Biology of Sport, Vol. 25 N°1, 2008

MINIMUM BLOOD LACTATE AND MUSCLE PROTEIN OF RATS DURING SWIMMING EXERCISE

F.A. Voltarelli, C.A. Gobatto, M.A.R. de Mello
UNESP, Sao Paulo State University, Brazil

Abstract. Few studies dealing with effort intensity during swimming exercise in rats have been reported in the literature. Recently, with the use of the lactate minimum test (LMT), our group estimated the minimum blood lactate (MBL) of rats during swimming exercises. This information allowed accurate evaluation of the effort intensity developed by rats during swimming exercise. The present study was designed to evaluate the effects of swimming exercise sessions in below, equivalent and above intensities to MBL, on protein metabolism of rats. Adult (90 days) sedentary male Wistar rats were used in the present study. Mean values of MBL, in the present study, were obtained at blood concentration of 6.7±0.4 mmol/L with a load of 5% bw. The animals were sacrificed at rest (R) or immediately after a single swimming session (30 min) supporting loads below (3.5% bw), equivalent (5.0% bw) and high load (6.5% bw) to AT. Blood samples were collected each 5 min of exercise for lactate determination. Soleus muscle protein synthesis (amount of L-[^{14}C] fenil alanyn incorporation to protein) and breakdown (tyrosin release) rates were evaluated. Blood lactate concentrations (mmol/L) stabilized with the below (5.4±0.01) and equivalent (6.4±0.006) to MBL but increased, progressively, with the high load. There were no differences in protein synthesis (pmol/mg.h) among rest values (65.2±3.4) and after-exercise supporting the loads below (61.5±1.3) and the equivalent (60.7±1.7) to MBL but there was a decrease with the high load (36.6±2.0). Protein breakdown rates (pmol/g.h) increase after exercise supporting the loads below (227.0±6.1), equivalent (227.9±6.0) and high (363.6±7.1) to MBL in relation to the rest (214.3±6.0). The results indicate the viability of the application of LMT in studies with rats since it detected alterations imposed by exercise.

(Biol.Sport 25:23-34, 2008)

Key words: Minimum blood lactate - Lactate minimum test - Protein synthesis - Protein degradation - Rats

---

Reprint request to: Prof. F.A. Voltarelli, Dept. of Physical Education, University of Sao Paulo State (UNESP), Campus of Rio Claro, 24-A Avenue, number 1515, Bela Vista, 13506-900, Rio Claro–State, São Paulo, Brazil.
Tel: (005519) 3526-4100; Fax: (005519) 3526-4321; E-mail: faunesp8@ yahoo.com.br

Introduction

The energy supply for muscular contraction during exercise may be derived from the aerobic and anaerobic metabolisms. During exercise, there is a transition zone in which a change on the predominance from aerobic metabolism to anaerobic metabolism occurs. This transition zone showed to be important in the evaluation of the physical conditioning and in the training prescription [22]. For this reason, several physiological evaluation protocols were developed in order to detect this metabolic transition zone, many of them employing blood lactate as marker. Among all procedures, the most used in practice are: the ventilatory threshold, observed by Wasserman and Mc. Ilroy [28], the anaerobic threshold [AT] identified through the increase on the blood lactate concentration, proposed by Kinderman *et al.* [12], the procedure that considers the blood lactate concentration of 4.0 mmol/L as the "beginning" mark of the blood lactate accumulation (OBLA), suggested by Sjodin and Jacobs [20], among others.

Tegtbur *et al.* [21] developed a test protocol for the metabolic transition identification, named minimum lactate test (MLT). This test involves the performance of supramaximal exercise for a short period of time with the objective of inducing hyperlactacidemia before the beginning of the standard test with progressive loads in treadmill. The minimum blood lactate (MBL) was defined as the speed in which the U-shaped curve obtained with the blood lactate values during progressive test reaches the nadir. This minimum blood lactate value supposedly indicates the metabolic transition [9,17,21,25].

For obvious reasons, a large number of researches involving physical exercises have been conducted with laboratory animals, especially rats, with blood lactate concentrations used for the effort intensity determination. As metabolic differences occur between human being and rats, it is reasonably to speculate about potential differences between species with regard to the lactate flow and other variables during exercise. Despite the relevance of the problem, studies approaching the lactate kinetics in rats during exercise are still unusual.

