DOUBLE EFFORT TEST FOR EVALUATION OF AEROBIC CAPACITY OF DIET-INDUCED OBESE RATS

EXERCISE AND SPORTS SCIENCES

ORIGINAL ARTICLE

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ABSTRACT

Introduction: The literature lacks studies about lactate actions and some limitations in studies involving healthy individuals or patients with some metabolic disorder. Objectives: This study aimed to evaluate the protocol of double effort test for obese-induced rats. Methods: Fourteen male Wistar rats were divided into two groups: Control (Con) and Obese (Obe). The control group was fed with standard chow and water ad libitum. The obese group was fed with standard chow, water ad libitum and hyperlipidic diet. Twelve weeks after the beginning of the hyperlipidic diet, insulin tolerance test, Maximal Lactate Steady State (MLSS) test and the double efforts test were performed. Results: The diet was effective to promote obesity. The obese group decreased insulin sensitivity in approximately 19% (Con = 2.156 ± 0.1187 AU vs Obe = 1.742 ± 0.1551 AU). The lactate concentration and velocity of anaerobic threshold at MLSS test were 3.780 ± 0.09 mmol/L e 18 $m.min^{-1}$ in both groups. The velocity of anaerobic threshold estimated by double efforts test was 15.59±0.653 $m.min^{-1}$ in Con group control animals and 16.42±0.672 $m.min^{-1}$ in Obe group. The double effort test underestimated around 13% and 8.7% the aerobic capacity in control and obese groups respectively, however, presented significant correlation with MLSS ($r = 0,88$; $P < 0,0075$ controls / $r = 0,92$; $P < 0,0031$ obese). Conclusion: So, the double effort test can be an interesting alternative to evaluate the aerobic capacity for both healthy sedentary and obese animals.

Keywords: obesity, lactic acid, running.

INTRODUCTION

In 1808, Barzelius observed that lactic acid was produced in the musculature of deers when they were hunted[1]. Approximately two centuries later, the lactate metabolism has not been well-elucidated and we need further understanding about its production, accumulation, removal and function (Lactate + hydrogen ions) during rest and muscular contraction[2,3].

Many researchers try to understand the lactate actions[4-6]. In 1964, Wasserman and McLlory introduced the term Anaerobic Threshold which could characterize the inflexion point of the lactacidemic curve, a momenta t which the transition zone between aerobic and anaerobic metabolism is found where the concentrations of this acid are approximately at 4.0 mmol/L[7]. Since then, many tests have been developed to measure aerobic capacity, such as the maximal lactate steady state (MLSS), which may be defined as the highest intensity at which the lactate concentrations are kept steady in exercise of long duration, being the balance point between lactate production and removal[8]. The protocol which evaluates the MLSS consists in the application of many tests at different intensities with 30-minute duration performed on two distinct days. For each intensity, blood samples are collected at every five minutes of exertion[9]. This test is able to measure in an individual and reliable manner, the metabolic transition moment, being hence considered gold-standard for validation of other protocols of anaerobic threshold test[10,11].

Besides the MLSS, other invasive tests – minimal lactate[8], *onset blood lactate* (OBLA) obtained by the lactate 4mmol/L steady concentration[12], double effort tests[13] – and non-invasive – Critical Power[14] – are able to evaluate aerobic capacity. However, due to the strong need to improve knowledge on the lactate activities and some limitations in the investigations with humans, the application and validation of protocols which evaluate aerobic capacity in experimental models using rats is increasing[15,16].

In 2011, Manchado-Gobatto[17] *et al.* validated the non-exhaustive double effort protocol on treadmill using sedentary Wistar rats. This test proposed by Chassain[13] and adapted by Manchado-Gobatto[17] consists in the performance of two five-minute efforts at each intensity, separated by two minutes of recovery between them, with blood collection for lactacidemic analysis at the end of the first and second efforts, calculating the null delta lactate with these values. We believe that since it is a test which does not lead to body exhaustion, its validation and applicability in populations which present any limitation in performing physical efforts or any chronic-degenerative disease is very relevant. The main causes for increase in weight as fat are related to environmental factors such as bad eating habit and physical inactivty[18,19]. Therefore, the main measures to be taken for obesity and related diseases prevention and/or treatment are eating reeducation and regular practice of physical exercise[20,21]. Thus, the aim of the present study was to test the use and applicability of the protocol of double effort test proposed by Chassain[13] and adapted by Manchado-Gobatto[17] in obese rats induced by hyperlipidic diet.

