months of resistance training on inflammatory markers, purinergic system components, hemodynamic and anthropometric parameters in hypertensive woman.

Methods: A total of 31 hypertensive group and 28 normotensive (control group) middle-aged sedentary women were submitted to 6 months of resistance training. All measurements and blood collection were carried out before (pretest), after 3 months and after 6 months (posttest) of training. Purinergic enzymes [nucleoside triphosphate diphosphohydrolase (NTPDase) and adenosine deaminase] were assessed in lymphocytes; IL-6, IL-10, ATP and C-reactive protein levels were measured in serum.

Results: Six months of resistance training was able to significantly reduce blood pressure (BP), IL-6, C-reactive protein, ATP levels as well as NTPDase and adenosine deaminase activities in hypertensive group. Physical training was also able to increase IL-10 levels in hypertensive group. A positive correlation was found between BP, enzyme activities and levels of ATP and IL-6. A negative correlation was found between BP and IL-10. Positive correlation was found between NTPDase and IL-6 levels ($P < 0.05$) as well as ATP levels and IL-6 levels.

Conclusion: Our findings demonstrated the relationship between purinergic signaling and inflammation in hypertension and suggests that resistance training serve as tool to reduce inflammation in hypertensive woman by modulating purinergic system.

Keywords: ectonucleotidases, hypertension, inflammation, purinergic system, resistance training

Abbreviations: ADA, adenosine deaminase; AMP, adenosine monophosphate; BP, blood pressure; CRP, C-reactive protein; EAH, essential arterial hypertension; HR, heart rate

INTRODUCTION

Essential arterial hypertension (EAH) is a multifactorial clinical condition characterized by elevated and sustained levels of blood pressure (BP) [1,2]. According to the WHO, one in four men and one in five women, that is, 22% of the world population over 18 years are affected by this disease, which represents an important risk factor for other cardiovascular comorbidities – such as stroke, acute myocardial infarction, heart failure, aneurysms and chronic kidney disease [1,2]. In addition, diseases associated with hypertension have high social and health costs and are associated with a decrease in life expectancy, making prevention essential [1–3]. Recent studies point out to a close relationship between EAH and inflammation [4–8]. High BP levels promote tissue damage and, consequently, a chronic inflammatory condition in the body, triggered by increased production of proinflammatory cytokines and by increased recruitment and activation of lymphocytes through vascular remodeling. These changes are involved in the increase in the BP levels and development of EAH [7–9]. In addition, this inflammatory process can be aggravated in the presence of excess body fat and a sedentary lifestyle [10].

The purinergic system has emerged as a new cellular mechanism that plays an important role in the regulation of inflammatory processes [11–13]. This system is mainly composed of adenine nucleotides: ATP, ADP, adenosine monophosphate (AMP), adenosine and inosine, purinergic receptors (P1 and P2), and enzymes known as ectonucleotidases [ecto-nucleoside triphosphate diphosphohydrolase (NTPDase) (also known as CD39), ecto-5′nucleotidase (also known as CD79) and adenosine deaminase (ADA)] [14,15]. It is already known that the ATP released by different cell types is recognized by specific purinergic receptors as a danger signal, thus leading to a variety of inflammatory responses [8,13]. After being released into the extracellular medium, ATP can be rapidly hydrolyzed to ADP and AMP by ecto-NTPDase. The formed AMP can be converted into adenosine

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by the action of ecto-5′nucleotidase and the adenosine can be further hydrolyzed to inosine by ADA [14,15]. These enzymes are found in the cell membrane of immune system cells, especially in lymphocytes [11,13].

The action promoted by the purinergic system will vary accordingly to the concentration and interaction of these adenine nucleotides and their binding to their corresponding receptors [11,13]. When ATP concentration increases, it binds to its receptors in lymphocytes and triggers proinflammatory responses, such as the release of IL-6, the activation and recruitment of lymphocytes to the site of injury. Conversely, adenosine acting via its receptors promotes suppression of inflammatory processes, for example: increase the release of IL-10, an anti-inflammatory mediator, by lymphocytes [12,16,17]. These events may be closely related to the hypertensive condition, since patients with EAH have increased levels of inflammatory markers, such as C-reactive protein (CRP) and IL-6 and decreased levels of anti-inflammatory cytokines, such as IL-10 [9,18] when compared with normotensive participants.

The complex network of enzymes that regulate nucleotide concentration are now considered to play a critical role in regulate immune responses in different tissues and the activity of ecto-NTPDases and ADA are particularly important for balancing the proinflammatory and anti-inflammatory effects of ATP released to extracellular milieu [13,19]. Purines are emerging as powerful extracellular signaling molecules rather than just energy molecules, which can modulate the onset, development and magnitude of the inflammatory response. Therefore, changes in purinergic signaling may play an important role in disease characterized by low-grade inflammation, such as EAH [4,7].

Several meta-analysis studies have reported that physical activity is recommended as the first-line option to treat many diseases, including EAH [3,10,20–23]. In fact, some studies point out that major exercise interventions are as effective as some antihypertensive drug interventions [20,24]. The protective effect of a physically active lifestyle against chronic inflammation-associated diseases may be closely ascribed to an anti-inflammatory effect of exercise [3,10,23,25,26].

Our previous studies have already demonstrated that physical exercise modulates the purinergic system from hypertensive animal models, favoring the anti-inflammatory properties from this system [4,27,28]. Recent studies show that the resistance training alone has excellent results in reducing BP in hypertensive and prehypertensive individuals, serving as an excellent possibility of treating hypertension [23]. However, little is known about the complexity of purinergic regulation in EAH, especially in humans. In this context, the aim of the current study is to investigate the correlation between EAH and purinergic signaling in lymphocytes from hypertensive patients, as well as the contribution of resistance training to lower BP through this system.

MATERIAL AND METHODS

Participants

An applied experimental study with a quantitative approach was carried out. The sample of the current study consisted of female sedentary volunteers aged between 40 and 60 years with and without diagnosis of hypertension. The selection of the sample was nonprobabilistic by quotas, considering the eligibility criteria.

The sample size was calculated in 50 participants (25 hypertensive and 25 normotensive), considering a mean SD of the main outcome variables of ten units, with a statistical power of 90%, a significance level of 0.05 (two-tailed distribution), a detectable difference between treatment of 10.6 unit. The initial sample consisted of 77 women (41 hypertensive and 36 normotensive). Data from women who missed three consecutive sessions or more than five sessions over the 6-month duration of the intervention protocol were also excluded from the analysis. Considering the exclusion criterion of missing sessions, 10 hypertensive and eight normotensive women were excluded from the data analysis.