Once the MBL determination only requires a test performed in a single day, it could be adequate for rats during swimming exercises. Thus, our group has recently developed studies with the objective of standardizing a protocol for the identification of the metabolic transition in rats during swimming exercises based on the LMT principles established by Tegtbur *et al.* [21]. In our study, the average MBL was obtained at load of 4.95±0.10% of the body weight, while the blood lactate average concentration was obtained in this load was of 7.17±0.16 mmol/L [23]. There are yet, other points of the LMT still unclear waiting for explanations before employment, with practical purposes in rats.

Objective: The present study had as objective to evaluate the effects of swimming exercise performed at intensities below, equivalent and above the MBL on the protein metabolism of inactive rats, aiming at inferring on the feasibility of MLT application in studies involving rats.

Materials and Methods

Animals: All experiments of the present study involving animals followed the specific Brazilian resolutions of the Bioethics of Experiments with Animals (law No 6.638 of May 8th 1979; Decree No 24.645 of July 10th 1934). Adult male Wistar rats (90 days of age), from the Central Bioterium – São Paulo State University (UNESP) / Botucatu were used. The animals remained in collective cages (5 rats per cage) and were fed with commercial chaw (Purina®) for rodents and water ad libitum, being kept under periodic 12 h bright-dark cycle at average temperature of 25±2°C.

Adaptation to the liquid environment: The adaptation consisted of maintaining the animals in shallow water at temperature of 32±2°C during 3 weeks, 5 days a week for 30 min. At the 2nd and 3rd adaptation weeks, the animals supported sinker overloads inserted into cotton "knapsacks" fastened with Velcro ® and attached to the thorax with the aid of an elastic [23]. The objective of such adaptation was to reduce the stress of animals in face of the exercise performed in the water [7].

LMT adapted to the rat's condition: Initially, the animals were placed in the tank full of water supporting overload equivalent to 50% of the body weight performing anaerobic exercises (jumps) during 6 min (30 s of exercises interrupted by 30 seconds of rest) for the elevation of the circulating blood lactate concentration. After 9 min of rest, the animals started the swimming exercise with intensities progressively higher (4.0; 4.5; 5.0; 5.5 and 6.5 % of the body weight) with duration of 5 min for each load. Before the beginning of the test (rest) and at each load change, blood samples were collected (25µl) for the lactate determination in the YSL® electrochemical analyzer model 1500 SPORT, Yellow Spring, OH, USA [23].

Swimming Test with Fixed Load: After the LMT, the mean MBL for the entire group (n=24) was determined at load of 5.16±0.17% of the body weight (bw) in the blood lactate concentration of 6.72±0.36 mmol/L. Based on this information, part of the animals were submitted to swimming tests (single session) 48 h later, supporting fixed load below (3.5% of bw; n=8), equivalent (5% of bw; n=8) and above (6.5% of bw; n=8) the MBL during 30 min [7]), in order to verify the blood lactate stabilization, once the MBL theoretically coincides with the maximum

lactate steady state [26]. Blood samples were collected during the test for the lactate determination each 5 min of exercise. The animals were sacrificed through decapitation with the aid of a guillotine in rest (n=8) or shortly after tests for the evaluation of the protein synthesis and degradation rates in the soleus muscle, as described below:

Protein synthesis: Longitudinal muscle strips (70 mg) were pre-incubated for 30 min in RPMI-1640 medium (with glutamine and without red phenol and sodium bicarbonate), supplemented with fat-free serum bovine albumin (BSA) (0.1%) and insulin (100u/ml) and saturated with gaseous mixture (95% of O_2 and 5% of CO_2). Next, the muscle strips were transferred into a new RPMI medium with the same supplementation containing ^{14}C phenylalanine (0.05 uCi/ml) and incubated for 2 more h. At the end of the incubation, the muscle strips were homogenized in trichloride acetic acid (TCA) 5% and centrifuged at 2000 rpm for 15 min at 4ºC. The TCA-insoluble material was washed 3 times with TCA 3%. The resulting precipitated was dissolved in SDS 10%, at room temperature for 30 min, for the determination of the protein content and the radioactivity incorporated to the muscular proteins. The muscular protein content was determined through the folin phenol method, proposed by Lowry *et al*. [13], and the radioactivity incorporated to the muscular protein was measured with the aid of a cintilator (PACKARD Tricarb 2100). The protein synthesis was calculated by dividing the incorporated radioactivity by the specific radioactivity of the phenylalanine in the incubation medium, being expressed as nanomoles of phenylalanine per milligram of protein for 2 h.

