

Original Article (short paper)

## Evaluation of maximal lactate steady state in middle-aged hypertensive women

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**Abstract — Aim:** The aim of this study was to investigate the lactate response in physically inactive hypertensive women submitted to the treadmill maximal lactate steady state (MLSS) protocol. **Methods:** Twenty-two hypertensive women (40 – 64 years) performed a familiarization period of walking on the treadmill following by one incremental test for estimating the initial workload for exercise testing. MLSS protocol was composed by walking in a treadmill during thirty minutes with fixed velocity in 5.5 km/h. Incline was used for determination of the intensity of each volunteer. Blood samples were collected from the ear lobe in the rest period, minute 10<sup>th</sup> and at the end of the test (minute 30<sup>th</sup> or at exhaustion time point) for lactate analysis. **Results:** Hypertensive women showed a lower lactate concentration at MLSS ( $3.25 \pm 0.81$  mmol/L) as compared with data obtained in the literature (4 mmol/L), approximately 18.8%. Neither inclines nor age affected MLSS parameters in the population. A positive and strong correlation was found between incline and MLSS, as well as incline and lactate level at minute 30<sup>th</sup>, even when adjusted by age factor. **Conclusion:** Physically inactive hypertensive women show a lower MLSS than the average established in the literature but within the range of variations previously reported. Furthermore, a higher MLSS incline correlates positive and directly with higher lactate concentrations for the same aerobic capacity regardless of age.

**Keywords:** aerobic performance, maximal lactate steady state, hypertensive women

### Introduction

Stroke, myocardial infarct and coronary artery disease are the main complication of arterial hypertension, representing a high cost for the public health system<sup>1</sup>. Recommendations for blood pressure control include pharmacological and non-pharmacological treatments. The later involves adoption of healthy lifestyle habits, as regular practice of physical exercise. Indeed, a plethora of studies has shown the effectiveness of physical exercise in reducing systolic and diastolic blood pressure<sup>1-4</sup>, collaborating directly with the reduction of cardiovascular risk.

The knowledge about the metabolic predominance during different intensities and types of physical exercise constitutes a factor of great importance for the individualized prescription of training<sup>5</sup>. This becomes fundamental and relevant in rehabilitation programs for hypertensive individuals, given the need in adopting safe intensities for the practice as well as promoting effective benefits on the cardiovascular system. Several studies have investigated distinct methodology that could clearly and accurately characterize the transition between the metabolic stages during exercise<sup>6-9</sup>. Among these methods, the analysis of the lactate concentration and determination of its respective threshold has been widely used because it is considered one of the best biomarker in the analysis of the individual responses from the metabolic point of view, allowing to determine aerobic and anaerobic predominance as well as the evolution of the individual response to exercise training programs<sup>10</sup>.

Currently considered the gold standard for determination of metabolic transition intensity in continuous exercise, the maximum lactate steady state (MLSS) is characterized as the highest intensity where the lactate concentration varies by less than 1 mmol/L during the final twenty minutes of constant workload, that is, where the release and removal of lactate occurs in a balanced way<sup>11</sup>. Studies have shown that the average of accumulation of lactate is around 4 mmol/L<sup>12</sup>. However, there is a great variety of protocols using different populations, types of exercises and intensities for MLSS determination, and therefore, it is expected that there will be a large interindividual variation since its determination is associated to the lactate concentration in the blood.

Previous studies in experimental model and clinical studies have shown that lactate concentration in the steady state is independent of resistance capacity<sup>13-15</sup>. In humans, particularly, Beneke, Hütler, Leithäuser<sup>11</sup> verified that MLSS as well as MLSS intensity are independent of performance, however, subjects with higher performance have higher MLSS workloads. Most of studies examining the lactacidemic responses were performed in athletes or healthy subjects with low physical fitness whereas studies investigating hypertensive subjects, mainly women, are scarce. In this sense, it is important to evaluate such parameters in hypertensive women using walk on treadmill, which is particularly interesting for this population when considering characteristics of the disease, especially with respect to the benefits on cardiovascular system promoted by moderate-intensity aerobic exercise widely proven in this type of ergometer when prescribed at appropriate intensities<sup>16</sup>.

