Physiological and biochemical responses to three interval aerobic training protocols in young men

Respuestas fisiológicas y bioquímicas a tres protocolos de entrenamiento aeróbico interválico en hombres jóvenes

Authors

Joni Marcio de Farias[1]
Leandro de Oliveira Carpes[1]
Ricardo Teixeira Quinaud[1]
Priscila CustódioMartins[1]
Tiago Locks Silveira[1]
Marcos Paulo Campos Assis[1]
EduardaValimPereira[1]
Paulo Locks Silveira[1]

[1] University of Southern Santa Catarina – UNESC, Criciúma, Brazil

Corresponding author:
Joni Marcio de Farias
E-mail: jmf@unesc.net

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Abstract

Introduction: The physiological benefits of physical exercise are well documented in health promotion and preventing diseases, although the intensity and duration of such exercise may alter the efficiency of certain variables.
Objective: To investigate intensity and volume effects in interval training both practically and biochemically.
Methodology: Young military personnel were randomly assigned to three interval aerobic exercise groups at light, moderate, and intense intensities, with progressively lower training volumes prescribed as exercise intensity increased (G1, G2, and G3, respectively) measured by their residual heart rate for 12 weeks. Pre/post data of body composition and non-acute biochemical markers from the study population were collected.
Results: After 12 weeks, all groups showed significant reductions in body fat mass, whereas only G1 exhibited significant reductions in BMI, body fat percentage, and resting heart rate. G2 and G3 showed significant increases in lean mass percentage. Regarding oxidative stress, significant changes were limited to DCF, GSH, and NO, with differences between groups depending on intensity and duration, while protein carbonylation, SOD, and sulfhydryl group content remained unchanged.
Discussion: Interval training improved body composition regardless of intensity, with light-intensity training showing additional benefits for BMI and resting heart rate, while moderate- and high-intensity training favored lean mass gains.
Conclusion: Effects on redox balance were more limited and intensity-specific, suggesting that exercise intensity should be considered when targeting oxidative stress outcomes in military populations.

Keywords

Body weight; oxidative stress; physical exercise; young adult.

Resumen

Introducción: Los beneficios fisiológicos del ejercicio físico están bien documentados en cuanto a la promoción de la salud y la prevención de enfermedades, si bien la intensidad y la duración de dicho ejercicio pueden alterar la eficacia de ciertas variables.
Objetivo: Investigar los efectos de la intensidad y el volumen en el entrenamiento por intervalos, tanto desde una perspectiva práctica como bioquímica.
Metodología: Se asignó aleatoriamente a personal militar joven a tres grupos de entrenamiento aeróbico por intervalos de intensidad ligera, moderada e intensa (G1, G2 y G3, respectivamente), estableciendo volúmenes de entrenamiento progresivamente menores a medida que aumentaba la intensidad —determinada según la frecuencia cardíaca residual— durante un periodo de 12 semanas. Se recopilaron datos previos y posteriores a la intervención sobre la composición corporal y marcadores bioquímicos no agudos de la población de estudio.
Resultados: Tras 12 semanas, todos los grupos mostraron reducciones significativas en la masa grasa corporal; sin embargo, solo el G1 presentó reducciones significativas en el IMC, el porcentaje de grasa corporal y la frecuencia cardíaca en reposo. Los grupos G2 y G3 mostraron aumentos significativos en el porcentaje de masa magra. En cuanto al estrés oxidativo, los cambios significativos se limitaron a los niveles de DCF, GSH y NO, observándose diferencias entre grupos según la intensidad y la duración, mientras que la carbonilación de proteínas, la actividad de la SOD y el contenido de grupos sulfhidrilo permanecieron inalterados.
Discusión: El entrenamiento por intervalos mejoró la composición corporal independientemente de la intensidad; el entrenamiento de intensidad ligera aportó beneficios adicionales para el IMC y la frecuencia cardíaca en reposo, mientras que los entrenamientos de intensidad moderada y alta favorecieron el aumento de masa magra.
Conclusión: Los efectos sobre el equilibrio redox fueron más limitados y dependientes de la intensidad, lo que sugiere que esta debe tenerse en cuenta al abordar los resultados relacionados con el estrés oxidativo en poblaciones militares.

