RBFEx
ISSN Online: 2675-1372

Revista Brasileira de Fisiologia do Exercício

Original article

Effects of a CrossFit® session on redox state markers

Efeitos de uma sessão de CrossFit® sobre marcadores do estado redox

Brenna Mirelle Barbosa Bastos, João Henrique Gomes, Ana Mara de Oliveira e Silva, Renata Rebello Mendes

1. Universidade Federal de Sergipe, Aracaju, SE, Brazil

ABSTRACT

Background: CrossFit® is a type of high-intensity functional training that may have health benefits. The modality has also been criticized due to the hypothesis that it could increase the risk of injuries due to oxidative stress generated by the intensity of the exercises. However, there are few studies evaluating oxidative stress in its practitioners. Objective: To evaluate the redox state in trained and non-trained adults, of both sexes, submitted to a high-intensity protocol named 'Cindy'. Methods: We evaluated 19 participants of a Crossfit® program, divided into beginners and experienced, women and men. For characterization, we evaluated body composition, maximal strength and aerobic capacity. For redox state evaluation, participants performed Cindy and had blood samples collected at pre-exercise and 30 minutes post-exercise, through biomarkers such as: SOD, GPx, FRAP and TBARS. Results: At the post-30 moment, there was a significant increase of GPx in the general population and, according to gender, this increase was in women (PRE 40.0 ± 3.9 and POS 46.7 ± 8.1), but not among men (PRE 36.4 ± 8.7 and POS 40.7 ± 5.7); we observed significant reduction of SOD, especially in novices (PRE 3273.1 ± 414.8 and POS 2378.1 ± 781.9); FRAP increased significantly (PRE 84.09 ± 20.49 and POS 106.27 ± 28.64), being this phenomenon observed in both sexes and experience levels; TBARS remained unchanged. Conclusion: A Cindy session promoted GPx and FRAP increase, SOD reduction and TBARS maintenance in its practitioners.

Keywords: oxidative stress; circuit-based exercise; high-intensity interval training.

RESUMO

Introdução: CrossFit ® é um tipo de treinamento funcional de alta intensidade que pode trazer benefícios à saúde. A modalidade também tem sido criticada devido à hipótese de que poderia elevar risco de lesões decorrentes do estresse oxidativo gerado pela intensidade dos exercícios. Porém, há poucos estudos avaliando estresse oxidativo em seus praticantes. **Objetivo:** Avaliar o estado redox em adultos treinados e não treinados, de ambos os sexos, submetidos a protocolo de alta intensidade denominado 'Cindy'. **Métodos:** Foram avaliados 19 participantes de programa de Crossfit®, divididos em novatos e experientes, mulheres e homens. Para caracterização, avaliamos composição corporal, força máxima e capacidade aeróbia. Para avaliação de estado redox, os participantes realizaram Cindy e tiveram amostras de sangue coletadas nos momentos pré-exercício e 30 minutos pós-exercício, por meio de biomarcadores como: SOD, GPx, FRAP e TBARS. **Resultados:** No momento pós-30, houve aumento significativo de GPx na população geral e, de acordo o sexo, esse aumento se deu nas mulheres (PRE 40,0 ± 3,9 e POS 46,7±8,1), porém não entre os homens (PRE 36,4 ± 8,7 e POS 40,7 ± 5,7); observamos redução significativa de SOD, especialmente nos novatos (PRE 3273,1 ± 414,8 e POS 2378,1 ± 781,9); a FRAP aumentou significativamente (PRE 84,09 ± 20,49 e POS 106,27 ± 28,64), sendo esse fenômeno observado em ambos os sexos e níveis de experiência; TBARS permaneceram inalterados. **Conclusão:** Uma sessão de Cindy promoveu aumento de GPx e FRAP, redução de SOD e manutenção de TBARS em seus praticantes.

Palavras-chave: estresse oxidativo; exercícios em circuitos; treinamento intervalado de alta intensidade.

Recebido em: 6 de julho de 2024; Aceito em: 30 de agosto de 2024.

Correspondence: Renata Rebello Mendes, remendes@academico.ufs.br

Introduction

CrossFit® is a type of high-intensity functional training consisting of aerobic and anaerobic stimuli, performed through fast movements and with little or no rest between sets [1], which has been linked to improved cardiovascular, metabolic, and cognitive health, thus reducing mortality risks [2,3]. However, this modality has been the target of scrutiny due to concerns related to the high risk of injuries, hypothetically caused by the high intensity at which training sessions are performed, insufficient rest between exercises, and oxidative stress (OS) [4-8].

