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Correlation Between Blood Lactate and Salivary Biomarkers During Exercise in Women with Hypothyroidism Treated With L-Thyroxine

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Abstract

Background: Hypothyroidism reduces circulating levels of thyroid hormones metabolic enzymatic activities, and the skeletal muscle's work capacity and lactatemia increase during exertion.

Objectives: To analyze salivary biomarkers and blood lactate obtained in exercise stages through exhaustion and investigate their correlation.

Methods: Eight females attended the University Clinical Hospital who showed a condition of subclinical hypothyroidism. Before they began replacing with L-thyroxine, they were submitted to an ergometric test to check their tolerance to effort. Eight weeks after treatment, all volunteers were again submitted to lab and ergometric tests. Saliva and blood were collected to determine total protein, nitric oxide, IgA, lactate, and lipid profile.

Results: The present study identified a correlation (0.8), so the behavior of the blood lactate could explain the behavior of the salivary of total proteins by 74.22% (R2 = 0.7422). Lactate production was minimized when women underwent L-thyroxine treatment in the first six stages of exercise. Still, total salivary proteins increased for pharmacotherapy between stages 1 and 8. While salivary nitric oxide had a percentage variation between pre- and post-exercise times of 96% before the start of treatment when the same exercise was performed after eight weeks of treatment with L-Thyroxine, this variation was 150%.

Conclusion: The measurement of STP is an essential marker of stress, and it is highly correlated with the most used blood marker, lactate. Regarding treatment with L-Thyroxine in women with subclinical hypothyroidism, after eight weeks of this therapy, it was possible to observe a reduction in blood lactate production after the incremental load test on a cycle ergometer, accompanied by a reduction in the concentration of SA. SNO and SIgA tended to increase in women undergoing treatment, and this treatment should be considered in subsequent studies as causing a different effect on these markers.

Keywords: Exercise, immunometabolism, sportomics, public health.

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INTRODUCTION

About 7 to 8% of all women aged more than 50 years and 3% of all men develop subclinical hypothyroidism at some point in their lives. Hypothyroidism is characterized by reduced circulating and tissue levels of the thyroidal hormones (L-thyroxine sodium, triiodothyronine); it may be classified as primary, secondary, or tertiary if the disease that causes it is located in the thyroid, hypophysis, or the hypothalamus, respectively (de Castro et al., 2001).

In cases of hypothyroidism, due to the reduced enzymatic activities that are part of the aerobic metabolism, there is a reduction in the skeletal muscle work capacity and increased lactatemia during effort (Kaminsky et al., 1991; Kaminsky et al., 1992; de Castro et al., 2001). In untreated hypothyroidism, there is reduced tolerance to physical efforts due to the reduction of inotropism caused by structural changes that take place in the myosin ATPase in the muscular cells. This modified enzyme determines a myocardial change that reduces the systolic volume and, thus, cardiac debit, a relevant factor in determining physical tolerance to effort (Klein & Ojamaa, 1998). Studies show that blood lactate accumulation was significantly smaller among patients suffering from subclinical hypothyroidism and peripheral hypo-vasodilation as compared to the control group, which suggests that early L-Thyroxine Sodium replacement may be helpful to improve these metabolic changes and, thus, perhaps ensure more tolerance to effort (Monzani et al., 1997).

Most tests used to determine improved physical performance or the changes in physical activity lead to blood sampling, the collection of which requires invasive procedures that always cause some discomfort to the patient and may even lead to the risk of contamination. Sampling saliva, on the other hand, requires no invasive procedures or special equipment as needed for tissue, blood, or plasma collection. In this regard, it has been shown that physical exercise is capable of leading to acute changes in the concentration of a few salivary biomarkers, such as nitric oxide (NO) (Panossian et al., 1999), IgA, and total protein (Steerenberg et al., 1997; Walsh et al., 1999; Hiscock & Pedersen, 2002; Bishop et al., 2006). NO levels in saliva after intense physical exercise increase significantly (Panossian et al., 1999; McAllister & Laughlin, 2006). The presence of NO may be quantified in the saliva as of the dosage of the nitrite and nitrate anions resulting from salivary gland stimulation (Bodis & Haregewoin, 1993).

The sympathetic nervous system's activity increases progressively with the intensity of the exercise, and it is the part responsible for the changes that take place in the salivary components during exercise (Chatterton et al., 1996; Bishop et al., 2000; Walsh et al., 2003). Human salivary alpha-amylase (HSA) is the most abundant protein in saliva. The salivary glands produce it, mainly synthesizing it by the parotid gland (Turner & Sugiya, 2002). The measurement of HAS activity may also indicate psychological stress since its secretion is regulated by the action of norepinephrine on the salivary glands (Nater et al., 2005; Yamaguchi et al., 2004).

Physical exercise may influence salivary immunoglobulin A levels (sIgA) and indicate the immunological function state in acute exercise (Steerenberg et al., 1997; Panossian et al., 1999; Hiscock & Pedersen, 2002; Bishop et al., 2006). The presence of sIgA in the saliva is one of the first barriers, protecting against the risk of infections, particularly in the upper respiratory tract.