Protein degradation: The release of tyrosine by the isolated muscle in the presence of cyclohexamide was used as protein degradation index, as previously described by Fulks *et al*. [4]. This method makes use of the fact that the amino acid tyrosine is not synthesized or degraded by the skeletal muscle. Longitudinal muscle strips (70 mg) were pre-incubated in Krebs-Ringer buffer (NaCl 1.2 mmol/L; KCl 4.8 mmol/L; $NaHCO_3$ 25 mmol/L; $CaCl_2$ 2.5 mmol/L; KH_2PO_4 1.2 mmol/L and $MgSO_4$ 1.2 mmol/L–pH 7.4), supplemented with glucose (5.5 mmol/L), BSA (1.34%), insulin (5u/ml) and cyclohexamide (5.0 mmol/L) and saturated with gaseous mixture (95% of O_2 and 5% of CO_2). Following, the strips were transferred into a new medium of same composition and incubated for 2 more h. At the end of the incubation, samples from the incubation medium were used for the tyrosine content determination, as described by Waalkes & Udenfriend [27].

Statistical analysis: Data were expressed as Mean ± Standard deviation. The statistical procedures included Analysis of Variance ANOVA followed by BONFERRONI, whenever required. The significance level adopted was of $p<0.05$.

Results

Fig. 1 shows the MBL mean values (blood lactate versus work load) during LMT for all animals (n=24). The mean MBL occurred at load of 5.2±0.2% of the body weight and blood lactate concentration of 6.7±0.4 mmol/L.

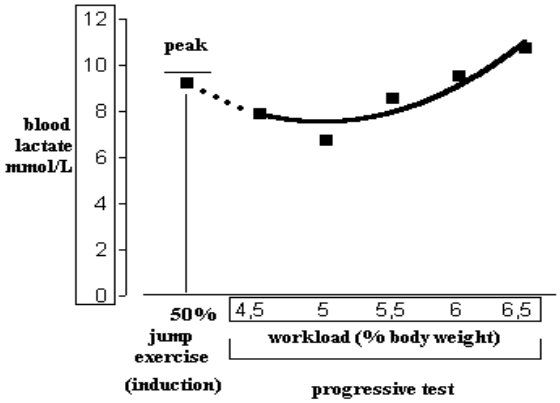

Fig 1
Blood lactate vs. workload curve during lactate minimum test. The mean minimum blood lactate for all animals (n=24) was obtained at load of 5.2±0.2% of the body weight and blood lactate concentration of 6.7±0.4 mmol/L

After MBL determination, the animals were submitted to swimming tests with loads below, equivalent and above the MBL. The results are presented in Figure 2. It is observed that the blood lactate concentrations stabilized from the 15th minute on during exercise with load below and equivalent to the MBL. On the other hand, at load above MBL, the blood lactate increased progressively.

