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BIOMEDICAL SCIENCES

Using training impulse and monotony methods to monitor aerobic training load in rats

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Abstract: This study is the first to apply training impulse (TRIMP) and Training Monotony (TM) methodologies, within the realm of sport science, in animal model studies. Rats were divided into Sedentary (SED, n=10) and Training (TR, n=13). TR performed a four-week moderate-intensity interval training with load progression. Lactate kinetics, lactate training impulse ($TRIMP_{Lac}$), maximal speed training impulse ($TRIMP_{Smax}$) and TM were utilized to develop and monitor training protocol. TR showed an 11.9% increase in time to exhaustion at the second maximum incremental test and a 17.5% increase at the third test. External work was increased by 17.8% at the second test and 30.3% at the third. There was a 10.6% increase in external work at the third test compared to the second for TR. No difference in $TRIMP_{Lac}$ between the 1st week (94±9 A.U) and 3rdweek (83±10 A.U) were seen. $TRIMP_{Smax}$ was 2400 A.U. in the 1st week, 2760 A.U. in the 2nd and 3rd weeks, and 3120 A.U. in the 4th week. The TM remained at 1.24 A.U throughout the protocol and there was no dropouts. $TRIMP_{Lac}$ and $TRIMP_{Smax}$ contributed to the development and monitoring loads, demonstrating their potential to improve the accuracy of training protocols in animal model research.

Key words: Training protocol, Training monitoring, Interval training, Animal model research.

INTRODUCTION

Animal model research is indispensable for examining precise morpho-functional adaptations and physiological mechanisms (Robinson et al. 2019). In the context of research interventions involving physical exercise, it is common to use rodents, particularly mice and rats, due to their adeptness to ergometers, such as the treadmill. These ergometers enable rigorous control and delineation of essential parameters in sport science, including training volume, training intensity, and training load. Precise manipulation of these parameters is pivotal in developing a training protocol grounded in fundamental exercise principles, most notably overload and individualization principles (Teixeira-Coelho et al. 2017, Kasper 2019). More specifically, the utilization of treadmill running protocols appears to be a more suitable choice than swimming, once treadmill running in rats enables the calculation and monitoring of external training load. However, survival strategies employed to avoid potential drowning, such as floating and attempts to escape from the swimming tank, can hinder the execution of planned training load and training load progression in swimming training (Poole et al. 2020).

The principle of training overload postulates a continuous increase in training load as a requirement to obtain chronic improvements in physical performance (Kasper 2019, França et al. 2022). This principle plays a critical role in

sustaining the promotion of positive adaptations as the organism adapts to the demands imposed by the training load, a process expounded upon by the General Adaptation Syndrome (França et al. 2022, Cunanan et al. 2018). However, an abrupt increase in training load, coupled with insufficient recovery intervals, can result in overtraining syndrome (OT), as showed by Hohl et al. (2009), potentially culminating in suboptimal performance, injuries, and dropouts (Chung et al. 2021, Cardoos 2015). Consequently, proper application of the overload principle necessitates individualized approaches.

The principle of individualization describes the relevance of considering the intra-group variability within the training protocol (Kasper 2019). Neglecting intra-group differences may lead to divergent training stimuli experienced by animals within the same experimental group, ultimately resulting in varying training loads upon completion of the training protocol. Therefore, it is essential to use internal and relative external intensity parameters, such as blood lactate concentration ([La-]) or maximal speed (Smax) percentage.

