Effect of Body Weight Variation on Swimming Exercise Workload in Rats With Constant and Size-Adjusted Loads

by ***Rodrigo Hohl[1,*], Renato Buscariolli de Oliveira[1], Rodrigo Luiz Perroni Ferraresso[1], René Brenzikofer[2] & Denise Vaz Macedo[1]***

[1]Laboratory of Exercise Biochemistry (LABEX), Biochemistry Department. Biology Institute, Campinas State University (UNICAMP), Campinas, São Paulo, Brazil

[2]Laboratory of Instrumentation for Biomechanics (LIB), Faculty of Physical Education (FEF) - Campinas State University, (UNICAMP), Campinas, São Paulo, Brazil

Summary

In swimming animal models, weights are added according to some percentage of body weight (%BW) or as a constant load (CL) to equalize the workload of each animal or to reduce the time in swimming-to-exhaustion endurance tests. The objective of the present study was to evaluate the effect of body weight variation on swimming exercise workload through the reliability analysis of swimming-to-exhaustion endurance tests. We examined the reliability by comparing the mean time to exhaustion (TEx) in trials performed on the 30th, 60th, 90th, 120th and 150th days of life of Wistar rats using three %BW and CL workloads (4%, 6% and 8% and 7 g, 11 g and 15 g, respectively). We also examined the within-subject variation of TEx over three trials of a CL test (15 g) within one week (when variability in body weight is minimal). The rats' body density was maintained during growth (mean (SD) 1.031 (0.026) g/ml – 1.026 (0.005) g/ml) despite their significant increase in body weight (mean(SD) 109.05(13.80) g - 442.92(29.39) g). Thus, the absolute loads in longitudinal %BW tests increased gradually, causing a decrease in TEx under all workloads. The CV confidence limits for TEx in CL tests showed high within subjects variation (17.1-111%) compared to the body weight variation (0.4-2.8%). We conclude that load adjustment based on %BW does not adequately equate to the workload between rats of different sizes. The methodology also showed high within-subject variation between trials (not related to body mass changes) that compromises the significance of small effects.

Introduction

Swimming in laboratory animals, such as small rodents, has been widely used to study the physiology and cellular metabolism of physical exercise. For this purpose, swimming has a number of advantages compared to treadmill running: the simple and inexpensive equipment that is required, the rodents' natural swimming abilities, the possibility of working at higher intensities and the strong survival instinct when exhaustion is imminent, ensuring a high performance level (*Dawnson & Horvat, 1970; Kramer et al., 1993; Matsumoto et al., 1996; Kregel et al., 2006*). In addition, swimming-to-exhaustion endurance tests have been extensively used to test performance before and after drug administration, long-term training regimens, acute exercise, diets and supplements (*Dawnson & Horvat, 1970; Kregel et al., 2006*). In this kind of endurance-performance test, the animal is placed in a tank filled with water and must swim with weights (i.e., lead) attached to its tail or chest until it is unable to remain on the surface.

Weights are used to reduce the time to exhaustion to a practical duration because unloaded rats can swim

**Correspondence:* Rodrigo Hohl
Laboratório de Bioquímica do Exercício (LABEX), Departamento de Bioquímica. Instituto de Biologia (IB), CP 6109, Universidade Estadual de Campinas – UNICAMP 13083-970, Campinas, São Paulo, Brazil
Tel +55 19 3521-6145 or +55 19 3521-6146
Fax +55 19 3521-6129
E-mail hohlrodrigo@gmail.com

Published in the Scandinavian Journal of Laboratory Animal Science - an international journal of laboratory animal science

for many hours in thermoneutral conditions (*Richter, 1957; Dawnson & Horvat, 1970*). To reduce the variation among individuals, added loads used in swimming exercises are commonly adjusted based on a percentage of body weight (%BW). This procedure is meant to standardize the amount of work performed by animals of different sizes (*McArdle & Montoye, 1966; McArdle, 1967; Dawnson & Horvat, 1970; Gobatto et al., 2001; Tanaka et al., 2003; Jung et al., 2004; Kregel et al., 2006).* However, load and endurance capacity (i.e., time to exhaustion) do not seem to be directly proportional, and heavier individuals are at a slight disadvantage in swimming tests that use %BW for load standardization in rats (*McArdle & Montoye, 1966; Naito & Griffith, 1977*) and humans (*Key, 1962*).