Although regular physical exercise is the only health behavior associated with a decrease in all-cause mortality in humans [9], studies show that intense physical exercise can generate OS, characterized by situations in which reactive oxygen species (ROS) outweigh antioxidant compounds [10]. According to Powers *et al*. [9], this exercise-induced OS can culminate in beneficial and harmful outcomes for its practitioners, depending on the amount of ROS produced.

Muscle force production is affected by OS in a biphasic manner; an optimal level of ROS is required for muscle fibers to generate 100% of their maximum isometric force production. However, elevations in ROS concentrations in muscle fibers, above this "ideal point", result in a decrease in the muscle's ability to generate force [9]. Additionally, moderate concentrations of ROS produced during exercise have been related to increased mitochondrial biogenesis, synthesis of oxidative enzymes, and greater activation of the Mammalian Target of Rapamycin (mTOR), the latter being an important factor for muscle hypertrophy [7]. However, high concentrations of ROS can result in significant damage to macromolecules, such as proteins, lipids, and DNA, which can lead to injuries, which have been so often associated with CrossFit® [11,12].

The research on OS related to CrossFit® is still vague and scarce. According to our research, to date, only one study has evaluated OS in CrossFit® practitioners [6]. In this study, the CrossFit® 'Cindy' protocol was compared to a 20-minute session of high-intensity treadmill running. Plasma lipid hydroperoxides (LOOH), ferric reducing antioxidant power (FRAP), and protein carbonyl (PC) were evaluated as biomarkers at four different time points, and the authors concluded that oxidative stress was similar in both modalities.

Therefore, it is clear that there is a need for more studies evaluating OS in CrossFit® practitioners, a modality with a growing number of practitioners. Thus, the present study aimed to assess the redox state in adult CrossFit® practitioners undergoing a high-intensity training protocol and compare the outcomes between both sexes and the level of training experience in the modality.

Methods

Experimental design

This study comprised a total of five visits. After approval of the research project by the Research Ethics Committee (no. 3,087,955/2018) and recruitment carried out through the social network of a CrossFit® box in the city of Aracaju, the first visit happened. On this occasion, the aim was to clarify the objectives and procedures that would involve the study to coaches and athletes, as well as to deliver the free and informed consent form (ICF) for later reading and signing by those who met the inclusion criteria and who were interested in participating. On the second visit, after receiving the signed ICF, an anthropometric assessment was carried out to characterize the participants and familiarize them with the 'Cindy' training protocol. On the third visit, as part of the ongoing sample characterization process, a maximum strength test was conducted (one repetition maximum - 1RM). On the fourth visit, an aerobic capacity test (Yo-yo test) was performed, finalizing the characterization of the participants (the participants were already familiar with the 1RM and yo-yo tests). After 48 hours of rest (no physical training allowed), the fifth visit happened, in which data collection took place: one hour after consuming a standardized breakfast, the participants underwent the 'Cindy' training protocol and blood collection before and 30 minutes after exercise (Figure 1).