Palabras clave

Adulto joven; estrés oxidativo; ejercicio físico; peso corporal.

Introduction

Physical exercise is understood as an effective, economical, and healthy strategy for health promotion, preventing new worsening cases and reducing sedentary behavior which is considered a risk factor for various diseases[1,2]. Aerobic exercise promotes important physiological and metabolic adaptations[3], including improvements in body composition, cardiorespiratory fitness, endothelial function, and oxidative balance[3]. Despite the well-established benefits of aerobic exercise, a considerable proportion of adults fail to achieve recommended exercise levels, and dropout rates from structured exercise programs remain high[4]. Among the factors associated with poor adherence, lack of time is consistently reported as one of the most common barriers to regular exercise participation[5].

In this context, the development of time-efficient exercise strategies has become an important topic in exercise science. High-intensity interval training (HIIT) has emerged as a potential alternative to traditional continuous exercise because it combines short periods of intense effort with recovery intervals, allowing exercise sessions to be completed in less time[6]. Additionally, the intermittent and dynamic characteristics of HIIT may increase exercise enjoyment and motivation, potentially contributing to better adherence[7], with cardiovascular benefits similar to moderate continuous training even with lower total training duration[8].

Different interval exercise intensities may promote distinct physiological and biochemical responses, influencing variables related to body composition and oxidative metabolism[9]. While higher intensities are commonly associated with greater metabolic demand and changes in body composition[10], lower intensities may induce important adaptations related to oxidative balance and cellular protection[11]. The importance of training with load control is anchored by the increase and/or decrease in the production of reactive oxygen species (ROS) in the muscle during performance, attributed to fatigue, following the hormesis concept (supercompensation). However, new studies challenge this idea, suggesting that overtraining is strongly correlated with performance loss predisposed by the oxidative imbalance of markers such as glutathione (GSH)[12]. According to Flockhart et al. (2021)[13], under an excessive training protocol, ROS levels increase but are balanced by the natural production of antioxidants.

Previous studies comparing aerobic exercise intensities have primarily focused on body composition and cardiometabolic outcomes, particularly in overweight or sedentary populations. However, there is still limited evidence comparing the effects of different interval aerobic exercise intensities on both body composition and biochemical markers related to oxidative stress in physically active young adults. Therefore, the present study aimed to compare the effects of 12 weeks of interval aerobic exercise performed at light, moderate, and high intensities on body composition and biochemical markers related to oxidative stress and redox balance in physically active young men.

It should be noted that interval training protocols used in applied settings frequently differ not only in exercise intensity but also in session duration, work-to-rest ratio, training density, and total exercise volume. Rather than attempting to isolate intensity under experimentally equated workloads, the present study compared three ecologically valid interval training models commonly used in practice. Therefore, the objective was to compare the overall physiological and biochemical adaptations associated with these distinct training configurations.

Method

Study design and Settings

This randomized, parallel-group, examiner-blinded superiority-controlled trial was performed in Brazil. Participants were randomly assigned, using concealed allocation, to one of three 12-week intervention groups: G1—light-intensity interval, G2—moderate-intensity interval, and G3—high-intensity interval. All participants consented in a written document to participate in the training program, and the protocol was approved by the local research ethics committee from the University of Extreme South of Santa Catarina – UNESC under the number 75101719.0.0000.0119. This study followed the Consolidated Standards of Reporting Trials (CONSORT) guidelines14.