METHODS

Male Wistar rats weighing around 200 g were allocated in the Animal Facility and divided in groups of seven animals per cage, under mean temperature of 22 ± 2ºC and 12h-light/dark cycle with

light cycle initiating at 07h00min a.m.. The experimental procedures used in the present study were approved by the Ethics Committee in Animal Experimentation of UNESP – Presidente Prudente Campus, file # 74/2009.

Obesity induction

Fourteen rats were distributed in two groups with seven animals – Control (Con) and Obese (Obe). The control animals were fed with standard chow (SUPRA LAB – Alisul Ind. Alimentos Ltda., São Leopoldo/RS; with composition of 25% of proteins, 3% of lipids, 18% of fibers, 11% of mineral material, 2% of calcium and 0.5% of phosphorus) and they were offered water *ad libitum*. The grou of obese rats was fed hyperlipidic diet composed of bacon, bologna, wieners, cookies, soda and standard chow, in a proportion of approximately 2:2:2:1:1:1, respectively, in a composition of 28% of carbohydrates, 13% of proteins and 59% of lipids, from the second month of life[22]. The animals were weekly weighed for follow-up of the body weight evolution.

Insulin tolerance test

The test consists in the administration of regular insulin, and the glucose decrease rate was evaluated for 25 minutes. The test was performed at the end of 12 weeks of diet, before the protocols which evaluated the aerobic capacity of the rats. The animals remained six hours at water, food and diet fasting. A small section was performed on the distal extremity of the animals' tail for blood samples collection. The first collection was performed before the intraperitoneal basal insulin administration. 1 U/kg of body weight of regular insulin (Novolin 100U/ml) with saline solution 0.9% + BSA 0.25% was administered. The blood collections were performed at times baseline (0 minute), five, ten, 15, 20 and 25 minutes, after insulin administration. Glycemia was verified with gluco tabs and a glucometer (Biocheck TD-4225/Bioeasy Diagnóstica Ltda. /MG, Brazil). This procedure was always performed in the afternoon shift to maintain the same insulin sensitivity status of the animals, and was performed exactly the same way for all groups. Subsequently, the decrease constant was calculated (kITT expressed in %/min.) from the linear regression of the glycemia concentrations obtained during the test[23].

Adaptation to treadmill

After 12 weeks of hyperlipidic diet, prior to the performance of the Maximal Lactate Steady State test and the non-exhaustive of double efforts test (Chassain), it was necessary to previously select the 'running' rats to compose the sample with duration of seven days. Each animal ran for five minutes per day at 10 m.min velocity^{-1}. The animals which were able to successfully finish between nine and 10 sessions were selected. After the selection period, the running animals underwent an adaptation process to the treadmill. This process occurred during two weeks with exercise sessions, three times per week, at increasing velocities (5-15 m.min^{-1}), and maximum duration of 15 minutes each session, being the protocol adapted by Manchado-Gobatto *et al.*,[17]. After the adaptation period, the animals performed the tests for identification of the anaerobic threshold intensity.

Maximal lactate steady state (MLSS)

The rats were submitted to five continuous tests at velocities equal to ten 10, 15, 18, 22 m.min^{-1}. Each animal performed the four tests with 48-hour interval between them. The velocities sequence was randomly distributed. For each velocity, the animals remained in continuous running for 25 minutes. Blood samples were extracted from the tail of the rats in six moments: baseline, five, ten 10, 15, 20 and 25 minutes of test. After the lactacidemic analysis, a plotting chart was designed, and the highest running velocity at which there was increase equal or lower than 1 mmol/L from the 10th to the 25th minute of exercise was considered as equivalent to MLSS[10,24].

Chassain test

The test was composed of two efforts of five minutes of duration with passive recovery between them at velocities ten 10, 15 and 20 m.min^{-1}. Each animal performed the test at the three intensities, randomly chosen, with 48-hour interval between them[16].

The critical load was determined using the results of the Chassain test (figure 1).

This value was calculated for each animal which performed the test. The values c abe seen in tables 1 and 2 in the results section.

Lactacidemic analysis

The lactacidemic analysis was performed from 25 µl blood samples which were collected from the distal proximity of the animal's tail in a heparinized capillary after the first effort and after the second effort at each velocity mentioned above. These samples were immediately transferred to 1.5 ml tubes containing 50 µl of NaF solution at 1% and stored in ice for subsequent electroenzymatic reading (YSL 2700 STAT, Yellow Springs Co., USA).