The final sample of this study included 59 middle-aged women of varying ethnicities, predominantly white and from different neighborhoods in the city of Chapecó (Brazil). The hypertensive group ($n = 31$) composed of hypertensive, sedentary women who use antihypertensive medication, submitted to resistance training; and the normotensive group ($n = 28$) composed of sedentary and normotensive women, also submitted to resistance training. The study was approved by the ethics committee of the Federal University of Fronteira Sul (UFFS), protocol 1.916.904. The study groups were designed based on other studies that have very high impact on this field [23,29,30] and which the pretest data collection is used as the control group without exercise influence. That is, the same participant is its own control. This type of study design is the most appropriate for our study, since more groups imply more participants and each person has different socioeconomic, physical and emotional characteristics, consequently, creating numerous new variables to interfere in the results. Therefore, the data collection performed in the pretest is the best possible control for the normotensive group and the hypertensive group that performed the exercise, since they are the same people, without new variables, which makes our results much more reliable.

The hypertensive group included women diagnosed with EAH and were undergoing clinical treatment, with BP levels below the levels considered severe for this disease, with SBP lower than 160 mmHg and DBP lower than 120 mmHg [1]. It is important to highlight that for the analyses of the hypertensive group we selected women undergoing clinical treatment for the disease for 5 years or more, with no changes in the use of medication in the last 2 years and with stable pressure levels, below 139 mmHg of SBP and 89 mmHg of DBP, and not decompensated to the treatment, that is, clinically stable [1,31]. Thus, it is not expected that these antihypertensive drugs (used for years) could have new actions on the patients' metabolism, due to the fact that these drugs reach an effective and stable treatment regimen (even when is used more than one medication in association), within 6–8 weeks after the start of use [1,31]. Thus, is not expected that the drugs could influence our results.

The normotensive group included women without a diagnosis of EAH, whose SBP and DBP values, measured

on two nonconsecutive days and at different times, were equal to or lower than 120 and 80 mmHg, respectively [1].

The study excluded women who presented comorbidities that represented a clinical contraindication to participate in the research, who used any medication that may have influenced the results of the study and women who smoked.

Anthropometric analysis was carried out before and after 6 months of resistance training. Hemodynamic analysis and blood samples collection were taken at three different times: before the start of the resistance training protocol, after 3 months, and after 6 months of performing the resistance training. Lymphocytes and serum were separated and analyzed, as described below.

Experimental protocol

Before the beginning or the resistance training, all the volunteers attended to the gym of the Community University of the Region of Chapecó (Unochapecó) and held a familiarization exercise session, which included all the exercises that would be used in the protocol's intervention. Both the normotensive and hypertensive groups underwent the intervention of the training protocol carried out twice a week, in two classes, one on Mondays and Wednesdays and another on Tuesdays and Thursdays. The exercise intensity was characterized as moderate intensity according to the Borg Intensity Scale [32], the duration of the activities was from 45 to 60 min of continuous physical activity per day, the type of exercise chosen being the resistance training accompanied by specific heating.

Both the normotensive group and the hypertensive group underwent the intervention of the following protocol, divided into three phases:

1. *Adaptation phase:* 3 weeks before the beginning of the 6-month resistance training protocol, involved six sessions in which emphasis was placed on learning the technique, self-awareness of the body strength intensity of each of the participants and on the coordination of movements;
2. *Basic phase:* the initial phase of the 6-month resistance training protocol, 17 weeks and 34 sessions, the objective of which was to increase muscle strength. This phase was characterized by maintaining the training volume and increasing the intensity throughout the phase;
3. *Specific phase*: final phase of the resistance training protocol, 10 weeks and 20 sessions, to intensify the process of strength gain and muscle hypertrophy. The training sessions in this phase were characterized by reductions in volume and increases in training intensity, with the inclusion of the method of progressive loads in the sessions.

During the intervention protocol, the following exercises were used: pec fly, hip adductor, lateral pulldowns, leg extensions, leg curls, alternate dumbbell curls, leg presses 45, triceps pulley, side elevations, leg curls and sit ups. When necessary, adaptations were made over the months of intervention. If the individual had any restriction or difficulty in performing the proposed exercise, an adaptation to the movement was performed, always trying to maintain the same muscle group, thus avoiding leaving the proposed protocol. Replacement example: a lateral pulldown exercise replaced by a low row, both exercises for the dorsal muscle.

Anthropometric evaluation

All anthropometric parameters were assessed according to the recommendations of the International Society for the Advancement of Kinanthropometry [33].

1. *Height:* the total height of the participants was determined by measuring the distance between the soles of the feet and the vertex of the head using a portable stadiometer (Gofeka, Criciúma, SC, Brazil) with an accuracy of 0.1 cm.
2. *Body mass:* body weight was determined by the individual's total body mass using a digital scale (Urbano OS-100, Canoas, RS, Brazil) with an accuracy of 50 g.
3. *Evaluation of the BMI:* the BMI was calculated by dividing the body mass of each participant by the square of their height expressed in meters, as recommended by the Brazilian Association for the Study of Obesity and Metabolic Syndrome (2010) [34].

Body fat percentage

To assess the fat percentage, a bioimpedance scale (Tanita BC-601 body composition model) was used. The patients remained at rest for 10 min and then electrodes were attached to the feet and hands, from which a low-frequency electrical current was triggered to obtain the results.

Blood pressure assessment

For BP measurements, a mercury column sphygmomanometer (Erkamater – E300; Berlin, Germany) was used. The procedures were performed according to the recommendations in the '7th Brazilian Guideline for Hypertension' [1], with measurements taken while participants were at rest. As a routine for monitoring, a BP measurement was performed before a training session, at least once a week, for all research participants, with an automatic BP monitor (Omron HEM-742INT; Omron Co.,Ltd., Shanghai, China).

Heart rate assessment

Each participant remained at rest before starting the exercise protocol, seated for a period of 5 min, and after that time, their heart rate (HR) was checked using the same automatic BP monitor (Omron HEM-742INT).

Blood collections

Three tubes of 4 ml of blood were collected, two tubes with EDTA as anticoagulant and one without anticoagulant, by venipuncture of the antecubital vein using a vacuum system. The entire blood collection procedure was performed by trained and qualified professionals and in compliance with all recommended biosafety procedures for this purpose. After the collections, all samples were prepared, properly stored and sent to the Biochemistry, Microbiology and Immunology Laboratory at UFFS, Chapecó campus, for analysis.

Isolation of mononuclear cells

Mononuclear leukocytes were isolated from blood collected with EDTA and separated on Ficoll-Histopaque density gradients as described by Böyum [35].