Therefore, the aim of the present study was to investigate the lactate response in physically inactive hypertensive women submitted to the treadmill MLSS protocol. The hypothesis is whether this population has a lower concentration of lactate compared with healthy individuals available in the literature, and that different inclines would determine distinct concentrations in the MLSS even considering the age as possible factor influencer.

## Materials and Methods

### *Study participants*

Twenty-two hypertensive women were eligible for the study. Their age varying between 40-64 years old, fifteen were on antihypertensive therapy and seven were untreated, physically inactive (less than 150 minutes of moderate physical activity or less than 75 minutes of vigorous physical activity), nonobese (body mass index < 30 kg/m<sup>2</sup>), non-prediabetic (fasting blood glucose < 100 mg/dL), nonsmoking, nonalcoholic, and without historic of chronic disease. All procedures were revised and approved by the Ethical Committee from the Institute of Biosciences at the São Paulo State University (UNESP) (n<sup>o</sup> 908.577/2015). Volunteers were informed of the procedures of the study and provided written informed consent.

### *Familiarization and incremental protocol*

Volunteers performed a familiarization period of walking on the treadmill, composed of 4-6 sessions of 20 minutes each aiming to learn all the procedures related to the subsequent tests of the study. After that, women performed one incremental protocol in treadmill in the last session of familiarization for estimate the initial intensity of maximal lactate steady state test, which supposedly could be close of the maximal steady state.

### *Maximal Lactate Steady State (MLSS)*

MLSS test protocol was consisted by walking in treadmill with fixed velocity in 5.5 km/h, and duration of thirty minutes. Initially, was collected a blood sample of ear lobe for future lactate analysis in resting condition. After, the test was started with five minutes of warm-up followed by thirty minutes of walking. New blood samples for lactate analysis as well as heart rate (HR) and subjective perception of effort (SPE) (Scale 6 - 20)<sup>17</sup> were obtained at minute 10 and at the final test, which could be at the minute 30 or when the volunteer could no longer sustain the exercise until the end. The intensity of exercise sessions of each volunteer was determined by the incline of the treadmill. MLSS was defined as the highest sustained walking intensity where the lactate concentration did not increase more than 1 mmol/l between minute 10 and 30<sup>18</sup>. When the lactate concentration was higher than 1 mmol/l at the end of the test, walking on treadmill in the next test session was performed at an incline below that previously performed. Plasma lactate was

measured by enzymatic assay as previously describe<sup>19</sup>. Each volunteer performed a minimum and maximal of 2 and 5 sessions, respectively, for determination of the MLSS. Tests were performed with interval minimum of 48 hours between them.

### *Submaximal VO<sub>2</sub> test*

Peak oxygen consumption was determined indirectly by one mile test (1.609 meters), originally proposed by Kline et al.<sup>20</sup> and later adapted to treadmill by Widrick, Ward, Ebbeling, Clemente, Rippe<sup>21</sup>. All volunteers performed warm up of five minutes previously. After that, a maximal velocity of walking was chosen, which should be maintained until the end of the test (approximately 12-20 minutes). Heart rate was monitored (Polar FS1) during all time. Peak oxygen consumption was calculated according to the following equation proposed by Pober, Freedson, Kline, McInnis, Rippe<sup>22</sup>:

$$\text{VO}_2 \text{ peak} = 92,08 - 0,10 (\text{body weight in pounds}) - 0,34 (\text{age in years}) + 9,72 (\text{gender: male} = 1; \text{female} = 0) - 1,01 (\text{walking time in minutes and hundredths of minutes}) - 0,13 (\text{heart rate in the end of the test}) + 0,86 (\text{physical activity level}).$$