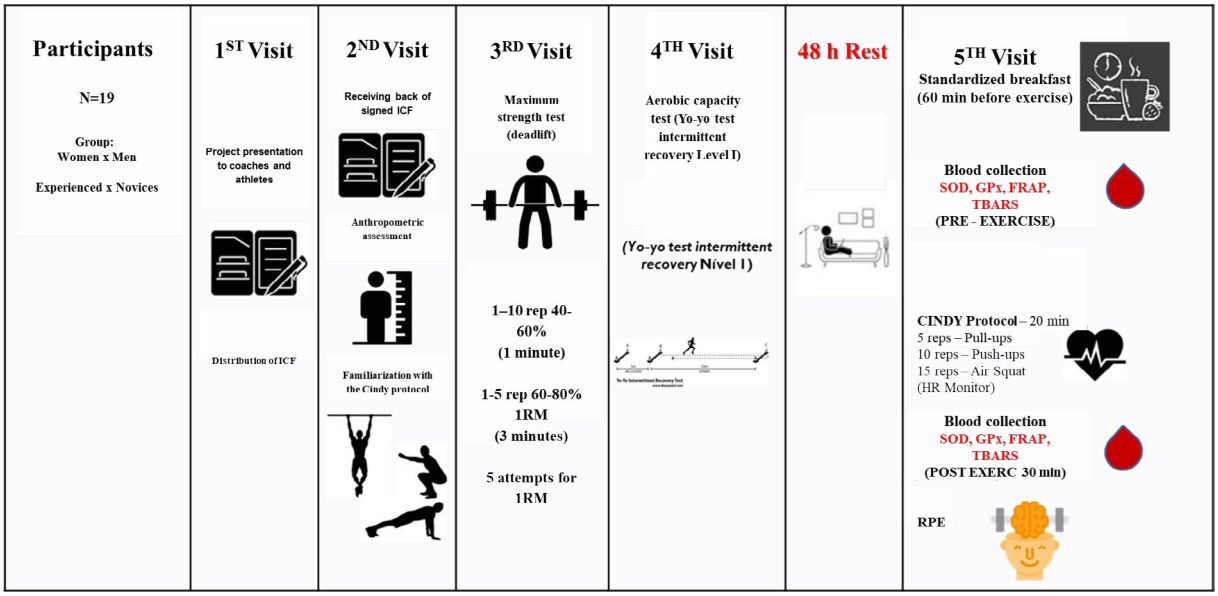


Figure 1 - Experimental design of the study

Participants

Healthy volunteers were recruited from a CrossFit®-affiliated gym (CrossFit® Quest, Aracaju/SE, Brazil). Inclusion criteria for participant selection were having at least three months of experience in a HIFT program and the ability to perform the 'Cindy' training protocol. Novice (NOV) participants were defined as those who had between 3 and 8 months of experience, while experienced (EXP) participants were

those who had more than 18 months of experience (Table 1). These time intervals were selected to ensure a significant gap in experience level between the two groups of participants, as suggested by Butcher *et al.* [13].

Although some participants had more than 18 months of experience, all were classified as recreational CrossFit® practitioners, mainly because they had never participated in an official competition (except those organized by the gym). Participants typically performed three to five training sessions per week.

Participants were excluded if they had: a) between nine and 17 months of experience; b) any injury or motor disability that prevented them from performing the tests and training protocol; c) any cardiovascular, metabolic, or neurological diseases; d) the use of any medication or drugs to enhance performance; e) the use of supplements containing antioxidant compounds in the last six weeks, as well as those considered ergogenic [14], such as caffeine, creatine, beta-alanine, nitrate and bicarbonates, in the last four months; f) not completing the 20 minutes of the 'Cindy' workout on visit five; g) not consuming the standardized breakfast before the 'Cindy' workout on visit five.

Table I - Participant characteristics (M ± DP)

Variables	N = 19	Mulheres	Homens	Novatos	Experientes
Age (years)	30.7 ± 4.8	28.6 ± 4.4	32.8 ± 4.3	31.1 ± 5.4	30.3 ± 4.5
Gender	9 H / 10 M				
Body weight (kg)	74.3 ± 14.1	63.5 ± 8.9	85.0 ± 9.3	77.6 ± 15.0	71.6 ± 13.6
Stature (m)	1.70 ± 0.10	1.63 ± 0.05	1.77 ± 0.04	1.70 ± 0.10	1.70 ± 0.10
BMI (kg/m²)	25.5 ± 3.0	24.0 ± 2.9	26.9 ± 2.4	26.8 ± 2.7	24.4 ± 2.9
Body Fat Percentage (%)	20.0 ± 5.4	22.7 ± 3.9	17.3 ± 5.4	22.1 ± 5.6	18.3 ± 4.8
Deadlift (kg)	116.9 ± 37.9	-	-	-	-
Yo-yo RL1 (m)	415.6 ± 188.2	-	-	-	-
VO_{2max} (mL.kg.min^{-1})	39.9 ± 1.6	-	-	-	-
HR_{max} (bpm)	188.1 ± 9.4	-	-	-	-

Notes: M: Mean; SD: standard deviation.
M: men; W: women; BMI: body mass index; Yo-yo RL1: yo-yo recovery test level 1; VO_{2max}: maximum oxygen consumption; HR_{max}: maximum heart rate.

Body composition

The anthropometric assessment covered data such as body mass, body height, and skin folds, which were later used to calculate the percentage of fat mass (%FM) and lean mass, according to Jackson and Pollock [15] and Jackson, Pollock, and Ward [16].

