

Article

Acute CrossFit® Workout Session Impacts Blood Redox Marker Modulation

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Abstract: We aimed to analyze the impact of a single CrossFit® session “workout of the day” (WOD) on plasma redox. Ten CrossFit®-experienced subjects volunteered to participate. Oxygen uptake (VO₂) during WOD and treadmill running (TR), performed at the same VO₂ and time as WOD, were continuously monitored. Venous blood samples were collected before (baseline—BL) and after both exercises, for lactate concentration, total antioxidant capacity, thiol content, and DNA damage measurements. Total antioxidant capacity decreased after both exercises (WOD and TR) vs. BL, with no differences between exercises. Thiol content increased after WOD; however, no differences between exercises were observed. DNA damage increased after both WOD and TR, although more exuberantly after WOD than TR. Much higher lactate levels were detected in WOD compared to TR. Our findings suggest that WOD induces an increased condition of oxidative injury and affects total antioxidant capacity in experienced CrossFit® performers.

Keywords: oxidative stress; free radicals; WOD



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1. Introduction

Extreme physical fitness programs have increased in popularity, especially CrossFit® training [1]. CrossFit® workouts comprise functional movements, performed at high-intensity (the so-called “workout of the day”—WOD [2]) and are typically composed of gymnastic-type exercises (involving body weight), “metabolic”-related conditioning, and weightlifting [2]. These exercise routines are performed in a circuit format, with short or no resting periods [2]. The goal is to finish the WOD in the “best possible time” (rounds for time) or to achieve the highest possible number of repetitions/rounds over periods of 10–20 min (as many rounds as possible) [3].

Although similar to circuit training, CrossFit® workouts do not typically provide structured resting periods, which entails participants to significantly elevate physical and physiological stress levels [4]. In fact, CrossFit® training programs could jeopardize the concept of wellness [5], eventually triggering significant elevation of several stress mediators in the plasma, including those related to redox state [6]. Like many other exercise-induced stress conditions, it is expectable that when facing increased oxidative stress, practitioners adapt to these homeostatic alterations by enhancing the expression of antioxidants, therefore creating a redox balance that aims at mitigating possible transient increased oxidative stress induced by exercise in some cellular compartments [7–9].

Nevertheless, depending on the specific features of the used exercise programs, namely its mechanical and/or metabolic exuberant prevalence [10–13] and/or the established relationship between exercise and recovery routines, an exacerbated oxidative stress condition may result. In fact, the augmented levels of produced oxidized molecules may overwhelm the ability of the antioxidant system and oxidative injury might occur at different levels of cellular organization, including damage to DNA, proteins and lipids [14–16]. Among the different methods to evaluate the impact of oxidant production under exercise conditions, the ability of the endogenous thiol-related glutathione and thioredoxin systems to counterbalance the potential enhanced oxidative stress [17], as well as the DNA damage [9,18] have been extensively used.

As previously reported by our group [10–12,19,20] and others [21–23], the prevalence of distinct metabolic and mechanical demands of a certain sport modality naturally triggers different main contributor sources of reactive oxygen species (ROS) during and after exercise. Actually, both expected high cardiovascular and metabolic load inferred, for example, by relatively long periods performing at elevated percentages of maximal heart rate (HR_{max}) and the simultaneous elevated neuromuscular demands in CrossFit® training sessions could potentially target several distinct sources and mechanisms associated with the possible increased oxidative stress condition. In addition, given the known regulatory role of the reactive oxygen and ROS in the cellular physiology through its signaling action, it is of utmost importance to ascertain whether a typical WOD induces alterations in redox-related mediators, a subject so far uncovered by the literature and the main goal of the present study.

Furthermore, is not yet known whether intense neuromuscular stimuli-induced ischemia/reperfusion-like events characterizing CrossFit® are decisive contributors to the possible increased redox changes associated with this modality. Considering that oxygen uptake (VO₂) at mitochondrial level is known to be associated with electron leak from electron transport chain complexes, thus resulting in ROS generation, and that WOD comprises both intermittent high neuromuscular demanding actions possibly implicating xanthine dehydrogenase/oxidase (XDH/XO) derived mechanisms in ROS production.