Another variable to be considered in the liquid environment is buoyancy, which is related to the body density of the animal. In a transversal analysis, Hohl *et al*. (*2007*) found no significant difference in the body density of Wistar rats of different masses and ages. Thus, the influence of systematic effects can be evaluated in a longitudinal paired analysis (using the same group of animals throughout their growth period) of endurance trials with loads adjusted by %BW. It is thought that in %BW tests, the weight attached must be directly proportional to the body weight to achieve a similar time to exhaustion in animals of different sizes. Moreover, the use of a constant absolute weight without regard for differences in body size means that a constant physical load is supported throughout growth. Therefore, if buoyancy is constant (i.e., body weight and volume increase proportionally), the influence of increasing body weight on time to exhaustion throughout the growth process can be experimentally verified through an endurance test using a constant load (CL) in a paired longitudinal study.

However, the variation among animals in time to exhaustion in swimming tests may be very high under both methods of load addition (*McArdle & Montoye, 1966; Dawnson & Horvat, 1970*). This variation may be reflected in the reproducibility of time to exhaustion between repeated trials using the same animal, even with little variation in body weight, which would compromise the reliability of the test. Knowledge of the reproducibility and variability of performance in an exercise test is important for the correct interpretation of performance data using the minimum number of animals. The more reliable the performance data, the more precise the measurements; furthermore, fewer animals will be required to observe significant treatment effects. A reliable measurement of performance is characterized by small systematic changes in the mean and minimal within-subject variation between repeated trials (*Hopkins, 2000*).

The purpose of this study was to examine (1) the appropriateness of using %BW and CL workload adjustment, by comparing the mean time to exhaustion in trials performed on the 30th, 60th, 90th, 120th and 150th days of life of Wistar rats and (2) the within-subject variation of time-to-exhaustion measurements over repeated trials of a CL test within one week, when the increase in body weight was minimal. This longitudinal paired study complements previous studies of exercise load in swimming rats (*McArdle & Montoye, 1966; Hardin, 1968; Dawnson & Horvat, 1970*) and provides further discussion about experimental refinement and reduction in animal use.

Materials and Methods

Animals

Forty-two heterogenic male albino Wistar rats (origin: Zentralinstitut für Versuchstierzucht (ZFV), Hannover, Germany, 1987) were divided into four experimental groups: G1, G2, G3 (n= 10 each) and G4 (n=12). All rats were 21 days old at the beginning of the experiment. The animals were housed in fours in plastic cages (60 x 50 x 22 cm, polypropylene) in acclimatized airflow racks [air-flow: 15 changes/hour, temperature: 25ºC (±1ºC) and humidity: 55% (±10%)] with inverted control of the light/dark cycle for 12 hours (lights on at 6 p.m.) with *ad libitum* food (Nutrival, CR-1, Nutrival Nutrients, Curitiba, PR, Brazil) and pretreated water. Bedding (sterilized aspen chips) was changed twice a week. All animals were acclimatized to the water and to swimming before the beginning of the tests by placing them in 35ºC (± 1ºC) water for ten minutes three times per week. This procedure was intended to prevent interference caused by stress without provoking training adaptation. The experimental protocols were pre-approved by the Animal Experimentation Ethics Committee (Medicine Faculty, UNICAMP) and were in accordance with the Guide for the Care and Use of Laboratory Animals (1996) and The Council of European Convention ETS 123.

Endurance-Test Conditions

The tests were conducted in an 85-cm x 65-cm x 100-cm tank with four divisions. The water was kept in thermoneutral conditions (35±1ºC) with a controlled chlorine level (2-4 ppm) and depth (90 cm). The body weight of each animal was measured with a digital scale (Gehaka; model BG 1000, division: 0.01 g; maximum capacity: 1010 g). Loads were added by placing lead weights in small bags attached to the trunk of each rat. The animals were always placed in the tank in pairs without contact. The performance parameter was the time needed to reach exhaustion, which was established when an animal remained underwater for ten seconds (*McArdle & Montoye, 1966*). The tank dimensions prevented the rats from touching the bottom and returning to the surface. The tests were carried out by the same two investigators for the duration of the study and were always performed in the afternoon (1 p.m. – 6 p.m.).

