Chinese Journal of Physiology 61(3): 144-151, 2018
DOI: 10.4077/CJP.2018.BAG548

A Novel Swimming Performance Test in Rats

Aristidis S. Veskoukis[1, 2], Antonios Kyparos[2], Vassilis Paschalis[3, 4], and Michalis G. Nikolaidis[2]

[1]Department of Biochemistry and Biotechnology, University of Thessaly, Viopolis, Mezourlo, 41500, Larissa, Greece
[2]Department of Physical Education and Sports Science at Serres, Aristotle University of Thessaloniki, Serres, Greece
[3]School of Physical Education and Sport Science, National and Kapodistrian University of Athens, Ethnikis Antistasis 41, 17237, Athens, Greece
and
[4]Department of Health Sciences, School of Sciences, European University Cyprus, Nicosia, Cyprus

Abstract

Swimming is an advantageous exercise modality since it induces limited muscle damage. Performance is a crucial endpoint measurement of physiological relevance in exercise physiology and clinical settings alike. To our knowledge, the literature lacks a comprehensive and widely accepted swimming performance protocol without suffering from high variability in time to exhaustion. Thus, the present study presents an easily carried out, two-phased swimming performance incremental test exhibiting low variability in the time to exhaustion among rats. All nine rats managed to complete the first 12 min-part of the test (phase 1) with gradually increased loads attached at the base of their tails equal to 2%, 3.5% and 5% (for 4 min each). All rats reached exhaustion at the 10% final load (phase 2). The mean swimming time until exhaustion, as a measure for defining exercise performance, was 865 ± 59 s. In conclusion, we have presented in detail a novel protocol for practically and satisfactorily measuring swimming performance in rats characterized by low variability in the time to exhaustion. This protocol, with the appropriate modifications, can be applied to a wide spectrum of experimental treatments.

Key Words: exercise, performance, rats, swimming, test, variability

Introduction

Rodents, especially rats and mice, are the most commonly used animal models in biomedical experiments. The use of rats probably surpasses mice in exercise physiology because of the larger blood volume and the grater tissue size that can be collected allowing the assessment of a larger number of variables. Indeed, exercise physiology has been largely profited by research in rat experimental models, as indicated by some important studies of the field (1, 18, 32, 36, 37).

Exercise is an intervention usually applied into different, multifaceted research areas in order to disturb cell and tissue redox equilibrium (34). Swimming is an exercise modality that has been extensively utilized in rat experiments. It is a preferable physiological stimulus able to disrupt tissue redox homeostasis, given that rats have the inherent ability to be excellent swimmers (23). As compared to running exercise models including treadmill or uphill running, swimming is undoubtedly, on the whole, an advantageous exercise modality (8). Indeed, swimming is a natural

Corresponding author: Dr. Aristidis S. Veskoukis, Department of Biochemistry and Biotechnology, University of Thessaly, Viopolis, Mezourlo, 41500, Larissa, Greece, Tel: +30 2410565286, E-mail: veskoukis@gmail.com
Received: July 19, 2017; Revised (Final Version): March 19, 2018; Accepted: April 14, 2018.
 ISSN : 0304-4920. http://www.cps.org.tw

ability for rats and, as such, it induces minimal anxiety and psychological stress on the animals. It is characteristic that the animals do not need electrical or other kinds of stimulation in order to continue swimming, which is in contrast to all types of treadmill running protocols (2, 25). Furthermore, it has been reported that swimming affords greater benefits to cortical and trabecular bone than uphill treadmill (49). Additionally, the muscular forces applied in swimming produce greater bone adaptations than running in rats (42). Based on the above, it becomes apparent that swimming is probably a slightly better method to measure exercise performance but it is not an ideal exercise intervention since the rats are forced to swim. Still, swimming provides a more uniform, whole body continuous exercise as it does not necessarily involve "stop and go" activity, like running, thus it ensures reaching exhaustion (23). Therefore, the stress during the experiments is minimal and does not confound the effects of the implemented treatments. Additionally, swimming induces limited muscle damage and does not cause foot injuries being a much less traumatic exercise model (23). Thus, the alterations observed in the measured variables are mostly attributed to exercise *per se* and not to muscle damage, as is the case for eccentric exercise modalities, such as downhill walking/running on the treadmill, that cause tissue trauma. Indeed, eccentric exercise has been associated with microtrauma, structural abnormalities of myofibrils (45) and disruption of the plasma membrane (29) that can cause functional deficiencies in skeletal muscle (3). Furthermore, it has been reported that horizontal treadmill running can also induce muscle damage particularly when applied to unaccustomed subjects (35, 38, 46).