Here, we analyzed the impact of both WOD and treadmill running (TR) sessions matched for mean WOD VO₂ and duration on plasma redox markers. Considering the different mechanical and metabolic involvement of both exercise modalities, we hypothesized that intensive CrossFit® exercises led to higher levels of oxidative stress when compared to running at similar VO₂-related intensity and duration.

2. Results

Anthropometric and physiological characteristics of CrossFit® participants are presented in Table 1. The intensity of WOD and TR sessions measured as percentage of VO₂ is presented in Figure 1. As can be depicted, during large part of the WOD and TR sessions VO₂ was maintained elevated ranging values correspondent to vigorous intensity. The levels of total antioxidant capacity (AOC) significantly decreased from baseline (BL) to the end of the WOD and TR sessions (BL: 256.5 ± 28.7 vs. WOD: 211.7 ± 29.1 and TR: 223.9 ± 41 µM, respectively; $p < 0.05$) (Figure 2, left panel), with no differences between exercises. Regarding the levels of total thiols, a significant increase from BL was observed only after the WOD (0.41 ± 0.04 and 0.38 ± 0.03 nmol TNB/mg protein; $p < 0.05$), while no differences were found between the two protocols (Figure 2, center panel). When compared to baseline values, DNA damage increased both after the WOD and TR sessions (2.5 ± 0.6 vs. 6.9 ± 2.5 and 4.3 ± 1.4%, respectively; $p < 0.01$); however, the CrossFit® session induced higher levels of damage than those observed after the TR protocol ($p < 0.05$) (Figure 2, right panel). Furthermore, the CrossFit® session elicited higher mean heart rate (HR) (WOD: 155 ± 6 vs. TR: 129 ± 16 b min⁻¹, $p < 0.001$), HR_{max} (WOD: 182 ± 5 vs. TR: 151 ± 12 b min⁻¹, $p < 0.001$) and lactate concentration ([La⁻]) (WOD: 16.5 ± 3.6 vs. TR: 2.4 ± 3.4 mM, $p < 0.001$) values than those obtained in the TR session, although

both protocols had the same duration and were performed at similar average VO_2 (WOD: 25.9 ± 3.0 vs. TR: $26.8 \pm 3.3 \text{ mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$, respectively).

Table 1. Physical characteristics of the CrossFit® performers.

	(n = 10)
Age (years)	30.8 ± 5.6
Training experience (years)	3.6 ± 0.2
Height (cm)	169.5 ± 10.3
Weight (kg)	73.5 ± 13
Body mass index (kg/m^2)	26 ± 2
Fat mass (%)	19.9 ± 3.9
Lean Mass (kg)	55 ± 12
Maximal oxygen uptake ($\text{mL} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	44.9 ± 7.2
Maximal respiratory quotient	1.1 ± 0.9
Maximal heart rate ($\text{b} \cdot \text{min}^{-1}$)	181 ± 11
Data are mean standard deviation (SD)	

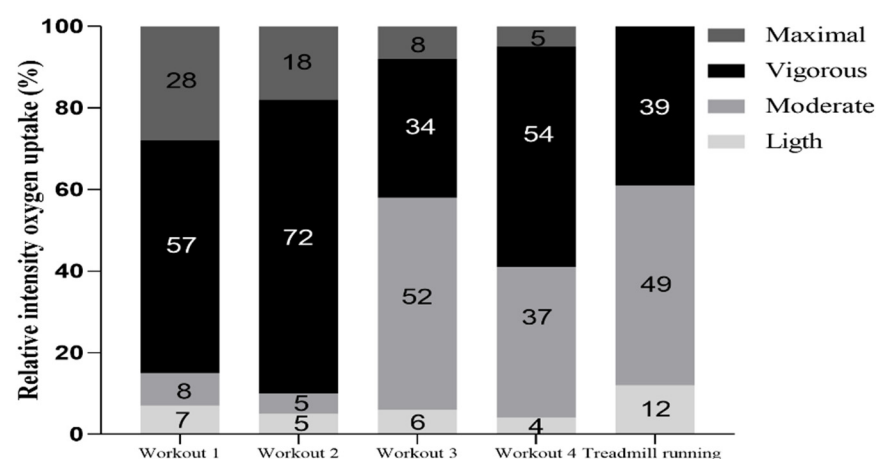


Figure 1. Mean values of the times sustained at relative intensity of VO_2 (rounded to the nearest unit, %) obtained during WOD workouts and treadmill running.