Monitoring training loads constitutes a mandatory prerequisite when utilizing exercise as a research intervention. This involves monitoring internal responses to training and tracking the progression of training load (Foster et al. 2001, 2017, Mujika 2017). Blood lactate concentrations [La-] commonly serve as biomarkers for evaluating training intensity, given their robust correlation with exercise intensity (Manchado et al. 2005, de Araujo et al. 2007, 2010, 2015, Beck et al. 2012, Teixeira-Coelho et al. 2017). The Training Impulse (TRIMP) offers a practical approach to monitoring training load (Foster et al. 2001). Additionally, Foster et al. (1998) introduced the concept of Training Monotony (TM), a training toll that aims to assess training load variability to mitigate the risk of overtraining. Although these tools are commonplace in human studies, their original intensity parameters are centered on heart rate and the rating of perceived exertion. So far, there has been no prior attempt to adjust these tools for application in animal model research.

As described above, the conceptualization, execution, monitoring, and analysis of a training protocol constitute a multifactor process replete with variables capable of influencing study outcomes, data interpretation, and study reproducibility. Therefore, our objectives were: A) Propose the use of TRIMP for monitoring training load in animal model studies; B) Implementing the concept of training monotony in an animal model study; C) Provide recommendations for the control and monitoring training protocol variables within animal model studies; D) Propose a moderate-intensity interval training protocol suitable for formulating exercise research interventions in animal model studies. Given the widespread use of exercise-based interventions in animal model studies, it is crucial that the scientific community uses tools for accurate control of training intensity. This effort not only increases the reproducibility of the results, but also reduces the chance of misattribution of the adaptations induced by physical training.

MATERIALS AND METHODS

Animals

Twenty-three male albino Sprague-Dawley rats, aged 70 days and weighing between 250 and 350 g at the beginning of the experiment, were housed at a controlled temperature of 22 ± 2ºC under a standard 12:12-hour light-dark cycle (lights off from 06:00 p.m to 06:00 a.m). They were provided ad libitum access to laboratory rat chow (NUVILAB CR, Quimtia, Brazil) and water.

Research protocol

All procedures adhered to the guidelines set forth by the Ethics Committee on the Use of Animals in Research (protocol nº 2016.1.462.90.7) and were in accordance with the "Principles of laboratory animal care" as outlined in national regulations (CONCEA publication, no. 11.794.2008). The study was conducted in compliance with the ARRIVE guidelines.

The research protocol was performed for five weeks. Initially, all animals performed a one-week familiarization period on a treadmill, each in individual stalls. Subsequently, they were subject to a maximal incremental running test on the last day of the familiarization phase and were then assigned to one of two groups: the Sedentary group (SED, n = 10) and the Training group (TR, n = 13). The TR group commenced the four-week training protocol on the following Monday after the incremental test. Detailed descriptions of the familiarization period, the incremental running test, and the training protocol are provided in the following sections and summarized in Figure 1.

Treadmill familiarization protocol

During the first week of the experimental protocol, all animals underwent an exercise familiarization period on a treadmill. This familiarization period is crucial for minimizing "non-runner" behavior, as established by Kregel et al. (2007). Load progression during this period involved increases in speed and total time spent on the treadmill, as detailed in Table I. Animals that did not maintain a running or standing pattern at the end of the treadmill were removed to prevent injuries to their paws and tails and were allocated in SED group (3 animals during this familiarization period). The treadmill did not employ shock grids at the end of the tracks.

Maximum incremental running test

Animals exhibiting a running pattern were considered capable of undergoing the maximum incremental running test. The initial test speed was set at 11.6 meters per minute (m/min), with increments of 1.6 m/min every 2 minutes until reaching a treadmill speed of 20 m/min. Beyond 20 m/min, the speed increased by 3.2 m/min every two minutes. Exhaustion was determined

