






Evaluation of serum arginase in overweight women under the acute effect of physical exercise - exploratory study

Avaliação da arginase sérica em mulheres com excesso de peso sob o efeito agudo do exercício físico - estudo exploratório

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ABSTRACT

Introduction: Obesity is a multifactorial condition related to the increase of serum arginase, favoring endothelial dysfunction. Physical exercise is a protective factor for endothelial homeostasis. Thus, knowing whether the level of arginase is modified by a physical exercise session is important. **Objective:** To verify the level of arginase in women with excess weight before and after a physical exercise session and evaluate the association between the level of arginase and the BMI of these patients. **Methods:** Exploratory study, which used the serum bank of a randomized clinical trial, in which sedentary women 18 to 30 years old, BMI > 24.9 kg/m² were included. Women with diseases that could alter the levels of arginase were excluded. The volunteers were randomized to the intervention group - a light intensity exercise session (EG) and a control group (CG). The serum arginase of 11 women in the CG and 9 in the EG was measured before and 24h after the intervention. Data analysis was performed using the SPSS 20 program using the unpaired Student's t-test and the Mann-Whitney U test. Statistical significance is defined as p < 0.05. **Results:** The patients' BMI was = 29 ± 4.7 kg/m². The intra-group analysis showed no variation in serum arginase before and after the intervention, as well as in the intergroup comparison using the arginase delta. There was no correlation between the level of arginase and BMI. **Conclusion:** A session of physical exercise did not change the levels of serum arginase in overweighted women.

Keywords: obesity; physical exercise; arginase.

RESUMO

Introdução: A obesidade é uma condição multifatorial apresentando relação com a elevação de arginase sérica, favorecendo a disfunção endotelial. O exercício físico é fator protetor para homeostase do endotélio. Assim, conhecer se o nível de arginase é modificado por uma sessão de exercício físico é de suma importância. **Objetivo:** Verificar o nível de arginase sérica em mulheres com excesso de peso antes e após uma sessão de exercício físico e avaliar a associação entre o nível de arginase sérica e o IMC dessas pacientes. **Métodos:** Estudo exploratório, que utilizou a soroteca de um ensaio clínico randomizado, no qual mulheres sedentárias com idade entre 18 e 30 anos, IMC > 24,9 kg/m² foram incluídas. Excluídas mulheres com doenças que pudessem alterar os níveis de arginase. As voluntárias foram randomizadas para grupo intervenção - uma sessão de exercício de leve intensidade (GE) e grupo controle (GC). A arginase sérica de 11 mulheres do GC e 9 do GE foi dosada, antes e 24h após a intervenção. Análise dos dados foi realizada no programa SPSS 20 através do teste t de Student não pareado e Teste de Mann-Whitney. Significância estatística definida como p < 0,05. **Resultados:** O IMC das pacientes foi = 29 ± 4,7 kg/m². A análise intragrupo não demonstrou variação da arginase sérica antes e após a intervenção, bem como na comparação intergrupo através do delta de arginase. Não se observou correlação entre nível de arginase e IMC. **Conclusão:** Uma sessão de exercício físico não modificou os níveis de arginase sérica em mulheres com excesso de peso.

Palavras-chave: obesidade; exercício físico; arginase.

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Introduction

Obesity, according to the World Health Organization (WHO), can be understood as a condition of multifactorial origin (historical, ecological, economic, social, cultural, political, and genetic), resulting from a positive energy balance, favoring the accumulation of fat and adding health risks due to its metabolic implication in blood pressure, cholesterol levels, serum triglycerides and insulin resistance [1,2].

Overweight and obesity are found with high prevalence, since the age of 5, in all Brazilian regions [3]. According to data from Vigitel 2017, 53% of women in Salvador are overweight (BMI ≥ 25 kg/m²), and 20.4% are obese (BMI ≥ 30 kg/m²) [4]. Until the end of 1980, this condition was growing modestly, but tripled in the last 20 years and, in the last 6 years, the increase is higher than 1% per year. The intake of high energy density foods and the non-use of these calories by the human body are essential parts of the metabolic imbalance, denoting the importance of both food reeducation and physical exercise [3].

The increase in the body mass index is related to the serum elevation of arginase [5], an enzyme that is responsible for the hydrolysis of L-arginine, the principal substrate for the formation of nitric oxide (NO) [6-9]. NO has many functions in the body, including its contribution to endothelial relaxation [10]. Once in a reduced quantity, as in the overweight population, there will be endothelial dysfunction due to the absence of nitric oxide, in addition to an increase in vasoconstrictor factors [11,12].