Body density

To monitor body density, we measured the volume and mass of each G2 animal in each experimental week from the 30th day until the 150th day of life (as shown in Table 1). We used the apparatus proposed by Hohl *et al*. (*2007*) to measure body volume in live, non-anesthetized rats by hydrostatic weighing. Briefly, the apparatus consists of two communicating cylindrical vessels linked by a flexible hose. The rat is placed in one vessel while water-level changes are measured by hydrostatic weighing of a cylindrical test tube with lead ballast placed in the other vessel. The connecting flexible hose is of size to attenuate the transmission of disturbances caused by animal movements. The recommendations for calibration and animal measurement were followed.

Longitudinal reproducibility of %BW and CL time-to-exhaustion endurance tests

Experimental groups G1, G2 and G3

From 30 days of age onward, thirty rats underwent two distinct tests of exhaustion each month: one test with the load adjusted according to %BW and the other test with a CL. The animals were divided into three experimental groups (G1, G2 and G3), initially comprising ten animals each, and were subjected to three levels of overload. The performance tests were

Table 1. Schedule used during the five experimental weeks, once a month from the 30th to the 150th day of life of the rats.

	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
Morning	-	-	-	-	Volume measuring	-
Afternoon	**G1** CL - 7g	**G2** CL -11g	**G3** CL -15g	**G1** %BW- 4%	**G2** %BW- 6%	**G3** %BW - 8%

CL: Constant-load endurance test; %BW: Percentage-of-body-weight endurance test; G1, G2 and G3: Experimental Groups.

performed on the 30th, 60th, 90th, 120th and 150th days of life following the schedule in Table 1. Thus, in each experimental week, the G1 rats carried a load of 7 g in the CL test and 4% of their body weight in the %BW test, while the G2 rats carried 11 g in the CL test and 6% in the %BW test. Finally, the G3 rats carried 15 g in the CL test and 8% in the %BW test. In the %BW test, the loaded bags and animals were weighed immediately prior to the beginning of the test. In the CL test, the same loaded bags were used from the beginning to the end of the experiment, guaranteeing that each animal was swimming with exactly the same load.

The groups with their respective loads (%BW and CL) were kept constant in all experimental weeks. The interval between tests performed on the same group was at least 72 hours, which is sufficient for replenishment of muscular glycogen (*Nakatani et al., 1997; Conlee et al., 1978*). In weeks without performance tests, the rats swam for ten minutes at 35ºC (± 1ºC) three times per week (*Jung et al., 2004*) to maintain their familiarity with the aquatic environment and handling (*McArdle & Montoye, 1966*).

Three G1 rats and one G2 rat did not demonstrate continuous swimming behavior throughout the study. These rats were removed. Data analyses were conducted with seven rats in G1 and nine rats in G2.

Within-subject variation of time-to-exhaustion and in body weight between repeated CL trials

Experimental group G4

The rats in experimental group G4 (n=12) performed three CL endurance tests during the same week at the 60th, 90th, 120th and 150th days of life. The interval between the tests was 72 hours, and a constant load of 15 g was used. Fifteen grams was chosen for this analysis because it caused the shortest time to exhaustion and exhibited similar longitudinal behavior as with the 7g and 11g weights. In weeks without performance tests, the rats swam for ten minutes at 35ºC (± 1ºC) three times per week to maintain their familiarity with swimming and handling and without significant physical training (*Jung et al., 2004*).

Statistical Analysis

Raw time-to-exhaustion (TEx) data from the longitudinal %BW and CL tests are presented as mean values and standard errors (SEM) on a logarithmic (log) scale. Because the magnitude of TEx was very different for each experimental group (G1, G2 and G3) and each experimental week, we used the log scale to describe the change in performance over the months of the experiment on a scale that would encompass all the experimental groups. Repeated-measures analysis of variance (ANOVA) with Tukey's *post hoc* test for significant differences was used to identify significant differences in mean values. Statistical significance for all analyses was accepted as $P \leq 0.05$.