Statistic analysis

Data normality was confirmed by the Shapiro-Wilk test. The ANOVA test with repeated measures was used for comparison of the body weight values. Insulin sensitivity was evaluated with the non-paired Student's *t* test. The Person correlation test was used to evaluate the correlation between the Anaerobic Threshold tests. The differences between groups were considered significant when *P* value was < 0.05. The statistical package used was IBM SPSS Statistics 20.0 for Windows.

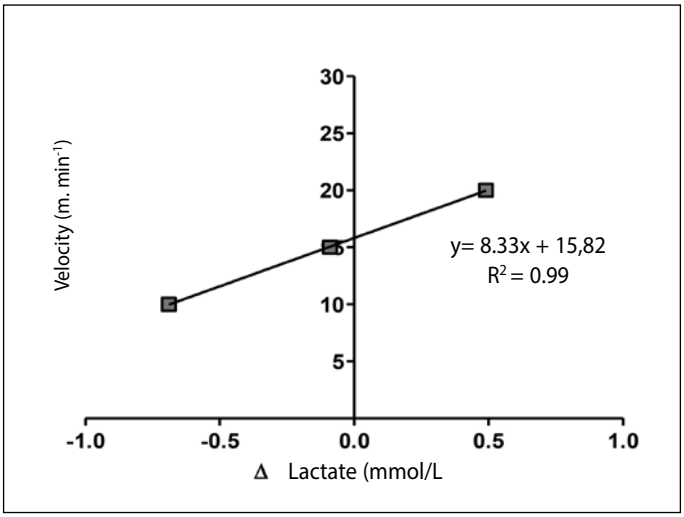

Figure 1. Example of determination of critical load by the Chassain test. The points represent the delta lactate obtained at each intensity. A linear regression was plotted and threshold velocity corresponds to the Y value (16.22 m.min^{-1}).

Table 1. Delta lactate of the intensities (10, 15, 20 m.min^{-1}), individual estimation of critical load (intercept Y) and linear e coefficient (R^2) of the control animals – Chassain Test.

Animal	Δ Lactate			Threshold velocity (m.min^{-1})	R^2
	10 m.min^{-1}	15 m.min^{-1}	20 m.min^{-1}		
	mmol/L	mmol/L	mmol/L		
1	-0.15	0.00	0.15	15.65	0.98
2	-1.86	-0.71	0.39	18.22	0.99
3	-0.25	0.00	1.65	12.92	0.84
4	-0.48	-0.33	0.77	15.08	0.83
5	-1.87	-0.12	0.53	16.89	0.93
6	-0.30	-0.15	0.69	14.30	0.86
7	-0.68	-0.09	0.51	16.13	0.99
Mean	-0.798	-0.200	0.670	15.59	0.917
SEM	0.282	0.094	0.180	0.653	0.027

Table 2. Delta lactate of the intensities (10, 15, 20 m/min^{-1}), individual estimation of the critical load (intercept Y) and linear coefficient (R^2) of the obese animals –Chassain Test.

Animal	Δ Lactate			Threshold velocity (m.min^{-1})	R^2
	10 m.min^{-1}	15 m.min^{-1}	20 m.min^{-1}		
1	-0.78	-0.6	0.24	18.26	0.87
2	-0.42	-0.33	0.09	18.75	0.87
3	-0.95	-0.48	1	15.67	0.91
4	-0.27	-0.42	0.09	17.62	0.47
5	-0.95	0	0.81	15.26	0.99
6	-0.68	0.42	0.72	14.01	0.90
7	-0.38	-0.03	0.42	15.42	0.97
Mean	-0.632	-0.205	0.480	16.42	0.854
SEM	0.105	0.134	0.138	0.672	0.066

RESULTS

Obesity was induced by hyperlipidic diet initiated when the animals were two months old. Increase of body weight was observed in the group obese from the fourth week of the diet (figure 2).

The obese group presented reduction in the insulin sensitivity of approximately 19% after 12 weeks of the diet (figure 3) and before performing the training protocol. .