In contrast, physical exercise, both acute and chronic, increases NO concentration, promoting positive adjustments in the cardiovascular, hepatic, skeletal muscle systems, among others [10]. This increase occurs through several mechanisms, but most of the studies that explain them were carried out in animal models, such as those carried out by Lee-Young et al. [13], Chies et al. [14], Faria et al. [15], and Long et al. [16]. Thus, it becomes necessary to investigate the topic in a more specific population of human beings, searching for both biomolecular and genetic markers [10].

Since arginase is an enzyme that is elevated in obese individuals and is related to the increase in morbidity and mortality because of cardiovascular diseases due to increase in total peripheral resistance in consequence of deficient endothelium-dependent vasodilation, in addition to the scarcity of studies in humans, knowledge of their levels in this population before and after an exercise session is necessary. Thus, this study aimed to verify the level of serum arginase in women with an excess weight under the acute effect of physical exercise, as well as to evaluate the association between the level of basal serum arginase and the BMI in overweighted women.

Methods

Population and study design

This study used the serum bank of a randomized clinical trial, registered in the Clinical Trial under the protocol NCT03170973, with an accessible population of

the School-Clinic at Faculdade Adventista da Bahia, Cachoeira/BA, Brazil. It was submitted to the Research Ethics Committee of Faculdade Adventista da Bahia, approved under protocol 34017514.5.0000.0042. The collections were performed from September 2015 to May 2016, and throughout the study, the guidelines on research with human beings, Resolution 466/2012 of the National Health Council, were observed.

The study population that generated the serum bank was 66 volunteers, selected randomly and invited to participate in the study. The inclusion criteria used were: female gender, 18 to 30 years old, BMI > 24.9 kg/m², and physical inactivity. The latter determined based on the International Physical Activity Questionnaire - long version [17]. The exclusion criteria were: women with parenchymal cardiovascular, metabolic, and renal diseases, hypothyroidism or diabetes mellitus, history of alcoholism or smoking, lipid-lowering drugs, corticosteroids, diuretics, beta-blockers, and contraceptives usage.

Sample selection and intervention protocol

The group of 66 women selected according to these criteria was randomly divided, based on a draw, into two groups, exercise, and control, both with 33 volunteers. For this study, considering its initially exploratory character, about one-third of the initial samples of each group were selected at random. In the exercise group, the first blood collection occurred after 12 hours of fasting in the antecubital vein. After 12 hours of this collection, the patients underwent a physical exercise session on a treadmill, divided into 3 times: warming up, conditioning, and cooling down. The warm-up was 7 minutes, the conditioning time corresponded to the energy expenditure of 250Kcal [18], and the cool-down lasted 5 minutes, with the participants' average time in the physical activity being 37 ± 8 min.

The intensity control was determined by speed, the treadmill inclination was maintained at 0° throughout the activity, and the intensity used was light, based on Borg's perception of effort [19], that is, on the original scale corresponded to a value between 9 and 11. For a better understanding of this scale, anchoring was carried out before the day of the exercise, accustoming the volunteers to respond appropriately when asked about the exercise intensity.

A polar cardio-frequency meter was used, which measured energy expenditure based on body mass, sex, and age, and after the exercise session, they were instructed to return home and maintain their usual diet. After fasting for 12 hours, they returned to the laboratory to collect the post-exercise blood sample, with a 24-hour interval between collections. The women in the control group were submitted to the same protocol as the experimental group, but they did not perform the exercise in the two previous days or 12 hours after the first and second collections, respectively. The blood collection and exercise protocol are shown in Chart 1, described in the control and exercise groups.

Chart 1 - Blood collection and exercise in the Exercise and Control groups

	1 st Sample collection	Exercise on a treadmill (12h after the 1 st collection)			Post-exercise	2 nd Sample collection
Exercise (n = 33)	12h Fasting; Antecubital vein	Light intensity; treadmill inclination at 0°; Mean time of 37 ± 8 min			Usual diet at home	12h Fasting; Antecubital vein
		Warm up	Conditioning	Cool down		
		7 minutes	Energy expenditure of 250Kcal	5 minutes		
Control (n = 33)	12h Fasting; Antecubital vein	7 minutes				12h Fasting; Antecubital vein

The patients, both in the exercise group and in the control group, were evaluated regarding the diet of the day before the exam using a 24-hour food record, made through an interview conducted at the time of blood collection. The volunteers reported what they had consumed the previous day in and between the three main meals.