### *Statistical analysis*

Data are presented as means  $\pm$  standard deviation. The normality of the data was verified by Kolmogorov-Smirnov test. Analysis of variance (Anova) for repeated measures was performed comparing lactate curve during the moments of the MLSS test. One sample t-test was used for comparison between MLSS from volunteers of our study and the reference values reported in literature (4 mmol/l). To verify the incline effect on lactate concentration at MLSS and at minute 30 was performed Anova one-way followed by Bonferroni post hoc when appropriate. After confirmed the absence of incline effect on age and the homogeneity of parameters of interaction incline and age, was performed Ancova analysis to verify the incline effect on MLSS and lactate concentration final using age as co-variable, followed by Bonferroni post hoc. Differences in HR and SPE were verified through application of paired t-test for comparison between 10 and 30 minutes in each variable. Analyses were performed using statistical software for Windows (IBM SPSS Statistics 23).  $P < 0.05$  was considered as statistically significant.

## Results

General characteristics of the participants are showed in Table 1. Hypertensive women were overweight and the peak of O<sub>2</sub> consumption was in a normal range for physically inactive middle-aged women. Of the total medications used, 52.8% was for hypertension control and the remainder (47.2%) was for other types of treatment (depression, thyroid, gastritis, cholesterol, among others).

Table 1. General characteristics of the volunteers

Parameters	Mean±SD
Volunteers, n	22
Age, years	52.36 ± 6.57
BMI, kg/m <sup>2</sup>	27.14 ± 2.32
SBP, mmHg	128.71 ± 14.30
DBP, mmHg	83.12 ± 6.78
Peak O <sub>2</sub> consumption, ml.kg <sup>-1</sup> .min <sup>-1</sup>	29.96 ± 3.75
Glycemia, mg/dL	93.14 ± 5.33
Medications	
ARBs, n(%)	11(23.90)
Beta blockers, n(%)	4(8.70)
Diuretics, n(%)	4(8.70)
CCBs, n(%)	2(4.35)
ACE inhibitors, n(%)	2(4.35)
Others non antihypertensives, n(%)	23(50)

Data are mean ± standard deviation. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; ARBs, angiotensin receptor blockers; Beta blockers, beta-adrenergic blockers; CCBs, calcium channel blockers; ACE inhibitors, angiotensin converting enzyme inhibitors.

Table 2. Values of the maximal lactate steady state test

Parameters	Resting	Minute 10	Minute 30	MLSS	Repeated measures Anova	Paired t-test
Lactate, mmol/l	0.96 ± 0.18 (0.57 - 1.36)	3.25 ± 0.75 a (1.73 - 4.73)	3.24 ± 0.94 a (1.34 - 4.90)	3.25 ± 0.81 b (1.54 - 4.82)	F <sub>1,32,27,73</sub> = 134.410; p<0.05	-
HR, beats/min	-	145.95 ± 15.77 (115 - 177)	150.91 ± 16.42 c (120 - 174)	148.43 ± 15.83 (117.5 - 174)	-	t21 = -3.983; p<005
SPE	-	13.64 ± 1.50 (11 - 17)	15.82 ± 2.00 c (12-19)	14.73 ± 1.46 (12-18)	-	t21 = -5.202; p<005

Data are mean ± standard deviation (minimum - maximal). HR, heart rate; SPE, subjective perception of effort. a = P < 0.05 compared with resting; b = P < 0.05 compared with MLSS reference value available in the literature (4.0 mmol/l); c = P < 0.05 compared to minute 10.

Table 3. Relationship between incline effect and lactate concentration.