Typical measurement error, represented by the coefficient of variation as a percentage (CV), was calculated to analyze the random within-subject variation of TEx and body-weight measurements among three repeated CL trials during the same week (experimental group G4). CVs were calculated from log (TEx) because the raw data displayed non-uniform error (heteroscedasticity). The CV is equal to 100*SD (standard deviation) of the differences between Trial 2 and Trial 1 and between Trial 3 and Trial 2. The rationale for using the CV as a measurement of reliability has been discussed by Hopkins (*2000*).

Results

Longitudinal body weight, volume and density (30-150 days): experimental group G2

The increases in mass and volume were proportional over the first five months of the rats' lives (30-150 days, Table 2), and body density did not differ significantly during this period. Body mass and volume increased strongly between days 30 and 60 and continued to increase at a lower rate until day 120. There was no statistical difference in mass and volume between days 120 and 150.

Table 2. Longitudinal body-weight, volume and body-density.

	30 days	60 days	90 days	120 days	150 days
Body Weight (g)	109.05 (13.80)*	270.03 (17.59)*	352.67 (15.09)*	427.72 (30.69)	442.92 (29.39)
Volume (ml)	105.87 (14.23)*	263.11 (14.13)*	341.97 (17.23)*	415.06 (30.91)	433.18 (29.93)
Body Density (g/ml)	1.031 (0.026)	1.026 (0.01)	1.032 (0.010)	1.031 (0.011)	1.026 (0.005)

Mean (SD) of body weight (g), volume (ml) and density (g/ml) of G2 rats (n = 9) on different days of life. * Significant difference (ANOVA, $P<0.001$) between all days. Density showed no significant difference.

Reproducibility of TEx in %BW and CL tests: experimental groups G1, G2 and G3

Figure 1 (A, B) shows the behavior of TEx measurements in the %BW and CL tests, respectively [means (SEM) are presented on a log scale].

In Figure 1A, TEx decreased across the first three %BW tests (30, 60 and 90 days of life) and stabilized after 90 days. TEx values for the same experimental group differed significantly between days 30 and 60 ($P<0.001$) and between days 90, 120 and 150 ($P<0.001$). The same behavior was observed in all experimental groups [G1 (4%), G2 (6%) and G3 (8%)].

Figure 1B shows that TEx values behaved differently in the CL test compared to the %BW Test. Here, the TEx increased significantly in G2 (11 g) and G3 (15 g) from 30 to 60 days (P < 0.001), and then stabilized. In the CL test for G1, which had the lowest constant load (7 g), there was a significant difference in TEx between days 30 and 90 (P <0.05).

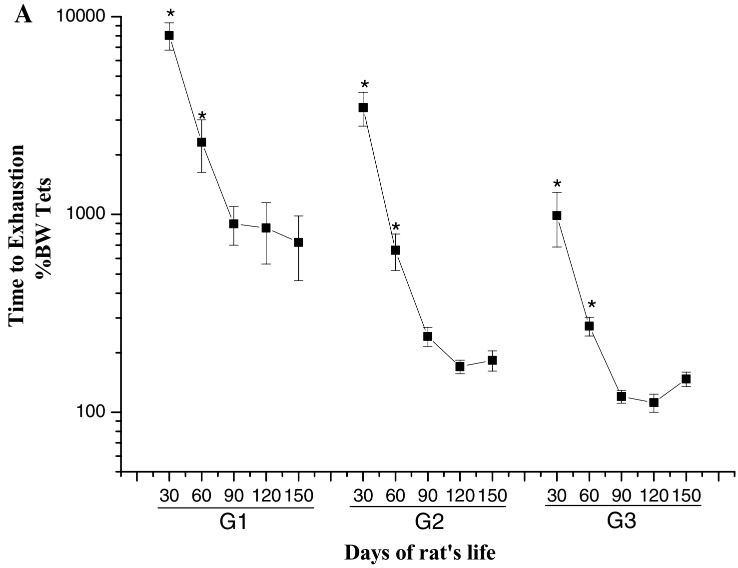
Within-subject variation in TEx and body weight in CL tests during one experimental week: experimental group G4

Table 3 presents the CV and 90% confidence intervals (CI) of TEx in the CL test (with 15 g) and body-mass measurements in experimental group G4. The largest CVs were observed in the experi-

Table 3. Typical measurement error as the coefficient of variation of the CL test (15 g) and body mass in each experimental week throughout the growth period of rats at 60, 90, 120 and 150 days of life.