Participants

The study population comprised 21 physically active military men, without physical or mental health restrictions preventing exercise participation. Considering the exploratory nature of this randomized trial and the limited availability of eligible military personnel, no power calculation was performed. Therefore, the findings should be interpreted as preliminary and hypothesis-generating. Participants were instructed to maintain their dietary habits and to avoid engaging in any structured exercise or vigorous physical activity on non-training days throughout the intervention period. After baseline assessments, participants were randomly allocated into the intervention groups through simple randomization using sealed and opaque envelopes prepared by a researcher not involved in the assessments or training supervision. The intervention team was blinded to the assessment results, and outcome assessors remained blinded to group allocation throughout the study to minimize potential bias. The participants and the researchers responsible for the training could not be blinded to the experimental groups due to the nature of the interventions.

Tests Protocols

The participants underwent a comprehensive health assessment, including a general health status anamnesis. Total body weight and height measurements utilized a Filizzolla scale, and body composition was assessed using Maltron BF906 bioimpedance, evaluating the percentage of body fat, fat mass in kilograms, body mass index (BMI), basal metabolic rate (kcal), and body water content (liters/%).

The selected biomarkers were chosen because they represent complementary components of redox homeostasis. Carbonyl proteins and DCF were used as indicators of oxidative damage and reactive oxygen species production, respectively. Sulfhydryl groups and reduced glutathione (GSH) reflect non-enzymatic antioxidant defense, whereas superoxide dismutase (SOD) represents enzymatic antioxidant capacity. Nitric oxide was included as an indirect marker of endothelial-related redox signaling and vascular adaptation. Together, these biomarkers provide a broader assessment of exercise-induced changes in oxidative balance rather than relying on a single biochemical pathway.

Oxidative stress

Protein oxidation of the blood samples was determined by quantifying carbonylated proteins through the reaction of carbonyl groups with dinitrophenylhydrazine. This reaction results in the formation of corresponding hydrazones. The carbonyl content was spectrophotometrically determined at 370 nm, as previously described by Levine15.

Hydroperoxide production was determined by intracellular formation of 2',7'-dichlorofluorescein (DCFHDA) from the oxidation of 2',7'-dichlorodihydrofluorescein diacetate (DCFHDA) by ROS LeBel16 with some modifications. To detect the total amount of thiol groups in the sample, the acid dithionitrobenzoic reagent (DTNB) was used. This reagent reduced the present thiol groups, resulting in the formation of a yellow derivative called 2-nitro-5-mercapto-benzoic acid (TNB). The amount of TNB formed (equivalent to the amount of sulfhydryl groups) was measured using a spectrophotometric device at a wavelength of 412 nm17.

Antioxidant defense

Superoxide dismutase (SOD) was measured by adrenaline oxidation inhibition adapted from Bannister and Calabrese18. Readings were taken for 180s at 10-s intervals and measured at 480nm, with values expressed in SOD units per milligram of protein (U/mg protein). Reduced glutathione (GSH) levels were measured in blood homogenate after protein precipitation with 1 mL of 10% trichloroacetic acid. A standard curve of reduced glutathione was used to calculate GSH levels in the samples. Absorbance was read at 412nm after 10 minutes19.

Vascular response

Nitric oxide (NO) included as an indirect marker of endothelial and vascular responses associated with exercise adaptations, was spectrophotometrically assessed through the stable metabolite nitrite. Nitrite content was calculated based on a standard curve from 0 to 100 nM prepared with sodium nitrite ($NaNO_2$) metabolite. Results were calculated in µmol nitrite/mg protein, and absorbance was measured at 540nm20.

Training Program

The physical training program took place on an official 8-lane athletics track in the afternoon, with respective groups organized based on effort intensity and distance covered. All groups were monitored using GPS-enabled heart rate monitors, with data extracted after each training session. The exercise model employed was interval training, and effort intensity was determined by the reserve heart rate. The group G1 (n=6) performed exercises at light intensity (40% to 60%) and completed 6 sets of 10 minutes with a 2-minute rest interval. Group G2 (n=8) engaged in moderate-intensity exercises (75% to 85%) and executed 5 sets of 7 minutes with a 5-minute recovery interval. And group G3 (n=7), undergoing high-intensity exercises (85% to 95%), conducted 8 sets of 20 seconds with a 10-second rest interval after each set. All groups adhered to individually determined and monitored training zones.