Little is known about the effect of physical exercise as evaluated based on salivary biomarkers in patients suffering from subclinical hypothyroidism. Therefore, the purpose of this study is to analyze total protein concentrations in the saliva and blood lactate as obtained in all exercise stages through exhaustion and compare the total protein concentrations, α-amylase activity, and nitric oxide and IgA present in saliva in the beginning, at the end of the incremental test, and five minutes after the sub-maximal effort test.

METHOD

Study Design and Participants

This is a cross-sectional, descriptive and observational study. This study's population was composed of patients attended at the University Clinical Hospital, who showed a condition of subclinical hypothyroidism identified based on the absence of signs and symptoms of hypothyroidism and by the analyses of the biochemical analyses of the total thyroid-stimulating hormone (TSH), free T4 (FT4), Triiodotironine (T3) and Tetraiodotironine (tiroxine) (T4). Eight female volunteers were selected. The inclusion criteria were: TSH concentrations above 4.4Um/dL, age between 40 and 60 years, IMC < 35 kg/m2. The exclusion criteria were: absence of clinical history of cardiovascular disease or dysfunction, chronic pulmonary failure, renal failure, diabetes mellitus.

The study commenced after the research project was approved by the Federal University of Uberlandia Ethics and Research Committee. After the initial procedures to confirm the subclinical hypothyroidism, the volunteers were invited to participate in the study and to sign an informed consent form. After these procedures, they were evaluated clinically to highlight any change that could prevent them from undergoing physical effort.

Research Instruments

Evaluation of the tolerance to effort

Before they began hormonal replacement with L-Thyroxine Sodium, the volunteers were submitted to an ergometric test to check their level of tolerance to effort. The tests were carried out in a cycle ergometer (Ergofit 167, Germany) from 9 AM to 11 AM with room temperature kept between 24 and 26ºC. The volunteers stretched and warmed-up briefly for two minutes with no load. The heart rate was measured continuously using a heart rate monitor (Polar S610TM, Polar Electro Oy, Finland). The test was started at 50 W, with 25 W increments in each stage (two minutes each) through exhaustion. Pedal rotation frequency was kept between 68 and 75 rpm. The tests were terminated: 1) voluntarily by the volunteer; 2) if it were not possible to maintain pedal rotation; or 3) when the heart rate surpassed 90% of maximum capacity.

Blood sampling and analysis

Using surgical gloves and after local asepsis with alcohol, the ear lobe was punctured with a discardable lancet. The first drop of blood was discarded to avoid contamination by lactate eliminated through the sweat produced by the sudorific glands, and then 25 mL of arterialized blood were collected using heparinized and calibrated glass capillaries. The blood that was collected was deposited in microtubes containing 50 mL of 1% sodium fluoride to inhibit the enolase glycolytic enzyme, interrupting the glycolytic activity and avoiding blood coagulation. The blood lactate was analyzed via the electro-enzymatic method in the YSI 1500 Sport L-Lactate analyzer (USI Inc, Yellow Springs, Ohio).

Hormone replacement with L-Thyroxine Sodium

As instructed by the endocrinologist that participated in the study, the patients started the treatment after the first test in the cycle ergometer receiving a 25-mcg pill of L-Thyroxine Sodium, orally, in the morning, and on an empty stomach. The initial dose was

increased, as required, until the TSH levels normalized. The L-Thyroxine Sodium dosage varied from 25 to 75mcg.

Re-Testing Procedures

Eight weeks after treatment was started with L-Thyroxine Sodium, on average, TSH normalization was achieved when all volunteers were once again submitted to lab tests and to the ergometric test to check tolerance to effort. The second test's procedures followed the same orientations as those used pre-treatment.

Saliva sampling and measurement

Stimulated saliva, by chewing gum (trident-menta™), was collected via the spitting method (Navazesh, 1993). Ten minutes before the physical test, stimulated saliva was collected in a graded test tube for three minutes. Immediately before this collection, the volunteers rinsed their mouths several times with distilled water to eliminate cellular debris and other elements. The volunteer was advised, a minute before each stage change, to swallow the saliva and start chewing the gum. Saliva collection started 30 seconds after each stage. The saliva was put in pre-cooled (4°C) mini-tubes. At around the maximum of three hours, the saliva was centrifuged at 12,000g, the sediment was discarded and the supernatant frozen at -80°C until the date of analysis. Total protein was measured using the Bradford method (Bradford, 1976), and the salivary amylase activity carried out via the kinetic method at 405 nm, using the 2 chloro-p-nitrophenyl-α-D-maltotrioside (CNP-G3) substrate according to the manufacturer's protocol (Amilasa 405, liquid line, Wiener lab, Argentina).

Salivary nitric oxide concentration dosage was carried out based on Granger's colorimetric method (Granger et al., 1995). Absorbance was measured in the microplate reader (Titertek multiskan plus MK11) through a 570-nm filter. The data were analyzed with the Microplate Manager software, version 4.0 (Bio-Rad Laboratories, USA).