Maximum strength assessment

Maximum strength was assessed using the deadlift exercise test, using an Olympic bar and 1.5 kg to 20 kg weight plates. The test involved warming up with 1 set of 10 repetitions with 40 to 60% of the estimated one-repetition maximum (1RM), and, after 1 min, another set of 5 repetitions with 60 to 80% of the estimated 1RM, and after 3 min, five attempts at a maximum voluntary action were performed until a 1RM for each subject was identified [17]. Rests (approximately 4-5 minutes) were taken between attempts to maintain maximum performance.

Aerobic capacity assessment

Aerobic capacity was indirectly assessed using the Yo-Yo Intermittent Recovery Level I (Yo-Yo) test. Running in a demarcated space consists of running a distance of 20 m twice ('round trip' = 40 m), separated by regular recovery periods of 10 seconds. The time to run the 40 m was progressively reduced, representing higher speeds at each stage. The subjects were instructed to complete as many stages as possible, reaching the end of the course at each sound signal. The test was interrupted when the volunteer was unable to complete the stage (being more than 3 m before the 20 m line on two consecutive sound signals) or reported an inability to complete the run. The test was performed in one attempt. Based on the distance and speed achieved during the test, aerobic capacity was determined, expressed by maximum oxygen consumption (VO_{2max}) in ml/kg/min (formula for predicting VO_{2max} = distance x 0.0084 + 36.4). The choice of Yo-yo Intermittent Recovery Level I was based on the study by Bangsbo, Iaia, and Krustrup [18].

Intervention: physical training

The type of exercise adopted as an intervention in the present study was a CrossFit® WOD known as 'Cindy' training [19,20].

The session began with a warm-up consisting of 5 min of low-intensity running and 5 min of joint mobility and dynamic stretching exercises. This WOD consisted of performing as many rounds as possible (AMRAP) of three exercises: 5 repetitions of pull-ups, 10 repetitions of push-ups, and 15 repetitions of air squats for 20 min.

To characterize exercise intensity, during the 'Cindy' training, subjects were monitored using a heart rate (HR) monitor (*Polar Team Pro*, *Kempele*, *Finland*). HR data were stored and later extracted from the Polar Team 2 Pro program. Additionally, the subjective perception of exertion (RPE), which has been strongly recommended for use in metabolic HIFT sessions [21–23], was obtained using the CR10 Borg scale [24]. Participants answered the following question: 'How hard did you think the exercise was?'. RPE measurement was performed 30 min after the 'Cindy' workout.

Standardization of breakfast on the day of data collection – visit 5

One hour before the training session, volunteers consumed powdered food supplements mixed with water as a standardized breakfast (approximately 320–350

calories). The breakfast consisted of protein, fat, and carbohydrates in the proportions of 20-35-45 (percentage of protein, fat, and carbohydrate). These percentages culminated in the ingestion of approximately 40 g of carbohydrates, 17.5 g of protein, and 13 g of fat. To avoid gastric discomfort on the day of the intervention, the same breakfast was offered in the familiarization session with the training protocol. The standardized breakfast was determined by a nutritionist specialized in sports nutrition.

Biochemical analyses

The biomarkers adopted in the present study were thiobarbituric acid reactive substances (TBARS), superoxide dismutase (SOD), glutathione peroxidase (GPx), and ferric-reducing antioxidant power (FRAP).

Determination of lipid peroxidation by the TBARS method

The determination of lipid peroxidation in plasma was performed by quantification of TBARS, according to the method described by Ohkawa, Ohishi, and Yagi [25], with minor modifications. A standard curve of 1, 1', 3, 3'-Tetraethoxypropane - TEP (0.5 - 8.0 nmol) was prepared, and the results were expressed in nmol of TEP/mL of plasma.

Antioxidant capacity via FRAP (Ferric-Reducing Antioxidant Power) method

Plasma was used to determine total antioxidant capacity according to the method described by Benzie and Strain [26] in 96-well plates using the FRAP reagent. Absorbance was read at 595 nm, and the results were expressed in mM eq. of Trolox/mL of plasma.

Determination of superoxide dismutase activity

SOD activity was assessed according to the methodology of McCord and Fridovich [27], which verifies the production of superoxide anion produced by the xanthine/xanthine oxidase system. The superoxide anion produced reduced cytochrome C, and this reduction was measured by the increase in optical density at 550 nm at 25ºC. The results were expressed in U/g of hemoglobin. One unit (U) is considered to be the activity of the enzyme that promotes 50% inhibition of the xanthine reaction at 25ºC at pH 7.8.