Dependent variable	One way Anova		Ancova	
	Incline	Age	Incline	
Lactate, mmol/l				
MLSS	F <sub>7,14</sub> = 2.335; p > 0.05	F <sub>1,13</sub> = 0.888; p > 0.05	F <sub>7,13</sub> = 2.256; p > 0.05	
Minute 30	F <sub>7,14</sub> = 3.539; p < 0.05	F <sub>1,13</sub> = 2.170; p > 0.05	F <sub>7,13</sub> = 3.774; p < 0.05	

Data are mean ± standard deviation (minimum - maximal). MLSS, maximal lactate steady state.

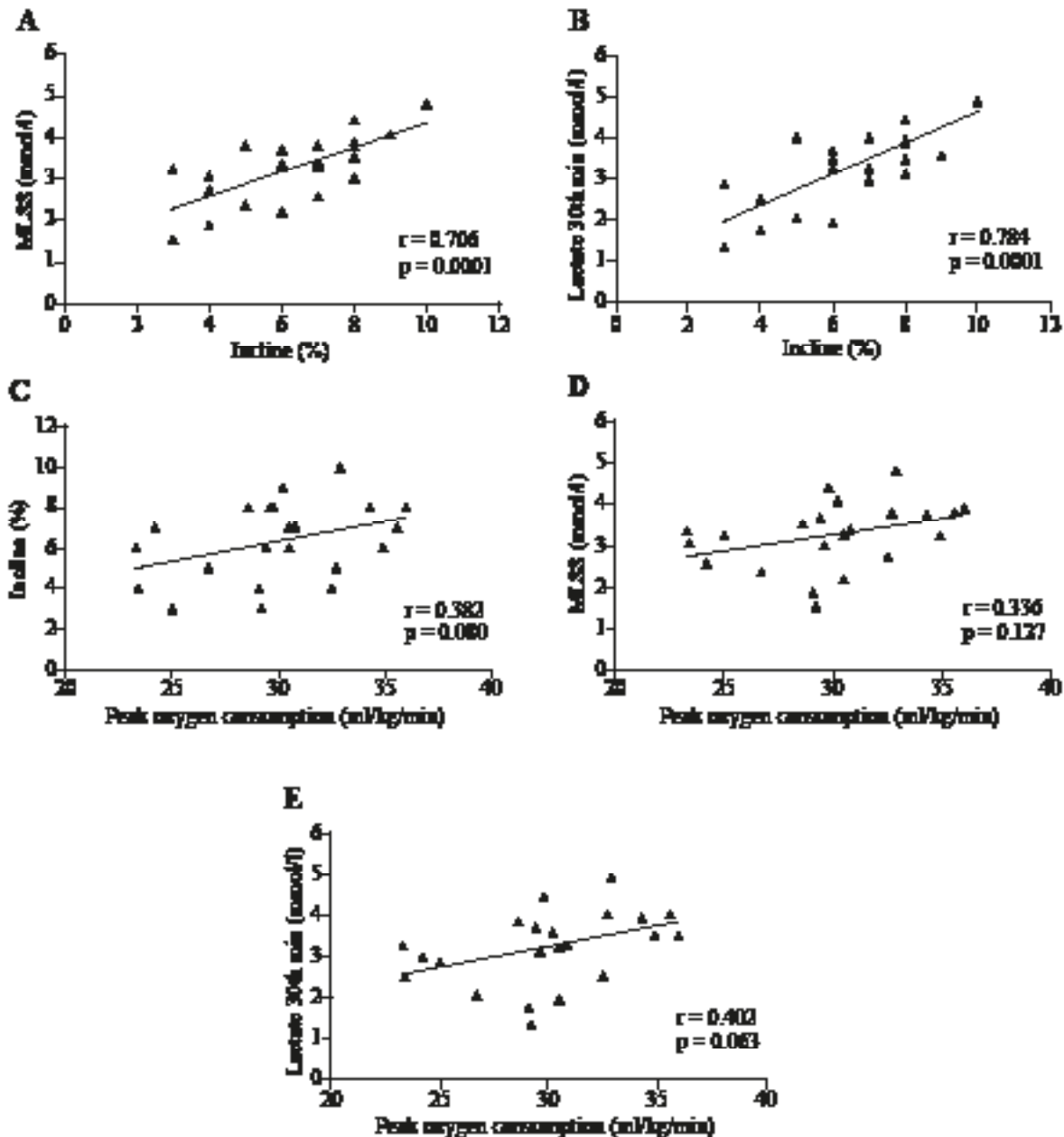
Positive and strong correlations were found between incline and MLSS (r = 0.706; p < 0.05) and incline and lactate concentration at the end of the test (r = 0.784; p < 0.05). In addition, peak oxygen consumption showed positive and