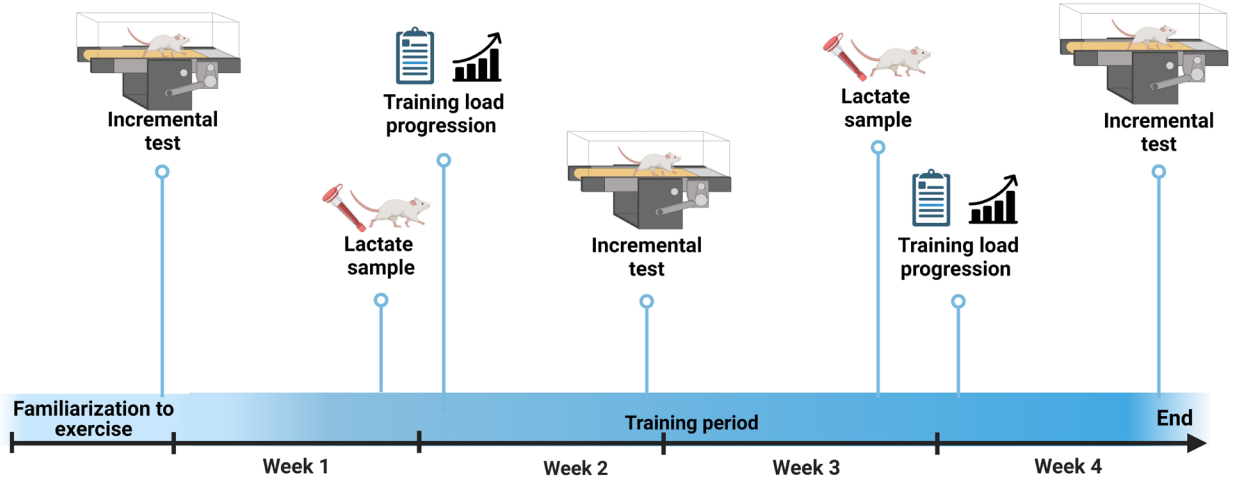

Figure 1. Timeline of the experimental protocol. The animals were adapted for one week and then trained for four weeks. Maximum incremental tests were performed at the end of the adaptation period, in the second week, and in the fourth week. Blood lactate samples were taken at the last training session of the first and third weeks. A training load progression was applied at week 2 and week 4. Created with BioRender.com.

when an animal touched the end of the track five times within one minute. At this point, the animal was removed from the treadmill, and the speed at which exhaustion occurred was recorded as the peak speed. The Smax was calculated using the following equation (adapted from Kuipers et al. 1985):

$$Smax = S1 + (S2 \times t/120)$$

where S1 = speed of the last complete stage of the test, S2 = increment in the stage where exhaustion occurred, and t = time at the stage where exhaustion occurred in seconds.

The animals that did not show a sufficient running pattern or that performed below average were allocated to the SED group. The Smax percentage (%Smax) was employed to prescribe training intensity. Since the treadmill used was a collective ergometer (with 10 tracks), it was necessary to cluster animals with similar fitness levels to conduct the training session at the respective %Smax speed. As a result, there were two distinct physical training subgroups, each running at speeds that matched their %Smax. Additionally, a maximum incremental test was conducted in week 2 to assess fitness level and verify that the prescribed speed still corresponded to %Smax.

Training protocol

The TR group engaged in three training sessions on alternate days (Monday, Wednesday, and Friday) between 11:00 a.m. and 2:00 p.m. As showed in Figure 2, each training session consisted of a warm-up period, the main exercise segment, and a cooldown period. The warm-up consisted of 5 minutes, followed by 18 minutes of the main exercise segment and a 5-minute cooldown. The main exercise segment comprised 3 sets, each of 2 minutes at intensity A (60-70% Smax), followed by 4 minutes at intensity B (25-35% Smax), characterizing the training sessions as moderate-intensity efforts (Manchado et al. 2005, Contarteze et al. 2008). Training load was quantified using the equation: Training load = Training Volume (total time in minutes) × Training Intensity (%Smax).