Determination of glutathione peroxidase activity

GPx activity was determined using the standardized methodology developed by Sies *et al*. [28]. This method is based on the measurement of the optical density decay at 340 nm caused by the oxidation of NADPH at 30 °C during the reduction of oxidized glutathione (GSSG) catalyzed by the enzyme glutathione reductase. The results were expressed in U/g of hemoglobin. One unit (U) of the enzyme was defined as the activity of the enzyme that oxidizes 1 µmol of NADPH per minute at 30°C at pH 7.0.

Statistical analysis

Normality and homogeneity of variances were verified by the Shapiro-Wilk and Levene tests, respectively. Data are presented as mean and standard deviation. The paired t-test (times) was used to compare the mean values of descriptive variables of all subjects. Two-way repeated measures analysis of variance (ANOVA) (group interaction [EXP x NOV and MEN x WOM] × time) was used to compare blood analyses, followed by the Bonferroni post hoc test to identify differences. All analyses were performed with SPSS-22.0 software (*IBM, SPSS Inc., Chicago, IL, USA*). Significance was set at $p < 0.05$.

Ethical approvals

Ethical approval was obtained from the Research Ethics Committee (Research Ethics Committee of the Federal University of Sergipe, process no. 3,087,955/2018). Participation was voluntary, and all participants signed the Free and Informed Consent Form before participating in the study

Results

The training session adopted in the present study was performed at high intensity, as shown in Table II.

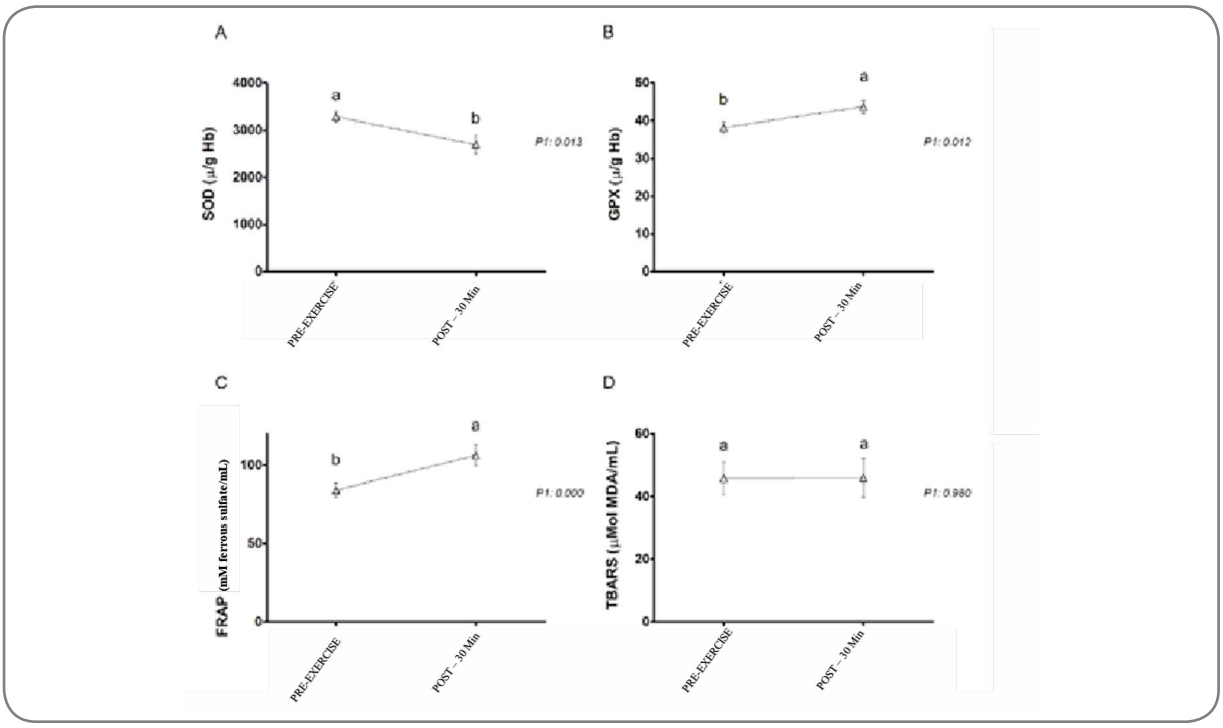
Table II - Characterization of the intervention - Cardiovascular responses, performance, and perceived exertion of participants undergoing the 'Cindy' protocol (M ± SD)

Variables	N = 19
Number of rounds	13.1 ± 3.5
HRx (bpm)	171.6 ± 9.4
%HR_{max}	93.2 ± 2.1
RPE (after 30min)	7.9 ± 1.1

Notes: M: mean; SD: standard deviation.
HRx: average heart rate; bpm: beats per minute; %HR_{max}: percentage of maximum heart rate; RPE: subjective perception of effort.