Experiments with rats have been previously used to address diverse research questions in exercise biochemistry and physiology including physical performance (5, 47, 48). Performance is a crucial endpoint measurement/biomarker of physiological relevance in exercise physiology that can provide mechanistic answers. The effects of an exercise stimulus on intermediate metabolism or a nutritional supplement on exercise adaptations can be appropriately monitored by measuring exercise performance (31). Moreover, exercise performance can reflect pathological conditions and diseases. In this sense, it has been correlated with disability and prediction of mortality in the elderly (14) and it is considered as a predictor of functional capacity after stroke (43), whereas impaired performance correlates with pulmonary hyperinflation and dyspnoea (11). Furthermore, reliable assessment of exercise performance can be a predictor of disability and disease onset in general both in rats (6) and humans (39, 50).

The water temperature and the familiarization protocol are two of the most crucial parameters to be met for reliably measuring exercise performance in rodents. It has been previously reported that the water temperature implemented in this study (*i.e.* strictly between 33.5°C and 34.5°C) is suitable for swimming as the animals do not experience any cardiovascular or other side-effects that would compromise their performance (9). Interestingly, if the water temperature is much higher than rat's body temperature (*i.e.* above 42°C), performance diminishes as the rat becomes hyperthermic, whilst when the water temperature is much lower than the rat's body temperature (*i.e.* below 20°C), hypothermia reduces performance and may even cause death (9). The swimming protocols currently used differ from each other regarding water temperature [28°C (52), 30°C (51) and 30-32°C (30)]. With regard to familiarization protocol, it has been demonstrated that animals exposed to novel stressors have intense stress responses, which are successfully diminished by familiarization (41). Therefore, during familiarization the rats become accustomed with water, handling and swimming under loads attached to their tails (19).

Exercise protocols to evaluate the effects of diverse treatments on swimming performance have been widely applied. However, there are substantial differences among these protocols and are often inadequately described. Indeed, Lamou *et al*. (24) and Narkhede *et al*. (33) have measured performance using a constant load (*i.e.* 10% of body weight) and water temperature in relatively low levels (*i.e.* 24-26°C and 23-27°C, respectively) without applying any familiarization protocol. Additionally, Mozorov *et al*. (30) used swimming with load equal to 8–9% of body weight added to the rats' tails and the total duration of the exercise was approximately 40 min. In addition, the majority of the exercise tests suffer from high variability in the time to exhaustion, which increases the need of using a large number of animals (16). Tables 1 and 2 present a detailed comparison between our protocol and other relevant studies regarding the most critical parameters as well as the variability in time to exhaustion.

From all the above it becomes apparent that the available protocols for measuring swimming performance have many discrepancies and lack homogeneity. To our knowledge, the relevant literature lacks of a detailed, reliable and widely accepted swimming performance protocol. Therefore, the aim of the present study was to describe in detail a novel and reliable swimming performance test in rats characterized by low variability in the time to exhaustion. This specifically designed for rats swimming protocol has been successfully applied to our previous studies, as

Table 1. Comparison of the most critical parameters of the present novel performance test with protocols of other studies that measured swimming performance in rats. NR: not reported; s: seconds, g: grams.

Performance protocol	Swimming familiarization	Water temperature (°C)	Individual swimming	Rapid and equal intervals between different loads (s)	Pre-load phase	Load (% of body weight)	Body weight (g)	Strain
Present	yes	33.5-34.5	yes	15-18	yes	2, 3.5, 5 and 10	350-400	Wistar
Goutianos *et al.* 2016 (13)	yes	33.5-34.5	yes	15-18	yes	1.5-2.5	353-407	Wistar
Narkhede *et al.* 2016 (33)	no	23-27	no	no	no	10	100-120	Wistar
Lamou *et al.* 2016 (24)	no	24-26	no	no	no	10	130-132	Albino
Wu *et al.* 2014 (51)	no	30	no	no	no	3	NR	Sprague-Dawley
Lima *et al.* 2013 (25)	yes	30-32	yes	no	no	13	180-250	Wistar
Veskoukis *et al.* 2012 (47)	yes	33-36	yes	no	no	4	280-290	Wistar
Swamy *et al.* 2011 (44)	no	NR	no	no	no	no	120-130	Albino
Yildiz *et al.* 2009 (52)	yes	28	no	no	no	no	190-250	Albino
Veskoukis *et al.* 2008 (48)	yes	33-36	yes	no	no	4	210-230	Wistar
Morozov *et al.* 2003 (30)	no	30-32	no	no	no	8-9	200-250	Albino
Derevenco *et al.* 1986 (10)	no	36	no	no	no	5	NR	Wistar

Table 2. Comparison of the variability in time to exhaustion between the present novel performance test and protocols of other studies that measured swimming performance in rats. SD: standard deviation; NR: not reported; s: seconds.