Training monitoring parameters

Blood lactate kinetics and lactate TRIMP

Blood samples (25 µL) were collected from the distal end of the animals' tails with previously calibrated glass capillaries containing 50 µL of sodium fluoride (NaF 1%). These samples were homogenized and stored at -4ºC. [La-] was determined using a Yellow Spring Instruments Electrochemical Lactimeter (YSI), model 2300 Stat. Blood samples were collected during the third training session of the first week and the third week of the training protocol. The TR group had 5 blood samples collected for each of these training sessions: Rest; 17th minute; 19th minute; 23rd minute; and 28th minute (Figure 2).

Blood Lactate Kinetics were employed as an internal parameter to assess training intensity during both the third training session of the first week and the third week of the training protocol. The lactate TRIMP ($TRIMP_{Lac}$) was calculated by the following equation: Lactate TRIMP = Training session main segment volume * mean of the

Table I. Progression of animals` adaptation to exercise training.

Weekdays	1	2	3	4	5
Speed (m/min)	5	5	10	10	Maximum incremental test
Duration (min)	30	60	30	60	

tree [La-] collected during the training session main segment.

Training impulse

Training impulse (TRIMP) was evaluated using two intensity parameters: Lactate TRIMP ($TRIMP_{Lac}$), which utilized lactate concentration to monitor the internal training load, and ($TRIMP_{Smax}$), which employed %Smax to monitor the external training load and was calculated by multiplying the training volume (training session per week, time spent at each exercise intensity) by %Smax.

Training monotony

It was calculated using the equation proposed by Foster (1998): TM = mean of weekly loads divided by the standard deviation of the training load values. TM was maintained below 2.0 U.A. throughout the entire training protocol.