Table 2 shows the values obtained from the lactate concentration curves of the volunteers submitted to the MLSS tests. Hypertensive women showed MLSS at 3.25 ± 0.81 mmol. After confirmed differences between the moments rest, 10<sup>th</sup> and 30<sup>th</sup> minute (p < 0.05), Bonferroni post hoc showed that only the 10<sup>th</sup> and 30<sup>th</sup> minutes did not differ from each other. When applied one sample t-test, we found that MLSS was significantly lower (18.8%) for this population (t21 = -4.332; p < 0.05) as compared with data adopted in the literature (MLSS: 4 mmol/L). As expected, HR and SPE were higher at minute 30<sup>th</sup> when compared with the minute 10<sup>th</sup> (3.4% and 16.0%, respectively).

Regarding incline, no effect on MLSS was observed (p > 0.05) in all participants. On the other hand, the incline affected significantly lactate concentration at the end of the test (p < 0.05), however, this was not confirmed through post hoc analysis. When excluded the age as confounding factor, no effect was observed of this co-variable on MLSS (p > 0.05) as well as no effect was observed for incline corrected on MLSS (p > 0.05). Similarly, the co-variable age showed no effect on lactate concentration at the end of the test (p > 0.05). Interestingly, there was effect of the incline on lactate concentration at 30<sup>th</sup> minute (p < 0.05) when the age factor was corrected, although this effect has not been observed in post hoc analysis. Data are shown in Table 3.

weak correlation with incline (r = 0.382; p > 0.05) and MLSS (r = 0.336; p > 0.05), and positive and moderate correlation with lactate concentration at minute 30 (r = 0.402; p > 0.05) (Figure 1, panels A- E).

Figure 1 - Correlation between incline, peak oxygen consumption and lactate concentration.



A: incline and MLSS; B: incline and lactate at 10<sup>th</sup> minute; C: peak oxygen consumption and incline; D: peak oxygen consumption and MLSS; E: peak oxygen consumption and lactate at 10<sup>th</sup> minute. MLSS, maximal lactate steady state.

When adjusted by the age factor, incline showed a strong correlation for the variables MLSS ( $r = 0.707$ ;  $p < 0.05$ ) and incline and lactate at minute 30 ( $r = 0.792$ ;  $p < 0.05$ ), while peak oxygen consumption showed correlations moderate for incline ( $r = 0.435$ ;  $p < 0.05$ ) and weak for MLSS ( $r = 0.270$ ;  $p > 0.05$ ) and lactate concentration at minute 30 ( $r = 0.327$ ;  $p > 0.05$ ) (Figure not showed).