Statistical analyses

Outcomes were analyzed according to the intention-to-treat principle using a full analysis set that included all randomized participants. The statistician remained blinded to participant recruitment and group allocation throughout the analyses. Data normality was assessed using the Shapiro–Wilk test, supported by visual inspection of histograms and Q–Q plots. Continuous variables are presented as mean ± standard error (SE) with corresponding 95% confidence intervals (95% CI). Primary and secondary outcomes were analyzed using generalized estimating equations (GEE) for repeated measures, with adjustment for baseline values of each outcome and baseline body fat percentage, as this variable differed between groups and could potentially influence the intervention response. When significant effects were identified, post hoc comparisons were adjusted using the sequential Bonferroni procedure. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using IBM SPSS Statistics for Windows (version 22.0; IBM Corp., Armonk, NY, USA).

Results

Twenty-one military men were randomized to one of three interval training groups: low-intensity (G1, $n = 6$; age, 23 ± 7 years), moderate-intensity (G2, $n = 8$; age, 25 ± 8 years), or high-intensity (G3, $n = 7$; age, 28 ± 9 years). All randomized participants were analyzed according to the intention-to-treat principle. No adverse events occurred during the intervention.

The anthropometric and heart rate data are presented in Table 1. GEE analysis revealed no significant intervention × time interactions for the anthropometric or hemodynamic variables (all $p > 0.05$). At baseline, the G1 presented lower body fat percentage ($p = 0.004$) and body fat mass ($p = 0.002$), as well as higher lean mass percentage ($p = 0.002$), compared with the G3 ($p < 0.05$). This difference between the groups persisted at the post-intervention time point for body fat percentage ($p = 0.047$) and body fat mass ($p = 0.030$).

Within-group analyses showed reductions in BMI ($p < 0.001$), body fat percentage ($p = 0.019$) and body fat mass ($p = 0.004$) in the G1, decreases in body fat mass and increases in lean mass percentage and lean mass in the G2, and reductions in body fat mass together with increases in lean mass percentage in the G3. Resting heart rate ($p = 0.035$) decreased only in the G1, whereas body weight remained unchanged throughout the intervention ($p > 0.05$).

Figure 1. Showed no significant differences between groups or across time for protein carbonylation, SOD, or sulfhydryl content (all p > 0.05).

Table 1. Anthropometry and hemodynamic measurements at baseline and after the 12-week intervention program

Variables	Light-intensity interval (G1)				Moderate-intensity interval (G2)				High-intensity interval (G3)			
	Pre	Post	Δ	*P* value	Pre	Post	Δ	*P* value	Pre	Post	Δ	*P* value
Anthropometry												
BMI (kg/m²)	23 ± 1	22± 1	-1 (-1 to 0)	**<0.001**	25 ± 1	25 ± 1	0 (-0 to 0)	1.000	27 ± 1	26 ± 1	-1 (-1 to 1)	0.126
Body weight (kg)	70 ± 5	71 ± 4	1 (-3 to 6)	0.659	75 ± 4	75 ± 4	0 (-1 to 1)	0.913	82 ± 4	82 ± 4	0 (-1 to 0)	0.371
Body Fat (%)	11 ± 3*	10 ± 3*	-1 (-1 to -0)	**0.019**	17 ± 2	16 ± 2	-1 (-2 to 0)	0.060	22 ± 2	20 ± 3	-2 (-4 to 1)	0.057
Body Fat (kg)	16 ± 3*	15 ± 3*	-1 (-2 to -0)	**0.004**	23 ± 2	21 ± 2	-2 (-3 to -0)	**0.035**	27 ± 1	24 ± 2	-3 (-5 to -0)	**0.027**
Lean Mass (%)	84 ± 3*	84 ± 3	0 (-1 to 1)	0.776	77 ± 2	79 ±2	2 (0 to 3)	**0.035**	73 ± 1	76 ±2	3 (0 to 5)	**0.027**
Lean Mass (kg)	55 ± 2	55 ± 2	0 (-1 to 1)	0.728	57 ± 3	58 ± 2	1 (0 to 2)	**0.027**	60 ± 2	61 ± 1	1 (-1 to 3)	0.286
Hemodynamic												
HR at rest (bpm)	82 ± 6	72 ± 4	-9 (-18 to -1)	**0.035**	81 ± 4	81 ± 4	0 (-8 to 8)	0.947	77 ± 1	71 ± 5	-6 (-14 to 3)	0.183