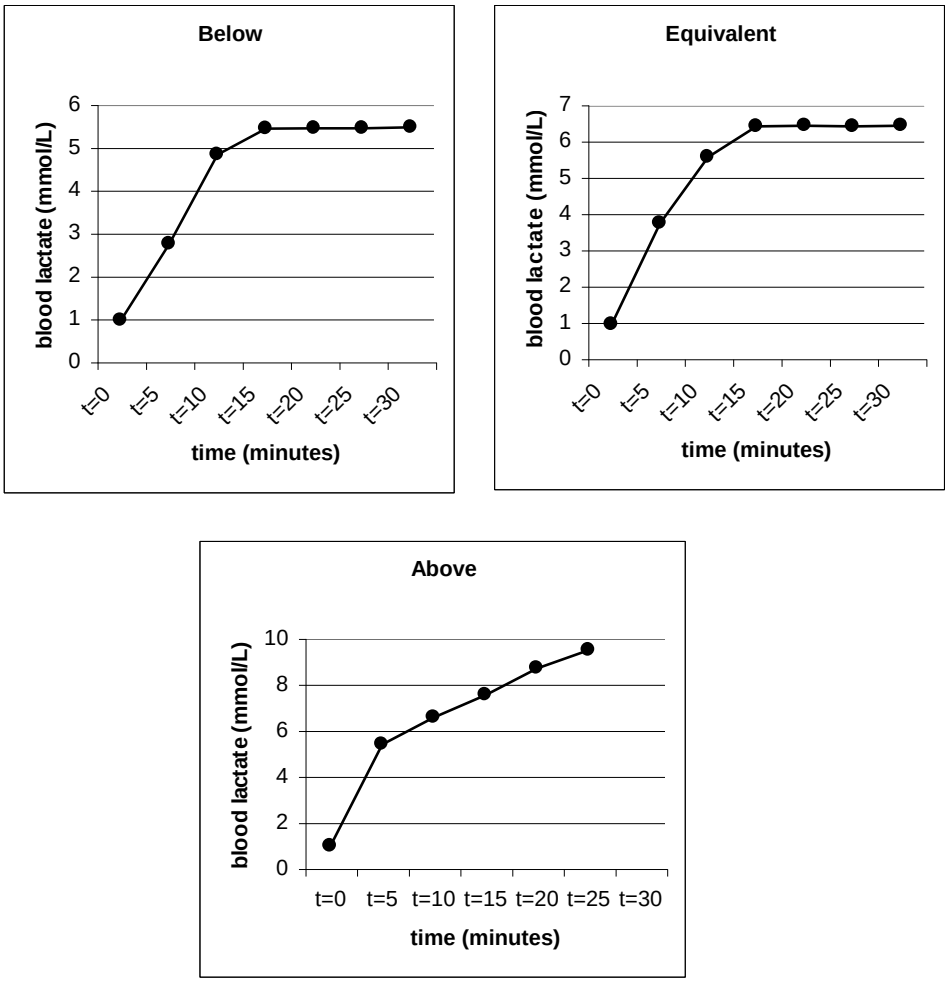

Fig. 2
Blood lactate mean values *vs.* time of exercise during fixed load test performed by animals with overloads below (n=8), equivalent (n=8) and above (n=8) the minimum blood lactate. The mean blood lactate concentrations stabilized in 5.4±0.01 and 6.4±0.006 mmol/L of blood lactate at loads below and equivalent to the MBL, respectively. At the load above, the mean blood lactate concentration increased progressively

Table 1 presents the individual and mean values of the protein synthesis rates in the soleus muscle of rats sacrificed in rest and after the swimming test with loads below, equivalent and above the MBL. The protein synthesis mean rates (pmol/mg.h) presented after exercise with loads below and equivalent to MBL showed no significant differences when compared with values in rest. On the other hand, the protein synthesis presented significant reduction after exercise with load above the MBL.

Table 1
Protein synthesis rates (pmol/mg.h) in the soleus muscle of rats sacrificed in rest and after the swimming test with loads below (3.5% of the body weight), equivalent (5% of the body weight) and above (6.5% of the body weight) the minimum blood lactate

	Rest (n=8)	Below (n=8)	Equivalent (n=8)	Above (n=8)
M±	65.2±	61.5±	60.7±	36.6±*
SD	3.4	1.3	1.7	2.0

*p<0.05 in relation to the other situations

The protein degradation rates, presented as individual and mean values, in the soleus muscle of rats sacrificed in rest and after a single session of swimming exercise with loads below, equivalent and above the MBL, are shown in Table 2. After exercise and at all loads, a significant increase on the protein degradation was observed, which was more intense after exercised with load above MBL.

Table 2
Protein degradation rates (pmol/mg.h) in the soleus muscle of rats sacrificed in rest and after the swimming test with loads below (3.5% of the body weight), equivalent (5% of the body weight) and above (6.5% of the body weight) the minimum blood lactate

	Rest (n=7)	Below (n=8)	Equivalent (n=8)	Above (n=8)
M±	214.3±	227.0±*	227.9±*	363.6±*
SD	6.0	6.1	6.0	7.1

*p<0.05 in relation to rest situation

Discussion

Despite the large number of attempts, the physiological bases for the blood lactate accumulation during exercise have not yet been fully elucidated. In different studies, the hypothesis that the lactate formation during submaximal exercise is due to tissue hypoxia has been questioned. In fact, these studies suggest that the existing experimental data may be interpreted alternatively and that the lactate formation during submaximal exercise is a result of the reduction on the oxygen availability in the mitochondria [2,11]. Notwithstanding, the determination of "thresholds", in other words, the determination of the exercise intensity in which the transition from aerobic metabolism to anaerobic metabolism occurs, seems to be an useful tool in the prescription of exercises [1,10,19,20].