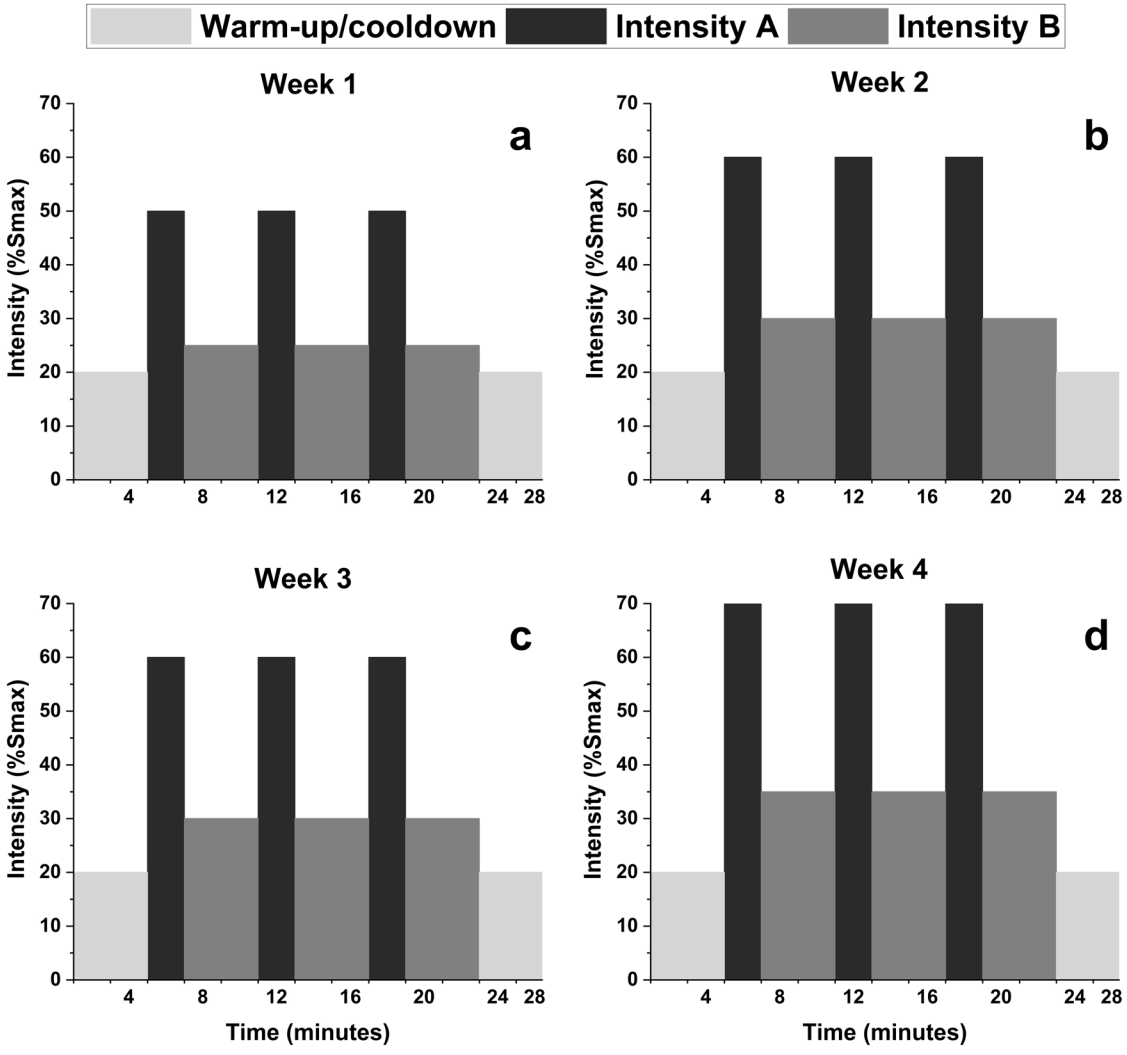

Figure 2. Training volume and intensity distribution in week 1 (panel A). Training volume and intensity distribution in week 2 (panel B). Training volume and intensity distribution in week 3 (panel C). Training volume and intensity distribution in week 4 (panel D). Intensity A: Highest intensity of effort; Intensity B: lowest intensity of effort; Smax: maximal speed.

Performance evaluations

Absolute performance

Time to exhaustion and peak speed reached in the maximum incremental tests were used to assess the effect of the training protocol on absolute performance.

External work

External work was calculated in Joules as bm × g × s × sinθ × t, where bm is the animal's body mass (kg), g is the acceleration of gravity (9.8 m/s^2), s is the treadmill speed (m/min), θ is the angle of treadmill inclination, and t is the time spent in each stage. Workload values were calculated for each stage of incremental exercise, including the incomplete stage, and were then summed to obtain the total external work (Soares et al. 2019).

Statistical analysis

The statistical analyses were conducted using GraphPad Prism 5 and IBM SPSS 20 for Windows. The normality was assessed by applying the Shapiro Wilk test on residuals. The box test of equality of covariance matrices was utilized to verify homoscedasticity and Mauchly's test was applied to test the assumption of sphericity to identify differences in the investigated parameters, a mixed-model two-way ANOVA followed by the Bonferroni post hoc test. Values of $p < 0.05$ were considered significant, and data were presented as mean ± standard error.

RESULTS

Lactate kinetics

No differences in [La-] were observed between the final training sessions of week 1 and week 3. Both training sessions exhibited consistent [La-] kinetics characterized by an initial increase during the warm-up phase, reaching peak [La-] levels during the main training segment, followed by a subsequent decrease during the cool-down phase. In week 1, [La-] levels were measured as follows: 2.33 ± 0.11 mmol L^{-1} at moment 1, 2.39 ± 0.26 mmol L^{-1} at moment 2, 3.12 ± 0.35 mmol L^{-1} at moment 3, 2.54 ± 0.32 mmol L^{-1} at moment 4, and 2.06 ± 0.21 mmol L^{-1} at moment 5. In week 3, the [La-] levels observed were: 1.95 ± 0.11 mmol L^{-1} at moment 1, 1.77 ± 0.15 mmol L^{-1} at moment 2, 2.06 ± 0.17 mmol L^{-1} at moment 3, 2.28 ± 0.44 mmol L^{-1} at moment 4, and 1.89 ± 0.33 mmol L^{-1} at moment 5 (Figure 3a).