The total IgA analysis used the adapted ELISA test (MacKinnon & Jenkins, 1993; Silva et al., 2001). The polystyrene plates (Maxi-Sorp, Nunc, Wohlen) were sensitized with the human anti-IgA antibody (Sigma Chemical, Buchs), diluted to the ideal concentration with a carbonate buffer, 0.06M (pH 9.6) for 12 hours at 4°C. The plates were washed and blocked with a specific o-phenylenediamine dichloride (OPD) buffer. The saliva samples were diluted beginning at 1:2 in 1% BSA-PBS-T and incubated for 1 hour at room temperature. After the rinse, biotinilated anti-IgA conjugated marked with diluted

peroxidase diluted to the concentration to be used was added. The H2O2 + OPD enzymatic substrate (chromogen buffer) was incubated at room temperature for 1 hour. For individual plate analysis, the results were expressed in ELISA indices (IE). The optic density (OD) values were determined in the microplate reader at 405 nm. After obtaining the total IgA results, this was divided by the total protein value in the saliva and the specific IgA value determined.

Anaerobic threshold determination

The anaerobic threshold was determined based on a bisegmented regression model (Toler & Burrows, 2010). Dmax method was employed here to determine anaerobic threshold for both biomarkers blood lactate and total protein of whole saliva. In according with Cheng et al., (1992) Dmax takes the first and the last point (x, y) of the exercise protocol to get a straight-line equation through these points, verifying the distance from all points to the straight line. The anaerobic threshold is represented by the biggest distance of these points (Bortolini et al., 2009).

Data Analysis

To estimate models that, although simplifications of reality present adequate adherence between absolute and estimated values, different researchers can choose different statistical models from the same database (Silberzahn & Uhlmann, 2015). Machine learning techniques and the new concept in metabolic studies suggested by sportomics may dictate the future pace of research in immunometabolism (Bassini & Cameron, 2014; Khoramipour et al., 2022).

Initially, descriptive statistics were performed on the data, with measurements of position (mean, median, mode, and percentiles), dispersion (amplitude, variance, standard deviation, and stand-ard error), and form (kurtosis and skewness).

Afterwards, the univariate analysis of these data was performed using the Shapiro-Wilk normality test (because the sample was smaller than 30 individuals). The equal variance test would be applied if the Shapiro-Wilk test presented a result indicating normal distribution ($P>0.05$). For results with $P>0.05$, the paired T-Student test would follow; if $P\leq0.05$, the paired T-Student test would follow the non-parametric Mann-Whitney test. If the Shapiro-Wilk test presented a result indicating non-normal distribution ($P\leq0.05$), the non-parametric Mann-Whitney test would be applied directly.

Cohen's equations (Cohen, 1992) were used to calculate the effect size for all variables to obtain Cohen d and r values:

$$d = \frac{(M1 - M2)}{\sqrt{(SD1^2 - SD2^2 \div 2)}}$$

$$r = \frac{d}{\sqrt{[(D^2) + 4]}}$$

Where M represents the means of observations and SD their respective standard deviations.

Table 1. Values of effect size.

Effect size	Small	Medium	Large
Cohen r	0.10	0.30	0.50
Cohen d	0.20	0.50	0.80

Source: (Cohen, 1992).

Next, multivariate data analysis was performed using data mining and machine learning techniques. In this phase, in order to seek a bivariate measure between the data, because the observations contain quantitative values, the Pearson and Spearman correlation tests were applied, with the Pearson correlation being used for a visual analysis using correplot strategy and as an initial measure for the following machine learning analyses.

As exploratory models of machine learning: Pearson correlation. The Z score was previously applied to adjust observations measurement units, and the Fruchterman-Reingold algorithm was applied with Euclidian Similarity Index (Fruchterman & Reingold, 1991; Karmen et al. 2019). As supervised and inferential Machine Learning Tool: polynomial regressions were performed.

Calculations to find the similarity (Euclidian distance) and Z score are as follows:

$$ED = \sqrt{\sum_j K = 1\ (ZXjp - ZXjq)2}$$

$$Z = \frac{Individual\ Score - Mean}{Standard\ Deviation}$$

So, for a better interpretation of the data, the calculation of percentage variation was applied:

$$\Delta\% = \frac{(Final\ value - Initial\ Value)}{Initial\ Value}\ x\ 100$$

The softwares SigmaPlot 14.5 (Academic Perpetual License - Single User – ESD Systat® USA), R and R Studio (Free version for Windows) and Past 4.03 (Free version for Windows) were used to carry out the different statistical tests and produce the graphs.

RESULTS AND DISCUSSION

To facilitate a holistic and integrated observation of the results, tables 2 and 3 present descriptive statistics, with measures of position, dispersion, and shape, of the behavior of these analytes at each stage of exercise and workload, in addition to the value of P and effect size for the variables blood lactate (Table 1) and total salivary proteins (Table 2).

Table 3, in turn, outlines an analysis of the dependent variables outside of exercise time, indicating the results before and after treatment with the investigated pharmacotherapy. Means and deviations were shown, in addition to the effect size for the treatment.