Serum samples storage and arginase dosage

The samples consisted of 5mL of blood in tubes with EDTA, centrifugation at a speed of 3,000 rotations/min for 10 minutes after collection; the serum was aliquoted and frozen at -80 °C for further analysis. Among the 66 patients, 11 patients in the control group (17, 40, 45, 35, 46, 47, 42, 12, 33, 51, and 57) and 11 patients in the exercise group (24, 36, 30, 50, 20, 58, 56, 28, 64, 66, and 7) were randomly selected by the sample number. However, at the time of dosing, hemolysis was observed in samples 24 and 36. Serum arginase dosage was performed on the plasma samples at the NUPS Laboratory (Health Research Center) of the *Escola Bahiana de Medicina e Saúde Pública*.

The kit used was an enzymatic immunoassay, sandwich ELISA, which has high sensitivity and excellent specificity for detecting arginase. The duplicate method was used for the arginase curve. The serum samples were separated previously for thawing while the solutions were prepared. First, the standard solution was reconstituted with 1.0mL of standard diluent (SD), with a concentration of 1.600 ng/mL. This was then diluted to 200 ng/mL and is considered the highest standard [20]. Then, 7 tubes containing 0.5 mL of the SD were separated for a serial dilution starting from the sample with 200 ng/mL (tube 1 to tube 7) and the last one with 0ng/mL (tube 8). The concentrations obtained are described in Chart 2. Two detection reagents, A and B, diluted 100 times with their respective diluents were used. The washing solution (WS) was diluted in 10 mL to 190 mL of distilled water, resulting in 200 mL of WS [20].

Chart 2 - Dilution concentrations

Tube	*	1	2	3	4	5	6	7	8
ng/mL	1,600	200	100	50	25	12.5	6.25	3.12	0

For analysis, 14 wells were separated to place the standard solution, 2 for the blank, and 80 for the patient samples, as shown in Figure 1. 100 μ L of the respective substances were placed in each well, covered with the sealer, and incubated for 1 hour at 37°C. The liquid was removed but without washing. Then, 80 μ L of detection reagent A was added to each well, and after closing with sealer, it was incubated for another 1 hour at 37°C. In this step, the washing was performed with 350 μ L of WS waiting 1-2min and drying with absorbent paper, repeating the process 3 times [20].