Performance protocol	Swimming time to exhaustion (s)	Variability (SD)	Variability (%)
Present	865	59	7
Goutianos *et al.* 2016 (13)	1740	270	16
Narkhede *et al.* 2016 (33)	660	300	45
Lamou *et al.* 2016 (24)	89	68	76
Wu *et al.* 2014 (51)	2800	632	23
Lima *et al.* 2013 (25)	100	37	37
Veskoukis *et al.* 2012 (47)	2766	360	13
Swamy *et al.* 2011 (44)	720	140	19
Yildiz *et al.* 2009 (52)	720	60	8
Veskoukis *et al.* 2008 (48)	3360	480	14
Morozov *et al.* 2003 (30)	2440	NR	-
Derevenco *et al.* 1986 (10)	132	54	41

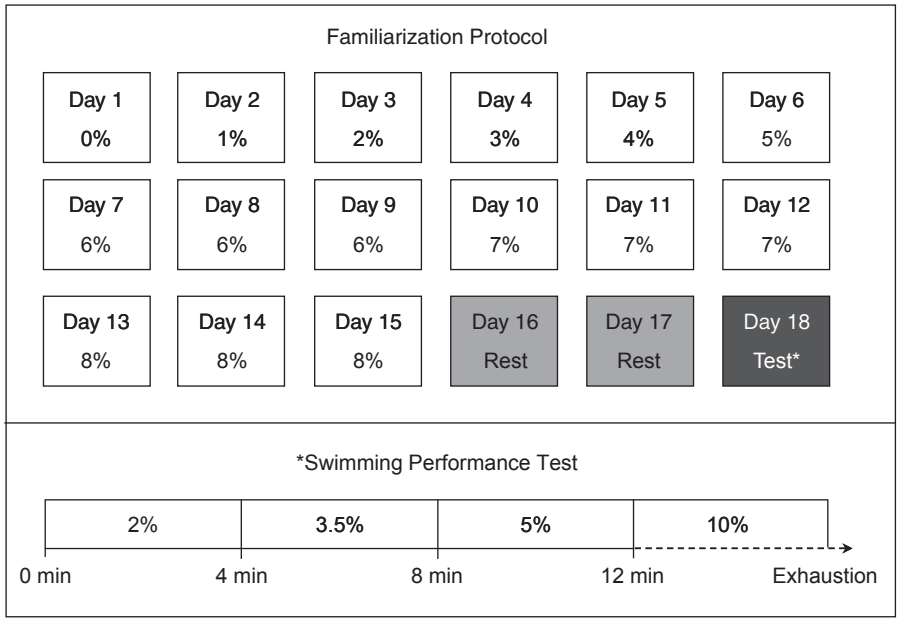

Fig. 1. The familiarization protocol and the performance test.

a performance assessment tool.

Materials and Methods

Rats

Nine, 4 months old male Wistar rats (Rattus norvegicus) weighing 350-400 g were used in the present study. The rats were group-housed in cages of three under a 12 h light:12 h dark cycle, controlled temperature (21-23°C) and controlled humidity (50-70%). Commercial rat chow and tap water were provided *ad libitum*. All rats were acclimatized in the animal facility room by remaining there for five days before the experiment was started and familiarized to swimming, using the familiarization protocol presented below. The animal facility room, where the rats were housed at their cages was right next to the room of the experiment. During familiarization protocol, one rat at a time was transported to the experimental room using a transport cage, familiarized and then returned back to its cage. That was the case during the exercise performance protocol, as well. All procedures were in accordance with the European Union guidelines for the care and use of laboratory animals (Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes), as well as the "Principles of laboratory animal care" (National Institutes of Health, NIH publication No. 86-23, revised 1985). The project was reviewed and approved by the institutional review board and the appropriate state authority.

The Water Tanks

Rats individually swam in deep water tanks of the same dimensions (*i.e.* diameter: 1.2 m, height: 1.1 m). The tanks were constructed of non-transparent plastic material to avoid the distraction of the rats' attention by the ambient conditions. The depth of the water was settled at 0.7 m to prevent rats from jumping outside and from touching the bottom of the tanks with their tails. A shallower tank would not be appropriate, as it would affect time of exhaustion and reduce the reproducibility of the experiment. The water tanks were marked with permanent pen to ensure that the water level was consistent for every rat across experiment. We used two identical water tanks in order each rat to swim into clean water.

The Water Temperature

The temperature of the water in both familiarization protocol and performance test was strictly set between 33.5°C and 34.5°C. Thus, the water tank was placed on a plastic insulating surface for restraining the temperature changes. After each rat completed the test, the water temperature fell approximately 0.5°C-1°C, therefore, it was settled before the next animal started swimming. The maintenance of the water temperature slightly lower than rat's body temperature (*i.e.* between 33°C and 36°C) allows the animal to

maintain its core temperature throughout the test (4).