Based on the analysis of serum lactate behavior (Table 2), it was possible to observe that there was a small effect of pharmacotherapy for stages 2, 3, 8, and 9, a medium effect for stage 1, and an effect considered large for stages 5 and 6 from the physical effort test.

Table 2. Holistic analysis of the behavior of blood lactate (mmol/L) during exercise stages.

	Before treatment						After treatment						D and E	
Stages	**AV**	**Me**	**SD**	**SE**	**Ku**	**Sk**	**AV**	**Me**	**SD**	**SE**	**Ku**	**Sk**	**P**	**D**
1 (15W)	0.9	0.8	0.40	0.10	10.4	3.01	1.5	1.0	1.28	0.32	5.55	2.56	0.002	0.62^{M}
2 (30W)	1.5	1.4	0.41	0.10	0.23	1.15	1.6	1.3	1.03	0.26	3.19	2.05	0.720	0.26^{S}
3 (45W)	1.9	2.2	0.43	0.11	-0.98	-0.70	1.8	1.5	0.77	0.19	0.09	1.09	0.136	0.25^{S}
4 (60W)	2.6	2.6	0.36	0.09	-0.88	-0.47	2.1	2.0	0.75	0.19	-0.44	0.66	0.049	0.72^{M}
5 (75W)	3.6	4.5	1.00	0.25	0.02	-0.07	2.5	2.1	0.92	0.23	-0.85	0.69	0.007	1.19^{L}
6 (90W)	4.4	4.6	1.30	0.32	0.77	-0.75	3.0	2.7	0.88	0.22	0.07	0.95	0.001	1.26^{L}
7 (105W)	5.0	5.1	1.40	0.36	0.26	-0.46	5.1	5.1	1.24	0.31	0.94	0.70	0.831	0.08
8 (120W)	6.0	5.9	1.17	0.31	0.05	-0.61	5.8	5.8	1.07	0.28	0.07	-0.03	0.530	0.24^{S}
9 (135W)	7.0	7.0	-	-	-	-	6.7	6.3	0.88	0.23	-1.32	0.74	-	0.39^{S}
10 (150W)	-	-	-	-	-	-	7.0	7.5	0.87	0.50	-	-1.73	-	-
Post 5 min	6.1	6.1	1.07	0.27	0.60	0.91	6.3	6.5	1.34	0.33	7.30	-2.21	0.250	0.14

AV = Average; Me = Median; SD = Standard Deviation; SE = Standard Error; Ku = Kurtosis; Sk = Skewness; P = P value (T Student test or Mann-Whitney); d = Effect Size (Cohen d); D and E = Difference and Effect. S = Small effect; M = Medium effect; L = Large effect.

Carrying out the same analysis as in the previous paragraph, but this time in terms of total salivary proteins (Table 3), a small effect was observed for the measurement five

minutes after the end of the exercise, a medium effect for stages 2, 3, 5, 7 and 8, and a large effect for stages 1, 4 and 9 of the exercise.

Table 3. Holistic analysis of the behavior of salivary total proteins (mmol/L) during exercise stages

	Before treatment						After treatment						D and E	
Stages	**AV**	**Me**	**SD**	**SE**	**Ku**	**Sk**	**AV**	**Me**	**SD**	**SE**	**Ku**	**Sk**	**P**	**D**
1 (15W)	0.8	0.9	0.13	0.03	-1.02	-0.51	0.9	0.9	0.11	0.03	-1.19	0.24	0.056	0.80^{L}
2 (30W)	0.9	0.8	0.14	0.03	-1.44	0.05	1.0	0.9	0.12	0.03	-0.88	0.69	0.059	0.78^{M}
3 (45W)	0.9	0.9	0.13	0.03	-1.61	-0.12	1.0	0.9	0.12	0.03	-0.90	0.63	0.054	0.71^{M}
4 (60W)	0.9	0.9	0.12	0.03	-1.41	-0.02	1.0	1.0	0.12	0.03	-0.82	0.52	0.021	0.86^{L}
5 (75W)	0.9	1.0	0.26	0.06	6.83	-2.34	1.0	1.0	0.12	0.03	-0.34	0.11	0.070	0.69^{M}
6 (90W)	1.1	1.0	0.13	0.03	0.46	0.60	1.0	1.1	0.15	0.04	-0.90	-0.18	0.758	0.11
7 (105W)	1.1	1.1	0.13	0.03	1.29	0.85	1.2	1.2	0.10	0.02	-0.77	-0.49	0.038	0.78^{M}
8 (120W)	1.1	1.1	0.13	0.03	1.91	1.45	1.2	1.2	0.12	0.03	-0.75	-0.02	0.052	0.75^{M}
9 (135W)	1.6	1.6	-	-	-	-	1.3	1.2	0.12	0.03	-0.51	-0.03	-	3.89^{L}
10 (150W)	-	-	-	-	-	-	1.3	1.2	0.15	0.09	-	1.69	-	-
Post 5 min	1.1	1.0	0.10	0.02	0.40	0.64	1.1	1.1	0.11	0.03	-1.49	0.09	0.193	0.47^{S}

AV = Average; Me = Median; SD = Standard Deviation; SE = Standard Error; Ku = Kurtosis; Sk = Skewness; P = P value (T Student test or Mann-Whitney); d = Effect Size (Cohen d); D and E = Difference and Effect. S = Small effect; M = Medium effect; L = Large effect.