Determination of the levels of inflammatory markers

CRP was assessed by the immunoturbidimetry method, based on the serum of patients in the hypertensive and control groups. The analysis of IL-6 and IL-10 was performed using the electrochemiluminescence method from the frozen serum of patients in both groups. These measurements were performed by the laboratory 'Diagnósticos do Brasil' in the city of Chapecó/SC.

Protein determination

Protein was measured by the Coomassie blue method according to Bradford [36], using serum albumin as standard.

Determination of ATP levels

The quantitative determination of ATP in serum was developed using the commercial bioluminescence assay kit with recombinant firefly luciferase and its substrate D-luciferin in the serum of the hypertensive group and in the control group. The assay is based on ATP luciferase, with a maximum emission of ~560 nm at pH 7.8 [37]. The reaction components, as follows, were combined to make a reaction solution pattern and adjust volumes according to each requirement. Each reaction contained 1.25 μg/ml of firefly luciferase, 50 μmol/l of D-luciferin and 1 mmol/l DTT in 1× reaction buffer. After 15 min of incubation, luminescence was measured.

Ectonucleotidase-nucleoside triphosphate diphosphohydrolase activity determination

After lymphocyte isolation, ecto-NTPDase activity was determined as described by Leal *et al.* [38] wherein the reaction medium contained 0.5 mmol/l $CaCl_2$, 120 mmol/l NaCl, 5 mmol/l KCl, 6 mmol/l glucose and 50 mmol/l Tris–HCl buffer at pH 8.0, with a final volume of 200 ml. Twenty microliters of the intact mononuclear cells suspended in physiological saline solution were added to the reaction medium (2–4 mg of protein) and preincubated for 10 min at 378 °C and incubation proceeded for 70 min. The reaction was initiated by the addition of substrate (ATP or ADP) to a final concentration of 2.0 mmol/l and stopped with 200 ml of 10% TCA. The released inorganic phosphate (Pi) was assayed by Chan *et al.* method (described in Cardoso *et al.* [28]) using malachite green as the colorimetric reagent and KH_2PO_4 as the standard. Controls were carried out by adding the enzyme preparation after TCA addition to correct for nonenzymatic nucleotide hydrolysis. All samples were run in triplicate and the specific activity is reported as nmol Pi released per min/mg of protein.

Adenosine deaminase activity determination

ADA activity from lymphocytes was determined according to Giusti and Galanti [39] on the basis of the Bertholet reaction, that is the formation of a colored indophenol complex from ammonia released from adenosine and quantified spectrophotometrically. Briefly, 25 ml of lymphocytes reacted with 25 ml of 21 mmol/l of adenosine pH 6.5 and was incubated at 378 °C for 60 min. This method is based on the direct production of ammonia when ADA acts in excess of adenosine. The protein content for lymphocytes experiment was adjusted between 0.1 and 0.2 mg/ml. Results were expressed in U/l. One unit (1 U) of ADA is defined as the amount of enzyme required to release 1 mmol of ammonia per minute from adenosine at standard assay conditions.

In-vitro tests

When the NTPDase, and ADA activities were tested *in vitro* in the presence of diuretics, angiotensin-converting enzyme inhibitors, beta blockers and AT1 antagonists' drugs, the drugs were diluted in 100% water, to a final concentration according to bioavailability, and added to the assay tubes.

Western blot of ectonucleotidase-nucleoside triphosphate diphosphohydrolases 1 and 2

Electrophoresis was performed with 12% polyacrylamide in a 'Bio-Rad Mini-Protean III' device. The peripheral blood lymphocytes were lysed in internal microtubes containing an extraction buffer (50 mmol/l of Tris–HCl, 1 mmol/l of EDTA, 1 mmol/l of phenylmethylsulfonyl fluoride, pH 7.5), with glass beads and in vortex for 1 min, twice, on ice. The samples were centrifuged at 10 000 × *g* for 20 min at 4 °C. The proteins present in the supernatant, determined by colorimetric assay [24], were diluted (1 : 1, v : v) in the Bio-Rad Laemmli sample buffer (62.5 mmol/l Tris–HCl, pH 6.8; glycerol a 25%, 2% SDS, 0.01% bromophenol blue) and then loaded (10 mg) and separated by size in 15% SDS–PAGE (100 V). The running buffer used contained 25 mmol/l of Tris, 192 mmol/l of glycine and 0.5% SDS. The proteins were transferred to a polyvinylidene difluoride membrane for 1 h (Bio-Rad, Hercules, California, USA) in buffer containing 25 mmol/l of Tris, 192 mmol/l of glycine and 20% methanol. Subsequently, the membrane was incubated with polyclonal antibody antiecto-NTPDase1 and antiecto-NTPDase2 (Québec, Canada; http://ectonucleotidases-ab.com) at room temperature overnight. Primary antibodies were used at a 1 : 400 dilution. The amount of protein was corrected to load a fixed protein concentration (1 mg) in 12% SDS–PAGE, and was determined based on experiments using different protein concentrations. The membranes were developed using the phosphatase substrate, nitro blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate [40].

Statistical analysis

First, the data were submitted to the Shapiro–Wilk test to verify its normality. As the data follows a normal distribution, the difference between the means was statistically analyzed by the two-way analysis of variance using the statistical program GraphPad Prism version 8.0 (GraphPad Software, San Diego, California, USA). Correlations were performed by using Pearson's correlation test. The differences were considered significant when the P value was less than 0.05. The variables are presented as mean ± SD.

RESULTS

The average age of participants in the normotensive group was 53.6 ± 3.6 years, while the mean height was 1.65 ± 0.08 m. In the hypertensive group, the mean age of the participants was 55.17 ± 4.3 years and the average height was 1.64 ± 0.08 m. After 6 months of resistance training, there was a change in the BMI of the normotensive group from the overweight range to the normal weight range (25.2 ± 4.5 vs. 24.5 ± 4.4). physical activity was also able to promote a significant reduction in SBP levels in the group of hypertensive patients (128.1 ± 3.7 mmHg in the pretest vs. 121.1 ± 1.7 mmHg after 6 months, $P < 0.05$) (Table 1). There were no significant changes in body mass, body fat percentage, HR and DBP of both groups, as well as in the SBP of the normotensive group and in the BMI of the hypertensive group, which is in the overweight range (Table 1). However, it is important to note that in hypertensive group the BMI reduced ∼3 unit (from 29.85 to 27.97) and body fat percentage reduced ∼2% (from 36.2 to 34.7).