The Familiarization Protocol

The familiarization protocol and the performance test are depicted in Fig. 1. The rats were familiarized according to an appropriately modified protocol that was previously presented by our group (47, 48). The familiarization protocol lasted 15 consecutive days and the daily swimming time was 5 min. In day 1, the rats swam free of load in order to avoid exacerbation of the anticipated stress caused by their first contact with water. In day 2, load equal to 1% of the rats' body weight was adjusted at the base of their tails and it was gradually incremented by 1% each day until day 6. In days 7 to 9, a constant 6% load was adjusted. It was increased to 7% in days 10 to 12 and finally to 8% the last three days of the familiarization protocol. Then, the rats rested at their cages for two days before the performance test took place. It is important that every rat must swim individually in the tank, otherwise, the proposed test produces false performance data.

The Swimming Performance Test

The swimming performance test presented herein is a graded exercise protocol with incremental loads adjusted at the base of the rats' tails in order to achieve continuous exercise. A load equal to 2% of the rats' body weight was initially adjusted and, following, it was gradually increased to 3.5% and then to 5%. The swimming time after the adjustment of each load was 4 min. After the first 12 min of exercise elapsed, the load was increased to 10% and the rats left to swim until exhaustion. The necessary time intervals required for the removal of the rats from the water tank and the changes of the loads were lasted from 15 s to 18 s. During the test, trained research personnel of our group observed animals performing the exercise performance protocol. The personnel were experienced and familiar to the normal behavior of the swimming rats and, thus, were able to immediately detect motor control impairments due to exhaustion. An animal was considered to have reached exhaustion when it exhibited loss of coordinated movements and failure to return to the surface within 10 s for three consecutive times (47, 48).

Results

The mean swimming time until exhaustion for the nine rats was 865 ± 59 s (mean ± SD). All rats managed to complete the first 12 min-part of the test (phase 1) with gradually increased loads attached at the base of their tails equal to 2%, 3.5% and 5% (for 4 min each). All nine rats reached exhaustion at the 10% final load (phase 2), after 145 ± 59 s (mean ± SD) (Fig. 2).

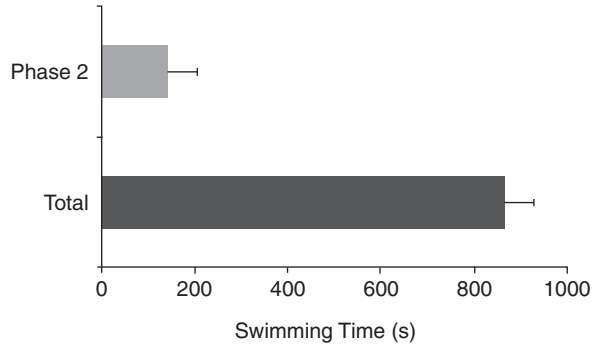

Fig. 2. The swimming time of the rats (mean ± SD) following phase 2 and the completion of the performance test.

Discussion

In the present study, we present a novel swimming performance test in rats that can reliably assess exercise performance. The prominent advantage of this protocol is the low variability in time to exhaustion among the rats. Noteworthy, small differences in variability of time to exhaustion characterize a reliable exercise performance test (17). Indeed, all nine rats successfully accomplished the first 12-min exercise part of the test with loads equal to 2%, 3.5% and 5% (phase 1) and then reached exhaustion at the final 10% stage of the protocol (phase 2). The low variability is of utmost importance because it enables to make direct numerical and statistical comparisons among differentially treated animals, as it has been demonstrated in a recent study of our research group (21).

The present swimming performance test has the following advantages on five critical issues:

i) Familiarization. It is probably the major criterion for a successful performance protocol. The familiarization protocol diminishes the stressful effects of water on rats' behavior. The animals become accustomed to both water and swimming with adjusted load at the base of their tails. Therefore, they swim normally without feeling any danger or stress allowing researchers to reliably measure time to exhaustion. In the present investigation, the familiarization protocol could probably be considered as a training protocol, which might be the main reason why the present novel performance test is characterized by low variability. However, according to the relevant literature, the duration of exercise training protocols must be equal or exceed 6 weeks in order to induce adaptations at the physiological or molecular level in rats. Specifically, it has been demonstrated that 6 weeks of swimming for 60 min per day induce ad-

aptations to oxidative stress and insulin sensitivity (53) and mitochondrial composition (25), as well as 14 weeks of treadmill running for 90 min per day enhance tissue antioxidant capacity (40). Based on the above, the familiarization protocol adopted in the present investigation could not be considered as training protocol since it lasted for only 15 days while the duration of each session lasted 5 min. Therefore, we believe that the well designed familiarization protocol of the present investigation is the main reason for the low variability reported in time to exhaustion.