Unlike the previous tables, the one below proposes an observation of the times outside the exercise stages, pointing out the influence of this effort on the basal values of nitric oxide, amylase, and salivary IgA but still seeking the effect of the pharmacotherapy used for eight weeks, and the metabolism lipid levels without exercise measured by total cholesterol (TC), high-density lipoproteins (HDL), very low-density lipoproteins (VLDL), low-density lipoproteins (LDL) and triglycerides (Tag).

Thus, it was clear that L-Thyroxine therapy for eight weeks caused a small effect on triglycerides, VLDL, and LDL, which are the plasma lipoproteins that require greater attention due to the chance of causing atherosclerosis, and a large effect on total cholesterol, indicating a modulation of lipid metabolism.

Table 4. Analysis of dependent variables measured outside of exercise time (average ± SD).

	Before treatment			After treatment		Effect Size (Cohen d)				Δ%
Variable	**Pre-ex**	**Post-ex**	**Post-5m**	**Pre-ex**	**Post-ex**	**Post-5m**	**Pre-ex**	**Post-ex**	**Post-5m**	**Pre x Post**
SNO (μM)	95.4 ± 24.15	187.1 ± 73.38*	237.0 ± 69.43*#	89.42 ± 18.63	224.1 ± 61.68*&	244.3 ± 58.83*	0.28^{S}	0.55^{M}	0.11	96%BT 150%AT
SA (U/L)	1956.8 ± 608.03	2525.1 ± 423.98*	2018.7 ± 544.79#	2037.6 ± 465.2	2370.2 ± 323.4*	1887.7 ± 430.9#	0.15	0.41^{S}	0.27^{S}	29%BT 16%At
SIgA (mg/dl)	10.9 ± 1.98	21.9 ± 2.72*	22.1 ± 2.41*	11.1 ± 1.71	23.0 ± 3.30*	22.8 ± 2.97*	0.10	0.36^{S}	0.25^{S}	100%BT 106%AT
Tag (mg/dl)	130.9 ± 48.18	-	-	118.8 ± 50.64	-	-	0.24^{S}	-	-	
HDL (mg/dl)	60.9 ± 9.30	-	-	61.4 ± 10.32	-	-	0.05	-	-	
VLDL (mg/dl)	24.4 ± 6.44	-	-	21.9 ± 5.5.3	-	-	0.42^{S}	-	-	
LDL (mg/dl)	83.4 ± 17.98	-	-	76.9 ± 12.12	-	-	0.42^{S}	-	-	
TC (mg/dl)	179.2 ± 28.87	-	-	159.9 ± 12.15	-	-	0.85^{L}	-	-	

SNO = Salivary Nitric Oxide; SA = Salivary Amylase; SIgA = Salivary Immunoglobulin A; Tag = triglycerides; HDL = High Density Lipoprotein; VLDL = Very Low-Density Lipoprotein; LDL = Low Density Lipoprotein; TC = Total Cholesterol; Pre-ex = Pre-exercise; Post-ex = Post-exercise; Post-5m = five minutes post-exercise. S = Small effect; M = Medium effect; L = Large effect; * = *Different ($P < 0.05$) in relation to Pre-ex; #Different ($P < 0.05$) in relation to Post-ex and &Different ($P < 0.05$) in relation to the same time between groups; AT = After treatment; BT = Before treatment.

The calculation presented in the methods, aiming to homogenize the units, followed by the Pearson correlation coefficient. After this data manipulation, the results obtained were presented as a correplot (Figure 1 and Figure 2), with the two main study variables measured at all stages of the exercise (lactate and total salivary proteins) presented in Figure 1 and the variables measured outside the times of exercise in Figure 2.

For logical reasons, as the workload was increased by 15W at each exercise stage, these two variables showed maximum positive correlation. Intriguingly, blood lactate and total salivary proteins showed a high positive correlation (0.8) with a P value < 0.001 (Figure 1).

Figure 2 revealed a negative correlation between the behavior of salivary amylase and total cholesterol (-0.6) and a positive correlation between the behavior of salivary nitric oxide and LDL (0.7), both with $P < 0.05$.