Lactate TRIMP and relative external load TRIMP

There was no statistically significant difference in $TRIMP_{Lac}$ between the final training sessions of week 1 and week 3, with values of 94 ± 9 A.U. in the first week and 83 ± 10 A.U. in the third week (Figure 3b). The planned execution of $TRIMP_{Smax}$ was successful, with TR achieving 2400 A.U in the first week, 2760 A.U in the second and third week, and 3120 A.U in the fourth week (Figure 3c).

Training monotony

Only one animal from the SED group withdrew the research protocol. Animals from TR group completed the entire training protocol without any dropouts, maintaining a TM value of 1.24 A.U.

Time to exhaustion

The TR group presented a superior time to exhaustion during the incremental tests compared to the SED group [$F(2,42) = 21.96$; $p < 0.001$]. The TR group exhibited a 11.9% improvement in time to exhaustion in the second maximum incremental running test ($p = 0.004$) and a 17.5% improvement during the third maximum incremental running test ($p = 0.001$) compared to the initial one. Nevertheless, no significant difference in time to exhaustion was observed within the TR group between

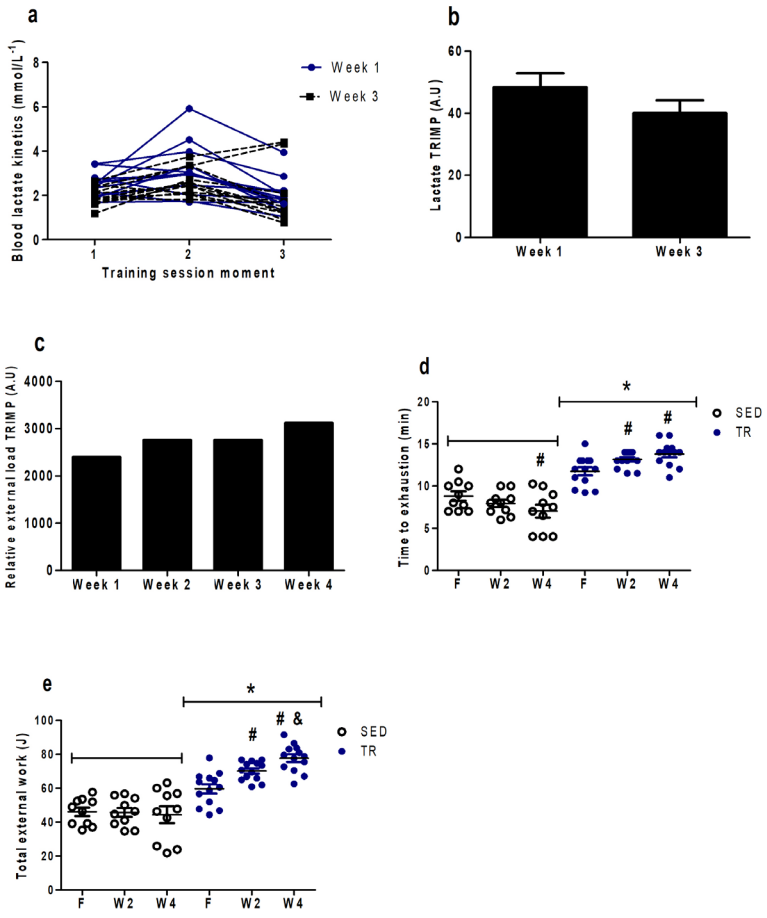

Figure 3. Blood lactate kinetics during a training session at week 1 and week 3 (mmol.L^{-1}: millimoles per liter) (3a). Lactate TRIMP at week 1 and week 3 (3b). Relative external load TRIMP in arbitrary units (A.U.) (3c). Total distance in meters (m) performed during the incremental test of the Sedentary (SD) and Training (TR) groups at familiarization week, week 2 (W2) and week 4 (W4) (3d). External work(J) of the SD and TR groups at familiarization week, week 2, and week 4 (W4) (3e). Mixed-model two-way ANOVA, followed by the Bonferroni post hoc test. Values of $p < 0.05$ were considered significant, and data were presented as mean ± standard error. *Different between groups; #Different intragroup from adaptation week; &Different between 2nd and 3rd test.