ii) Water temperature. The strict maintenance of water temperature between 33.5°C and 34.5°C averts the appearance of cardiovascular or other health problems that potentially affect swimming performance. Maintaining a water temperature slightly lower than rat's body temperature allows the animal to maintain its core temperature throughout the test eliminating a crucial factor that can compromise animal's health and protocol reliability.

iii) Individual swimming. According to personal observations, the presence of more than one rat in the tank causes distraction and abnormalities in swimming behavior, finally, producing a falsified measurement of performance.

iv) Rapid and equal intervals between different loads. The test managed to convert an otherwise interval-scaled exercise into an almost continuous-scaled exercise protocol due to the optimization of the loading procedure. The time that the rats remained outside the water before changing each load was almost identical and negligible. Thus, the low variation in time to exhaustion is ensured. This short time interval, which was almost identical for each animal did not permit the rats to rest and regain some of their lost energy assuring the accurate and reliable performance measurement.

v) The pre-load 12-min phase. The low variability in time to exhaustion is basically the result of the fact that the rats followed the same pre-load swimming phase. They all managed to successfully swim the first 12 min of the test with the relatively low loads adjusted to their tails (*i.e.* 2%, 3.5% and 5%) and reached exhaustion at the final phase of the protocol, when the high load (*i.e.* 10%) was adjusted to their tails. According to our findings, the variability in time to exhaustion (*i.e.* the SD, which is the output measure showing how our performance test probably improves the relevant published protocols) equals to 6.8%, which is a much lower variability compared to previous studies of our group. Specifically, variability was equal to 14% in Veskoukis *et al*. (48) and 16% in Goutianos *et al*. (13). This fact indicates a substantial improvement in the measurement of swimming performance. Furthermore, the SD of the time to exhaustion presented here is lower compared to other studies, where it approximately equals to 50% in Narkhede *et al*. (33), 41% in Derevenco *et al*. (10), 40% in Lamou *et al*. (24), 19% in Swamy *et al*. (44) and 9 % in Chang *et al*. (7).

In Table 1, a more thorough comparison is presented between the present protocol and the tests used by other investigators regarding the aforementioned five critical issues. Table 2 provides detailed comparison of time to exhaustion variability between the present novel performance test and the protocols of relevant studies. According to the data presented in the Tables, we believe that the increased variability in time to exhaustion observed in several other studies is mainly attributed to the differences in the above mentioned five methodological parameters. Specifically, most of the tests did not use a swimming familiarization protocol, they applied different water temperature and they did not force the animals to individual swimming. Moreover, in these studies neither applied rapid and equal intervals between different loads nor adopted a pre-load phase.

One of the major limitations of tests measuring time to exhaustion is the comparison of groups with differences in body weight and muscle/fat composition due to diverse buoyancy levels. It is known that the load adjusted at the rats' tails is negatively correlated with the time to exhaustion (28). In this sense, there is experimental evidence indicating that the ability of an animal to swim is not maintained as its size increases, thus hampering its buoyancy (28), while this finding has also been demonstrated in humans (20). In support of these data, swimming endurance has been negatively correlated with body mass and, hence, with the load at the animal's tail (8, 12, 15, 22, 26, 27). These results imply that the reliability of a swimming protocol is compromised if the variability in time to exhaustion is increased. This, however, is not the case in the present performance protocol where the variability in time to exhaustion is relatively low. Nevertheless, it should be mentioned that the performance test we present in this study is directly applicable only to rats of the particular exercise capacity, body weight and body composition. Therefore, it could not be readily applied without the necessary adjustments, to aged, obese or diseased rats, conditions that affect both swimming capacity and buoyancy. In these cases, pilot experiments are needed in order to specify the stages, the durations and the loads of the modified performance tests. For example, if a group of rats exhibits a 50% decrease in exercise capacity compared to the rats used in the present study, then the 2%, 3.5%, 5% and 10% loads could be decreased by 50% and substituted by 1%, 1.75%, 2.5% and 5%, respectively.

In conclusion, we have presented an easily carried out swimming performance test characterized by low variability in the time to exhaustion among rats. This novel exercise test has been repeatedly used by our research group for assessing swimming performance in rats reliably and accurately, thus enabling to obtain precise measurements without the need of using a large number of animals. The performance test can be appropriately modified by other researchers so that it can be applied to a wider spectrum of experimental treatments.

Conflict of Interest

The authors declare no conflict of interest.