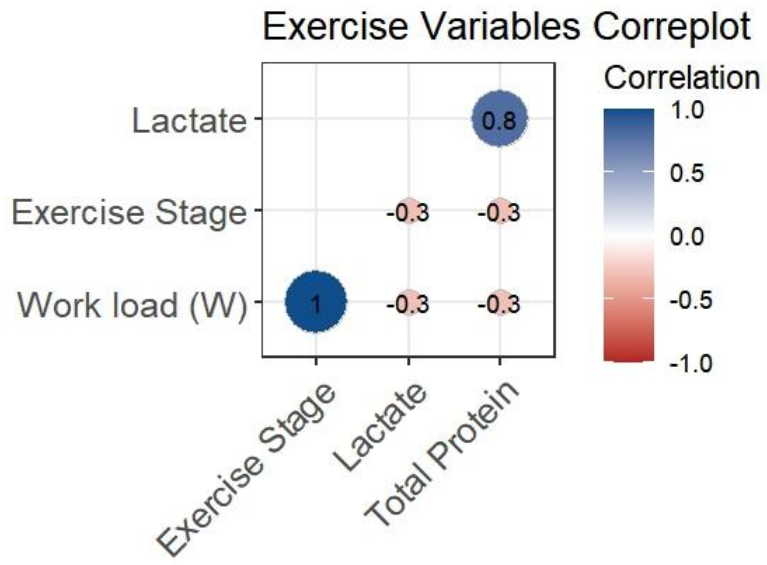

Figure 1. Correplot with the correlation coefficients between the dependent variables (Pearson) for variables during exercise

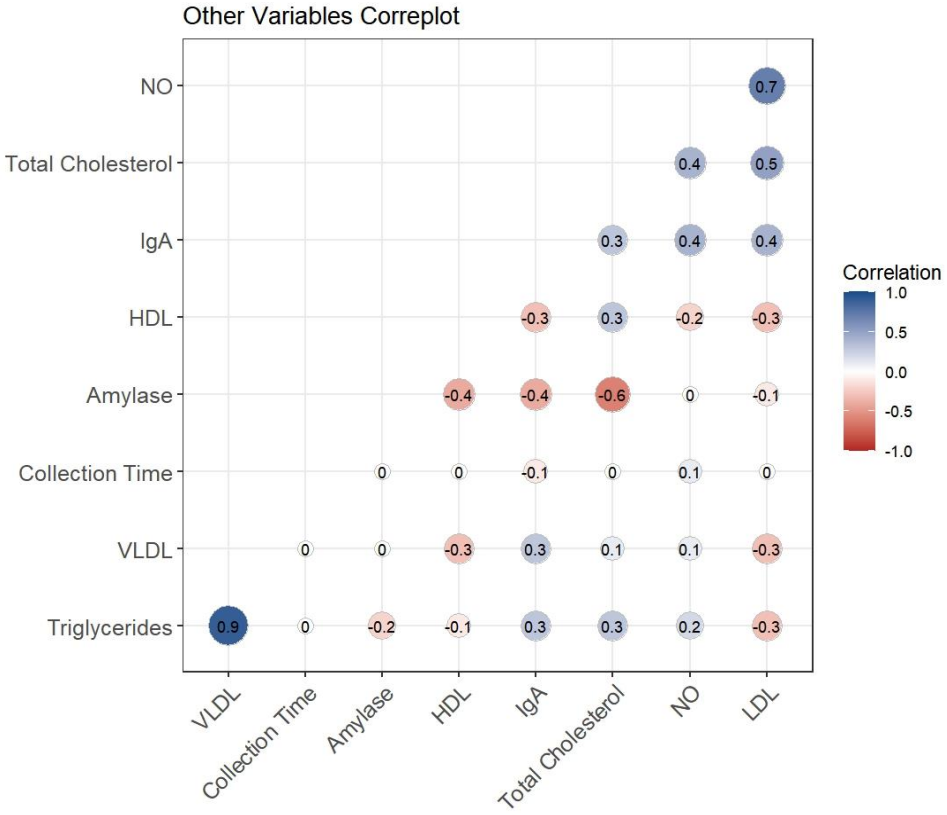

Figure 2. Correplot with the correlation coefficients between the dependent variables (Pearson) for other variables

Maintaining the logical line proposed by the main objective of observing a possible association between serum lactate and total salivary proteins, which, as they are quantitative data, were evaluated by correlation, and also, based on the outputs obtained, a second unsupervised and exploratory machine learning strategy, where the data treated by Z Score were subjected to the Euclidean similarity index and plotted with a dendrogram.

In this way, the findings gave robustness to the thesis of a possible correlation between the explanatory variable lactate and the dependent variable total salivary proteins (Figure 1), with a high similarity between them (Figure 3).

