



Metabolic Responses to Exercise

Managing Editor

Tommy Boone, PhD, MPH

Editor-in-Chief

Jon K. Linderman, PhD

Review Board

Todd Astorino, PhD

Julien Baker, PhD

Tommy Boone, PhD

Larry Birnbaum, PhD

Eric Goulet, PhD

Robert Gotshall, PhD

Alexander Hutchison, PhD

M. Knight-Maloney, PhD

Len Kravitz, PhD

James Laskin, PhD

Derek Marks, PhD

Cristine Mermier, PhD

Chantal Vella, PhD

Ben Zhou, PhD

Official
Research Journal of
the American Society of
Exercise Physiologists
(ASEP)

ISSN 1097-9751

Endurance Swimming Periodized Training in Rats

Dos-SANTOS, JW¹, de MELLO, MAR²

Department of Physical Education, UNESP - Univ Estadual Paulista, FITES-Lab Physiology Applied to the Sportive Training, SP, Brazil.

¹Bauru, ²Rio Claro.

ABSTRACT

Dos-Santos, JW, de Mello, MAR. Endurance Swimming Periodized Training in Rats. *JEPonline* 2010;13(5): 29-43. The aim of this study was to develop an experimental protocol for endurance swimming periodization training in rats similar to high performance training in humans, and compare it to continuous training. Three groups of male Wistar rats (90 days old) were allocated to Sedentary Control (SC); Continuous Training (CT); and Periodized Experimental Training (PET) groups. PET and CT trained 5 days/week, over five weeks, CT: continuous training supporting a 5% body mass (bm) load for 40 min/day; PET: training subdivided into basic, specific, and taper periods, with overload changed daily (volume-intensity, continuous, and interval training). Total training overload was quantified (% bm X exercise time in training session) and equalized for the two trained groups. Glucose (³H]2-deoxyglucose) uptake, incorporation to glycogen (synthesis), glucose oxidation (CO₂ production), and lactate production from [U-¹⁴C]glucose by soleus muscle strips incubated in presence of insulin (100μU/mL) were evaluated 48h after the last training session. The load equivalent at 5.5mM blood lactate concentration ([La-5.5]) was determined in the incremental test. Lactate production was similar in all groups. PET presented higher glucose uptake (59%) than SC, and higher glycogen synthesis (51 and 22%) and glucose oxidation (147 and 178%) than SC and CT, respectively. CT presented higher glycogen synthesis rates (23%) than SC. Load [La-5.5] was similar between trained groups and higher than SC. PET presented higher values for glucose metabolism than CT and SC. These results open up new perspectives for studying training methods used in high performance sport through swimming exercise in rats.

Key Words: Exercise, Glucose, Lactate, Periodization, Rat.

INTRODUCTION

High performance sport training is subdivided into periods and is organized according to overload variation (volume/intensity/recovery) to obtain maximal performance in competition. This method of organizing training during a season is called periodization, a concept initially introduced by Matveyev in the 1960s (23) and still in use now. However, intervention in high performance training for scientific research is quite difficult to attain. When possible, it is done in only part of the training periodization (9,20,25,35) or even longitudinally over a season (26).

An alternative way of studying the effects of physical training is to use experimental protocols in rodents. Different aerobic evaluation and training protocols studied in humans have been reproduced in rats; these include intensive interval training (4,8,37,38), anaerobic threshold evaluation (31,39), maximal lactate steady state (11,22), and training prescription by maximal oxygen uptake (41) and anaerobic threshold (6). Recently, periodization training in running exercise was used in rats (7,18). However, the periodization model used in these two studies was a simple progressive endurance protocol with one day of recovery in the week. This partially reproduces the periodization model used in high performance sports training, as overload (volume/intensity/recovery) was not changed daily. To our knowledge, however, there are no studies that have reproduced high performance training periodization in a swimming exercise experimental model using rats. Obviously, muscle architecture, relative activation of individual muscles, biomechanics, and movement efficiency are different between humans and rats, however, similar training may present similar responses in both species.

Several biomarkers can be used to verify the effects of endurance training. Of these, blood lactate response in an incremental test is one of the most commonly used in humans (15) and has also already been tested in rats (6). In experimental exercise rat models, several studies have shown that endurance training improves glucose metabolism, that is, glucose transport and uptake (40), glycogen synthesis, and lactate production (16). Muscle metabolism *in vitro* studies in rats allow the effects of training to be verified in more detail. However, comparison between different endurance training methods has not yet been studied in experimental exercise rat models.

Since the organization of sports training is made through periodization training models and some aerobic evaluation and training protocols have been reproduced in experimental rat models, our hypothesis is that it is possible to develop a periodized experimental training model for swimming exercise training in rats and that this training protocol has the best effects on blood lactate response in the incremental test and on the muscle glucose metabolism. Thus, the aim of this study was to develop an endurance periodization experimental training model in rats and to compare it to linear continuous training through muscle glucose metabolism and blood lactate response evaluations.

METHODS

Animals and Treatment

Twenty-three male, 90-day-old, Wistar rats were kept in cages (37 x 31 x 16 cm), 3-5 rats per cage, in a controlled room with 12h light/dark cycles at 22±1°C with free access to water and food (Purina rodent chow). Animal body mass was measured at the beginning of each week. Food uptake of each group was measured on first day of each week (difference between pre and post 24h in each cage) and in the last week 48h before the end of experimentation. The calculation made was based on an uptake of 100g per animal per cage and multiplied by the body mass of each animal and expressed in grams. The rats were subdivided into three groups: Sedentary Control (SC, n=9), kept inactive; Continuous Training (CT, n=7), and Periodization Experimental Training (PET, n=7). In the last week of the experiment, the animals performed an incremental test to determine aerobic fitness. All animals were sacrificed by decapitation and CT and PET groups were killed 48h after their last

training session. The experimental procedures were carried out in accordance with Brazilian College of Animal Experimentation (COBEA) guidelines and approved by the Science School Ethics Committee of Univ Estadual Paulista (Process nº: 1501/46/01/08).

Training Overload Quantification

Overload (OV) for each training session was adjusted at the beginning of each week according to body mass (bm) and expressed as percentage body mass (% bm). The daily training load in each training session consisted of a light fabric (tactel) “mini bag” with an adjustable elastic band fixed to the anterior part of animal’s thorax and a Velcro opening to add lead pieces (Figure 1). Attachment of the bag to each rat took between two and three seconds. This was done on dry land and the training session only began when all rats had their bags attached.