For practical purposes, the identification of the metabolic transition has been performed by submitting subjects to efforts with loads progressively higher and using the blood lactate as marker. The threshold determination may be performed based on the deviation of the circulating lactate concentration base line (AT) or on the work intensity corresponding to a fixed circulating lactate concentration (OBLA). The first method is based on the fact that the non-linear increase on the blood lactate concentration in relation to the exercise intensity indicates metabolism transition, according to definition of Kinderman *et al*. [12]. The second procedure takes the principle that up to a given circulating lactate concentration, a balance between the muscular lactate production and the removal of this substrate from the blood stream occurs, according to Sjodin & Jacobs [20] and Heck *et al*. [8].

The threshold determinations also seem valuable in clinical studies [15,28]. Once there are obvious limitations in researches involving human beings, especially in clinical studies, animal models have provided important information with regard to the performance of physical exercises under several experimental conditions including obesity [18,24], diabetes [14], malnutrition [5,16] and fast [26].

In the LMT originally described by Tegtbur *et al*. [21] for human beings, the initial lactic acidosis was induced in athletes by means of two consecutive exhaustive runs. The second run was followed by 8 min of recovery (slow walking). Following, the run progressive test took place.

Based on these information, a test to determine the minimum blood lactate during swimming exercise was developed in our laboratory using the LMT basic principles proposed by Tegtbur *et al*. [21], adapted to the rat's conditions [23,26]. The MBL of inactive rats in the first study [23] was obtained at mean load of 4.9±0.1% of the body weight and blood lactate average concentration of

7.2±0.2mmol/L. The physical training increased the load (5.9% of the body weight) and reduced the blood lactate concentration (5.9±0.1 mmol/L) in which MBL was obtained [26].

The exercise intensity and blood lactate mean values corresponding to the MBL of all animals in the present study are presented in Figure 1. As verified, the exercise load and the blood lactate concentration equivalent to the MBL seemed to be similar to values obtained by Voltarelli *et al*. [23]. Such results suggest potential application of this procedure in studies aimed at the evaluation of the physical conditioning and the prescription of swimming exercises in rats. However, before the adoption of this test with these purposes, it is recommended to know better the metabolic responses resulting from the application of this technique.

The main objective of the present study was to evaluate the effects of the swimming exercise performed at intensities below, equivalent and above the MBL on the protein metabolism of rats during a single session of swimming exercise.

Fig. 2 presents the blood lactate concentration values of rats during swimming tests (30 min) with loads below, equivalent and above the MBL. These tests were performed with the objective of checking the blood lactate stabilization during exercise performed at these intensities, according to protocol proposed by Gobatto *et al*. [7].

Voltarelli *et al*. [23] performed procedure similar to procedure presented in the present study (single session of swimming exercise, where inactive and eutrophic animals supported load equivalent to the MBL) and observed stabilization of the blood lactate concentration in 6.4±0.3 mmol/L at intensity of 5.0% of the body weight. Similar mean values of exercise load and blood lactate concentration stabilization (5.0% of bw at 6.4±0.006 mmol/L) were observed in animals that performed exercise at the MBL intensity in the present study. When animals swam with load below MBL, the blood lactate stabilized in lower concentrations (5.4±0.01). On the other hand, the blood lactate stabilization was not observed when the exercise was performed at intensity above MBL. It is also worthy emphasizing that none of the animals submitted to exercise in the last situation were able to complete the test. Fatigue occurred at 25 min.

This suggests that the blood lactate production and removal rates are similar during effort performed at intensities below and equivalent to MBL and presented unbalance when the load applied was higher than the MBL. Such findings allowed characterizing intensities applied in the present study as below (3.5% of the body weight), equivalent (5.0% of the body weight) and above (6.5% of the body weight) the metabolic transition more safely.