Table 2 shows the antihypertensive drugs used by participants in the hypertensive group. Twenty-one participants (67.74%) use diuretics, 20 participants (64.51%) use Angiotensin-converting enzyme inhibitors, six patients (19.35%) use beta blockers and five participants (16, 12%) use AT1 antagonist. In-vitro tests by using the antihypertensive medicines taken by the hypertensive women did not show statistical differences (data not shown) in the purinergic system enzymes activities.

In the analysis of the inflammatory profile (Fig. 1), it can be observed that the plasma levels of IL-6 (Fig. 1a) were significantly elevated in the hypertensive group when compared with the normotensive group in the pretest (6.5 ± 0.7 vs. 4.3 ± 0.3 pg/ml, $P < 0.05$), which remained elevated in the hypertensive group, but showed a tendency to decrease after 6 months of resistance training (6.5 ± 0.7 vs. 5.5 ± 0.5 pg/ml). The plasma levels of IL-10 (Fig. 1b) showed a significant increase after 6 months of resistance training in the normotensive group when compared with the pretest (7.3 ± 0.4 vs. 6.1 ± 0.3 pg/ml, $P < 0.05$) and in the hypertensive group when compared with the pretest (7.1 ± 0.3 vs. 5.5 ± 0.4 pg/ml, $P < 0.05$), in the same period. Regarding the CRP analysis (Fig. 1c), it can be seen that hypertensive patients had high serum CRP levels compared with the normotensive group in the pretest (1.22 ± 0.1 vs. 0.63 ± 0.2 mg/l, $P < 0.05$). After performing resistance training, a reduction in CRP levels can be observed in the hypertensive group when compared with the pretest period (0.95 ± 0.1 vs. 1.22 ± 0.1 mg/l, $P < 0.05$). In the normotensive group, no other changes were observed. It is important to highlight the positive correlation found between IL-6 and SBP as well as other proinflammatory parameters (Fig. 2).

TABLE 2. Antihypertensive medicines taken by the hypertensive group

Medicine	Number of participants	Percentage (%)
Diuretics	21	67.74
Angiotensin-converting enzyme inhibitors	20	64.51
Beta blockers	6	19.35
AT1 antagonists	5	16.12

With the analysis of serum ATP levels (Fig. 1d), it can be seen that in the pretest the hypertensive group has higher levels of ATP compared with the normotensive group (0.34 ± 0.0 vs. 0.3 ± 0.0 μmol/ATP, $P < 0.05$). In the hypertensive group, after 6 months of resistance training, a reduction in serum ATP levels can be observed when compared with the pretest (0.29 ± 0.0 vs. 0.34 ± 0.0 μmol/ATP, $P < 0.05$). There were no changes in the normotensive group.

Regarding the ATP hydrolysis (Fig. 3a), it can be noted that in the pretest the hypertensive group shows an increase in hydrolysis when compared with the normotensive group (8.48 ± 0.7 vs. 6.28 ± 0.3 nmol Pi/min/mg ptn, $P < 0.05$). After 6 months of resistance training, a reduction in ATP hydrolysis can be observed in the hypertensive group when compared with the pretest (6.76 ± 0.5 vs. 8.48 ± 0.7 nmol Pi/min/mg ptn, $P < 0.05$). There were no significant changes in the normotensive group.

According to the hydrolysis of the ADP (Fig. 3b), it can be perceived that the hypertensive group presents, in the pretest, an increase in the levels of hydrolysis of the ADP compared with the normotensive group in the same period (4.35 ± 0.3 vs. 3.24 ± 0.4 nmol Pi/min/mg ptn, $P < 0.05$). However, in the hypertensive group, after 6 months of resistance training, there was a reduction in the hydrolysis of ADP when compared with the pretest (3.26 ± 0.3 vs.

TABLE 1. Sample characterization

	CT (pretest)	HYP (pretest)	CT (posttest)	HYP (posttest)
Age (years)	53.64 ± 3.6	56.17 ± 4.3		
High (m)	1.63 ± 0.1	1.62 ± 0.1		
Body mass (kg)	66.61 ± 2.6	71.21 ± 3.5	63.45 ± 3.3	67.76 ± 4.4
BMI	25.22 ± 4.5	29.85 ± 4.9	24.59 ± 4.4	27.97 ± 4.7
SBP (mmHg)	118.9 ± 1.7	128.1 ± 3.7[a,b]	115.4 ± 1.6	121.1 ± 1.7[a,b]
DBP (mmHg)	78.6 ± 1.2	84.7 ± 1.6	76.9 ± 1.3	82.2 ± 1.8
Body fat percentage (%BF)	32.3 ± 9.7	36.2 ± 10.1	30.6 ± 8.2	34.7 ± 7.7
Heart rate	75.67 ± 9.7	79.50 ± 10.5	69.13 ± 5.1	74.56 ± 7.8

Age, anthropometric and hemodynamic parameters of control group (normotensive woman) and hypertensive group in the pretest and posttest. Values of age, high, body mass, BMI, SBP, SBP, body fat percentage and heart rate evaluated in both control group (normotensive woman) and hypertensive group at the pretest (before RT) and posttest (after 6 months of RT). Data are presented as mean ± SD. Statistical analysis two-way ANOVA, considering $P < 0.05$. ANOVA, analysis of variance; CT, control group; HYP, hypertensive group; RT, resistance training.
[a]Indicates statistical difference between groups at the same moment.
[b]Indicates statistical difference between pretest and posttest in the same group.