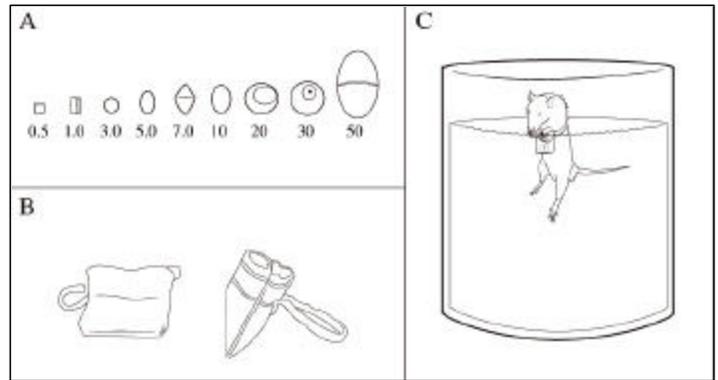
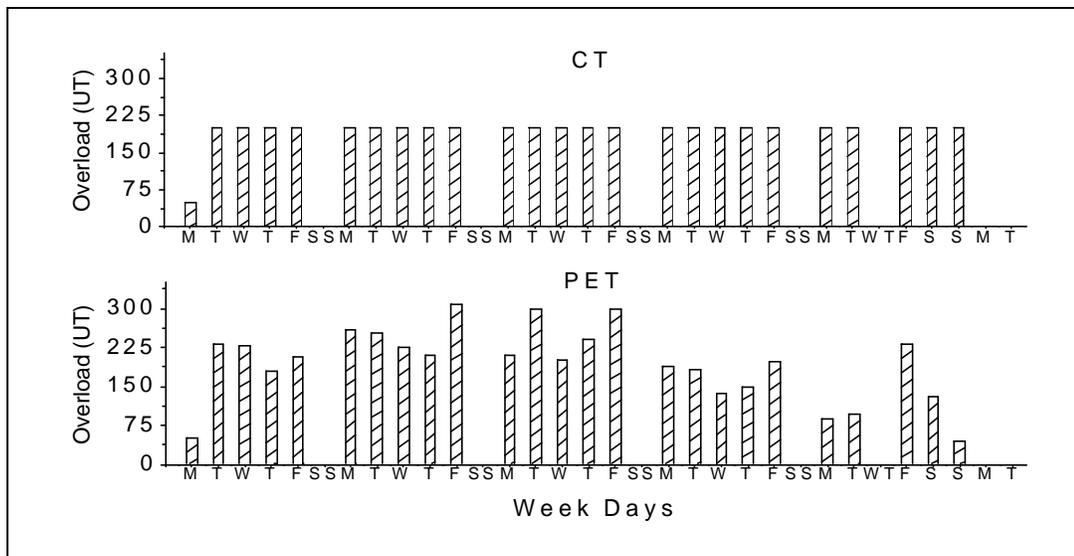


Figure 1. Characteristics of swimming exercise model in rats. A: graduated lead pieces (g) to increase the overload; B: backpacks with openings to insert load, and elastic band to fix it to the animal’s thorax; C: the rat with the backpack in swimming exercise position, with total overload, backpack and lead pieces.



done based on the relation of the exercise time and on the maximal oxygen uptake $\dot{V}O_{2max}$ or the blood lactate concentration ($[La]$), respectively. Therefore, we adapted the calculation of OV to the swimming exercise in rats, employing the total exercise time in the training session (ET, in minutes) and the intensity (%bm, in grams) to obtain the value of OV in each training session ($OV = ET \times \%bm$). The sum of OV of all of the training sessions of each protocol corresponded to the total overload of training (OV_{total}), as follows:

$$OV_{total} = (ET_1 \times \%bm_1 + ET_2 \times \%bm_2 \dots + ET_n \times \%bm_n) [UT]$$

Where: OV_{total} = total training overload during the five weeks of specific training; ET = total exercise time, in minutes; % bm = percentage body mass; UT = arbitrary units of training.

Once training load for CT was the same everyday, PET load was adapted to have the same total overload as CT. The OV_{total} for both groups, CT and PET, was 4850 UT, considering all training sessions in the five weeks for the respective training protocols (Figure 2).

Training protocols

The animals performed swimming training in tanks with individual partitions (25cm diameter PVC tubes); water depth was 45cm and temperature $31 \pm 1^\circ C$. For PET interval training (IT) sessions, an apparatus was used to raise and keep the animals on the water surface during the recovery period. It consisted of two sets of six PVC tubes with a metal net on the bottom and a cable fixed in each set. It was raised manually using a pulley system where the two cables were fixed to a wooden handle. After elevation, it was kept in the raised position by a cable fixed to a hook at the base of the tank, the objective being animal recovery (Figure 3).

Both CT and PET groups trained 5 days/week over 5 weeks. Prior to the five weeks specific training, there were four weeks of adaptation training (progression training) with both groups. This adaptation period was to help prevent high stress, mainly in PET rats who performed intensity training. Briefly, the adaptation period was: first week, increasing exercise time 5 min/day (20-40 min); second and third weeks: exercise time kept constant (40 min) and intensity raised by 3.5 and 5% bm per week, respectively (5 min/day, i.e. 20 to 40min); fourth week: animals in both groups swam 40 min/day with 5% bm. In the fifth week the animals in CT started receiving continuous and PET periodized training as per their respective protocols. The CT group protocol included continuous swimming exercise with a 5% bm load for 40 min/day (except the first day, Figure 2). In the last week, training days were changed (recovery on Wednesday-Thursday and training on Saturday-Sunday), because of the TAPER week employed in the PET protocol (Figure 2). TAPER is a procedure used in human swimming training that consists of reducing the amount of training before competition to achieve peak performance in the competition (25). In PET, training sessions were mainly by interval