With the objective of determining the protein synthesis (incorporation of ^{14}C phenylalanine into protein) and degradation (tyrosine release) rates in the soleus muscle, the rats were sacrificed in rest or shortly after a single swimming exercise session with loads below, equivalent and above the MBL. Such procedure was important to evaluate whether MBL estimated through the protocol here described is sensitive to other metabolic alterations imposed by exercise.

As shown in Table 1, the protein synthesis mean rates (pmol/mg.h) obtained after exercise at load below (n=8; 61.5±1.3) and equivalent (n=8; 60.7±1.7) to MBL showed no significant differences when compared to values in rest (n=8; 65.2±3.4). However, this index was significantly reduced when exercise was performed with load above MBL (n=8; 36.6±2.0). This suggests that the MBL is a sensitive indicative of alterations on the protein metabolism imposed by exercise.

The protein degradation average rates (pmol/mg.h) in the soleus muscle of rats sacrificed in rest and after a single swimming exercise session with intensities below (n=8; 227.0±6.1), equivalent (n=8; 227.9±6.0) and above (n=8; 363.6±7.1) the MBL were higher than those observed in rest (n=8; 214.3±6.0) (Table 2). The highest values were obtained when the animals were exercised at load above the MBL. These results reinforce the hypothesis that the MBL is an indicative sensitive to alterations on the muscular protein metabolism resulting from exercise.

It is worthy emphasizing that the procedure for the protein metabolism analysis here employed was performed in vitro and in the presence of insulin, hormone that stimulates the muscular protein synthesis [6]. The mean rates of the protein metabolism obtained in the soleus muscle of rats submitted to a single swimming exercise session showed that the synthesis was reduced and the degradation was increased in animals that exercised at loads above the MBL. Such fact may be a result of exercise being performed at intensity above the transition from aerobic to anaerobic metabolisms, thus generating inhibition on the insulin-activated signalization cascade and reducing the synthesis and allowing the muscular protein degradation [3]. Further studies must be conducted in order to elucidate the mechanisms involved in the effects of physical exercises performed at intensities below, equivalent and above the MBL on the muscular protein metabolism of rats.

Conclusions

- The set of results of the present study shows an adaptation of the MLT protocol applied to rats during swimming exercises;
- The responses related to the protein metabolism indexes in the skeletal muscle of rats showed that swimming physical exercises performed by animals with load

above the MBL further the reduction on the synthesis and the increase on the muscular protein degradation.

References

1. Billat V.L., P.Sirvent, J.P.Koralsztein, J.Mercier (2003) The concept of maximal lactate steady state: a bridge between biochemistry, physiology and sport science (review). *Sports Med.* 33:407-426

2. Brooks G.A (2000) Intra-and extra cellular lactate shuttles. *Med.Sci.Sports Exer.* 32:790-799

3. Dardevet D, C.Somet, M.Balage, J.Grizard (2000) Stimulation of in vitro rat muscle protein synthesis by leucine decreases with age. *J.Nutr.* 130:2630-2635

4. Fulks R.M, J.B.Li, J.L.Goldberg (1975) *J.Biol.Chem.* 250:290-298

5. Galdino R.S, C.C.A.Souza, E.Luciano, M.A.R.Mello (2000) Protein calorie malnutrition does not impair glucose metabolism adaptations to physical exercise. *Nutr.Res.* 20:257-270

6. Garlick P.J, I.Grant (1998) Amino acid infusion increases the sensitivity of protein synthesis to insulin: effects of branched chain amino acids. *Biochem.J.* 254:579-584

7. Gobatto C.A, M.A.R.Mello, C.Y.Sibuya, J.R.M.Azevedo, L.A.Santos, E.Kokubun (2001) Maximal lactate steady state in rats submitted to swimming exercise. *Comp.Bioch.Physiol.* 130:21-27

8. Heck H, A.Mader, G.Hess, S.Muck, W.Hollman (1985) Justification of the 4.0 mmol/L lactate threshold. *Int.J.Sports Med.* 6:117-130

9. Jones A.M, J.H.Doust (1998) The validity of the lactate minimum test for determination of the maximal lactate steady state and physiological correlates to 8 km running performance. *Med.Sci.Sports Exerc.* 30:1304-1313