## Discussion

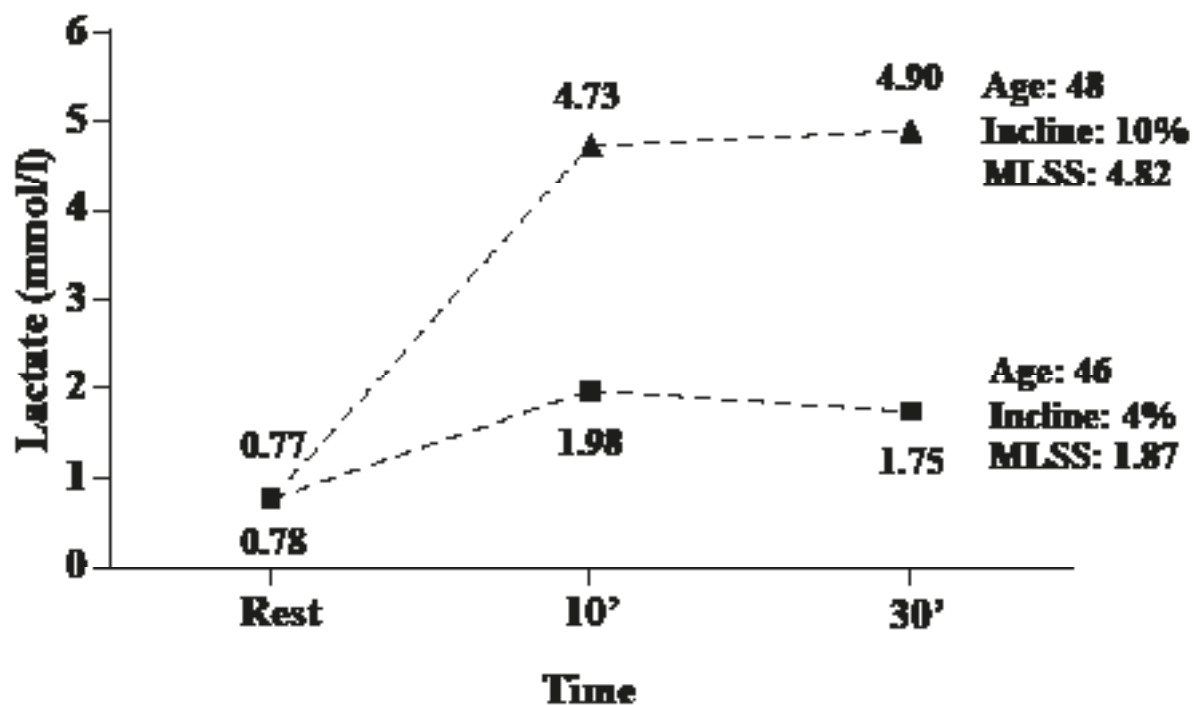
The prevalence of arterial hypertension increases abruptly during climacteric period and it is well established the relevance

of exercise training on blood pressure regulation<sup>23</sup>. In addition, the intensity of physical exercise is the primary variable for management hypertension in general population in an attempt to get the best beneficial responses on the cardiovascular system. As stated previously, studies examining hypertensive women during climacteric period is crucial to establish adequate exercise intensity for hypertension treatment. Indeed, previous study found lactate values in steady state ranging from 1.9 - 7.5 mmol/L (mean value in 4.9 mmol/L) evaluating adult males in cycle ergometer<sup>11</sup>. Our study showed that MLSS in hypertensive women was reached close to 3.25 mmol/L, below of the mean value and closer to the lower limit of variation for humans. Incline did not have any effect on lactate at its maximum steady state irrespective of age.

On the other hand, incline affected lactate concentration at the end of the test age-independently but this phenomenon could not be verified by post hoc analysis. In moderate-intensity exercises, an increase in lactate level is observed as compared with rest values, with slight changing in its concentration, but remaining constant when the workload is maintained<sup>18,24</sup>. Analyzing the obtained data individually in the studied population, even though the mean delta difference between the lactate concentrations of the two moments remained constant ( $\Delta = -0.3$  mmol/L), some volunteers presented increases of the concentration of lactate reaching up to 0.81 mmol/L between moments, which supposedly may have compromised the incline effect observed only in the final lactate analysis, since the MLSS is defined as the mean obtained between period of 20 minutes. On the other hand, strong correlations were found between incline and MLSS and lactate at the end

of the test. The fact that volunteers are physically inactive does not guarantee that the optimal aerobic training intensity would be the same for all. This could have been further strengthened if larger inclines were also translated into higher aerobic capacity as assessed by peak oxygen consumption in our study. Despite there was no observed strong correlation between peak oxygen consumption with incline and lactate, a comparison of some of our data shows the existence of different patterns involving incline. It is clearly observed discrepant values of lactate for a very close age group between two volunteers of the study (for more details please see figure 2). In this sense, the hypothesis of incline effect cannot be ruled out, and more studies are necessary to investigate this association (incline and lactate levels) in this particular population as well as in other populations to get conclusive information.