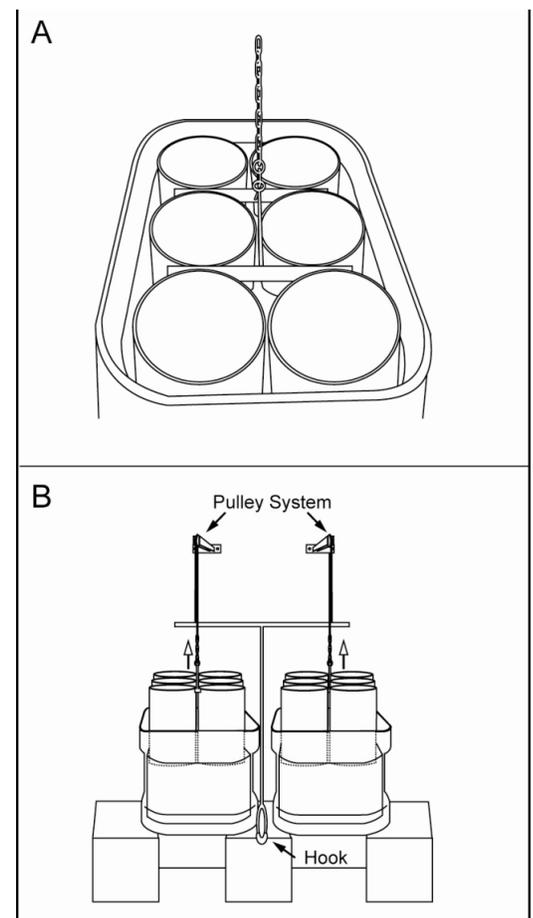


Figure 3. The Tanks with individual partitions. **A:** position of the equipment during exercise. **B:** equipment raised for animal's recovery during interval training carried out in PET group.

training (main training) ending with continuous training (complementary training) to equalize training overload with the CT group (Table 1).

PET Intensity Levels

In PET, training protocols were classified at five different intensity levels, according to aerobic demand (A-1, A-2 and A-3), and anaerobic metabolism (AN-1 and AN-2) adapted from swimming in

Table 1. Characteristics of the periodized experimental training (PET) in each daily training session, according to aerobic (A-1, A-2, A-3) and anaerobic (AN-1 and AN-2) intensity level, including the number of repetitions, exercise time, recovery time and the training overload.

Daily training session								
Training Periods	Week Days	Main training			Complementary training			
		Intensity level	Rep x time x rec	Overload (% bm)	Intensity level	Time (min)	Overload (% bm)	
BASIC	Mon	A-1	1 x 20 x (-)	2.5	-	-	-	
	Tue	A-2	1 x 30 x (-)	6.0	A-1	20	2.5	
	Wed	A-3	6 x 4.0 x 1.5	8.0	A-1	15	2.5	
	Thu	A-1	1 x 60 x (-)	3.0	-	-	-	
	Fri	A-2	4 x 6.0 x 0.5	6.5	A-1	20	2.5	
	Sat-Sun			Off training				
	Mon	A-2	1 x 35 x (-)	6.0	A-1	25	2.5	
	Tue	A-3	6 x 4.0 x 1.5	8.0	A-1	25	2.5	
	Wed	AN-2	6 x 0.5 x 3.0	50	A-1	30	2.5	
	Thu	A-3	6 x 2.5 x 1.0	9.0	A-1	30	2.5	
	Fri	A-2	6 x 6.0 x 0.5	6.5	A-1	30	2.5	
	Sat-Sun			Off training				
	SPECIFIC	Mon	AN-1	4 x 1.5 x 5.0	25	A-1	20	3.0
		Tue	A-2	5 x 6.0 x 0.5	8.0	A-1	20	3.0
Wed		A-3	5 x 2.5 x 1.0	10	A-1	25	3.0	
Thu		AN-2	6 x 0.5 x 3.0	50	A-1	30	3.0	
Fri		A-2	5 x 6.0 x 0.5	8.0	A-1	20	3.0	
Sat-Sun				Off training				
Mon		A-2	3 x 6.0 x 0.5	8.0	A-1	15	2.5	
Tue		AN-1	4 x 1.5 x 5.0	25	A-1	15	2.5	
Wed		A-3	4 x 2.5 x 1.5	10	A-1	15	2.5	
Thu		AN-2	4 x 0.5 x 3.0	50	A-1	20	2.5	
Fri		A-2	4 x 5.0 x 0.5	8.0	A-1	15	2.5	
Sat-Sun				Off training				
TAPER		Mon	AN-2	3 x 0.5 x 3.0	45	A-1	10	2.0
		Tue	A-3	3 x 2.5 x 1.5	10	A-1	10	2.0
	Wed	A-2	4 x 6.0 x 0.5	8.0	A-1	20	2.0	
	Thu-Fri			Off training				
	Sat	A-3	3 x 3.0 x 0.5	9.0	A-1	20	2.5	
	Sun	A-2	1 x 4.0 x (-)	8.0	A-1	14	1.0	

Microcycles = cycles of training (5-9 days); Rep = repetitions; Exe = exercise; Rec = recovery, expressed in minutes; (-) = without recovery, continuous training. The complementary training was carried out only by continuous training, in A-1 intensity. Note: In most of the PET training sessions the interval training was the main training and it was complemented immediately with continuous training (complementary training).

humans (21). For aerobic training a 5-6% bm load was used as reference, as it represents maximal lactate steady state (11) and anaerobic threshold (39), in rat swimming exercise; this intensity was called A-2. Intensities A-1 and A-3 were classified as below and above the anaerobic threshold as 1-3% and 8-10% bm, respectively. These aerobic intensity levels were used in more than one training protocol. Anaerobic training AN-1 corresponded to 25% bm for 1.5 min exercise with 5 min of recovery (8). In the four anaerobic training sessions AN-2 consisted of 0.5 min exercise with 3.0 min of recovery and only numbers of repetitions (3-6 repetitions) and intensity (45 or 50% bm) varied

(Table 1). Unlike aerobic training protocols, animals performing anaerobic exercise protocols did not swim the whole time on the surface of the water. Training was carried out with jumps, due to high overload. We counted number of jumps in one AN-1 (4 x 1.5 min with 5 min of recovery and 25% bm) and AN-2 (5 x 0.5 min with 3 min of recovery and 50% bm) training session. Animals performed most jumps impelled by all four paws. They performed 38 ± 3 jumps per set (CV=18%) in AN-1 and 16 ± 1 jumps per set (CV=19%) in AN-2. All PET training sessions are shown in Table 1. The proportion of the aerobic and anaerobic training at the five intensity levels is presented in Table 2. Total aerobic and anaerobic training were 84.2% and 15.8%, respectively.

The experimental periodized training protocol was based in the Matveyev's classical model (24), with a basic preparation period (BPP) and a specific preparation period (SPP), two weeks each. After the

Table 2. Absolute and relative total overloads of the PET group, in arbitrary units of training (UT), in each intensity level (A-1, A-2, A-3, AN-1 and AN-2).