Values are mean ± SE or mean (95% CI). BMI: Body Mass Index; HR: heart rate; Bold p-values indicate significant results ($p < 0.05$). *indicate significant results from G3 at the same time ($p < 0.05$).

For DCF, no significant differences were found between groups at Pre, but at Post, G3 (0.43 ± 0.01 U Fluorescence/mg protein) showed significantly higher levels than G2 (0.36 ± 0.01 U Fluorescence/mg protein; $p < 0.001$). Additionally, G3 showed a significant increase in DCF from Pre (0.38 ± 0.03) to Post (0.43 ± 0.01; $p = 0.026$).

For GSH, G1 (0.012 ± 0.001 U Fluorescence/mg protein) was significantly higher than G3 (0.007 ± 0.001 U Fluorescence/mg protein) at Post ($p = 0.009$), although no significant Pre-to-Post change was observed within any group for this marker.

For NO, G1 showed significantly lower levels than G3 at Pre (0.12 ± 0.03 vs. 0.24 ± 0.03 μmol/mg protein; $p = 0.024$). G1 also showed a significant increase in NO from Pre to Post (0.12 ± 0.03 to 0.32 ± 0.06 μmol/mg protein; $p = 0.003$), an effect not observed in G2 or G3.

Figure 2. Biochemical results obtained from pre and post 12-weeks interventions

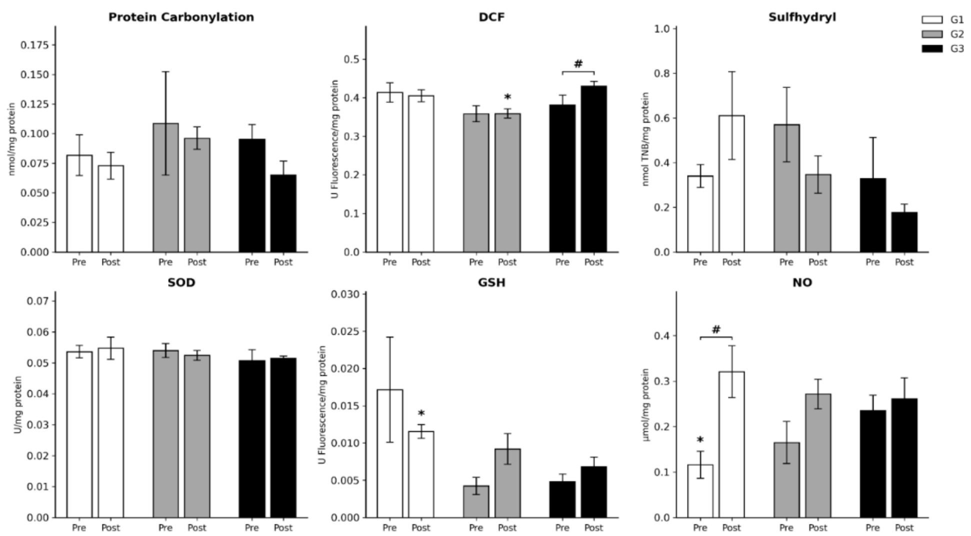

Figure 2. Effect of interval training on oxidative stress and antioxidant biomarkers across three groups: G1 (light-intensity), G2 (moderate-intensity), and G3 (high-intensity), measured before (Pre) and after (Post) the intervention. Panels show: Protein Carbonylation (nmol/mg protein); DCF (2′,7′-dichlorofluorescein, U Fluorescence/mg protein); Sulfhydryl groups (nmol TNB/mg protein); SOD (superoxide dismutase, U/mg protein); GSH (reduced glutathione, U Fluorescence/mg protein); and NO (nitric oxide, μmol/mg protein). Bars represent estimated marginal means ± standard error (SEM), obtained from Generalized Estimating Equations (GEE) models. Pairwise comparisons were performed with Bonferroni correction. *indicates a significant difference ($p < 0.05$) between groups at G3 in the same time point. #indicates a significant difference ($p < 0.05$) between Pre and Post within the same group.