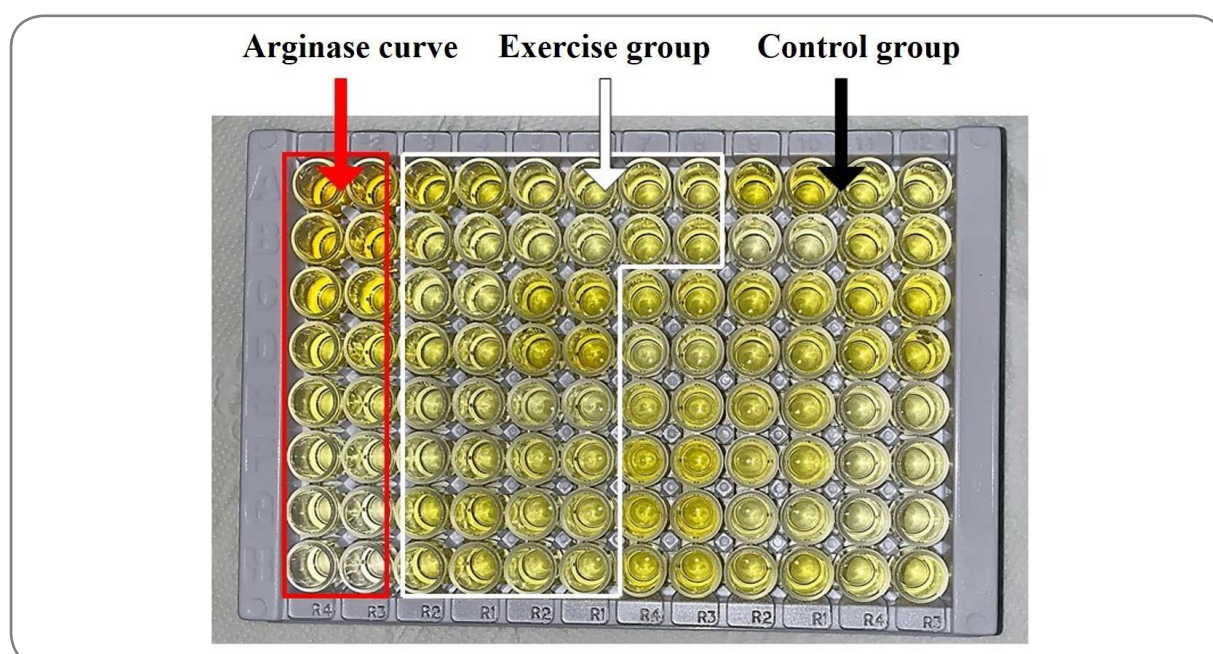


Figure 1 - ELISA plate kit for detecting arginase filled with the curve and samples from the exercise and control groups

Then, 80 μ L of detection reagent B was added to each well, closing again with the sealer and incubating for another 30 minutes at 37°C. New washings were performed 5 times. The substrate solution was added at a concentration of 90 μ L in each well, covered with a new sealer, and incubated for 10 to 20 minutes at 37°C. After the liquid turned blue [20], 50 μ L of stop solution was added to the wells, and the color changed to yellow. There were no water droplets or fingerprints on the underside of the plate, as well as bubbles on the liquid surface. Once this was done, the measurement was performed at 450 nm [20].

Statistical analysis

The continuous, parametric variables were described as means and standard deviations and compared using the Student's t-test for paired samples. To verify the ELISA test's reliability, the mean and standard deviation of the arginase curve was used, with $R^2 = 0.9932$.

The levels of intragroup arginase have been described in medians and interquartile ranges. For intergroup analysis, the arginase delta (baseline moment after intervention) was used. The Mann-Whitney U test compared the intragroup analysis' medians and associated the arginase levels with the patients' BMI, these tests being used for the analysis of non-parametric variables.

All analyzes were performed using SPSS (Statistical Package for the Social Sciences) version 20, Excel (2010 version), adopting a significance level with a value of $p < 0.05$.

Results

The study included a serum bank of 20 women, 11 from the control group and 9 from the exercise group, chosen at random. The mean age was 24 ± 3.4 years, and the BMI = 29 ± 4.7 kg/m² in the general population. Subdividing the groups, the mean age was 24.9 ± 3.7 years old and 24.2 ± 3.1 years old in the control and exercise groups, respectively, with a p-value of 0.365. The mean BMI varied between 30.2 ± 5.1 kg/m² in the control group and 28.9 ± 4.6 kg/m² in the exercise group, with a p-value of 0.829, with no statistical significance. The variable laboratory values (total cholesterol, HDL, LDL, triglycerides, blood glucose, insulin, Homa-IR, and Homa-Beta) did not differ between groups. The clinical characteristics are described in Table I.

Table I - Laboratory characteristics of overweight women in the total sample and by group on the 1st day of blood collection