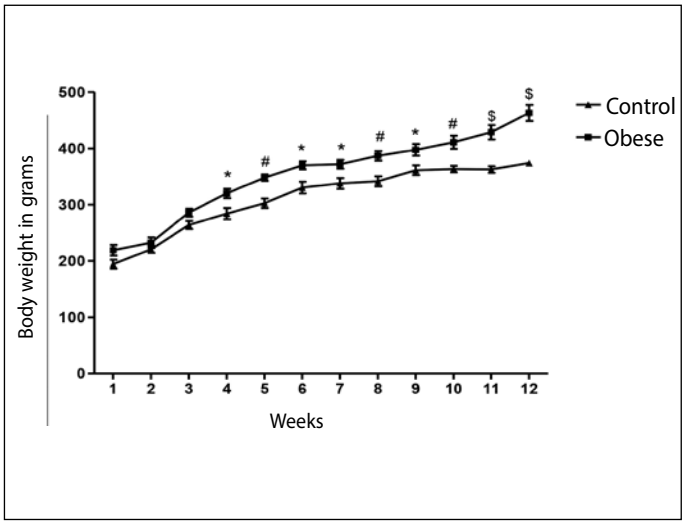

Figure 2. Body weight of the animals during 12 weeks. The data are presented as mean ± SEM. *$P < 0.05$ *versus* Control; #$P < 0.01$ *versus* Control; $P < 0.001$ *versus* Control (n = 7).

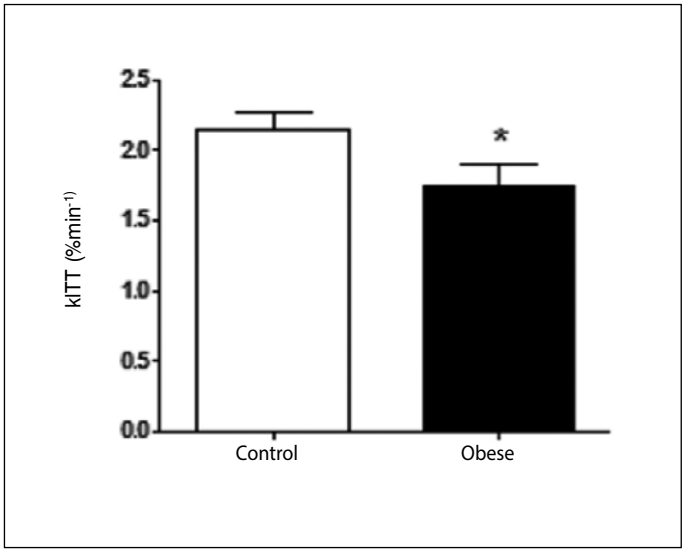

Figure 3. Glucose decrease per minute constant (kITT). Data are presented as mean ± SEM. *$P < 0.05$ *versus* Control (n = 7).

The delta lactate values progressively increased according to the effort velocity increments. Statistic difference has not been observed between groups in the double efforts test. In the maximal steady state test, the lactate concentration and the anaerobic threshold velocity were 3,780 ± 0.09 mmol/L and 18 m.min^{-1}, respectively, for both groups (figure 4).

The threshold velocity estimated by the Chassain test was of 15.59 ± 0.653 m.min^{-1} (R = 0.917 ± 0.02) (table 1) for the animals from the control group. The threshold velocity estimated by the Chassain test was of 14.02 ± 0.565 m.min^{-1} (R = 0.86 ± 0.03) (table 2) for the animals from the obese group.

DISCUSSION

The use of experimental models with rats for evaluation of metabolic and physiological parameters has rapidly increased and has been extremely reliable for humans[15,24]. However, studies which evaluate the physical capacity of healthy animals and especially animals with any metabolic disorder are still scarce. The present study evaluated aerobic capacity through the method

proposed by Chassain[13] adapted by Manchado-Gobatto[17] in thin and obese rats by hyperlipidic diet.

Half of the animals in this study were submitted to a protocol of hyperlipidic diet with the aim to make them obese. After four weeks of exposure to this hyperlipidic diet, there was significant increase in body weight of the animals from the obese group and reduction in the insulin sensitivity when compared with the animals from the control group (figures 2 and 3), which only received standard chow as food, highlighting the obesogenic effect of the diet[22] and the correlation between obesity and metabolic alterations caused by fat excess[25]. The cytokines released by the excessive adipose tissue may generate a scenario of peripheral systemic inflammation[26,27], altering the insulin signaling and initiating a possible insulin resistant status[28].

Obesity, besides altering the physiological functions[28], may be considered as a limiting factor for exercise tests performance, even in animals. The majority of the protocols used for identification of effort intensity leads the animal to moments of physical exhaustion[24], which reduces applicability when animals with any kind of chronic metabolic or functional alteration are considered. Thus, the double effort test presents great advantage in the evaluation of aerobic capacity of animals suffering from any pathology since it is not exhaustive.