Discussion

The present study compared the effects of 12 weeks of interval aerobic exercise performed at different intensities and volume on body composition and biochemical markers related to oxidative balance in physically active young men. Our main findings indicate that all three exercise intensities promoted favorable changes in body composition, with reductions in body fat mass observed across all groups, although only the light-intensity protocol led to significant reductions in BMI, body fat percentage, and resting heart rate. In contrast, the moderate- and high-intensity protocols were more effective in promoting gains in lean mass. With respect to oxidative stress and antioxidant biomarkers, the interventions had a more limited impact, with significant between-group and within-group differences restricted to DCF, GSH, and NO, while protein carbonylation, SOD, and sulfhydryl content remained largely unaffected.

Aerobic exercise has a causal relationship with β-oxidation, regardless of intensity21-22. All groups showed significant reductions in absolute fat mass, whereas only the light-intensity interval training group (G1) significantly reduced body fat percentage and BMI. Likewise, a recent dose-response meta-analysis including 116 randomized trials demonstrated that reductions in body fat are primarily associated with greater weekly exercise duration rather than exercise intensity. These findings may explain why the light-intensity interval training group in the present study showed a significant reduction in body fat percentage despite exercising at a lower intensity, suggesting that longer exercise duration may compensate for lower exercise intensity in promoting favorable changes in body composition.

Another alteration observed in physiological variables was a lean mass increase in kilograms and percentage; the group that underwent high-intensity training (G3) showed a significant gain ($p<0,05$). Timmons24 subjected sedentary young men to an intense training protocol for 8 weeks, also increasing lean mass. Meanwhile, the 8-week intervention study by Kazior25 demonstrated that adding HIIT during the interval pauses of resistance training would stimulate tissue anabolism more than catabolism, allowing for positive outcomes in muscle mass. However, the mechanisms underlying these responses were not directly investigated in the present study.

The oxidative stress-related findings should be interpreted considering the interaction between reactive oxygen species production and antioxidant defense mechanisms, which collectively contribute to exercise-induced physiological adaptations26. This interpretation is consistent with Molina-López et al. (2022), who reported that professional soccer players presented increased oxidative stress following training and suggested that antioxidant strategies may attenuate redox imbalance, although inflammatory markers were less responsive. In the present study, distinct responses were observed across nitric oxide and hydroperoxide (DCF) according to exercise intensity, suggesting different adaptations in oxidative balance following the intervention. The light-intensity group demonstrated changes in NO levels accompanied by reductions in resting heart rate, whereas the high-intensity group presented increased hydroperoxide levels. Since DCF is associated with oxidative processes 27 these findings suggest that interval exercise performed at different intensities may elicit distinct oxidative responses. Additionally, nitric oxide plays an important role in vascular regulation and exercise-related physiological adaptations27,29. However, as vascular function was not directly assessed in the present study, no mechanistic interpretation can be established. Together, these findings reinforce that exercise-induced oxidative responses should be interpreted considering the interaction between oxidative challenge and antioxidant defense systems rather than isolated biomarkers, highlighting distinct biochemical adaptations according to exercise intensity following the 12-week intervention.