10. Jones A.M, H.Carter (2000) The effect of endurance training on parameters of aerobic fitness. *Sports Med.* 29:373-376

11. Khatz A, K.Shalin (1990) Role of oxygen in regulation of glycolysis and lactate production in human skeletal muscle. *Exerc.Sports Sci.* 19:1-28

12. Kinderman W, G.Simon, J.Keul (1979) The significance of the aerobic-anaerobic transition for the determination of work load intensities during endurance training. *Eur.J.Appl.Physiol.* 42:25-34

13. Lowry O.H, N.J.Rosebroug, A.L.Farr, R.T.Randall (1951) Protein measurement with the folinphenol reagent. *J.Biol.Chem.* 193:265-275

14. Luciano E, M.A.R.Mello (1999) Atividade física e metabolismo de proteína em músculos de ratos diabéticos. *Rev.Paul.Educ.Fís.* 12:202-209

15. Neiva C.M, V.Bunc, M.A.R.Mello (1999) Glicemia, insulinemia e trigliceridemia de ratos diabéticos experimentais e alimentados com dieta hiperlipídica, submetidos a treinamento físico por aeróbio e anaeróbio. *Rev.Cient.Univ.Franca* 201-210

16. Prada F.J.A, F.A.Voltarelli, C.A.M.Oliveira, C.A.Gobatto, D.V.Macedo, M.A.R.Mello (2004) Condicionamento aeróbio e estresse oxidativo em ratos treinados por natação em intensidade equivalente ao limiar anaeróbio. *Rev.Bras.Ciência Mov.* 29-34

17. Ribeiro L, P.Balikian, P.Malachias, V.Baldissera (2003) Stage length, spline function and lactate minimum swimming speed. *J.Sports Med.Phys. Fitness* 43:312-318

18. Scalfani A (1988) Animal models of obesity: classification and characterization. *Zut. J. Obes.* 8:497-508

19. Simões H.G, C.S.G.Campbell, M.H.Tango, F.Mello, D.C.Maziero, V.Baldissera (2000) Lactate minimum test in swimming relationship to performance and maximal lactate steady state. *Med.Sci.Sports Exerc.* 32:S161

20. Sjodin B, I.Jacobs (1981) On set of blood lactate accumulation and marathon running performance. *Int.J.Sports Med.* 2:23-26

21. Tegtbur U, M.W.Busse, K.M.Braumann (1993) Estimation of individual equilibrium between production and catabolism curing exercise. *Med.Sci.Sports Exerc.* 25:620, 627

22. Tegtbur U, H.Machold, H.Meyer, D.Storp, M.W.Busse (2001) Determining the extent of extensive physical performance in patients with coronary heart disease. *Zeit.Kardiol.* 90:627-645

23. Voltarelli F.A, C.A.Gobatto, M.A.R.Mello (2002) Determination of anaerobic threshold in rats using the lactate minimum test. *Braz.J.Med.Biol.Res.* 35:1389-1394

24. Voltarelli F.A, W.M.S.Nunes, V.Santhiago, J.R.Pauli, D.R.Garcia, C.E.M.Romero, A.S.Silva, C.A.Gobatto, M.A.R.Mello (2003) Determinação do Limiar Anaeróbio em Ratas Obesas com Glutamato Monossódico (MSG). *Rev.Logos* 11:84-93

25. Voltarelli F.A, M.A.R.Mello, C.A.Gobatto (2004) Glicogênio muscular e limiar anaeróbio determinado em ratos durante natação. *Rev.Motriz.* 10:35-40

26. Voltarelli F.A, M.A.R.Mello, C.A.Gobatto (2004) Limiar anaeróbio determinado pelo teste do lactato mínimo em ratos: efeito dos estoques de glicogênio muscular e do treinamento físico. *Rev.Port.Ciências Desp.* 4(3):16-25

27. Waalkes T.P, S.Udenfriend (1957) Tyrosine in plasma and tissues. *J.Lab.Clin.Med.* 50:733-736

28. Wasserman K, M.B.McIlroy (1965) Detecting the threshold of anaerobic metabolism in cardiac patients during exercise. *Am.J.Cardiol.* 14:844-852

Accepted for publication 19.04.2006