	Typical error of measurement as CV(%) (Confidence Interval 90%)							
	60 Days		**90 Days**		**120 Days**		**150 Days**	
	(2-1)	**(3-2)**	**(2-1)**	**(3-2)**	**(2-1)**	**(3-2)**	**(2-1)**	**(3-2)**
Time to exhaustion w/ 15g	61.9 (43.4 - 111.1)	45.1 (32.0 - 78.1)	37.1 (25.1 - 71.2)	31.5 (21.4 - 59.3)	24.2 (17.1 - 42.6)	15.8 (11.6 - 27.3)	33.0 (23.5 - 57.6)	27.7 (19.8 - 47.7)
Body Weight	1.0 (0.7 - 1.8)	1.2 (0.9 - 2.1)	1.0 (0.8 - 1.6)	1.3 (1.0 - 2.0)	0.8 (0.6 - 1.4)	0.5 (0.4 - 0.8)	0.6 (0.4 - 0.9)	1.8 (1.3 - 2.8)

Three CL (15 g) trials were performed on the experimental group G4 (n=12) during the same week at days 60, 90, 120 and 150. Body weight was measured before each trial. The CV was calculated as 100*SD of the differences between the three trials using log-transformed data: Trial 2 compared to Trial 1 (**2**-**1**) and Trial 3 compared to Trial 2 (**3**-**2**). CV: coefficient of variation.



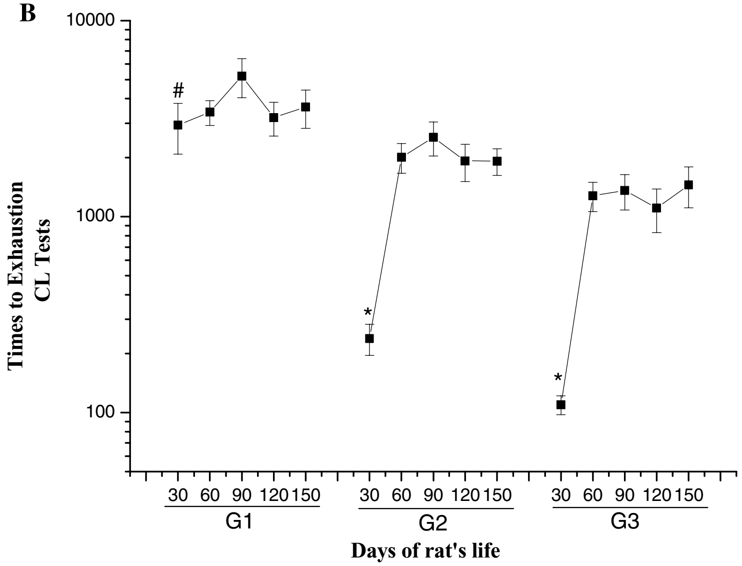


Figure 1. A. Time to exhaustion in %BW tests from the 30th to the 150th day of life. Loads were adjusted to 4% (G1, n= 7), 6% (G2, n= 9) or 8% (G3, n= 10) of body weight. * Significant difference from all other days of life (P <0.001). **B** – Time to exhaustion in CL tests from the 30th to the 150th day of life. Constant loads were set at 7 g (G1, n= 7), 11 g (G2, n= 9) or 15 g (G3, n= 10). * Significant difference from all other days of life (P <0.001). # Significant difference at day 90 (P <0.05). %BW: Percentage of body weight. CL: constant load. G1, G2 and G3: experimental groups. Raw data are presented as means (SEM) on a log scale.

mental week beginning on day 60, with the highest upper confidence limit of 111.1%. The CV tended to decrease from day 90 onward, although the 90% CI upper limits ranged from ~27% to ~71%. Moreover, the variation in mass over the same period was small in all cases; the highest upper limit of the 90% CI was only 2.8% [150 days (3-2)].