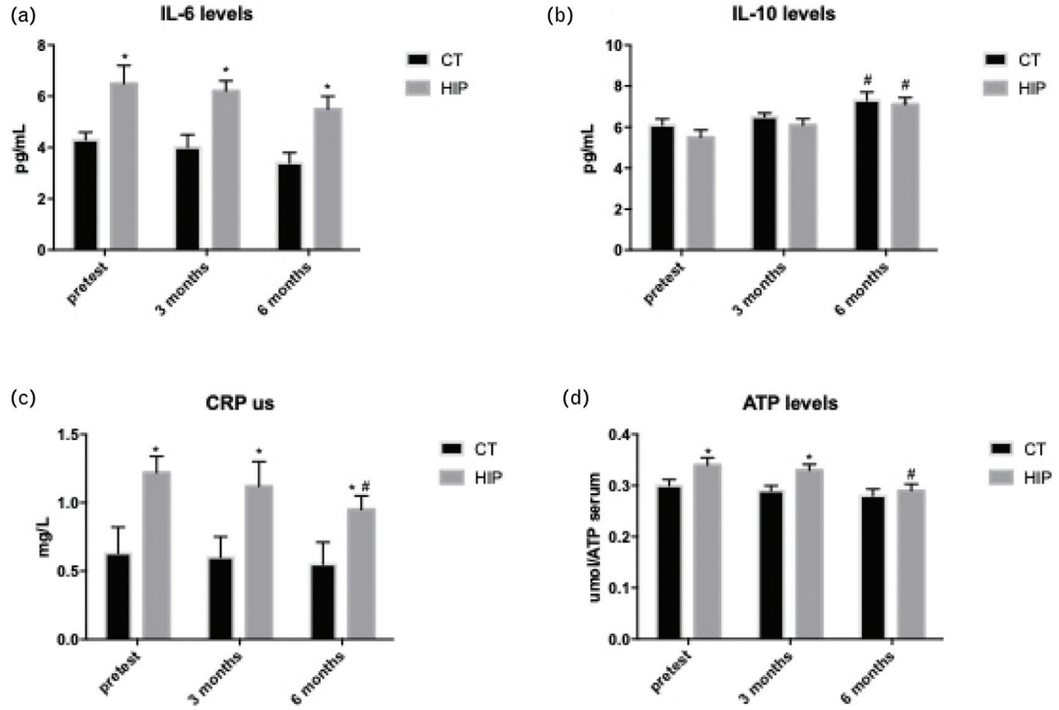

FIGURE 1 Inflammatory parameters evaluated before (pretest), after 3 months and after 6 months (posttest) of resistance training protocol in control group [(CT); normotensive woman] and hypertensive group (HIP). (a) Serum levels of IL-6, (b) serum levels of IL-10, (c) serum levels of C-reactive protein, (d) serum levels of ATP. Data are presented as mean ± SD. Statistical analysis two-way analysis of variance, considering $P < 0.05$. *Indicates statistical difference between groups at the same moment. #Indicates statistical difference between pretest and posttest in the same group.

4.35 ± 0.3 nmol Pi/min/mg ptn, $P < 0.05$). There were no significant changes in the normotensive group.

Regarding the activity of ADA (Fig. 3c), it is observed that the hypertensive group has increased activity of the enzyme in relation to the normotensive group in the pretest (4.69 ± 0.3 vs. 2.68 ± 0.3 U/l, $P < 0.05$). Although, after 6 months of resistance training, a reduction in ADA activity was observed in the hypertensive group when compared

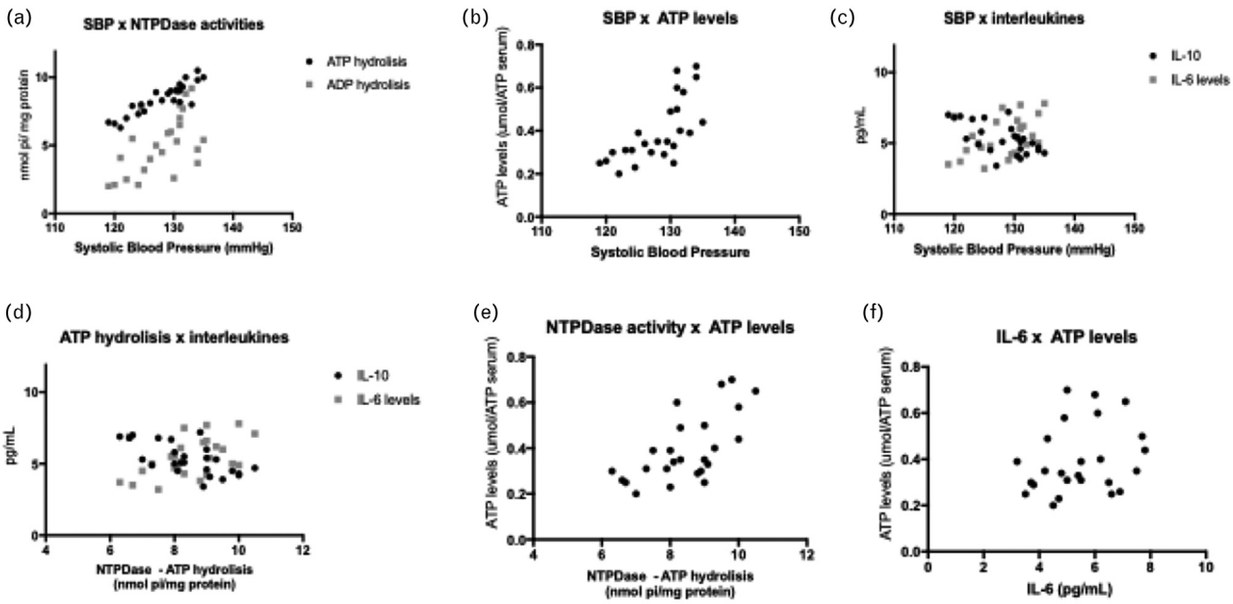

FIGURE 2 Pearson's correlations between variables at the pretest moment in hypertensive group. (a) Positive correlation between SBP and nucleoside triphosphate diphosphohydrolases activities ($r = 0.8917$ for ATP hydrolysis and $r = 0.5713$ for ADP hydrolysis). (b) Positive correlation between SBP and ATP levels ($r = 0.6999$). (c) Positive correlation between SBP and IL-6 levels ($r = 0.4442$); negative correlation between SBP and IL-10 levels ($r = 0.6169$). (b) Positive correlation between nucleoside triphosphate diphosphohydrolase (hydrolyzing ATP) and IL-6 levels ($r = 0.4511$) and negative correlation between nucleoside triphosphate diphosphohydrolase (hyprolyzing ATP) and IL-10 levels ($r = -0.4772$). (e) Positive correlation between nucleoside triphosphate diphosphohydrolase (hydrolyzing ATP) and ATP levels ($r = 0.5522$). (f) Positive correlation between ATP levels and IL-6 levels ($r = 0.4959$). $P < 0.05$ for all analysis.