Overload	A-1	A-2	A-3	AN-1	AN-2	TOTAL
Absolute (UT)	1406.5	1776.0	900.0	300.0	467.5	4850
Percent (%)	29.0	36.6	18.6	6.2	9.6	100

preparation period, a taper microcycle (TAPER) was performed as used in human swimming training (21,25) to obtain "peak performance" at the end of the experiment. During the first week of BPP, there was an increase in volume and a small increase in intensity (introductory microcycle); in the second week, peak volume reached 66 min in one training session (development microcycle). In SPP, week three, there was a small reduction in training volume but intensity was increased reaching its peak. This week had greater training overload (shock microcycle). In the fourth week, both intensity and volume decreased to provide recovery (recovery microcycle). The final PET period was in the last two weeks (9 days). This was proposed to provide training supercompensation and to reach peak performance (TAPER); it consisted of a reduction in total training volume (10.3%), AN-2 (10%), and A-1 (24%) training, and maintenance of A-2 and A-3 training (Figure 2).

Aerobic Fitness Test

At the end of the experiment animals performed an aerobic test to determine the load (% bm) equivalent to a blood lactate concentration of 5.5mM ([La-5.5]). Evaluation consisted of an incremental test with four stages of three minutes, separated by five minutes of recovery. The sedentary animals performed the test with loads of 0.7, 2.3, 3.9 and 5.5% bm and trained animals with loads of 5.0, 7.5, 10.0 and 12.5% bm. One blood sample was collected immediately after each stage for lactate analysis. Aerobic fitness was determined by the load (% bm) equivalent to a blood lactate concentration of 5.5mM [La-5.5] from the lactate vs load curve, through linear regression with the two points closest to [La-5.5], as [La-5.5] corresponded to maximal lactate steady state intensity in rat swimming (11). Linear regression was used because the lactate vs load curve did not present exponential growth, but demonstrated a linear response in most animals (sedentary = 55% and trained = 36%).

Blood Collection and Lactate Analyze

Blood samples (25 μ L) for lactate analysis were collected from a cut at the tip of the tail, diluted in 50 μ L NaF (1%), frozen (-20°C), and subsequently analyzed. Concentrations were determined by an electrochemical method in a lactate analyzer (YSL 1500 STATS, USA).

Soleus Muscle Glucose Metabolism *in vitro*

Glucose metabolism evaluated was as previously described (27). The soleus muscle was isolated with the minimum possible lesion and longitudinal strips weighing 25-35mg (wet weight) were first incubated for 30 min at 37°C in a Dubinoff water bath (FANEN, MODEL 145), inside glass scintillation vials containing 1.5mL of Krebs bicarbonate buffer (NaCl 0.9%; HEPES 6.64mM; KCl 0.032%; CaCl₂

1.14mM; KH_2PO_4 0.015%; NaHCO_3 0.19%; MgSO_4 0.03%) equilibrated with a mixture of 95% O_2 -5% CO_2 , pH7.4. Then the muscle strips were transferred to new glass scintillation vials (outer vials) containing 1.5mL of Krebs bicarbonate buffer supplemented with glucose 5.5mM, containing [^{14}C]glucose (0.25mCi/mL) and [^3H] 2-deoxiglucose (2DG, 0.5mCi/mL) and insulin (100 μU /mL). Inside these outer vials, other glass vials (inner vials), which were formed like a scoop with an upwards-directed straight shaft, containing 700 μL of thiamine 10-x were installed. The shafts of the inner vials were squeezed about 1cm through a small hole in a round rubber membrane. The outer vials were sealed with the rubber membrane and locked with plastic rings. This system, containing the muscle strips, was incubated in the Inoffensive water bath for 60 min. The release of CO_2 was stimulated by injecting 200 μL of trichloroacetic acid (TCA) 25% into the outer vials and CO_2 was trapped in thiamine 10-x during a further 3h incubation at 37°C. Lactate production was determined by measuring the radioactivity of the ^{14}C in the outer vial incubation medium after separating the substrate using Dowex-2 ion exchange columns. Glucose incorporation to glycogen (glycogen synthesis) was determined by measuring the radioactivity of the ^{14}C in precipitate obtained during the muscle glycogen extraction process (34). Glucose oxidation was estimated by measuring the radioactivity of ^{14}C in the inner vial liquid. Glucose (2DG) uptake was evaluated in the alkaline phase obtained during muscle glycogen extraction process, by measuring the radioactivity of ^3H . All radioactivity measurements were carried out in a PACKARD Patrica 2100 scintillation counter, in a TRITON X-100 toluene-based scintillate. The experiment was concluded with n=9, n=7 and n=7 in groups SC, CT and PET, respectively. However, two slices of the soleus muscle were removed of one of the animal from Group SC (9 +1) and three animals from Group CT (7 + 3) and PET (7 + 3). All animals were chosen randomly. Thus, the incubation process was made with 10 slices of soleus muscle from each group (n =10).

Statistical Analyses

Data normality was verified by the Shapiro-Wilk test. Therefore, parametric analysis was made for glucose uptake, glycogen synthesis, lactate production, glucose oxidation, [La-5.5] load, food uptake in the whole experiment and in the last week by one-way analysis of variance (ANOVA). The difference between body mass was analyzed by two-way ANOVA. Overload assessment in the two training protocols not presenting normality and it was analyzed by non-parametric statistical (Mann-Whitney test). Significant difference was determined by Newman-Keus *pos-hoc*, when appropriate. Based on the results of a pilot study previous, an a priori power analysis indicated that a sample size of 30 slices of soleus muscle resulted in a statistical power value of 0.97 for increases in glucose uptake. We used 10 slices of soleus muscle for each group. We beginning the experiment with 10 rats per group, but the experiment was conclude with n=9, n=7 and n=7 in groups SC, CT and PET, respectively. However, the incubation process was made with 10 slices of soleus muscle from each group (see above the Soleus Muscle Glucose Metabolism *in vitro* session). In the present study power values for glucose uptake, glycogen synthesis, lactate production, glucose oxidation and [La-5.5] in aerobic fitness test were 0.56, 0.99, 0.58, 0.87 and 0.99, respectively. Results are reported as means \pm SEM, and the significance level adopted was $p < 0.05$. All calculations were performed using Statistica 7.0 software.

Table 3. Body mass at the beginning and at the end of the experiment of sedentary control (SC), continuous training (CT) and periodized experimental training (PET) groups.