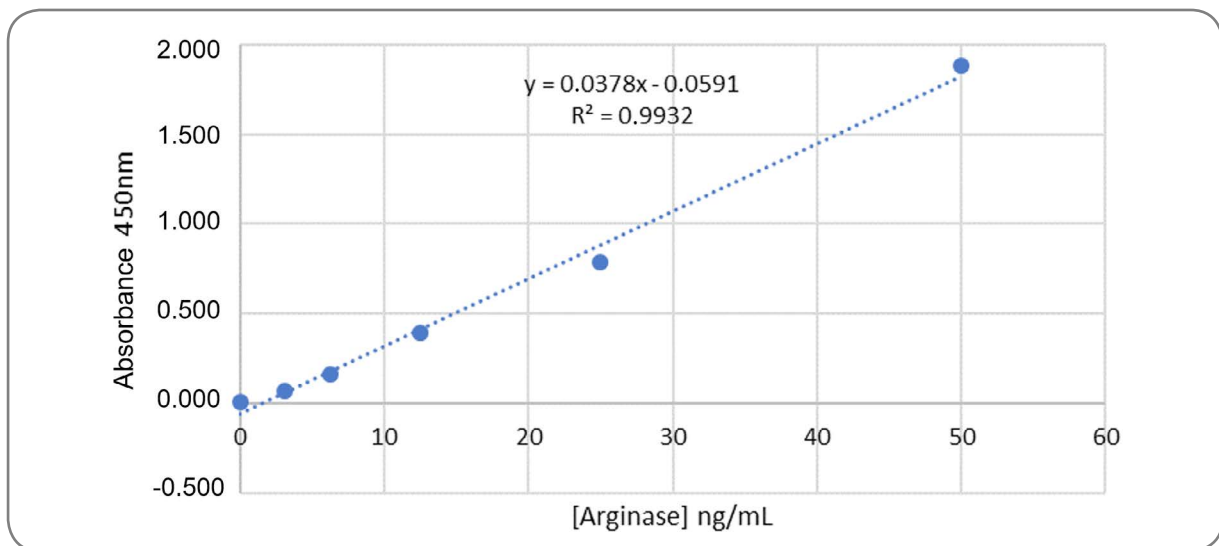
Variables	CG (n = 11)	EG (n = 9)	p
Triglycerides (mg/dL)	95.2 ± 44.7	128 ± 87.1	0.327
Total cholesterol (mg/dL)	174.8 ± 36.4	161.5 ± 33.5	0.408
HDL (mg/dL)	49.1 ± 9.4	48.8 ± 7.7	0.957
LDL (mg/dL)	106.7 ± 28.6	87.1 ± 26.3	0.128
Blood glucose (mg/dL)	84.6 ± 7	83.6 ± 11.5	0.837
Insulin (mIU/mL)	10 ± 4.3	9.9 ± 8.1	0.974
Homa-IR	2.4 ± 1	2.4 ± 2	0.996
Homa-Beta	34 ± 16.8	33.2 ± 29.9	0.939

CG = Control group; EG = Exercise group; p – Student's t-test.

The arginase curve obtained by diluting the standard solution in 8 samples of different concentrations serves as a basis for the analysis of the others. It is possible to observe in Table II and Graph 1, the mean and standard deviation of the curve, the reliability of the test being notorious, since the curve follows a linear and increasing pattern, with minimal deviation from the axis and with $R^2 = 0.9932$, thus configuring a method of good sensitivity.

Table II - Arginase curve

Concentration	OD. 1	OD. 2	Mean OD.	Standard deviation
200	2.467	2.452	2.460	0.011
100	2.049	2.054	2.052	0.004
50	1.968	1.799	1.884	0.120
25	0.774	0.808	0.791	0.024
12.5	0.426	0.358	0.392	0.048
6.25	0.199	0.123	0.161	0.054
3.125	0.129	0.008	0.069	0.086
0	0.018	0.000	0.009	0.013