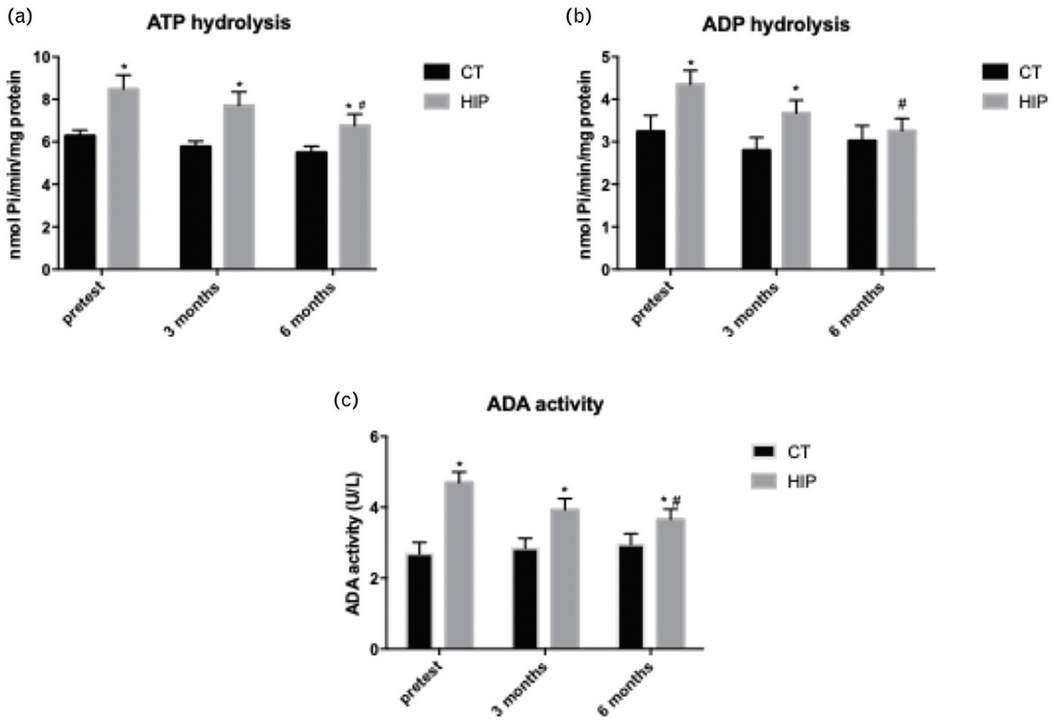

FIGURE 3 Ectonucleotidases activities evaluated before (pretest), after 3 months and after 6 months (posttest) of resistance training protocol in control group [(CT); normotensive woman] and hypertensive group (HIP). (a) ATP hydrolysis (nucleoside triphosphate diphosphohydrolase acitivity). (b) ADP hydrolysis (nucleoside triphosphate diphosphohydrolase activity). (c) Adenosine deaminase activity. Data are presented as mean ± SD. Statistical analysis two-way analysis of variance, considering $P < 0.05$. *Indicates statistical difference between groups at the same moment. #Indicates statistical difference between pretest and posttest in the same group.

with the pretest (4.69 ± 0.3 vs. 3.65 ± 0.3 U/l, $P < 0.05$). The expression of the enzymes NTPDase1 (Fig. 4a) and NTPDase2 (Fig. 4b) analyzed by means of western blot did not present significant alterations in the studied period.

Table 3 shows the correlations between almost all studied variables. The most important correlations results were plotted in Fig. 2. In hypertensive group, at pretest moment, there was a positive correlation between SBP and NTPDase activities ($r = 0.8917$ for ATP hydrolysis and $r = 0.5713$ for

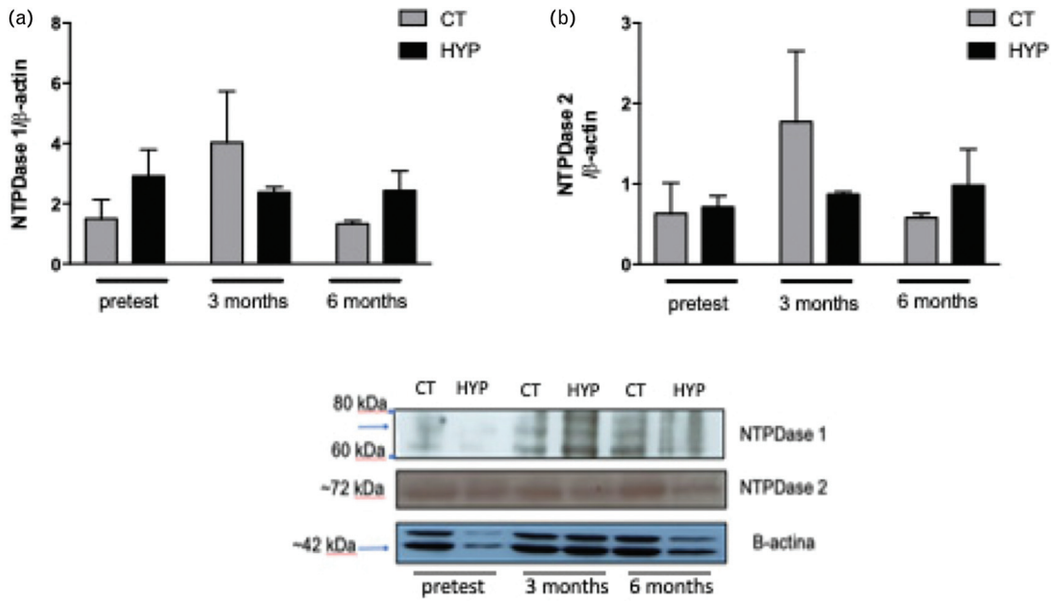

FIGURE 4 Nucleoside triphosphate diphosphohydrolases 1 and 2 expression (Western blotting) evaluated before (pretest), after 3 months and after 6 months (posttest) of resistance training protocol in control group (CT; normotensive woman) and hypertensive group. Data are presented as mean ± SD. Statistical analysis two-way analysis of variance, considering $P < 0.05$.

TABLE 3. Pearson's correlations between almost all variables at the pretest (before resistance training) and posttest (after 6 months of resistance training) moments in hypertensive group

	Hypertensive group before RT (pretest)	Hypertensive group after 6 months of RT (posttest)	Normotensive group before RT (pretest)	Normotensive group after 6 months of RT (posttest)
Variables	*r* value		*r* value	
SBP × NTPDase (ADP)	0.5713[a]	0.4923[a]	0.4233	0.3988
SBP × NTPDase (ATP)	0.8917[a]	0.8199[a]	0.4566[a]	0.4461[a]
SBP × ADA activity	0.3921	0.4162	0.3722	0.3619
SBP × ATP levels	0.6999[a]	0.7218[a]	0.5522[a]	0.5934[a]
SBP × IL-6 levels	0.4442[a]	0.5194[a]	0.4339[a]	0.4669[a]
SBP × IL-10 levels	−0.6169[a]	−0.5921[a]	−0.4211[a]	−0.5185[a]
SBP × CRP levels	0.4288[a]	0.4109[a]	0.3902	0.4081
NTPDase (ATP) × IL-6 levels	0.4511[a]	0.5239[a]	0.4323[a]	0.4138
NTPDase (ATP) × IL-10 levels	−0.4772[a]	−0.4955[a]	−0.4378[a]	−0.4562[a]
NTPDase (ATP) × ATP levels	0.5522[a]	0.4977[a]	0.4799[a]	0.4911[a]
NTPDase (ADP) × IL-6 levels	0.3798	0.3902	0.2989	0.3212
NTPDase (ADP) × IL-10 levels	−0.2845	−0.1199	−0.2349	−0.2187
NTPDase (ADP) × ATP levels	0.4188	0.3082	0.3399	0.3976
ADA activity × IL-6 levels	0.2734	0.3045	0.2987	0.3122
ADA activity × IL-10 levels	− 0.3911	−0.2908	−0.2166	−0.3141
ADA activity × ATP levels	0.2994	0.3853	0.2344	0.2956
ATP levels × IL-6 levels	0.4959[a]	0.5368[a]	0.4233[a]	0.4585[a]
ATP levels × IL-10 levels	−0.4333	−0.5110[a]	−0.5444[a]	−0.5114[a]
%BF × IL-6 levels	0.4111[a]	0.4200[a]	0.4109[a]	0.4803[a]
%BF × IL-10 levels	−0.3276	−0.4003	−0.2991	−0.3905
BMI × IL-6 levels	0.1976	0.2078	0.2110	0.1790
BMI × IL-10 levels	−0.1892	0.2888	0.1903	0.1994