Groups	Body Mass (g)	
	Initial	End
SC (n=9)	368.3 \pm 5.2	437.2 \pm 5.9*
CT (n=7)	367.9 \pm 12.2	447.6 \pm 13.6*
PET (n=7)	370.7 \pm 3.8	450.6 \pm 7.3*

Values are means \pm SEM. *Significant difference between the initial and end of experiment (ANOVA two-way, $p = 0.001$).

RESULTS

Body mass did not present significant difference between groups at the beginning and end of the experiment. The three groups differed only between the start and end of the experiment (Table 3). Food uptake was similar between groups throughout the experiment as well as in the last week, 48h before experimentation (Table 4). Mean weekly training overload values did not differ between training protocols (PET = 970 ± 130 and CT = 970 ± 30 UT, $p = 0.97$) and total OV in the two protocols was 4850UT for five weeks of specific training.

Table 4. Food uptake during all the experiment and at last week, 48 h before experimentation, of sedentary control (SC), continuous training (CT) and periodized experimental training (PET) groups.

Groups	Food uptake (g)	
	Mean of all experiment	Last week
SC (n=9)	18.7 ± 1.2	20.5 ± 0.2
CT (n=7)	20.1 ± 0.8	20.6 ± 0.6
PET (n=7)	21.0 ± 1.2	20.2 ± 0.4

Values are means \pm SEM of each group, calculated on one day of each week.

higher than the SC group (Figure 4).

DISCUSSION

Our main objective was to design a periodized experimental training (PET) model to improve endurance in rats. Despite the existence of other periodization models and coaching concepts, e.g., Tschien's intensity load, Aroseiev's pendulum, and Verkhoshansky's block periodization models (23), for swimming exercise conditions in rats, we chose for our endurance periodized experimental training, to adapt and combine Matveyev's classic periodization model (24) with procedures used in human swimming (21,25). Our PET protocol consisted of a basic (increase in volume) and specific (reduction in volume and increase in intensity training) preparatory period. After this preparation period, we had a taper microcycle (TAPER), the same as in human swimming training (21,25), to obtain "peak performance" at the end of the experiment. The specific period lasted five weeks. However, before that, there was a four-

The load equivalent to [La-5.5] did not differ between the two trained groups (PET = 6.6 ± 0.8 and CT = $7.4 \pm 0.7\%$ bm, $p = 0.43$) and both were higher than the sedentary group (SC = $2.9 \pm 0.6\%$ bm, $p = 0.002$ and $p = 0.001$, respectively). This confirms the efficiency of both training protocols.

There was a significant improvement in PET group glucose metabolism compared to the other groups. The PET group presented higher glucose uptake rates than the SC group, and higher glycogen synthesis and glucose oxidation rates than both SC and CT groups. In the CT group, only glycogen synthesis rates were

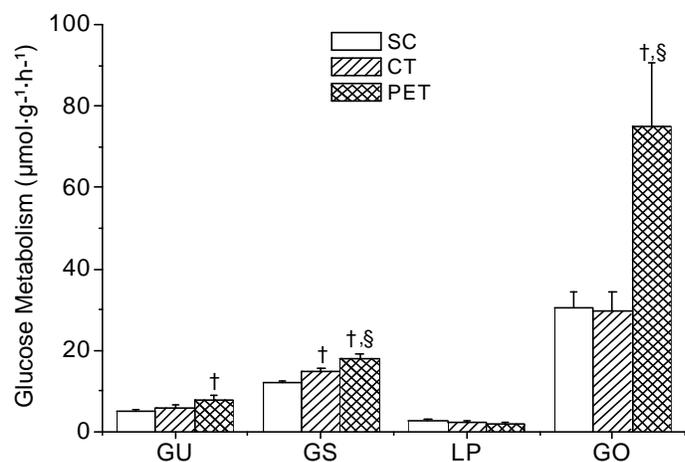


Figure 4. Glucose uptake (GU), glycogen synthesis (GS), lactate production (LP) and glucose oxidation (GO) rates (in $\text{mmol}\cdot\text{g}^{-1}\cdot\text{h}^{-1}$) by isolated soleus muscle for sedentary control (SC), continuous training (CT) and periodized training (PET) groups. Values are means \pm SEM of isolated muscle striped from the soleus muscle (n=10 slices per group). Significant difference ($p < 0.05$): [†] ? SC; [§] ? CT. GU: PET vs SC ($p = 0.03$); GS: PET vs SC ($p = 0.0002$); PET vs CT ($p = 0.01$) and CT vs SC ($p = 0.04$); GO: PET vs SC ($p = 0.02$) and PET vs CT ($p = 0.01$).

week adaptation period. We did not determine the effects of adaptation period because our objective was to develop an experimental protocol and evaluate its efficiency. We suggest that future experiments evaluate the effects of the adaptation period as well as studying other load progression models, e.g., using a shorter period.

The TAPER was proposed to provide a training supercompensation period and to reach peak performance at the end of experiment (simulating competition), in which there was a reduction in total training volume (10.3%), AN-2 (10%), and A-1 (24%) training, and maintained levels of A-2 and A-3 training. Evidence shows that TAPER improves swimming performance (25). The effects of TAPER were not separately tested in our study, nor were the basic and specific preparation periods; however, we believe that TAPER, in part, might have collaborated to the success of the PET protocol. Furthermore, it is interesting to note that the reduction in training overload over nine days did not impair aerobic fitness.

In PET, training protocols were classified at five different intensity levels, according to aerobic demand (A-1, A-2 and A-3), and anaerobic metabolism (AN-1 and AN-2). This was adapted from human (21,25) to rat swimming (8). A-2 aerobic training (5-6% bm) represents maximal lactate steady state (11) and anaerobic threshold (39) in swimming rats. Training at A-3 intensity level (~60% above the anaerobic threshold = 8-10% bm) was used to simulate an intensity close to $\dot{V}O_{2max}$, in the same way as is in human swimming. A-1 training intensity was used in PET because it is often used in competitive human swimming to complement training volume at the end of training sessions, to facilitate lactate removal after intensity training and muscle relaxation. To attain these objectives all PET A-1 intensity training sessions ended with 10-20 min at 1-3% bm load, called here complementary training.