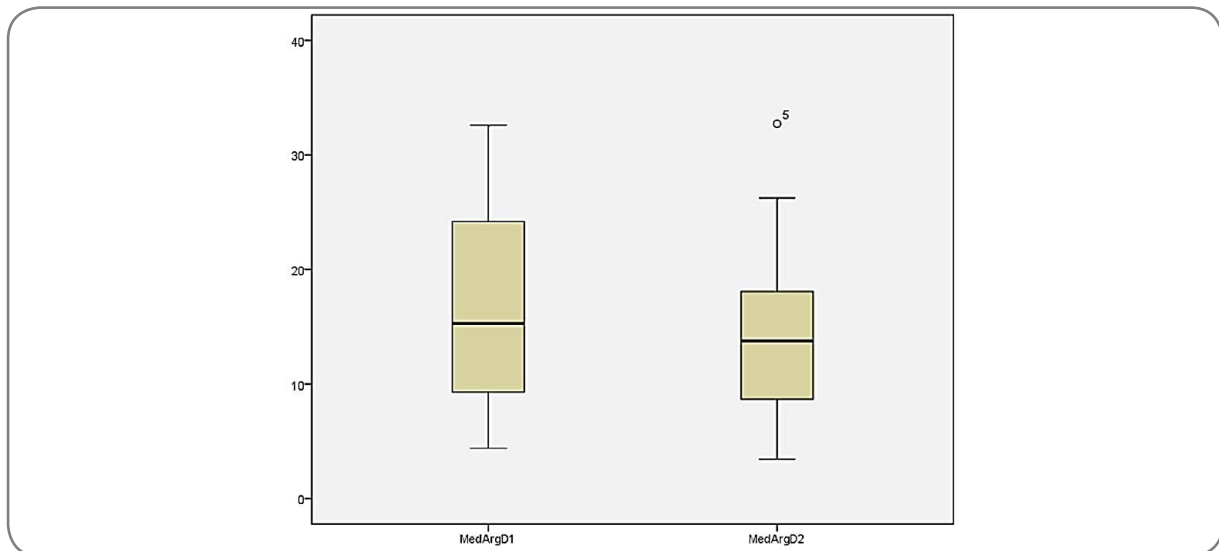
**Graph 1** - Arginase curve

The analysis of the comparison of the intra-group arginase level on the first and second day of the collection did not show variation in the concentration of serum arginase in the experiment group, as well as in the control group, as described in Table III and shown in Graphs 2 and 3.

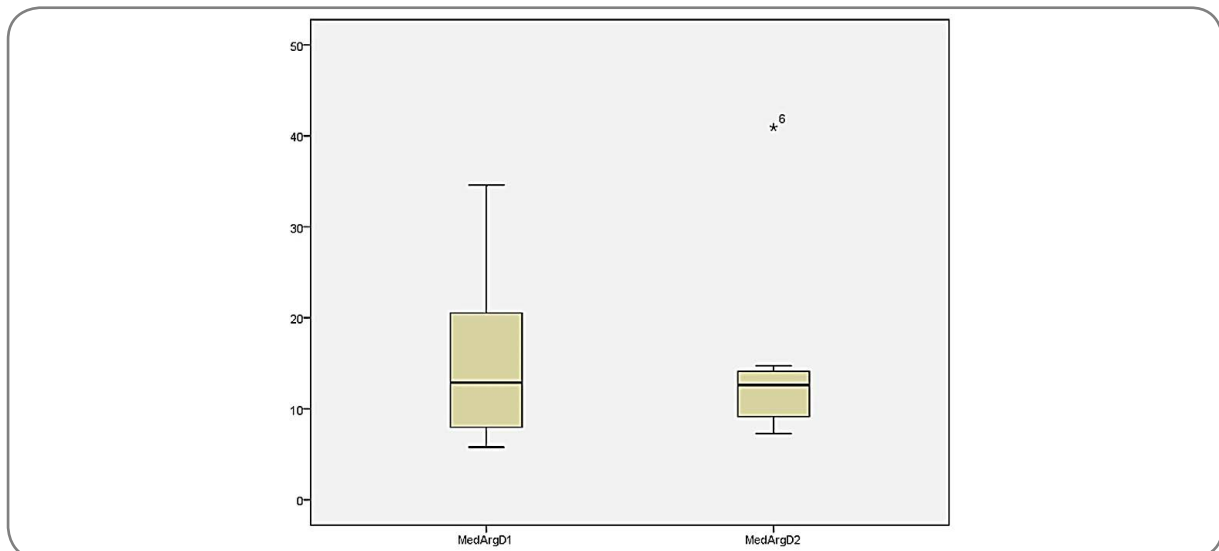
Table III - Intragroup analysis of serum arginase values in overweight women on the first and second days

	1 st dia	2 nd dia	p
Control group	20.1 (12.7-30.0)	14.8 (10.7-22.9)	0.477
Exercício group	12.8 (7.5 - 26.2)	12.6 (8.7-14.4)	0.953

*Median (Interquartile range). Mann-Whitney U test.



Graph 2 - Serum arginase levels on the first and second day of collection in the control group in overweight women



Graph 3 - Serum arginase level before (first day) and after (second day) of an exercise session in overweight women

In the intergroup comparison, the variation between the baseline and post-intervention moment (Δ Arginase) of each of the groups was used, with no difference in the levels of arginase between the control group and the exercise group $[-0.99 (-7.9 - 4.7)$ vs. $0.53 (-5.3 - 6.5)$; $p = 0.71$].

No association was observed between the levels of arginase and the BMI of the patients, separating them into overweight ($n = 12$) and obesity ($n = 8$), with an average of arginase of 14.76 ($SD = 9.89$) and 20.96 ($SD = 11.87$) respectively, with p -value = 0.436 .

Discussion

This study's results demonstrated that the performance of a single session of light physical exercise in overweight women did not alter the levels of serum arginase.