References

1. Aoi, W., Naito, Y., Takanami, Y., Kawai, Y., Sakuma, K., Ichikawa, H., Yoshida, N. and Yoshikawa, T. Oxidative stress and delayed-onset muscle damage after exercise. *Free Radic. Biol. Med*. 37: 480-487, 2014.
2. Araujo, L.C.C., Souza, I.L.L., Vasconcelos, L.H.C., Brito, A.F., Queiroga, F.R., Silva, A.S., Silva, P.M., Cavalcante, F.A. and Silva, B.A. Chronic aerobic swimming exercise promotes functional and morphological changes in rat ileum. *Biosci. Rep*. 35: e00259, 2015.
3. Armstrong, R.B., Ogilvie, R.W. and Schwane, J.A. Eccentric exercise-induced injury to rat skeletal muscle. *J. Appl. Physiol. Respir. Environ. Exerc. Physiol*. 54: 80-93, 1983.
4. Baker, M.A. and Horvath, S.M. Influence of water temperature on oxygen uptake by swimming rats. *J. Appl. Physiol*. 19: 1215-1218, 1964.
5. Booth, F.W. and Laye, M.J. Lack of adequate appreciation of physical exercise's complexities can pre-empt appropriate design and interpretation in scientific discovery. *J. Physiol*. 87: 5527-5539, 2009.
6. Carter, C.S., Sonntag, W.E., Onder, G. and Pahor, M. Physical performance and longevity in aged rats. *J. Gerontol. A Biol. Sci. Med. Sci*. 57: B193-B197, 2002.
7. Chang, Q., Miao, X., Ju, X., Zhu, L., Huang, C., Huang, T., Zuo, X. and Gao, C. Effects of pulse current on endurance exercise and its anti-fatigue properties in the hepatic tissue of trained rats. *PLoS One* 8: e75093, 2013.
8. Dawson, C.A. and Horvath, S.M. Swimming in small laboratory animals. *Med. Sci. Sports* 2: 51-78, 1970.
9. Dawson, C.A., Nadel, E.R. and Horvath, S.M. Cardiac output in the cold-stressed swimming rat. *Am. J. Physiol*. 214: 320-325, 1968.
10. Derevenco, P., Stoica, N., Sovrea, I. and Imreh, S. Central and peripheral effects of 6-hydroxydopamine on exercise performance in rats. *Psychoneuroendocrinology* 11: 141-153, 1986.
11. Foglio, K., Carone, M., Pagani, M., Bianchi, L., Jones, P.W. and Ambrosino, N. Physiological and symptom determinants of exercise performance in patients with chronic airway obstruction. *Respir. Med*. 94: 256-263, 2000.
12. Friedman, W.A., Garland, T.Jr. and Dohm, M.R. Individual variation in locomotor behavior and maximal oxygen consumption in mice. *Physiol. Behav*. 52: 97-104, 1992.
13. Goutianos, G., Veskoukis, A.S., Tzioura, A., Paschalis, V., Margaritelis, N.V., Dipla, K., Zafeiridis, A., Vrabas, I.S., Nikolaidis, M.G. and Kyparos, A. Plasma from exercised rats administered to sedentary rats induces systemic and tissue inflammation. *Physiol. Rep*. 4: e13087, 2016.
14. Guralnik, J.M., Simonsick, E.M., Ferrucci, L., Glynn, R.J., Berkman, L.F., Blazer, D.G., Scherr, P.A. and Wallace, R.B. A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission. *J. Gerontol*. 49: M85-M94, 1994.
15. Hardin, D.H. Reliability of selected swimming endurance tests for laboratory rats. *Res Q* 39: 405-407, 1968.
16. Hohl, R., de Oliveira, R.B., Ferraresso, R.L.P., Brenzikofer, R. and Macedo, D.V. Effect of body weight variation on swimming exercise workload in rats with constant and size-adjusted loads. *Scand. J. Lab. Anim. Sci*. 38: 145-154, 2011.