The AN-1 and AN-2 protocols combined swimming exercise with jumps, due to high overload. This can be seen as a limitation in the rat swimming training, but we do not see a problem in this procedure. Dry-land strength training and in-water resisted training (10) are used in human swimming training. There are also auxiliary training sessions in other sports using auxiliary equipment and performed in different places to those where the specific training and competitions occur. We counted the number of jumps in one training session in each AN-1 and AN-2 protocol. In AN-1 training (1.5 min sets) 38 jumps were performed and in AN-2 training (0.5 min sets) 16 jumps were performed. It is difficult to make a relationship between intensity relative to overload and the jumps because the animal jumps according to its own volition. There was a high variation in the numbers of jumps during sets (CV = 18 and 19%, respectively). Possibly, protocols standardizing numbers of jumps instead of fixed times could better quantify overload. For example, from our results we can suggest one training protocol for lactate production (4 x 30-35 jumps with 5 min recovery at 25% bm) and one for power training (3-6 x 10-12 jumps with 1-3 min recovery at 50% bm). One protocol with jumps in-water with a fixed number of jumps (4 x 10 jumps with 1 min recovery at 50% bm) was proposed by Rogatto and Luciano (33) in our laboratory. This has been used in other studies with positive responses, i.e., it induced an increase in number of monocytes in diabetics rats (29), and increased immune function, and decreased tumor growth and cancer cachexia (19).

Most training protocols used in intensity training levels in this study were tested by dos Santos (8) and presented similar [La] to the respective exercise intensities proposed by Maglischo (21) for swimming training in humans. Therefore, the training protocols used in this study can be applied as a good reference for studying the effect of different intensities in aerobic and anaerobic training in rats.

We compared PET with linear continuous training (CT) in swimming rats, because this model is often used in rat exercise models with several objectives; we suggest that the linear training model is not

the best training protocol for increasing endurance in swimming rats. In fact, the PET used in this study was more efficient in improving glucose metabolism than the CT protocol, both with equalized overload. This can be confirmed by the significantly higher glucose uptake rates observed in PET compared to SC ($p < 0.05$). The PET group also displayed higher, but not significant, values than CT (32%). Other parameters that show the efficiency of the PET protocol are the higher values of glycogen synthesis and glucose oxidation compared to the SC and CT groups. CT also presented an improvement in glycogen synthesis ($p < 0.05$) and glucose uptake (20%) compared to SC. There was a small, but not significant, reduction in lactate production in both trained groups. Body mass and food uptake did not differ between groups. In part, these parameters may also demonstrate the efficient organization of the PET protocol, because, despite several sessions of intensity training in PET, there were no differences in body mass and food uptake between groups. In humans, loss of body mass and depressed appetite are some of the symptoms of overtraining (36), a syndrome caused by excessive exercise training associated with inadequate recovery. Food uptake was analyzed only in the last week to verify whether it had any implications on performance and the interpretation of other results. Food uptake did not influence any parameter because analyzing it in the last week also found no difference between groups.

Recently, periodized training was used in running exercise to verify its effects on dendritic cells in rats (7,18). In these two studies, the training protocol raised intensity once a week, which in part reproduces the periodization model used in high performance sport training, since the overload component (volume/intensity/recovery) were not changed daily. This periodization model is similar to the so called linear periodized training (32), and very close to the continuous training protocol used in our study. In both cases, the load was raised once a week, except for the day in periodized training when intensity was reduced for recovery (7,18). In the same studies (7,18), periodized training presented better results than the sedentary control group, but it was not compared to another training protocol, which leaves questions as to its efficiency in comparison with other training model. In our study, complex non-linear periodized training (PET) was performed, in which the load varied daily throughout the whole training period. In human strength training, non-linear periodized training also presented better results than linear periodized training (32). Thus, in spite of the differences in exercise type and species, there is evidence that non-linear periodized training can also have better effects than the linear periodized training in swimming rats.

Improvements in glucose uptake (37,40), glycogen synthesis (16,37), and lactate production (16) were also observed in other *in vitro* studies. However, it is necessary to consider some methodological differences, such as the type of muscle evaluated (slow or fast twitch), the exercise intensity (aerobic or anaerobic), and the insulin concentration used in the incubation medium.

When comparing high-intensive interval training (10 x 20s with 10s recovery and 14% bm load) and low-intensity continuous training (2 x 3h with 45 min recovery and 2% bm load), glucose transport and GLUT-4 content in epitrochlearis muscle were higher than the control sedentary group, however, both trained groups presented similar results (37). In contrast, our periodized training presented better results than continuous moderate training, carried out separately.

The difference in insulin sensitivity in endurance and sprint trainings was studied in soleus muscle (21) and results showed that endurance training increased insulin responsiveness, glycogen synthesis, and lactate production, which did not occur in sprint training (16). One intensity interval training protocol in swimming rats, 45 min supporting 15% bm load and 30s:30s exercise:recovery ratio, also did not increase soleus muscle glucose uptake (4). Together, these results suggest that endurance training has a greater effect on the glucose metabolism than sprint training. However, the addition of aerobic and anaerobic intensity interval training did not impair the efficiency of PET training on glucose metabolism in our study.

Insulin concentration is determinant in glucose transport and in studies where an increase in insulin sensitivity was verified (21,40), insulin concentrations, 500 μ U/mL and 10,000 μ U/mL, respectively, were higher than in our study, 100 μ U/mL. In the study that used the same insulin concentration as our study, exercise training did not improve lactate production and glycogen synthesis (17).

Glycogen synthesis in our trained animals, CT and PET groups, was higher than in SC, which confirms results from another study (16). However, it is necessary to highlight that PET also presented higher glycogen synthesis than CT. Increase in glycogen synthesis seems to have a direct relation to insulin sensitivity (16), and glucose transport (14). This was observed in our results, mainly in the PET group which presented higher glucose uptake than SC and CT, and higher glycogen synthesis than SC. On the other hand, the slight increase (no significance) in CT glucose uptake compared SC, can be verified by the significant increase in CT glycogen synthesis. The main difference between the two training protocols was the aerobic and anaerobic intensity training in PET. AN-1 and AN-2 training were more intense and were performed with jumps. This was one differential in PET. However, it is not possible to attribute the success of PET training just anaerobic training alone. PET used different training sessions on each day and for this reason we believe that the higher glycogen synthesis, as well as the other improved parameters in PET, was caused by combination of stimulus (change in volume/intensity, combination of aerobic and anaerobic training, and adequate recovery).