%BF, body fat percentage; ADA, adenosine deaminase; CRP, C-reactive protein; NTPDase, nucleoside triphosphate diphosphohydrolase; RT, resistance training.
[a]Indicates statistical significance $P < 0.05$.

ADP hydrolysis), ATP levels ($r = 0.6999$), IL-6 levels ($r = 0.4442$) and levels CRP ($r = 0.4288$). A negative correlation was found between SBP and IL-10 levels ($r = 0.6169$). NTPDase (hydrolyzing ATP) positively correlates to ATP levels ($r = 0.5522$) and IL-6 levels ($r = 0.4511$) and negatively correlates to IL-10 levels ($r = -0.4772$). ATP levels ($r = 0.4959$) body fat percentage ($r = 0.4111$) positively correlates to IL-6 levels. In normotensive group, at pretest moment there was a positive correlation between SBP and NTPDase activity (hydrolyzing ATP $r = 0.4566$), ATP levels ($r = 0.5522$) and IL-6 levels. A negative correlation was found between SBP and IL-10 levels ($r = -0.4211$). NTPDase (hydrolyzing ATP) correlates positively with ATP levels ($r = 0.4799$) and IL-6 levels ($r = 0.4323$) and negatively correlates with IL-10 levels ($r = -0.4378$). ATP levels ($r = 0.4233$) and body fat percentage ($r = 0.4109$) are positively correlated with IL-6 levels. As can be seen in Table 3, after 6 months of physical exercise, the same scenario about correlations can be observed. For example, the more ATP levels, the more IL-6 levels; what confirms the relationship between analyzed variables, in both groups.

DISCUSSION

EAH is a chronic disease accompanied by an inflammatory condition, with vascular infiltration of immune cells. This scenario causes vascular changes, which actively participate in the mechanisms of elevating BP [7]. In contrast, physical activity shows positive results both in controlling BP levels and in reducing cardiovascular risk, mainly due to its anti-inflammatory effect [26,41]. High levels of muscle strength decrease the risk of death from all causes, becoming a physical component that should also be encouraged in the hypertensive population [23]. Recent studies showed that resistance training alone is able to reduce SBP and DBP in prehypertensive and hypertensive individuals, especially in the elderly, in addition to demonstrating the safety of this type of physical training [23].

Considering the importance of the purinergic system modulating immune functions and that physical activity is being considered one of the main lifestyle changes that contributes to the improvement of cardiovascular health, and that the medications for this disease are not being used properly, this study is pioneer in the investigation of the chronic effects of resistance training on the activity and expression of ectonucleotidases in lymphocytes, ATP levels and its association with classic inflammatory markers.

In our previous studies, we have already demonstrated the effects of aerobic physical exercise on purinergic system and hypertension in animal models [4,27,28]. This study, however, is the first carried out in humans to study the relationship of resistance training on purinergic signaling and inflammatory parameters in hypertensive women. Our main results demonstrate that the modulation of purinergic system components is one of the mechanisms by which resistance training exerts its anti-inflammatory properties and helps to treat hypertension. Since our study was carried out with a female population, it could be considered a limitation and other studies with a similar design would be interesting with men, to elucidate this relationship in males.

To exclude a direct effect of drugs used by patients with hypertension, in this study, we also tested NTPDase and ADA activities *in vitro* in the presence of antihypertensive drugs. All concentrations used in-vitro represented, approximately, the mean plasma values of the medications and the drugs were tested on lymphocytes from control

individuals. The results obtained demonstrate that NTPDase and ADA activities were not affected by the presence of the medications.

Our results have shown a reduction in SBP levels after 6 months of resistance training, corroborating the current literature on the hypotensive effect of resistance exercise [23] and corroborating to the information that physical exercise acts as an adjunct in the treatment of EAH [21]. We have found also a tendency to reduce the percentage of fat, body mass and BMI of both groups after resistance training – an important data, since adiposity is a risk factor for the development of complications related to EAH [42,43].

Regarding to inflammatory parameters, before resistance training, it was observed that the group of hypertensive women had increased levels of IL-6, CRP and extracellular ATP. These changes are very characteristic of the proinflammatory condition observed in EAH and these variables are interrelated, since the liver starts the production of CRP as a response to the release of IL-6 [18]. In blood vessels, CRP promotes activation of immune cells, in addition to promoting the expression of adhesion molecules and releasing signals to reduce the production of vasodilators, being an important part in the process of vascular remodeling [9,18]. In addition, the increase in IL-6 may have occurred as a result of increased levels of extracellular ATP, since ATP, when binds its purinergic receptors in lymphocytes, triggers signaling for greater production of proinflammatory cytokines [13,17,44]. Reinforcing the link between ATP release and increase of IL-6 levels, our results displayed a positive correlation between these two variables.

Corroborating the inflammatory process seen in hypertensive women, although not statistically significant, our study demonstrated a trend of the decrease in IL-10 levels in the pretest, when compared with the normotensive group. A study by Mattson [45] with hypertensive animal models, demonstrated the reduction of regulatory T lymphocytes, responsible for modulating the inflammatory response, in the group, decreasing the production of anti-inflammatory cytokines, such as IL-10, corroborating with our findings. We also observed a negative correlation between SBP and IL-10, showing that hypertension can be associated to low levels of IL-10 and high levels of ATP and IL-6. This imbalance establishes an inflammatory feedback that stimulates a cycle of chronic inflammation and worsening of the disease [9,45].