Figure 2 – Variations in the lactate concentration pattern of physically inactive hypertensive women submitted to different intensity exercise.



MLSS, maximal lactate steady state.

Increase in the number and size of mitochondria, increase in the capillary density and improving the use of energy sources by skeletal muscles are some of the factors that allow aerobically trained individuals to reach higher steady state levels<sup>25,26</sup>, that does not appear to be affected over the years<sup>27</sup>. Interesting study with 27 trained individuals distributed in 3 different age groups, Mattern, Gutilla, Bright, Kirby, Hinchcliff, Devor<sup>28</sup> found a decrease in  $VO_2$  in MLSS and MLSS intensity in older subjects ( $64.6 \pm 2.7$  years) when compared with younger ( $25.9 \pm 1.0$  years) and middle-aged adults ( $43.2 \pm 1.0$  years), however, there were no differences for lactate concentration in the maximal steady state, although there was a tendency to decrease with age (the elderly presented values of approximately 3.5 mmol/L, while values greater than 4

mmol/L were observed in young subjects). These results reinforce the weak correlation between peak oxygen consumption and lactate levels found in our study, suggesting that lactate levels do not seem to be directly associated with a decrease in peak oxygen consumption when evaluated throughout the test of one mile. When associated physical inactivity with aging, changes in cardiovascular, metabolic and muscular profile become more pronounced and preponderant for a lower exercise performance, however it is not known if the lactate responses and its maximum steady state would be affected by age or physical inactivity in this particular population.

Although our volunteers showed heterogeneity in the incline corresponding to MLSS, the protocol employed in this study

proved to be effective for the identification of MLSS in this population. As proposed in the literature, MLSS was determined at the highest intensity where there was no variation greater than 1 mmol/L after minute 10<sup>th</sup><sup>18</sup>. Given that walking is widely adopted as exercise training in rehabilitation programs, we chose to standardize the speed of the MLSS tests at 5.5 km/h for all volunteers because it was apparently the highest velocity in which no joggings occurred, being the stability of individual lactate achieved by increasing the incline of the ergometer. Likewise, we chose to perform two blood lactate collections during the test (minutes 10 and 30) due to the difficulty of collecting the volunteers on incline walking and to avoid major interruptions of exercise during the sampling moments of the blood sample, which could promote a greater lactate disappearance even though the collection time lasts just a few seconds.

It is not yet known whether menopause could influence MLSS. An interesting review showed contradictory results when it was compared different periods of the menstrual cycle submitted in an incremental protocol<sup>29</sup>. In our volunteers, seven were in premenopausal period, five were in transition phase and the other ten were in postmenopausal period, with four using menopausal hormone therapy. The stratified analysis of our data was not sufficient for concrete statements to be drawn. Indeed, a larger and specific sample size for this purpose are necessary. A possible explanation for the impact of menopause on MLSS would be related to the effects of estradiol on fatty acid oxidation and its inhibition on muscle glycogen metabolism. Kendrick, Steffen, Rumsey, Goldberg<sup>30</sup> observed longer running time until exhaustion as well as lower glycogen utilization during submaximal exercise in oophorectomized rats treated with estradiol. In this sense, it could be thought that greater aerobic intensities could be sustained by longer durations without increasing the participation of the anaerobic metabolism, for example in volunteers who could not complete the total exercise time. Indirectly, less glucose utilization would result in lower lactate production.