Lactate production did not present any differences between the groups studied. The anaerobic (AN-1 plus AN-2 = 15.8%) and in part, the aerobic intensive training (A-3 = 18.6%) used in the PET group, stimulated the anaerobic glycolytic pathway, as the A-3 intensity training protocols presented [La] =6-7mM (8), but this was not enough to raise soleus muscle lactate production. Most of the training sessions in the PET group were performed using light and moderate aerobic training (A-1 plus A-2 = 65.6%), which presented [La] <4mM (8). However, the CT training protocol has submaximal characteristics, [La] ~3.5mM (8), and has little stimulating effects on anaerobic glycolysis. The aerobic characteristics of the soleus muscle, a slow twitch muscle (2) with a higher capacity for lactate oxidation (3), and of the CT and PET protocols, may partly explain why lactate production was not altered in groups CT and PET. In spite of this, it is not possible to discard the hypothesis that the PET group did not have anaerobic metabolism or fitness altered, as intensive trainings were performed in the PET group. It is possible that evaluation of fast twitch fibers, e.g., extensor digitorum longus, epitrochlearis, or white gastrocnemius muscles could add more information about the anaerobic alterations.

Complete glucose oxidation (CO_2 production) was increased in PET, but not in CT. Just as in CT, an intensive interval training with 15% bm load, exercise:recovery ratio of 30s:30s, isolated performance, also did not increase the glucose oxidation rate (4). This demonstrates the efficacy of PET in comparing to these two exercise training models: linear continuous and intensive interval training. In studies with humans, well trained subjects presented a higher glucose oxidation rate than not so well prepared individuals during aerobic intensity exercise (5,28). In our experimental periodization model, we included 15.8% of anaerobic interval training (AN-1 and AN-2) and 55.2% of aerobic intensive interval training (A-2 and A-3), because training intensity in human studies shows stronger correlation with performance than training volume or frequency in swimmers (26) and the introduction of intensive training (~15%), improves performance in 40Km cycling (20,35), 10Km running (1), and skier rankings (9). Obviously, besides the differences between humans and rats, there are limitations in comparing results from the *in vitro* animal experimental model and *in vivo* exercise training studies in humans. However, if there is relationship between higher muscle glucose oxidation *in vitro* and higher glucose oxidation rates in aerobic exercise trained humans, we do not know. We can only

speculate that the higher glucose oxidation rate in the PET group may indicate a better aerobic fitness.

We did not determine performance in our study; however we carried out an incremental test to determinate the load equivalent to [La-5.5] (aerobic fitness). In humans, effort intensity equivalent to 4.0mM blood lactate concentration represents the anaerobic threshold and has been used to verify the effects of aerobic training (13). However, in swimming rats, Gobatto et al. (11) verified that the [La] equivalent for maximal lactate steady state is 5.5mM. The [La-5.5] equivalent loads for both trained groups were higher than sedentary group load; this did not differ between the PET and CT groups. It is interesting to observe that the [La] kinetics did not present the same pattern as seen in the incremental test in humans (15) and in running rats (31), i.e., a point of abrupt rise in [La]. The blood lactate vs load curve presented a linear response in most animals. Possibly, the incremental test in swimming rats is not sensitive enough to discriminate effects between animals with a similar training level, just as occurred in evaluation of the glucose metabolism.

Considering some limitations of swimming rats, it was possible to standardize a periodized training protocol (PET) for swimming exercise in rats, in the same way it is applied to high performance athletes. Although CT improved some aspects of the glucose metabolism, PET performed with the same total overload as CT ($OV_{total} = 4850$ UT), and organized in basic, specific, and taper periods combining aerobic continuous, and aerobic and anaerobic intensive interval training, was more efficient than continuous linear training, CT.

CONCLUSIONS

Both CT and PET work load at an intensity equivalent to [La-5.5] were similar and than SC. However, the periodized training PET presented better results for glucose metabolism compared to the SC and CT group. Therefore, the results of the PET group open up new perspectives for the study of different exercise training methods and periodized training through the experimental exercise model in rats, aiming at performance training or health fitness.

ACKNOWLEDGEMENTS

The authors gratefully thank Clarice Yoshiko Sibuya, Eduardo Custódio, José Roberto Rodrigues for their expert technical assistance and to Manoel Henrique Salgado, PhD, for assistance in statistical analyses.

Address for correspondence: Julio W dos Santos, PhD, UNESP, Universidade Estadual Paulista, Av Luiz Edmundo Carrijo Coube s/n, Bauru, SP, Brasil. Departamento de Educação Física, CEP: 17033-360. Phone: 55 14 31036082 FAX: 55 14 31036070 Email: santos@fc.unesp.br.

REFERENCES

1. Acevedo EO, Goldfarb AH. Increased training intensity effects on plasma lactate, ventilatory threshold, and endurance. *Med Sci Sports Exerc* 1989;21:563-568.
2. Ariano MA, Armstrong RB, Edgerton VR. Hindlimb muscle fiber populations of mammals. *J Histochem Cytochem* 1973;21:51-55.
3. Baldwin KM, Hooker AM, Herrick RE. Lactate oxidative capacity in different types of muscle. *Biochem Biophys Res Commun* 1978;83:151-157.

4. Braga L, de Mello MAR, Manchado FB, Gobatto CA. Continuous and intermittent exercise: Effects of training and detraining on body weight and muscle metabolism in obese rats. **Rev Port Cienc Desp** 2006;6:160-169.
5. Callow M, Morton A, Guppy M. Marathon fatigue: the role of plasma fatty acids, muscle glycogen and blood glucose. **Eur J Appl Physiol Occup Physiol** 1986;55:654-61.
6. Carvalho, FC, Masuda, OM, Pompeu, FAMS. Method for diagnosis and control of aerobic training in rats based on lactate threshold. **Comp Biochem Physiol A Mol Integ Physiol** 2005;140:409-413.
7. Chiang LM, Chen YJ, Chiang J, Lai LY, Chen YY, Liao HF. Modulation of dendritic cells by endurance training. **Int J Sports Med** 2007;28:798-803.
8. dos-Santos JW. **Protocolos de treinamento aeróbio intervalado e da periodização para natação com ratos** [dissertation]. Rio Claro, SP, Brazil; UNESP - São Paulo State University, 2004. 174 p.
9. Gaskill SE, Serfass RC, Bacharach DW, Kwilly JM. Responses to training in cross-country skiers. **Med Sci Sports Exerc** 1999;31:1211-1217.
10. Giroid S, Maurin D, Dugué B, Chatard JC, Millet G. Effects of dry-land vs. resisted- and assisted-sprint exercises on swimming sprint performances. **J Strength Cond Res** 2007;21:599-605.
11. Gobatto CA, de Mello MAR, Sibuya CY, Azevedo JRM, Santos LA, Kokubun E. Maximal lactate steady state in rats submitted to swimming exercise. **Comp Biochem Physiol A Mol Integ Physiol** 2001;130:21-27.
12. Gorostiaga EM, Walter, CB, Foster C, Hickson RC. Uniqueness of interval and continuous training at the same maintained exercise intensity. **Eur J Appl Physiol Occup Physiol** 1991;63:101-107.
13. Heck, H.; Mader, A.; Hess, G.; Mucke, S.; Müller, R.; Hollmann, W. Justification of the 4-mM lactate threshold. **Int J Sports Med**, 1985;6:117-130.
14. Henriksen EJ, Stump CS, Trinh TT, Beaty SD. Role the glucose transport in glycogen supercompensation in reweighted rat skeletal muscle. **J Appl Physiol** 1996;80:1540-1546.
15. Jacobs, I. Blood lactate: implications for training and sports performance. **Sports Med** 1986;3:10-25.
16. Langfort J, Budohoski L, Newsholme EA. Effect of various types of acute exercise and exercise training on the insulin sensitivity of rat soleus measured in vitro. **Pflugers Arch** 1988;412:101-105.
17. Leighton B, Blomstrand E, Challiss RA. Acute and Chronic effects os strenuous exercise on glucose metabolism in isolated, incubated soleus muscle of exercise-trained rats. **Acta Physiol Scand** 1989;136:177-184.