17. Hopkins, W.G. Measures of reliability in sports medicine and science. *Sports Med*. 30: 1-15, 2000.
18. Jonsdottir, I.H., Schjerling, P., Ostrowski, K., Asp, S., Richter, E.A. and Pedersen, B.K. Muscle contractions induce interleukin-6 mRNA production in rat skeletal muscles. *J. Physiol*. 528 (Pt 1): 157-163, 2000.
19. Jung, K., Kim, I.H. and Han, D. Effect of medicinal plant extracts on forced swimming capacity in mice. *J. Ethnopharmacol*. 93: 75-81, 2004.
20. Key, J.R. Relationship between load and swimming endurance in humans. *Res. Q* 33: 559-565, 1962.
21. Kourtzidis, I.A., Stoupas, A.T., Gioris, I.S., Veskoukis, A.S., Margaritelis, N.V., Tsantarliotou, M., Taitzoglou, I., Vrabas, I.S., Paschalis, V., Kyparos, A. and Nikolaidis, M.G. The NAD^+ precursor nicotinamide riboside decreases exercise performance in rats. *J. Int. Soc. Sports Nutr*. 13: 32, 2016.
22. Kramer, K., Dijkstra, H. and Bast, A. Control of physical exercise of rats in a swimming basin. *Physiol. Behav*. 53: 271-276, 1993.
23. Kregel, K.C., Allen, D.L., Booth, F.W., Fleshner, M.R., Henrikson, E.J., Musch, T.I., O'Leary, D.S., Parks, C.M., Poole, D.C., Ra'anan, A.W., Sheriff, D.D., Sturek, M.S. and Toth, L.A. *Resource Book for the Design of Animal Exercise Protocols*. American Physiological Society, 2006.
24. Lamou, B., Taiwe, G.S., Hamadou, A., Abene, Houlray, J., Atour, M.M. and Tan, P.V. Antioxidant and antifatigue properties of the aqueous extract of moringa oleifera in rats subjected to forced swimming endurance test. *Oxid. Med. Cell. Longev*. 2016: 3517824, 2016.
25. Lima, F.D., Stamm, D.N., Della-Pace, I.D., Dobrachinski, F., Carvalho, N.R., Royes, L.F.F, Soares, F.A., Rocha, J.B., Gonzalez-Gallego, J. and Bresciani, G. Swimming training induces liver mitochondrial adaptations to oxidative stress in rats submitted to repeated exhaustive swimming bouts. *PLoS One* 8: e55668, 2013.
26. Lindstedt, S.L. and Schaeffer, P.J. Use of allometry in predicting anatomical and physiological parameters of mammals. *Lab. Anim*. 36: 1-19, 2002.
27. Matsumoto, K.K., Ishihara, K., Tanaka, K., Inoue, K. and Fushiki, K. An adjustable-current swimming pool for the evaluation of endurance capacity of mice. *J. Appl. Physiol*. 81: 1843-1849, 1996.
28. McArdle, W.D. and Montoye, H.J. Reliability of exhaustive swimming in the laboratory rat. *J. Appl. Physiol*. 21: 1431-1434, 1966.
29. McNeil, P.L. and Khakee, R. Disruptions of muscle fiber plasma membrane. Role in exercise-induced damage. *Am. J. Pathol*. 140: 1097-1109, 1992.
30. Morozov, V.I., Pryatkin, S.A., Kalinski, M.I. and Rogozkin, V.A. Effect of exercise to exhaustion on myeloperoxidase and lysozyme release from blood neutrophils. *Eur. J. Appl. Physiol*. 89: 257-262, 2003.
31. Murase, T., Haramizu, S., Shimotoyodome, A., Nagasawa, A. and Tokimitsu, I. Green tea extract improves endurance capacity and increases muscle lipid oxidation in mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol*. 288: R708-R715, 2005.
32. Nakatani, A., Han, D.H., Hansen, P.A., Nolte, L.A., Host, H.H., Hickner, R.C. and Holloszy, J.O. Effect of endurance exercise training on muscle glycogen supercompensation in rats. *J. Appl.*