A study with a similar objective, however, with 41 individuals without a previous history of asthma, diabetes mellitus, and other comorbidities, showed that after continued exposure to moderate-intensity physical exercise (one hour of exercise bike/day, 5x/week, for one month) the level of NO increased while L-arginine was reduced, suggesting a direct relationship between physical exercise and improved endothelial function [21]. However, when dosing arginase, in agreement with this study, it did not change between pre and post-intervention, believing that there was a nitrosylation of the molecule, with activity regulated by the increase in NO [21]. It is worth mentioning that no other studies were found that analyzed arginase before and after exposure to physical exercise, being it in a single session or continuously. Thus, it is not possible to make other comparisons.

Arginase is an intracellular enzyme present in erythrocytes, liver, and kidneys, and is found in plasma mainly after inflammation, chronic organ damage, and hemolysis [22]. Inflammatory markers such as TNF- α and CRP can contribute to the induction of its activity [23], and in the overweight population, this component is relevant.

White adipose tissue, one of the constituents of adipose tissue, has 40% of macrophages in its composition, being a contributing factor to the increased availability of arginase in plasma since this enzyme is also induced by monocytes [24,25]. In addition, it is suggested that at the time of weight gain, hypertrophy of adipocytes occurs, followed by a mechanism of ischemia in the vessels of the adjacent region and hypoxia, triggering a local inflammatory process and chemotaxis of more macrophages to that region, promoting the elevation of TNF- α , IL-6 and CRP [25]. In this same population, oxidative stress also contributes to the inflammatory cascade [25], adding then one more factor that has been proven to be related to the activation and increased expression of arginase [5].

Other conditions that contribute to the upregulation of arginase are insulin resistance and liver damage secondary to non-alcoholic fatty liver disease, very common in the overweight population [5]. These factors may justify the elevated baseline serum levels of arginase in this population and are not amenable to reduction with a single session of light physical exercise.

The increase of this enzyme in this population of young women can also be detected in very young individuals. For example, when compared to a population of adolescents within normal weight and overweight, the average of arginase was 39.3 ± 26.9 ng/mL and 95.8 ± 68.2 ng/mL, respectively. In opposition to this study, there was an association between arginase and anthropometric markers such as weight, BMI, waist-to-hip ratio, and waist circumference, in addition to a family history of arterial hypertension, CRP, and TNF- α , which strengthens the biological plausibility of triad, obesity, inflammation and increased arginase [26].

When comparing non-obese and obese rats, the elevated serum arginase and the development of endothelial dysfunction in those overweight were well established, proving that the competitive relationship between arginase and NO by the

substrate promotes deficient NO-mediated vasodilation in this population. When administered with arginase or arginine inhibitors, the substrate itself, the endothelial response was considerably more satisfactory [4].

Given this competitive relationship and although it is proven that physical exercise is associated with the preservation of the functional capacity of the endothelium by promoting an increase in NO concentration after a single session [10], it is not possible to establish its impact with the reduction of levels of arginase without comparison with the variations that may occur in the serum level of NO.

It is important to note that the arginase's role in endothelial dysfunction in this population is due to its chronic maintenance at high levels. Faced with this, physical exercise promotes an immediate compensatory response from the body, demanding an increase in NO, which happens, even if in an imperfect way. The measurement of NO in this study would allow us to compare the endothelial response with the levels of arginase in patients in the exercise group, demonstrating how much this competition for the substrate could interfere with endothelial dysfunction.

Although there is scientific evidence in the literature that establishes a correlation between increased BMI and increased arginase, the present study did not find statistical significance when comparing these data. The study had some limitations that may have interfered with the results, such as small sample, low intensity and frequency of exercise, and absence of direct assessment of the NO level, as well as the evaluation of other factors that could potentially alter the enzyme, such as inflammation, liver damage, and insulin resistance. However, as this is a random sample, these possible biases are attenuated, and our results are not infeasible.

Conclusion

In this exploratory study, a single session of physical exercise was not able to modify the levels of serum arginase in overweight women.

Potential conflict of interest

No potential conflicts of interest relevant to this article have been reported.

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Author contributions

Conception and design of the research: Ladeia AMT, Wagnacker DS. **Data acquisition:** Wagnacker DS. **Data analysis and interpretation:** Nolasco HG, Ladeia AMT, Silva JJ, Souza AJ. **Statistical analysis:** Nolasco HG, Ladeia AMT, Silva JJ, Souza AJ. **Obtaining financing:** Ladeia AMT. **Writing of the manuscript:** Nolasco HG, Ladeia AMT. **Critical review of the manuscript for important intellectual content:** Ladeia AMT.

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