Related to anthropometric results, we did not observe significant reductions in the body fat percentage (%BF), body weight and BMI. It is important to state that resistance training improves lean body mass and, because of that, can have no effects on body weight and BMI [46]. However, we found a positive correlation between %BF and IL-6, demonstrating the association between reduction in %BF and reduction in IL-6, that is, demonstrating the association between obesity and inflammation, as well as reduction in adiposity and reduction of inflammation. These positive correlations were seen in both groups (hypertensive and normotensive) and corroborates to the literature [47,48]. Indeed, exercise per se is able to reduce proinflammatory cytokines and augment anti-inflammatory cytokines. In our study exercise was responsible for the augment in IL-10 in both groups.

In addition to the differences found in inflammatory parameters, our study also showed changes in the activity of ecto-NTPDases. In the pretest of the hypertensive group, the activity of ecto-NTPDases (hydrolyzing ATP and ADP) was high when compared with normotensive group. The augment in NTPDase (hydrolyzing ATP) positively correlates to SPB, IL-6 and ATP levels, reinforcing that enzyme activity is responding to the high ATP concentration in the extracellular milieu, and this augment in ATP is acting in lymphocytes inducing a proinflammatory response though IL-6 production [13,17]. This scenario is probably responsible to worse the disease, although further investigation is need to unveil the details.

Our results also demonstrate that ADA activity is increased in hypertensive woman before resistance training. This increase in ADA activity is probably resulting in less adenosine available in the medium. This fact explains the low levels of IL-10, since adenosine stimulates the release of IL-10 by T lymphocytes, acting as an anti-inflammatory molecule [8,13,15,17].

After performing the resistance training protocol, significant changes were observed in the inflammatory profile and in the behavior of ATP levels and ecto-NTPDases. The hypertensive group showed an increase in the plasma concentration of IL-10, in addition to a tendency to decrease levels of IL-6 and a reduction in plasma concentrations of CRP and ATP. With these changes, physical activity was able to reduce the inflammation seen in EAH, proving the immunomodulatory and adaptive effect of chronic physical exercise, considering that acute episodes of exercise promote a proinflammatory environment, promoting the transient increase in inflammatory mediators, including IL-6 [10,49]. Such immunomodulatory effects of exercise seem to be mediated by the exercise's ability to adjust and improve the number and function of regulatory T lymphocytes [50].

Literature states that the reduction of inflammatory stimuli of IL-6 contributes to the decrease in hepatic production of CRP [10,25]. We observed a positive association between IL-6 and NTPDase activity (hydrolyzing ATP) and ATP levels, which indicates that exercise acted on IL-6 levels in hypertensive patients by reducing serum ATP levels and its hydrolysis. This contributes to the reduction of purinergic receptors activation and reduces the inflammatory responses during EAH [8,10,17,26,42], The normotensive group also showed an increase in plasma concentrations of IL-10, a consequence of the modulating effect of chronic exercise [10,26,50].

Ecto-NTPDases also responded to resistance training. Responding to the decrease in serum ATP levels, there was a reduction in the activity of ecto-NTPDase and ADA, in the hypertensive group after resistance training. As a consequence, there is a reduction in the hydrolysis of ATP, ADP and adenosine [8,19], probably attenuating the responses mediated by the P2 receptor and amplifying the anti-inflammatory responses mediated by adenosine in the P1 receptors [15,19]. Adenosine binds to receptors on the cell membrane of lymphocytes, inhibiting their activation and proliferation, in addition to inhibiting the release of

pro-inflammatory cytokines and stimulating the production of IL-10 by regulatory T lymphocytes [11,13,17]. Ecto-NTPDases have the ability to mitigate inflammation, increasing the concentration of adenosine in the extracellular milieu and, consequently, decreasing the production of proinflammatory cytokines, such as IL-6 [12,13].

Pearson's correlations also show the interconnection between physical exercise, purinergic system, inflammation and hypertension. In the hypertensive group, the increase in SBP is correlated with the increase in ATP, CRP and IL-6 levels, as well as with the greater activity of NTPDases, hydrolyzing ATP and ADP. In addition to the increase in BP levels, the increase in ATP levels is also associated with increased levels of IL-6 and a high percentage of body fat correlates with higher levels of IL-6. On the other hand, the increase in IL-10 levels correlates with the decrease in SBP, as well as with lower levels of ATP and the decrease in its hydrolysis. Thus, IL-10, with its anti-inflammatory action, is able to slow the inflammatory response and decrease vascular remodeling, influencing the drop in BP levels [11,13].

In the normotensive group, although the results do not point out statistical differences, we observed a tendency toward a reduction in the hydrolysis of ATP, ADP and ADA, signaling the modulating effect of the purinergic system in these patients. This becomes more evident when looking at Pearson's correlation data, which show the interconnection between the effect of exercise on both inflammatory parameters and the purinergic system. We found that increased levels of IL-6 correlate with increased levels of ATP and with a higher percentage of body fat. In addition, we observed that the levels of IL-10 decrease with the increase in ATP hydrolysis and that high levels of ATP correlate with the decrease in levels of IL-10, proving that the anti-inflammatory effects are due to mechanisms related to modulation of purinergic signaling.

It is important to highlight that these correlations were observed in both groups. in the hypertensive group and in the normotensive group. It was observed that the increase in SBP was correlated with the same variables, both in the hypertensive group and in the normotensive group, and the same is seen with the variables correlated with the decrease in SBP. This makes evident a link between the immune system and the purinergic system and reinforces the anti-inflammatory modulating effect of the purinergic system both in patients in the hypertensive group and in healthy participants (normotensive group).

In conclusion, the effect of resistance training on ecto-nucleotidases may be one of the most important mechanisms revealed in this study in relation to the benefits of physical activity for the treatment of EAH. This study uses as a 'control group without exercise influence' the values of the variables found in the volunteers of both groups in the pretest when the patients were sedentary, as a way of reducing the interference that other two groups with different people may cause in the results. However, the lack of a control group (with different participants) without exercise protocol intervention could be a small limitation of this study, especially for the hypertensive individuals.

The enzymatic modulation promoted by resistance training regulates the levels of nucleotides and nucleosides in the extracellular environment and also regulates the function of immune cells. Thus, resistance training promotes an anti-inflammatory environment by increasing the availability of adenosine and decreasing inflammation, in addition to reducing BP levels and improving the anthropometric profile, in women. Further studies in the male population are needed to assess the clearest form of these results in men. This mechanism proves to be an important therapeutic tool to be explored for the management of EAH and for other diseases that cause chronic inflammation in the body.

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Conflicts of interest

This is an academic work; thus, authors have no conflict of interest.

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