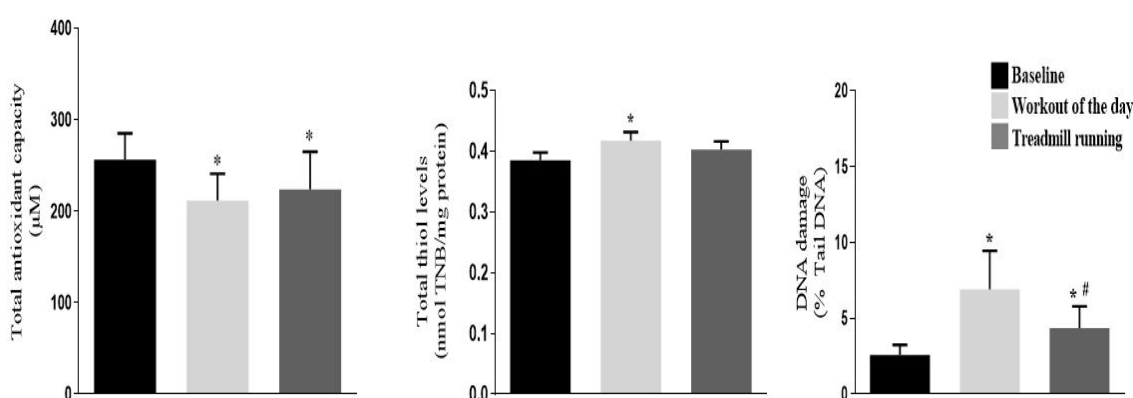


Figure 2. Effect of CrossFit® and treadmill running sessions on total antioxidant capacity, total thiol levels and DNA damage (left, center, and right panels). *,# different from baseline and WOD, respectively ($p < 0.05$).

3. Discussion

The aim of this study was to analyze the effect of a single CrossFit® WOD session on plasma redox biomarkers. Additionally, a treadmill running session matched for the same duration and VO_2 obtained during the WOD was considered in the experimental setup.

The results of our study suggest that the WOD session with the aforementioned characteristics promotes a more significant alteration on oxidative damage biomarkers and antioxidant capacity in the plasma and blood of CrossFit® performers, when compared to the correspondent 40 min running session.

The HR values observed during WOD vs. TR (155 ± 6 vs. 129 ± 16 b min⁻¹) as well as the characteristics of many typical CrossFit® movements performed during the WOD, particularly those involving intense muscular actions and of short duration interspersed with resting or less intense periods are typically associated, for instance, with decreased content of skeletal muscle adenine nucleotides, disruption of muscular calcium homeostasis, periods of ischemia followed by reperfusion. These are known as established conditions favoring the activation of the endothelial and muscular XDH/XO, resulting in additional ROS generation [10,24].

As an estimated 1–5% of the total VO₂ results in the formation of the oxygen radical superoxide (O₂⁻) [15,25] and given the high level of VO₂ accompanying CrossFit® session (Table 2), it is not surprising that the biomarkers of oxidative stress and damage had increased [12,26] and circulating neutrophil-induced oxidative burst [27] can contribute to the observed blood oxidative stress and damage. The influence of eccentric exercise-mediating muscular damage-like events on the formation of ROS has also been reported [19,28].

Table 2. Schematic representation of the CrossFit® WOD workouts.

Workouts	Duration	Repetitions	Recovery	Load (Men and Women)
Rowing ergometer	4 min	Maximum	4 min	—
Air bike	4 min	Maximum	8 min	—
Randy	6 min	Maximum	8 min	35 and 25 kg
Deadlift, toes to bar, dumbbell thruster and dumbbell walking lunges	6 min circuit	10 each exercise	—	Deadlift (80 and 55 kg), dumbbell thruster (30 and 20 kg) and dumbbell walking lunges (30 and 20 kg)