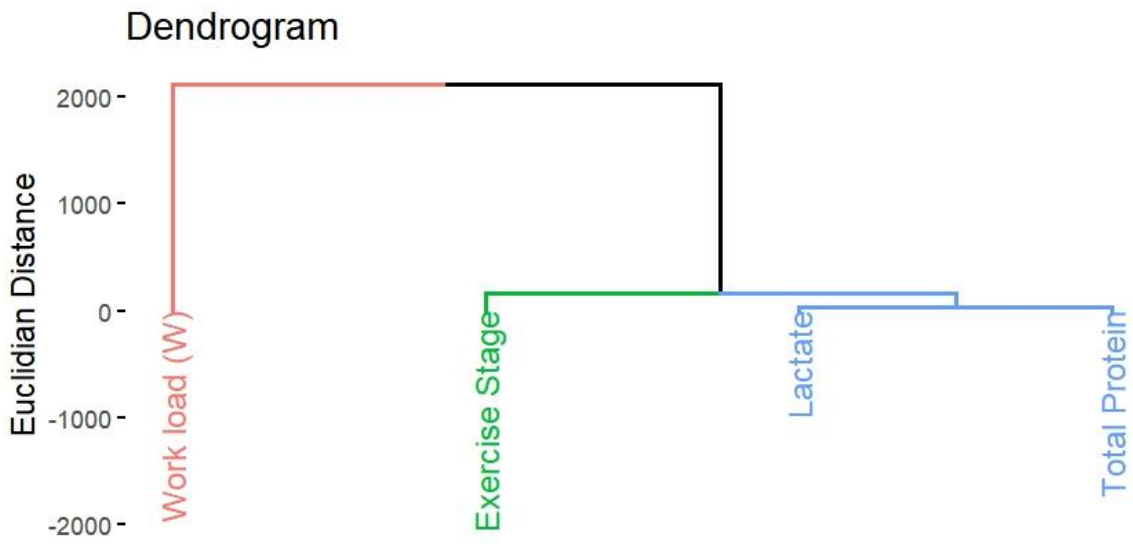

Figure 3. Euclidean similarity index applied after homogenization by calculating the Z score

After the data mining phase using different exploratory strategies, the main output went to the supervised and inferential machine learning phase, where through polynomial regression (Figure 4) an R2 value of 0.7422 was reached, indicating that even in In a small sample, the explanatory variable lactate was able to explain 74.22% of the behavior of the dependent variable total salivary proteins, indicating the latter as an excellent biomarker of stress in salivary fluids, which are non-invasive, devoid of risks to the subject and easy to conduct.

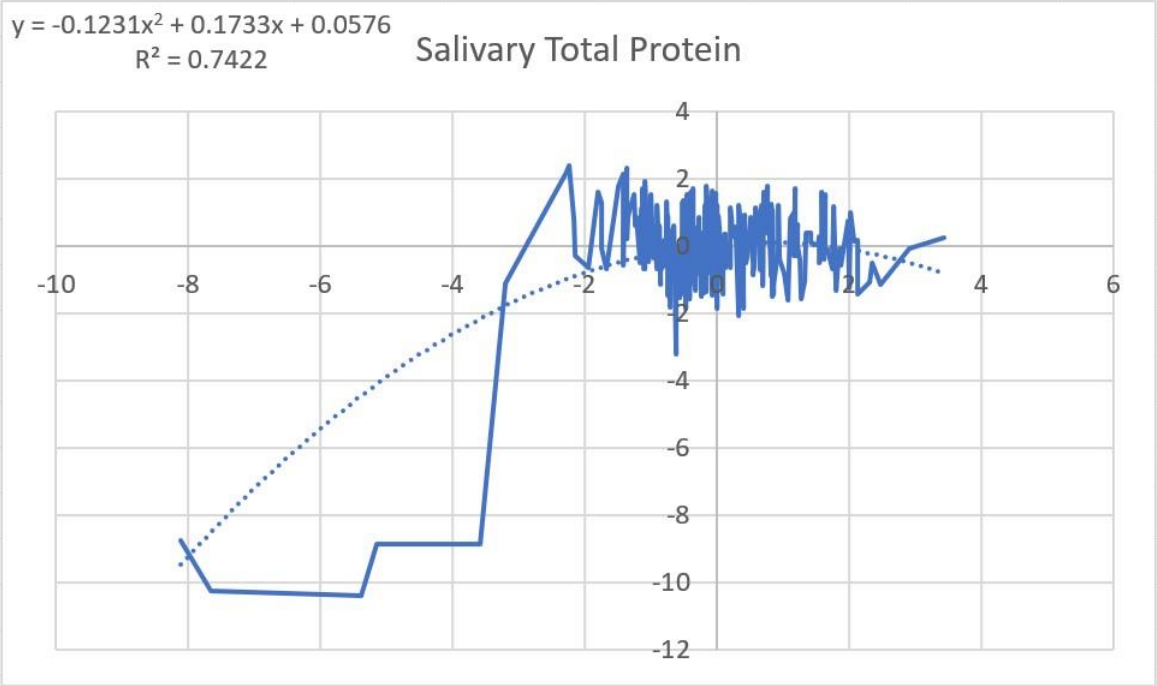

Figure 4. Polynomial regression between the explicative variable (lactate – x axis) and the dependent variable (total salivary proteins – y axis).

DISCUSSION

The present study identified a strong positive correlation (0.8) even in a relatively small sample, so the behavior of the blood lactate concentration could explain the behavior of the salivary concentration of total proteins by 74.22% (R2 = 0.7422). Another notable fact of the present study was that lactate production was minimized when women were undergoing L-Thyroxine treatment in the first six stages of exercise, this effect being small in stages 2 and 3, medium in stages 1 and 4, and large in stages 5 and 6. There was also a reduction with a small effect in stages 8 and 9. Still, about pharmacotherapy, total salivary proteins showed an increase between stages 1 and 8 (except stage 6), with the effects being considered medium in stages 2, 3, 5, 7, and 8 and large in stages 1, 4, and 9, and the therapy may have reduced the correlation between these variables when considering the entire experiment, as was the case. This antagonistic behavior between these variables when using this pharmacotherapy must be further investigated.