18. Liao HF, Chiang LM, Yen CC et al. Effect of a periodized exercise training and active recovery program on antitumor activity and development of dendritic cells. **J Sports Med Phys Fitness** 2006;46:307-314.
19. Lima C, Alves LE, Iagher F, Machado AF, Bonatto SJ, Kuczera D, Souza CF, Pequeto DC, Muritiba AL, Nunes EA, Fernandes LC. Anaerobic exercise reduces tumor growth, cancer cachexia and increases macrophage and lymphocyte response in Walker 256 tumor-bearing rats. **Eur J Appl Physiol** 2008;104:957-964.
20. Lindsay FH, Hawolwy JA, Myburgh KH, Schomer HH, Noakes TD, Dennis SC. Improved athletic performance in highly trained cyclists after interval training. **Med Sci Sports Exerc** 1996;28:1427-1434.
21. Maglischo EW. **Swimming fastest**. Human Kinectis, Champaign IL. 2003.
22. Manchado FB, Gobatto CA, Contarteze RVL, Papoti M, de Mello MAR. Maximal lactate steady state in running rats. **JEPonline** 2005;8:29-35.
23. Manso JMG, Valdivieso, MN, Cabalero JA. **Planificación del entrenamiento deportivo**. Madrid: Gymnos Editorial,1996.
24. Matveyev, LP. **Fundamentals of sports training. English translation of the revised Russian version**. Moscow: Progress, 1981.
25. Mujika I, Busso T, Lacoste L, Barale F, Geysant A, Chatard J-C. Modeled responses to training and taper in competitive swimmers. **Med Sci Sports Exerc** 1996;28:251-258.
26. Mujika I, Chatard JC, Busso T, Geysant A, Barale F, Lacoste L. Effects of training on performance in competitive swimming. **Can J Appl Physiol** 1995;20:395-406.
27. Nunes WMS, de Mello MAR. Glucose Metabolism in Rats Submitted to Skeletal Muscle Denervation. **Braz Arch Bio Technol** 2005;48:541-548.
28. O'Brien MJ, Viguie CA, Mazzeo RS, Brooks GA. Carbohydrate dependence during marathon running. **Med Sci Sports Exerc** 1993;25:1009-1017.
29. Oliveria CAM, Rogatto, GP, Luciano, E. Efeitos do treinamento físico de alta intensidade sobre os leucócitos de ratos diabéticos. **Rev Bras Med Esporte** 2002;8(6):219-224.
30. Overend TJ, Paterson DH, Cunningham DA. The effect of interval and continuous training on the aerobic parameters. **Can J Sports Sci** 1992;17:129-134.
31. Pilis W, Zarzeczny R, Langfort J, Kaciuba-Uscieko H, Nazar K, Wojtyna J. Anaerobic threshold in rats. **Comp Biochem Physiol A Comp Physiol**. 1993;106A:285-89.
32. Rhea MR, Ball SD, Phillips WT, Burkett, LN. A comparison of linear and daily undulating periodized programs with equated volume and intensity for strength. **J Strength Cond Res** 2002;16:250–255.
33. Rogatto, GP, Luciano, E. Efeitos do treinamento físico intenso sobre metabolismo dos carboidratos. **Rev Bras Ativ Fis Saúde** 2001;2:39-46.

34. Sjörgreen B, Nordenskjold T, Holmgren H, Wollerstron J. Beitrag zur Kenntniss des Lebernrhythms. *Pflugers Arch. Gesamte Physiol Menschen Tiere* 1938;240:47.
35. Stepto NK, Hawley JA, Dennis SC, Hopkins WG. Effects of different interval-training programs on cycling time-trial performance. *Med Sci Sports Exerc* 1999;31:736-741.
36. Stone MH. Muscle Conditioning and muscle injuries. *Med Sci Sports Exerc* 1990;22:457-462.
37. Terada S, Yokozeki T, Kawanaka K et al. Effects of high-intensity swimming training on glut-4 and glucose transport activity in rat skeletal muscle. *J App Physiol* 2001;90:2019-2024.
38. Terada S, Tabata I, Higuchi M. Effect of high-intensity intermittent swimming training on fatty acid oxidation enzyme activity in rat skeletal muscle. *Jpn J Physiol* 2004;54:47-52.
39. Voltarelli FA, Gobatto CA, de Mello MAR. Determination of anaerobic threshold in rats using the lactate minimum test. *Braz J Med Biol Res* 2002;35:1389-1394.
40. Yaspelkis BB III, Singh MK, Trevino B, Krisan AD, Collins DE. Resistance training increases glucose uptake and transport in rat skeletal muscle. *Acta Physiol Scand* 2002;175:315-323.
41. Wisloff U, Helgerud J, Kemi OJ, Ellingsen O. Intensity-controlled treadmill running in rats: $\dot{V}O_{2max}$ and cardiac hypertrophy. *Am J Physiol Heart Circ Physiol* 2001;280:H1301-H1310.

Disclaimer

The opinions expressed in **JEPonline** are those of the authors and are not attributable to **JEPonline**, the editorial staff or ASEP.