Some limitations should be considered when interpreting the present findings. The study included a relatively small sample size composed exclusively of physically active young military men, which may limit the generalizability of the results to other populations. However, the inclusion of participants with similar occupational physical activity demands and training backgrounds provided a more homogeneous sample, potentially reducing variability associated with differences in habitual physical activity levels. In addition, dietary intake was not controlled, and the 12-week intervention period may not have been sufficient to fully characterize longer-term physiological and biochemical adaptations. Training protocols varied not only in intensity but also across variables such as total session volume, duration, training density, and work:rest ratios. Due to these methodological configurations, it is not possible to isolate or attribute the physiological and biochemical outcomes solely to exercise intensity.Despite these limitations, the present study contributes to the understanding of exercise-induced adaptations by simultaneously evaluating body composition and biochemical markers related to oxidative balance following different interval exercise intensities. The comparison of light-, moderate-, and high-intensity protocols within the same intervention design provides relevant information regarding distinct physiological and biochemical responses to exercise training and may contribute to future investigations aimed at optimizing exercise prescription strategies.

Conclusions

In conclusion, a 12-week interval training program improved body composition in military personnel regardless of exercise intensity, with reductions in body fat mass observed across all groups. However, light-intensity training was uniquely effective in reducing BMI, body fat percentage, and resting heart rate, while moderate- and high-intensity training were more effective in promoting lean mass gains. Effects on oxidative stress and antioxidant status were more limited and intensity-specific, with significant changes restricted to DCF and NO. These findings suggest that different interval training configurations were associated with physiological and biochemical adaptations, the protocols differed in several training characteristics besides intensity, these findings should not be interpreted as the isolated effect of exercise intensity,induce adaptations between morphological and redox-related outcomes, reinforcing the concept that different physiological systems respond differently to exercise training stimuli. From a practical perspective, high-intensity interval exercise may represent a time-efficient strategy for optimizing body composition adaptations, although potentially accompanied by greater oxidative challenge depending on the training configuration. Collectively, the present findings highlight the importance of individualized exercise prescription according to specific physiological objectives and support the need for future investigations exploring the complex interaction between exercise intensity, oxidative balance, and long-term physiological adaptation in larger samples.

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Author Affiliations

Joni Marcio de FariasPhD. Professor; Postgraduate Program in Public Health / University of Southern Santa Catarina, Criciuma, Brazil.

https://orcid.org/0000-0003-2843-6482

Leandro de Oliveira CarpesPhD. Professor; Research Group on Health Promotion / University of Southern Santa Catarina, Criciuma, Brazil.

https://orcid.org/0000-0003-1886-251

Ricardo Teixeira Quinaud: PhD. Professor; Research Group on Health Promotion / University of Southern Santa Catarina, Criciuma, Brazil.

https://orcid.org/0000-0001-6043-3658

Priscila Custódio Martins: PhD. Professor; Research Group on Health Promotion / University of Southern Santa Catarina, Criciuma, Brazil.

https://orcid.org/0000-0002-6388-5550

Tiago Locks Silveira: MSc Professor; Physical Education Course / University of Southern Santa Catarina, Criciuma, Brazil.

https://orcid.org/0009-0005-2132-7533

Marcos Paulo Campos Assis: Master's Student; Postgraduate Program in Health Sciences, Criciúma, Brazil.

https://orcid.org/0000-0001-5334-8885

EduardaValimPereira: MSc. Professor; Research Group on Health Promotion / University of Southern Santa Catarina, Criciuma, Brazil.

https://orcid.org/0000-0002-5264-767X

Paulo Locks Silveira: PhD. Professor; Postgraduate Program in Health Sciences / University of Southern Santa Catarina, Criciuma, Brazil.

ttps://orcid.org/0000-003-4908-2257

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Authors and translators' details:

Joni Marcio de Farias	jmf@unesc.net	Author
Leandro de Oliveira Carpes	l.carpes@unesc.net	Author
Ricardo Teixeira Quinaud	ricardoquinaud@unesc.net	Author
Priscila Custódio Martins	priscila.martins@unesc.net	Author
Tiago Locks Silveira	tiagols@unesc.net	Author
Marcos Paulo Campos Assis	campos_pro@hotmail.com	Author
Eduarda Pereira Valim	eduardavalim@hotmail.com	Author
Paulo Locks Silveira	psilveira@unesc.net	Author