According to Figure 2, there was a significant reduction ($p < 0.05$) in SOD after 30 min when compared with the values observed in the pre-exam. GPx and FRAP showed a significant increase ($p < 0.05$) after 30 minutes. There were no changes in TBARS (Table III).

The comparison of results between genders showed that GPx increased significantly in women (time effect), with no differences between genders.



Letters compare means across time points; means followed by different letters are statistically different ($p \leq 0.05$), while means followed by the same letter or not followed by any letter do not differ ($p \geq 0.05$). P1: effect of time. SOD: superoxide dismutase; GPx: glutathione peroxidase; FRAP: ferric-reducing antioxidant power; TBARS: thiobarbituric acid reactive substances

Figure 2 - Variation of SOD (A), GPx (B), and FRAP (C) in 19 participants and TBARS (D) in 11 participants. Comparisons between pre-exercise and post-exercise (POST-30min) moments

Table III - Table III - Evaluation of biomarkers at different times for both sexes (M ± SD)

Variables	Women (PRE)	Women (POST)	Men (PRE)	Men (POST)
SOD	3267.7 ± 335.4	2727.7 ± 1083.9	3304.6 ± 628.3	2655.4 ± 718.3
GPx	40.0 ± 3.9	46.7 ± 8.1*	36.4 ± 8.7	40.7 ± 5.7
FRAP	72.1 ± 17.2	85.7 ± 21.1	96.1 ± 18.0	126.8 ± 20.2
TBARS	36.5 ± 14.7	38.5 ± 22.5	59.8 ± 13.2	57.0 ± 17.3

M = mean; SD = Standard deviation; SOD = Superoxide dismutase; GPx = Glutathione peroxidase; FRAP = Ferric reducing ability of plasma; TBARS = Thiobarbituric acid reactive substances

The comparison of results between genders showed that SOD was significantly reduced in novices (time effect) (Table IV).

Table IV - Assessment of biomarkers at different times for different levels of experience (M ± SD)

Variables	Novices (PRE)	Novices (POST)	Experienced (PRE)	Experienced
(POST)	3273.1 ± 414.8	2378.1 ± 781.9*	3296.6 ± 563.5	2942.4 ± 934.2
GPx	36.4 ± 6.4	42.6 ± 5.3	39.6 ± 7.0	44.6 ± 9.0
FRAP	85.2 ± 21.1	106.0 ± 31.6	83.2 ± 22.2	106.5 ± 29.4
TBARS	47.7 ± 18.8	48.0 ± 21.9	44.0 ± 19.1	43.8 ± 23.9

M = mean; SD = standard deviation; SOD = Superoxide dismutase; GPx = Glutathione peroxidase; FRAP = Ferric-reducing ability of plasma; TBARS = thiobarbituric acid reactive substances

Discussion

The main findings of the present study showed that a high-intensity 'Cindy' training session promoted an increase in antioxidant capacity (FRAP), a significant reduction in SOD, and a notable increase in GPx, without changes in TBARS.

The increase in FRAP levels was also observed in the study by Kliszczewicz *et al.* [6]; this phenomenon is expected after a high-intensity activity, whether predominantly aerobic or anaerobic [29], which corroborates the exercise protocol adopted in the present study, since our intervention was shown to be high-intensity, through monitoring of HR and RPE. The increase in FRAP after intense exercise has been explained by the joint and efficient work of the various antioxidants available in the body of its practitioners, which increase their efforts to combat the more significant production of free radicals caused by exercise [30].

When evaluating antioxidant activity, we observed an increase in GPx after 30 minutes of training. This fact can be explained by the concept that antioxidant enzymes respond adaptively, increasing their activities to combat free radicals and the damage caused by them after physical exercise, especially high-intensity exercise [31]. Santos *et al.* [32] also reported an increase in GPx after high-intensity exercise (RAST TEST) by trained athletes; the authors justified this increase as an attempt by the antioxidant system to combat the oxidative stress generated by the RAST TEST.