the second and third maximum incremental running tests. The SED group demonstrated a 20% decrease in time to exhaustion during the third maximum incremental running test in comparison to the initial test ($p = 0.006$) (Figure 3d).

External work

The TR group performed a superior external work compared to the SED group in all maximum incremental running tests [$F(2,42) = 17.520$; $p < 0.001$]. The TR group presented a 17.8% increase in external work during the second running test and a 30.3% increase during the third running test in relation to the first one ($p < 0.001$ and $p < 0.001$, respectively). Additionally, TR showed a 10.6% increase in external work ($p = 0.002$) during the third incremental test compared to the second test. No difference in the external work was observed in the SED group throughout the training protocol (Figure 3e).

DISCUSSION

The training protocol has effectively promoted positive performance-related adaptations. The TR group improved the external work throughout all maximum incremental tests. The training tools have efficiently assisted the development of the training protocol and provided the possibility to monitor the training performance. This study demonstrates the importance of reporting relative results, since an exclusive reliance on non-relative measures would result in an underestimation of the TR

group's performance and imply a decrement in the SED group.

To provide the necessary stress levels for positive adaptations, we implemented a training load progression in weeks 2 and 4, following the intensity guidelines proposed by Teixeira-Coelho and colleagues (2017). Their research has demonstrated that an intensity-based training load progression was more effective when compared to volume-based approaches. Additionally, we conducted a maximum incremental test at the end of week 2 to reevaluate and readjust the training intensity. This adjustment ensured concordance between the executed and the prescribe training intensity. By the end of the second week of training, the TR group had already manifested enhanced time to exhaustion, peak speed and work capacity. Consequently, not performing this reassessment could imperil the training load increment and the entire training protocol, potentially leading to an underestimation of the TR group's physical capacity and compromising study reproducibility, as the reported training load would not correspond to the actual training load performed.

Nevertheless, it is well established in literature that an excessive overload and insufficient recovery, particularly in conjunction with elevated TM values, can promote performance decrements and overtraining (Foster et al. 2001, Putlur et al. 2004, Hohl et al. 2009). For this reason, mitigating the risk of overtraining in animal model studies should be a critical concern, given that overtraining can compromise the results and lead to dropouts. That there were no dropouts throughout the entire training protocol, with a TM value of 1.24 A.U, which is within the 2.0 A.U range recommended by Foster (1998) for minimizing the risk of overtraining in humans. These results show the potential of incorporating TM in the development of a training protocol to effectively mitigate dropouts in animal models.

In this study, we have used the lactate kinetics as one of the methods for monitoring training intensity. The TR group consistently maintained the intended training intensity, as demonstrated by lactate concentration close to 3.9 $mmol \cdot L^{-1}$, a lactate threshold value proposed by Manchado and colleagues (2005). These findings indicate that neither the training load increment nor the training intensity readjustment induced undesirable spikes in lactate levels during training sessions. Nonetheless, it is pertinent to acknowledge that lactate levels are highly responsive to adrenergic hormones and neuromodulators (Gjedsted et al. 2011, Grip et al. 2015). Therefore, researchers must implement meticulous care during procedures, particularly during the initial placement of the animal on the treadmill, as undue stress at this moment could precipitate an adrenergic discharge, potentially compromising subsequent lactate measurements or resulting in a heightened perception of effort intensity, which could adversely affect the entire training session (Kunstetter et al. 2018).