Discussion

To date, no study has compared and verified the reliability of swimming-to-exhaustion tests with constant loads (CL test) and with loads based on a percentage of body weight (%BW test) in a longitudinal paired analysis over the growth period of Wistar rats. We examined this issue because we have found it difficult to equate swimming training protocols and time to exhaustion in performance tests using weights adjusted by %BW in animals of different sizes.

Our data show that the animals did not maintain consistent TEx values throughout the growth period, even though we attempted to equalize their effort by adjusting the loads based on %BW (Fig. 1A). There was a significant systematic decrease in mean performance when comparing 30 and 60 days to 90, 120 and 150 days. Systematic changes in the mean from consecutive trials without any apparent treatment or training could represent a learning effect, motivation or fatigue. This kind of interference must be eliminated for reliable evaluation of performance between trials on the same subjects (*Hopkins, 2000*). In this case, the cause of the systematic decrease in TEx seemed to be the adjustment of the load using %BW, which increased the exercise intensity in concert with body weight. As observed by Key (*1962*), McArdle & Montoye (*1966*) and Naito & Griffith (*1977*), this methodology does not guarantee similarity in time to exhaustion between subjects with different body weights.

In this context, McArdle & Montoye (*1966*) suggested that buoyancy should be taken into account when establishing swimming loads. To investigate this issue, we have developed an apparatus that can accurately and reproducibly measure body volume in live rats without anesthesia or fur shaving, allowing them to swim immediately after the procedure (*Hohl et al., 2007*). In the present study, the buoyancy (body density) of the animals remained constant despite a significant increase in body weight (Table 2). This means that the absolute workload of each rat throughout the growth period increased in the %BW test, causing a significant decrease in TEx during the maturation process as muscle mass increased. When the increase in body weight became less pronounced from 90 days onward (Table 2), the downward trend in TEx was no longer statistically significant (Fig. 1A).

Even though the SEMs of the 6% and 8% BW tests at 90, 120 and 150 days were smaller than those at 30 and 60 days (Fig. 1A), these small SEMs may not suggest a standardized physical effort but may reflect the higher absolute loads that rats reached in these tests (~26-32 g). In addition, the larger SEMs that were observed in the 4% BW tests after the trials carried out on days 90, 120 and 150 may have been due to lower absolute loads (~11-16 g) than the 6% and 8% BW tests in the same period. Our observations are consistent with those of Hardin (1968), who suggested that the end of a swimming-to-exhaustion performance test should be determined by physiological and metabolic differences and not by the absolute load that the rats can support while maintaining themselves on the surface of the water. In other words, the loads were so heavy that the physiological variability among rats lost significance, and this pattern does not indicate effort standardization. Effort standardization occurs when the load is adjusted to consider individual variability in the physical/physiological condition of the animal (i.e., individual anaerobic threshold, VO_2 or size).

Ratio standards, where physiological capacity (i.e., strength or VO_2) is divided by body mass or an alternative index of body size, have frequently been used in an attempt to remove the effect of body size (*Nevill et al., 1992*). This approach lacks empirical support and assumes that the rats' endurance capacity is directly proportional to their body mass. In addition, this approach implies a systematic error in the %BW test that could affect the sensitivity of any treatment effect on time to exhaustion in case of variation in body mass with maintenance of buoyancy. To control for this possibility, allometric scaling could be used to describe the disproportionate changes in physical capacity and body size (*Davies & Dalsky, 1997; Jaric et al., 2002*). Quantitatively, allometry is usually expressed in the form of power-law equations relating some physiological function (Y) as a dependent function of body mass (m) in the form $Y = a\ m^b$, where a and b are derived empirically (*Lindstead & Schaeffer, 2002*). When the load is adjusted based on %BW to reduce the time to exhaustion (which is related to a physiological function) to a viable experimental duration, it is assumed that $b = 1$ and $Y = a\ m$. In other words, even if the absolute added weight is different for animals of different masses, the physiological function will be equally stressed because it is thought to be directly proportional to body weight. Thus, time to exhaustion would be similar. This relationship has been accepted for decades, but we found *no experimental support* for this proportional relationship between body weight and time to exhaustion in rodent swimming tests.