Physiol. (1985) 82: 711-715, 1997.
33. Narkhede, A.N., Jagtap, S.D., Nirmal, P.S., Giramkar, S.A., Nagarkar, B.E., Kulkarni, O.P. and Harsulkar, A.M. Anti-fatigue effect of Amarkand on endurance exercise capacity in rats. *BMC Complement. Altern. Med*. 16: 23, 2016.
34. Nikolaidis, M.G., Kyparos, A., Dipla, K., Zafeiridis, A., Sambanis, M., Grivas, G.V., Paschalis, V., Theodorou, A.A., Papadopoulos, S., Spanou, C. and Vrabas, I.S. Exercise as a model to study redox homeostasis in blood: the effect of protocol and sampling point. *Biomarkers* 17: 28-35, 2012.
35. Radley-Crabb, H., Terrill, J., Shavlakadze, T., Tonkin, J., Arthur, P. and Grounds, M. A single 30 min treadmill exercise session is suitable for 'proof-of concept studies' in adult mdx mice: a comparison of the early consequences of two different treadmill protocols. *Neuromuscul. Disord*. 22: 170-182, 2012.
36. Rijkelijkhuizen, J.M., de Ruiter, C.J., Huijing, P.A. and de Haan, A. Low-frequency fatigue is fibre type related and most pronounced after eccentric activity in rat medial gastrocnemius muscle. *Pflugers. Arch*. 447: 239-246, 2003.
37. Sakamoto, K., Nosaka, K., Shimegi, S., Ohmori, H. and Katsuta, S. Creatine kinase release from regenerated muscles after eccentric contractions in rats. *Eur. J. Appl. Physiol. Occup. Physiol*. 73: 516-520, 1996.
38. Schill, K.E., Altenberger, A.R., Lowe, J., Periasamy, M., Villamena, F.A., Rafael-Fortney, J.A. and Devor, S.T. Muscle damage, metabolism, and oxidative stress in mdx mice: Impact of aerobic running. *Muscle Nerve* 54: 110-117, 2016.
39. Schneider, B.C. and Lichtenberg, P.A. Physical performance is associated with executive functioning in older African American women. *J. Aging Res*. 2011: 578609, 2011.
40. Sexton, W.L. and Laughlin, M.H. Influence of endurance exercise training on distribution of vascular adaptations in rat skeletal muscle. *Am. J. Physiol*. 266: H483-H490, 1994.
41. Simpkiss, J.L. and Devine, D.P. Responses of the HPA axis after chronic variable stress: effects of novel and familiar stressors. *Neuroendocrinol. Lett*. 24: 97-103, 2003.
42. Snyder, A., Zierath, J.R., Hawley, J.A., Sleeper, M.D. and Craig, B.W. The effects of exercise mode, swimming *vs.* running, upon bone growth in the rapidly growing female rat. *Mech. Ageing Dev.* 66: 59-69, 1992.
43. Stookey, A.D., Katzel, L.I., Steinbrenner, G., Shaughnessy, M. and Ivey, F.M. The short physical performance battery as a predictor of functional capacity after stroke. *J. Stroke Cerebrovasc. Dis*. 23: 13013-13015, 2014.
44. Swamy, M.S., Sivanna, N., Tamatam, A. and Khanum, F. Effect of polyphenols in enhancing the swimming capacity of rats. *Funct. Foods Health Dis*. 1: 482-491, 2011.
45. Takekura, H., Fujinami, N., Nishizawa, T., Ogasawara, H. and Kasuga, N. Eccentric exercise-induced morphological changes in the membrane systems involved in excitation-contraction coupling in rat skeletal muscle. *J. Physiol*. 533: 571-583, 2001.
46. Tsatalas, T., Giakas, G., Spyropoulos, G., Paschalis, V., Nikolaidis, M.G., Tsaopoulos, D.E., Theodorou, A.A., Jamurtas, A.Z. and Koutedakis, Y. The effects of muscle damage on walking biomechanics are speed-dependent. *Eur. J. Appl. Physiol*. 110: 977-988, 2010.
47. Veskoukis, A.S., Kyparos, A., Nikolaidis, M.G., Stagos, D., Aligiannis, N., Halabalaki, M., Chronis, K., Goutzourelas, N., Skaltsounis, L. and Kouretas, D. The antioxidant effects of a polyphenol-rich grape seed extract *in vitro* do not correspond *in vivo* using exercise as an oxidant stimulus. *Oxid. Med. Cell. Long.* 2012: 185867, 2012.
48. Veskoukis, A.S., Nikolaidis, M.G., Kyparos, A., Kokkinos, D., Nepka, C., Barbanis, S. and Kouretas, D. Effects of xanthine oxidase inhibition on oxidative stress and swimming performance in rats. *Appl. Physiol. Nutr. Metabol*. 33: 1140-1154, 2008.
49. Warner, S.E., Shea, J.E., Miller, S.C. and Shaw, J.M. Adaptations in cortical and trabecular bone in response to mechanical loading with and without weight bearing. *Calcif. Tissue Int*. 79: 395-403, 2006.
50. Wennie Huang, W.N., Perera, S., VanSwearingen, J. and Studenski, S. Performance measures predict onset of activity of daily living difficulty in community-dwelling older adults. *J. Am. Geriatr. Soc*. 58: 844-852, 2010.
51. Wu, R.M., Sun, Y.Y., Zhou, T.T., Zhu, Z.Y., Zhuang, J.J., Tang, X., Chen, J., Hu, L.H. and Shen, X. Arctigenin enhances swimming endurance of sedentary rats partially by regulation of antioxidant pathways. *Acta Pharmacol. Sin*. 35: 1274-1284, 2014.
52. Yildiz, A., Ozdemir, E., Gulturk, S. and Erdal, S. The effects of creatine long-term supplementation on muscle morphology and swimming performance in rats. *J. Sports Sci. Med*. 8: 516-522, 2009.
53. Zacarias, A.C., Barbosa, M.A., Guerra-Sá, R., De Castro, U.G.M., Bezerra, F.S., de Lima, W.G., Cardoso, L.M., Santos, R.A.S., Campagnole-Santos, M.J. and Alzamora, A.C. Swimming training induces liver adaptations to oxidative stress and insulin sensitivity in rats submitted to high-fat diet. *Redox Rep*. 22: 515-523, 2017.