During physical exercise, including activities in which the metabolism is predominantly anaerobic, or in isometric or explosive exercises, such as in CrossFit®, ischemia and reperfusion occur. In tissue reperfusion, O_2, together with hypoxanthine, promotes the synthesis of superoxide anion (O_{2^-}) and hydrogen peroxide (H_2O_2), species with a high reactive content [33]. Thus, in situations where there is an increase in pro-oxidant compounds, GPx acts together with SOD in an attempt to convert superoxide anion and hydrogen peroxide into water: through dismutation, SOD, being zinc-dependent, catalyzes the synthesis of hydrogen peroxide from superoxide anion; however, as hydrogen peroxide is reactive and can promote oxidative damage, GPx, a selenium-dependent enzyme, catalyzes the reduction of hydrogen peroxide into water (Figure 3) [34]. Thus, in the present study, GPx was likely elevated to counter a potential increase in hydrogen peroxide-induced by Cindy.

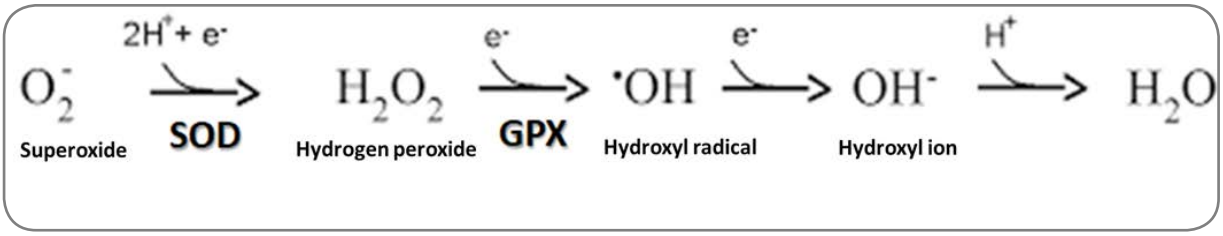


Figure 3 - Conversion of superoxide radical into water, through the action of SOD and GPx enzymes. Adapted from: Barreiros, David, and David (2006). [35]

It is worth noting that the increase in hydrogen peroxide may be the result of the action of SOD on the superoxide radical (Figure 3). Therefore, GPx would 'continue' the work of SOD. According to Groussard *et al.* [36], the accumulation of hydrogen peroxide can reduce SOD activity, corroborating our findings, which demonstra-

ted a drop in SOD 30 minutes after exercise.

Considering this hypothesis, it is possible to assume that, in the present study, at some point during and immediately after Cindy, SOD activity may have increased in an attempt to combat a possible increase in superoxide radicals, as has been observed in recent studies [37,33,38]; the action of SOD would culminate in greater formation of H_2O_2, and subsequently, the accumulation of H_2O_2 would have inhibited the action of SOD, characterizing the drop in SOD observed 30 min after the end of Cindy. However, to test this hypothesis, it would have been necessary to assess SOD activity at more time points than those tested in our study, which can be considered a limitation.

Another limitation is that the nutritional status of the participants with zinc and selenium was not assessed since possible deficiencies of these nutrients could interfere with the activity of the SOD and GPx enzymes, respectively [39].

There is also the hypothesis that the reduction in SOD could indicate that our intervention (Cindy) was not intense enough to cause significant production of reactive oxygen species, not requiring an increase in SOD activity to eliminate excess superoxide [40]. However, in this case, GPx activity and FRAP would not have been elevated either, which weakens this hypothesis. Likewise, the %HR and RPE data also rule out this hypothesis since both methods indicated high intensity.

Finally, in our study there was no change in TBARS concentrations, these compounds being considered markers of lipid peroxidation. Evaluating this result together with the other findings of the present study, it is possible to suggest the hypothesis that exercise (Cindy) caused greater formation of oxidative compounds, which were efficiently 'combated' by the participants' antioxidant system, which in this study was evaluated through SOD and GPx, but which may have been strengthened by numerous other mechanisms not assessed in the present study. All these antioxidant efforts would have culminated in an increase in the antioxidant capacity (FRAP), and this stronger defense would have been sufficient to prevent lipid peroxidation, thus justifying the maintenance of TBARS.