Still on the effect of treatment, while salivary nitric oxide (SNO) had a percentage variation between pre- and post-exercise times of 96% before the start of treatment when the same exercise was performed after eight weeks of treatment with L-Thyroxine, this variation was 150%. For salivary Immunoglobulin A, the behavior was agonistic to SNO,

but to a lesser extent, being 100% before treatment and 106% after. Salivary amylase had an antagonistic behavior, going from 29% before treatment to 16% after eight weeks.

It had previously been observed in a similar protocol that saliva provides a convenient and noninvasive matrix for assessing specific physiological parameters, including some biomarkers of exercise. Thus, total protein concentration of whole saliva provides a convenient and noninvasive matrix for determining the anaerobic threshold during incremental exercise tests (Bortolini et al., 2009).

However, if both are markers of stress intensity, among these forms of stress is physical exercise, why not investigate their correlation and the potential for a blood marker to explain the behavior of a salivary marker and propose non-invasive tests for this purpose?

Furthermore, the present study clearly shows that the total protein concentration of whole saliva correlates highly with the concentration of blood lactate during the cycle ergometer protocol with increasing load.

Sportomics studies, reproducing real exercise conditions, have provided essential and robust data and information about immunometabolism in different forms of stress. This strategy, combined with data mining and machine learning techniques, indicates the future of sports science (Gonçalves et al., 2012; Gonçalves et al., 2022; Galvão et al., 2023; Gomes et al., 2023; Nahon et al., 2023; Prampero et al., 2024).

Regarding metabolism, the glycolytic pathway leads to the conversion of glucose absorbed from food into pyruvate, later being reduced to lactate. Years ago, lactate was considered just a residue from anaerobic metabolism. Still, more recent studies show that its production does not cease under aerobic conditions and has become essential for providing energy and oxidation reactions. Lactate stands out in at least three functions: A source of cellular energy, a gluconeogenic precursor, and a signaling molecule. Furthermore, the literature shows a significant increase in lactate production due to physical exercise, with lactate used as muscle fuel (da Silva et al., 2023).

Observing lactate as a product of carbohydrate metabolism, the increasing behavior at each stage of exercise and load increment corroborates the literature and is nothing new (Mercier et al., 1989; Ahmaidi et al., 1992; Zajac et al., 2014; Kanniainen et al., 2023). However, the correlation between its blood measurement and the behavior of the concentration of total salivary proteins verified in the present study appears to be new, with

total salivary proteins only being used to correct the concentration of salivary lactate and subsequent comparison with serum lactate (Franco-Martinez et al., 2019) or as a salivary marker of exercise intensity (Rodrigues de Araujo et al., 2018). Moreover, Rodrigues de Araujo et al. (2018) identified saliva as a potential biological fluid for biochemical monitoring. In this study on football athletes, the authors identified that The HIIE protocol as a physical test on football athletes increased the salivary concentration of markers of exercise intensity, such as lactate, total protein, and cortisol, but did not affect Ig-A levels. Redox homeostasis in saliva appears to be more related to uric acid levels as a key factor in TBAR homeostasis, indicating an appropriate experimental stress model used in the present study.

Bocanegra et al. (2012) had already observed in swimmers that salivary lactate may represent a new biomarker of exercise intensity and a good predictor of salivary lactate kinetics. However, the kinetics of salivary biomarkers may have different behaviors depending on the sample, as in a previous study with obese and sedentary adults submitted to the same exercise model (de Souza et al., 2018), there was no change in salivary IgA, unlike the present study where this marker presented an increase of around 100% by HIIE. Therefore, studies with different populations and experimental conditions are essential to advance knowledge about the triad of exercise, biomarkers, and health conditions.

CONCLUSION

The noninvasive measurement of total salivary proteins is an essential marker of stress, and it is highly correlated with the most used blood marker, lactate. Regarding treatment with L-Thyroxine in women with subclinical hypothyroidism, after eight weeks of this therapy, it was possible to observe a reduction in blood lactate production after the incremental load test on a cycle ergometer, accompanied by a reduction in the concentration of salivary amylase. Nitric oxide and immunoglobulin A, both in saliva, tended to increase in women undergoing treatment, and this treatment should be considered in subsequent studies as causing a different effect on these markers.

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CONFLICT OF INTEREST

The author officially certifies that there are no conflicts of interest with any party with respect to this research.

AUTHOR'S CONTRIBUTION

Silva, Gonçalves, da Silva, Bortolini, Resende and Espindola: essential contributions to the conception and design of the study protocol; acquisition, analysis and interpretation of data; and involvement in drafting of the manuscript. Silva, Gonçalves and Magalhães-Neto: critical revisions for important intellectual content. All authors read and approved the final manuscript.

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