Moreover, the maintenance of buoyancy (Table 2) could justify the use of a constant weight in longitudinal CL tests. In this case, the maturation process that increases the body mass could explain the systematic increase in TEx values between 30 and 60 days (Fig. 1B) in the CL tests. This observation is consistent with the theoretical allometric equation $Y = a\ m^b$. Therefore, independent of the power b, it is expected that with increasing body weight, there will be a concomitant increase in Y that is related to TEx.

By performing CL tests with the same rats throughout their growth period, the effect of body weight on TEx can be examined by calculating the variables a and b of the allometric equation. However, the exponent b and the constant a will be accurate only if the CL test is reliable; otherwise, the variables a and b themselves present a random error that compromises the applicability of the allometric equation. In this study, TEx values in the CL test (Fig. 1B) were constant after day 60 day, suggesting that $b = 0$ and Y (related to TEx) = a. However, the apparent stabilization of TEx in the CL test may have been due to its high variability (Table 3), making this parameter insensitive to the significant increase in body weight between days 60 and 120 (Table 2).

The within-subject variation in TEx, which was evaluated in the G4 group (Table 3), was much greater (90% CI upper limits between 27.3 and 111.1%) than the variation in body mass (90% CI upper limits between 0.4 and 2.8%), indicating that some rats doubled their TEx between trials despite the insignificant variation in body weight. Therefore, the low reliability of the CL test was not due to variation in body weight but to other unknown effects.

Compared to human studies, the poor reliability in time-to-exhaustion tests may be due to the relationship between exercise duration and power. Small changes in power (on the order of 1%) can cause variations in time to exhaustion in the order of 10-20% (*Hinckson & Hopkins, 2005*). The pattern of movement and total work done by swimming rats may differ among tests because they are forced to exercise and must try to survive. For example, contact with the walls and submersion alter the pattern of motion, and the pattern of motion alters the speed of leg movement and the force required for water displacement, thus affecting the power exerted. When the interventions studied (i.e., drugs, physical training, diets and supplements) do not generate a response that exceeds the high random variability of swimming-to-exhaustion tests, the interventions can be considered innocuous due to the lack of sensitivity in measuring the time to exhaustion.

The variability of time to exhaustion should be considered when attempting to
refine experiments and reduce the number of animals using the methodology presented here. For example, if the experiment were performed on the 90th day of the rat's life using a longitudinal paired study during one week with minimal body-size variation,

the investigator should expect an effect greater than ~37% (see the 90-day CV in Table 3) on the time to exhaustion. In practice, according to Dell et al. (2002), if we assume that the researchers expect a 20% increase in time to exhaustion in a repeated paired study with a power (1-ß) of 90% and a significance level (α) of 5%, then the number of animals required will be about 40. This number can be reduced to about eight if the same researchers assume that a 50% increase is significant for their study. However, the CVs presented in Table 3 should be used under the conditions presented in this study; reliability analyses for each particular condition (i.e., rodent strain, swimming tank, age, load adjustment and body-size variation during the study) should be conducted before beginning the study.

Conclusion

Swimming-to-exhaustion tests performed with rats using constant loads or loads adjusted by %BW show poor reliability. The methodology can influence the statistical significance of comparative data when analyzing the time to exhaustion of treated and control groups, especially in experiments where changes in body weight are significant in a longitudinal paired study. Such changes can cause systematic errors when the %BW test is used; thus, changes in the mean may occur despite the effect of treatment.

We conclude that load adjustment by %BW does not adequately equalize the workload between animals of different sizes. This bias is attenuated only when animals of similar sizes are used in a transversal study. The adjustment of swimming loads in rodents is not accurate, and its application merits debate because physiological capacity is not only dependent on size. Finally, reliability analysis is essential to refine the experiment and reduce the number of animals; in this sense, swimming exercise to exhaustion in rodents, with load adjustment, shows excessive variability in the methodology that should be reported according to the conditions of a particular experiment.

Acknowledgements

The authors thank Ana Maria Marçal Porto for technical support. This study was supported by the Brazilian agencies FAPESP and CNPq.

References

Conlee R K, R C Hickson, W W Winder, J M Hagberg & J O Holloszy: Regulation of glycogen resynthesis in muscles of rats following exercise. Am. J. Physiol. 1978, *235(3)*, R145-R150.