The impact of exercise sessions on the TBARS concentration of its practitioners has been the subject of numerous studies, and the results are divergent, with some studies corroborating our findings [36,41-44] and others in which TBARS were elevated [45,46]. This divergence is understandable since it is believed that, in organisms in which antioxidant defenses are efficient, for several reasons, including nutritional status and sufficient to combat the reactive species generated by intense physical exercise, there will be greater protection of membrane lipids, culminating in less damage, marked by TBARS. On the other hand, in situations where antioxidant defenses are inefficient and situations where the formation of reactive species generated by intense physical exercise is very high, it will not be possible to 'guarantee' the protection of membrane lipids, culminating in greater damage, marked by elevated TBARS [47].

The low reliability and accuracy of the TBARS assessment method have also been considered a possible cause of the contradictory results in the literature [42,

48]. It is also important to highlight that in the assay to assess lipid peroxidation (TBARS), there was a partial loss of blood samples, and, for this reason, we had only 11 participants, a small population, which may increase the risk of bias.

Additionally, there is still the possibility that changes in TBARS after exercise occur later, beyond the 30 minutes adopted in the present study. However, to test this hypothesis, it would be necessary to have evaluated TBARS more times than those tested in our investigation, which can be considered a limitation.

As discussed previously, GPx reduces oxidative stress to prevent oxidative damage. Thus, according to Fortes *et al*. [49], women and men have significant physiological differences. For example, men's muscle fibers are larger than women's, which is why men tend to excel in exercises that require speed and strength. In addition, they have a lower fatigue threshold than women. Furthermore, in a certain study, women were shown to be more susceptible to muscle injuries caused by oxidative stress. Therefore, it can be thought that GPx increased only in women since they need a higher level of effort to reproduce the same exercise as men, consequently increasing ROS, oxidative stress, and GPx.

Regarding the decrease in SOD in the novice group, we can argue that the physical capacity of the experienced ones is more developed [50]. Therefore, novices need greater effort to perform the same exercises, thus increasing the level of oxidative stress and, consequently, superoxide anion molecules. However, as previously discussed, it may be that at the time of analysis, the biomarker in question had its levels reduced by the strong presence of hydrogen peroxide, which has been shown to decrease SOD concentrations.

Additionally, according to Powers *et al*. [9], exercise intensity depends on the functionality of the individual's cardiovascular system and fatigue, so the limitation of the cardiovascular system, with fatigue, alters the intensity and duration of the exercise. Thus, concerning novices, SOD decreased when compared to experienced ones. It can be assumed that the duration and intensity of the exercise were lower in this group, and after 30 minutes, there were no longer as many superoxides to be combatted. However, more blood samples and SOD analysis at different times would be necessary to confirm the hypothesis.

Finally, the oxidative stress caused by physical exercise, depending on its level, can be beneficial or harmful to health and performance [9]. Thus, analyzing our results, because the protocol applied was of high intensity, we saw that there was an increase in oxidative stress caused by the intensity of the physical exercise, but we did not find evidence of oxidative damage 30 minutes after its end. Thus, our findings suggest that performing Cindy would not increase the risk of undesirable outcomes, such as muscle injuries, loss of strength, impaired hypertrophy, or development of chronic non-communicable diseases.

As far as we have investigated, this is the first study to analyze the acute effects of a HIFT session on these biomarkers of oxidative stress. For this reason, our results may be useful to verify how the redox state of an individual behaves in a CrossFit® workout to assess whether or not there is oxidative stress and, in the future, to

encourage more in-depth discussions on the hypothesis that this modality is related to an increased risk of injuries. Such future discussions are fundamental; after all, according to Moran *et al*. [51], there has been an exponential increase in the number of HIFT practitioners worldwide.

Conclusion

A Cindy session promoted an increase in GPx and FRAP, a reduction in SOD, and maintenance of TBARS in CrossFit® practitioners, 30 minutes after its completion. GPx changes were observed in women, while SOD changes were observed among novices. FRAP was altered in all groups.

Conflicts of interest
There are no conflicts of interest.

Sources of funding
No funding.

Authors' contributions:
Conception and design of the research: Bastos BMB, Gomes JH, Silva AMO, Mendes RR; Data collection: Gomes JH, Mendes RR; Data analysis and interpretation: Bastos BMB, Gomes JH, Silva AMO, Mendes RR; Statistical analysis: Gomes JH; Manuscript writing: Bastos BMB, Mendes RR; Critical revision of the manuscript for important intellectual content: Bastos BMB, Gomes JH, Mendes RR

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