This study also introduced the utilization of the TRIMP proposed by Foster for animal model studies. We calculated TRIMP using [La-] and the %Smax. No significant difference was observed between $TRIMP_{Lac}$ values in week 1 and week 3. These findings suggest a training adaptation process within the TR group, as evidenced by the absence of a spike in A.U compared to the training load increment in week 2 and the training intensity readjustment in week 3. $TRIMP_{Lac}$ emerges as a promising tool for monitoring internal load in animal model studies, as it can be not very accessible to measure the heart rate in rats during the training session. The $TRIMP_{smax}$ proves to be a valuable toll in the training protocol planning, offering insights

into individual training load progression and facilitating the calculation of TM. In this study, both procedures were concomitantly applied, providing an integrated approach to address the limitations of each method while offering insight into both internal and external training responses.

Collectively, the set of monitoring tools employed in this study has allowed the application of the overload principle while maintaining low TM values, ensuring training load progression without altering the desired training protocol intensity characteristics and thereby mitigating the risk of underestimating or overestimating physical capacity.

Regarding overall performance gains, the TR group exhibited a longer time to exhaustion, and greater external work when compared to the SED. Furthermore, intragroup analyses revealed that the TR group improved external work in all maximum incremental tests. Nevertheless, when employing absolute methods for data analysis, the TR group did not exhibit significant performance improvements between the second and third incremental tests. These disparities underscore the potential pitfalls of exclusively relying on absolute measures for data analysis and reporting, as such an approach can lead to the overestimation or underestimation of results. Ghasemi et al. (2021) reported in their review that Sprague Dawley rats exhibit rapid growth until postnatal day 168. Furthermore, Andrade et al. (2022) observed an increase in rat's body mass between two trials separated by just 48 hours, highlighting the significant impact that variables can have on performance results. In our study, the TR animals exhibited approximately a 5% weight gain between familiarization and week 2 (516 ± 5 g *vs* 545 ±4 g), and a 6% weight gain between week 2 and 4 (545 ± 4 *vs* 575 ±6 g). As expected, the SED animals had a greater weight gain, which was 10% in the first period of the experimental protocol (536 ± 11 g *vs* 590 ± 11 g) and 20% in the second (590 ± 13 g *vs* 648 ± 17 g).

The performance data from this study align with previous animal model studies investigating the effects of training parameter manipulation on physical performance. These studies have explored various training methods and protocol durations (Daussin et al. 2008, Teixeira-Coelho et al. 2017, Carvalho et al. 2022), emphasizing the importance of rigorous monitoring of training intensity to provide a minimum of translational evidence within the field of sport science (Poole et al. 2020). Regardless of the chosen training method, it is evident that describing, controlling, and monitoring training parameters are critical factors for achieving reproducible results when using exercise as a research intervention.

This study has limitations, one of which is the absence of a training group without any specific training strategy concerning training load progression and TM. Nevertheless, future studies could include additional groups with equalized total training loads. This would enable investigation of: 1) Manipulating TM; 2) The effects of not applying adjustments to prescribed training intensity based on maximum incremental tests; 3) Implementing $TRIMP_{Lac}$ throughout the entire training protocol. The fact that the rats were not randomly allocated to the experimental groups and the lack of experiments with female rats are also limitations that can be addressed in futures studies.

CONCLUSION

The utilization of $TRIMP_{Lac}$ was effective in assessing the internal training load in animal model. Similarly, the application of $TRIMP_{Smax}$ can facilitate the development of training protocols, allowing for the visualization of training loads. $TRIMP_{Lac}$ and $TRIMP_{Smax}$ effectively contributed

to the development and monitoring of the training protocol, demonstrating their potential to significantly improve the accuracy of training protocols in animal model research.

Implementing the training monotony concept in animal models allows for progressive increases in training loads while mitigating the potential risk of overtraining. The moderate-intensity interval training protocol presented in this study demonstrated its effectiveness in enhancing physical performance in rats.

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