Davies M J & G P Dalsky: Normalizing strength for body size differences in older adults. Med. Sci. Sports Exerc, 1997. *29*, 713–717.

Dawson C A & S M Horvath: Swimming in small laboratory animals. Med. Sci. Sports. 1970, *2*, 51-78.

Dell R B, S Holleran & R Ramakrishnan. Sample size determination. ILAR J. 2002**,** *43(4)*, 207–13.

Gobatto C A , M A R De Mello, C Y Sibuya, J R M De Azevedo, L A Dos Santos & E Kokubum: Maximal lactate steady state in rats submitted to swimming exercise. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 2001*, 130(1)*, 21-27.

Guide for the Care and Use of Laboratory Animals. Institute of Laboratory Animal Research, Commission on Life Sciences, National Research Council. 1996. published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA

Hardin D H: Reliability of selected swimming endurance tests for laboratory rats. Res. Q. 1968, *39 (2)*, 405-407.

Hinckson E A & W G Hopkins: Reliability of time to exhaustion analyzed with critical-power and Log-Log modeling. Med. Sci. Sports Exerc. 2005, *37 (4*), 696-701.

Hohl R, R O Buscariolli, D V Macedo & R Brenzikofer: Apparatus for measuring rat body volume: a methodological proposition. J. Appl. Physiol. 2007, *102***,** 1229-1234.

Hopkins W G: Measures of reliability in sports medicine and science. Sports Med. 2000, *30(1)*, 1-15.

Jaric S, S Radosavljevic-Jaric & H Johansson: Muscle force and torque in humans require different methods when adjusting for differences in body size. Eur. J. Appl. Physiol. 2002, *87*, 304–307.

Jung K, I H Kim & D Han: Effect of medicinal plant extracts on forced swimming capacity in mice. J. Ethnopharmacol. 2004*, 93(1)*, 75-81.

Key J R: Relationship between load and swimming endurance in humans. Res. Q. 1962, *33(4),* 559-565.

Kramer K, H Dijkstr & A Bast: Control of Physical Exercise of Rats in a Swimming Basin. Physiol Behav 1993, *53*, 271-276.

Kregel K C, D L Allen, F W Booth, M R Fleshner, E J Henriksen, T I Musch, D S O' Leary, C M Parks, D C Poole, A W Ra'anan et al. (Committee to develop an APS Resource book for the design of animal exercise protocols): 2006, Swimming in rats. In: Resource book for the design of animal exercise protocols, 35- 40. The American Physiology Society. Available from . the-aps.org/pa/action/exercise/book.pdf (last access in 2010).

Lindstedt S L & P J Schaeffer: Use of allometry in predicting anatomical and physiological parameters of mammals. Laboratory Animals 2002, *36*, 1–19.

Matsumoto K, K Ishihara, K Tanaka, K Inoue & T Fushiki: An adjustable-current swimming pool for the evaluation of endurance capacity of mice. J Appl Physiol 1996, *81(4),* 1843-1849.

Mcardle W D: Metabolic stress of endurance swimming in the laboratory rat. J. Appl. Physiol. 1967, *22(1),* 50-54.

Mcardle W D & H J Montoye: Reliability of exhaustive swimming in the laboratory rat. J Appl Physiol 1966, *21(4)*, 1431-1434.

Naito H K & D R Griffith: Interaction of thyroxine, energy metabolism and swimming performance of rats. Life Sci. 1977, *20*, 1121-130.

Nakatani A, D H Han, P A Hansen, L A Nolte, H H Host, R C Hickner & J O Holloszy: Effect of endurance exercise training on muscle glycogen supercompensation in rats. J Appl Physiol. 1997, *82(2),* 711-715.

Nevill A M, R Ramsbottom & C Williams: Scaling physiological measurements for individuals of different body size. Eur. J. Appl. Physiol. Occup. Physiol. 1992, *65(2),* 110–117.

Richter C P: On the phenomenon of sudden death in animals and man. Psychosom. Med. 1957, *19*, 191-98.

Tanaka M, F Nakamura, S Mizokawa, A Matsumura, S Nozaki & Y Watanabe: Establishment and assessment of a rat model of fatigue. Neurosci Lett. 2003, *352(3)*, 159-162.