The use of certain classes of antihypertensive drugs also exerts effects on the practice of exercise, including on blood lactate levels. The literature has shown that the use of beta-blockers decreases lactate concentration during high and moderate intensity exercises<sup>31,32</sup>, with the responses varying for the use of selective and non-selective beta blocker<sup>31</sup>. Also, Minami et al.<sup>33</sup> verified that rats treated with perindopril, angiotensin converting enzyme inhibitors (ACE inhibitors), had greater tolerance to exercise due to delayed lactate increase when compared with control rats. On the other hand, Bergeron et al.<sup>34</sup> found higher lactate increases in adult males when submitted to a exercise protocol in cycle ergometer for 40 minutes at 50% maximal oxygen consumption followed by 30 minutes at 70% maximal oxygen consumption on enalapril therapy when compared with control group. In our study, only four women were on beta-blockers therapy and two were on ACE inhibitors treatment, minimizing a possible influence of these medications on the metabolic parameters. Regarding angiotensin receptor blockers (ARBs), studies show a direct influence on glucose metabolism and an increase in lactate concentration in resting conditions after administration of the drug<sup>35</sup>. When we compared eleven volunteers who were on ARBs therapy with those who were not on ARBs, no significant differences were found in lactate concentrations and MLSS incline (data not shown), suggesting that this class of drug has no influence on exercise performance.

The mean values for both HR and SPE in the MLSS reveal a moderate-high intensity, which is expected for a MLSS protocol. A perfect relationship between HR ( $\pm 148$  bpm) and SPE ( $\pm 14$ ) originally proposed in scale of 6 to 20, which would roughly represent HR variations between 60 and 200 bpm<sup>17</sup>, was verified. This further strengthens the use of this scale as an effective tool for intensity monitoring. It should be pointed out that exercise in MLSS does not represent a global physiological stability<sup>36,37</sup>, as represented in our protocol by the increase in HR and SPE during exercise session, suggesting that several mechanisms are triggered during exercise session in response to exercise intensity as well as for maintenance of the exercise including autonomic nervous system, metabolic parameters (lactate levels) and motor behavior.

It should be emphasized that for MLSS protocol is necessary two to five sessions as well as a long-lasting test to characterize the intensity, besides experience by the applicator. It is important to be attentive to the signals transmitted by each individual and by monitoring tools during the test. The voluntary withdrawal of a test, for example, should always be accompanied by hyperventilation and high HR and SPE values, that should be consistent with the subject's profile avoiding bias on intensity determination. In our study, a well-controlled of those variables was assessed assuring that MLSS was reached for each volunteer.

Based on our results, it is concluded that physically inactive hypertensive women show a lower MLSS than the average established in the literature but within the range of variations previously reported. Furthermore, a higher MLSS incline correlates positive and directly with higher lactate concentrations for the same aerobic capacity regardless of age. We reiterate the need for further research with this population and in large number for controlling other possible influencing factors such as menopause and replacement therapy as well as the use of antihypertensive medications.

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## ERRATUM

In the article “*Evaluation of maximal lactate steady state in middle-aged hypertensive women*”, published in volume 24, number 2, 2018: DOI: 10.1590/S1980-6574201800020013 and identification: e101896.

In the page 2:

### Where it was written

$P < 0.05$  was considered as statistically significant

### Should read:

Additionally, Pearson’s correlation analyses were performed to assess the relationship between incline and MLSS, incline and lactate at minute 30, peak oxygen consumption and incline, peak oxygen consumption and MLSS, and peak oxygen consumption and lactate at minute 30.  $P < 0.05$  was considered as statistically significant.

In the page 5:

### Where it was written

These results reinforce the weak correlation between peak oxygen consumption and lactate levels found in our study,

### Should read:

These results reinforce the weak and non significant correlation between peak oxygen consumption and lactate levels found in our study,