In order to validate the proposed method, the maximal lactate steady state test (MLSS) was applied, which is considered 'gold-standard' for the identification of aerobic capacity[17] (figure 4).

Manchado-Gobatto *et al*.[17] validated the double effort protocol on treadmill using healthy sedentary male Wistar rats. The velocity corresponding to the MLSS was of 20 m.min^{-1} (lactate concentration = 3.90 ± 0.03 mmol/L), with the Chassain protocol underestimating the aerobic capacity of the animals in 20%, according to considerations from the study itself. The results of this study (figure 4) were lower than those found by Manchado-Gobatto *et al*[17]. The intensity corresponding to the MLSS of the control group as in the obese group was of 18 m.min^{-1} (lactate concentration = 4.13 ± 0.139 mmol/L). The double effort test underestimated the aerobic capacity of the animals in 13% (control) and 8.7% (obese); however, significant correlation was found between the tests for the animals from the control group ($r = 0.88$; $P < 0.0075$) and obese group ($r = 0.92$; $P < 0.0031$) (tables 1 and 2). Other studies, such as the ones by Pilis *et al*.[29] and Langfort *et al*.[30], which evaluated the LAn using the progressive test of multistages, estimated the zone of metabolic transition through individual charts of the lactate concentrations versus velocity and found higher LAn intensities (25 m.mn^{-1}).

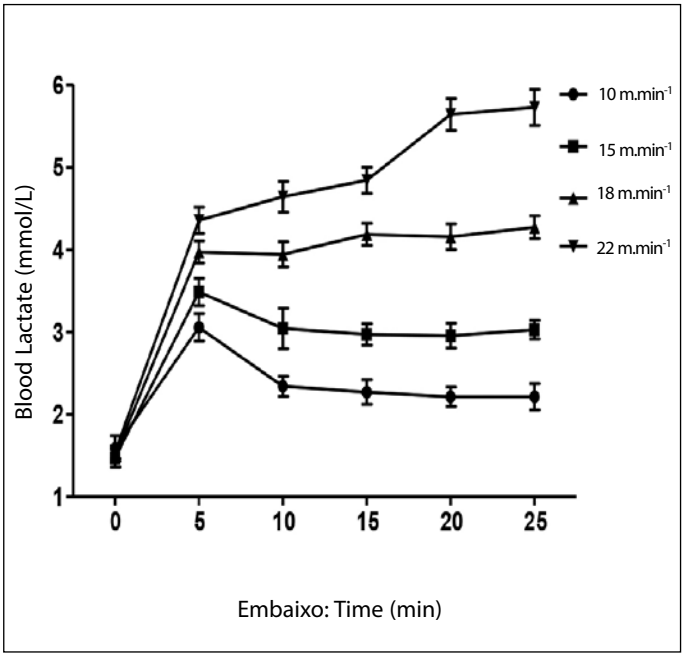

Figure 4. Bllod lactate values during the Maximal Lactate Steady State test in the times 0, 5, 10, 15, 20 and 25 minutes of test (n = 7). Data are presented as mean ± SEM.

The differences found in the MLSS lactacidemic concentrations and the double effort test also suggest possible protocol-dependence as observed in the study by Manchado-Gobatto *et al*.[17]; however, the double effort test presented high correlation with the MLSS both for the control and obese animals which presented alterations in insulin sensitivity, suggesting possible applicability in populations with any metabolic disorder.

The great limitation of this study was the fact the animals were not able to reach the 25 m.min^{-1} running velocity in the maximal steady state test, which caused alteration in the tests from 20 to 18 m.min^{-1} and from 25 to 22 m.min^{-1} performance velocities, being different from the ones suggested in the study by Manchado-Gobatto *et al*.[17]. Similarly, such fact caused exclusion of the 25 m.min^{-1} velocity used in the Chassain test, keeping only the 5, 10 and 20 m.min^{-1} velocities.

In fact, further investigation is necessary to understand the lactacidemic behavior in the different animal models and the several LAn evaluation protocols. It is also worth mentioning the importance to analyze tests models which individually evaluate LAn of the animals, since work intensity may alter the physiological responses. Thus, methods which individually estimate the aerobic capacity will present reliable results and will enable the prescription of a training protocol respective to the physical capacity of each animal.

CONCLUSION

The double effort test presented high correlation with the MLSS, indicating its possible applicability to evaluate the aerobic capacity both of healthy sedentary animals and obese animals with harmed insulin sensitivity.

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All authors have declared there is not any potential conflict of interests concerning this article.

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