Recently, it has been proposed that at least in some models of exercise, mitochondria are not as important source of ROS production during exercise as NADPH oxidases (NOX), being these last considered to be a major source of contraction-related ROS production and relevant mediators in redox-signaling and metabolism. It was suggested that lower intracellular pH that occurs during some muscle contraction turns mitochondria towards antioxidant rather than pro-oxidant state [29], as mitochondrial H₂O₂ consumption is pH sensitive, and it is higher in lower pH [30]. Both in vitro studies using NOX2 inhibitors [31,32] and in vivo exercise studies using NOX2 activity-deficient mice [33] support the relevance of NOX2 in ROS generation. Moreover, it was reported that skeletal muscle NOX2 activity increased after high-intensity interval training (HIIT) exercise, which might be interpreted as a required mechanism associated with HIIT adaptation related to antioxidant defense, glucose metabolism, and mitochondrial function [34].

Considering the specific physiological demands imposed by a WOD session, none of these potential sources should be ruled out in the current study. However, it is important to note that under the technical constraints of the present study, we cannot conclusively demonstrate a causal link between any of those potential sources and the increased plasma oxidative stress and damage found.

The results showed a decrease in plasma antioxidant capacity after performing both types of exercise ($p < 0.05$), despite no significant differences were observed between the two exercise protocols. Although studies reporting the acute and chronic effects of CrossFit® on antioxidant capacity are lacking, some have shown that single metabolic-based conditioning sessions based on CrossFit® can increase oxidative stress in a similar way to high-intensity exercise performed on a treadmill [35]. CrossFit® training, seen as a chronic stimulus, is part of a demanding fitness program as much as HIIT [1]. For example, an HIIT protocol, carried out for three weeks, resulted in a decrease in oxidative stress

markers and a significant increase in antioxidant status, being these adaptations achieved after nine exercise sessions [36].

The authors suggested that these responses of the pro-oxidant and antioxidant balance with HIIT could be considered to be an additional beneficial response for this type of training program. Therefore, our aforementioned results suggest that the WOD performed by the participants in our study might had a more severe impact on the analyzed markers if they were untrained subjects, as well-trained individuals may develop favorable redox adaptations in a short period of time to cope with WOD-induced increased oxidative stress and damage [15,17]. In fact, Shing et al. [37] demonstrated that only three consecutive sessions of anaerobic training resulted in a significant decrease of oxidative stress markers and an increase in antioxidant status.

Such oxidative and redox adaptations in several tissues, which occur in a short period of time compared to traditional endurance training programs, boost organ and body defense capacities to deal with stressful conditions, and make this type of exercise attractive, not only for increasing physical performance levels, but also to mitigate disease risk factors or develop health-related biomarkers within the populations [9,38]. One should notice the important physiological role of ROS, as biomolecules that are very important in the activation of numerous signaling pathways involved in the increased cellular and sub-cellular defense capacity, including the antioxidant, thus justifying the systematic physical exercise practice [15,17,22], i.e., the moderate and systematic increase in the production of these species results in the activation of cascades of cell and sub-cellular signaling and transcription factors that are decisive in the biosynthesis of enzymes and other molecules with antioxidant potential [9,15].

Another important marker of oxidative stress, more specifically the oxidation of proteins and compounds containing sulfhydryl group (-SH), is the content of total thiols. The levels of this marker increased after the WOD protocol ($p < 0.05$) with no significant differences between the exercises. The -SH groups of proteins can be oxidized by free radicals, thus compromising the functioning of these proteins [16,39]. A possible explanation for our results would be the increase in stress proteins induced by exercise, as these proteins have the function of controlling cellular homeostasis, protecting against excessive oxidation. Additionally, as -SH content was measured in plasma and under acute exercise-induced oxidative stress and damage liver export reduced glutathione (GSH), an important tripeptide with antioxidant properties containing an -SH group, to the blood to cope with tissue (mainly delivered to skeletal muscles) redox challenges [15,24]. Therefore, we should not exclude that the observed (possibly transient) increased thiol content could have resulted from the possible increased GSH export from the liver after the intense WOD.

Intense exercise-induced oxidative stress leads to DNA damage [9,40]. The present results demonstrated a significant increase in the levels of DNA damage after both WOD and treadmill running sessions ($p < 0.05$). However, when compared to WOD, the levels of DNA damage observed after treadmill running were significantly lower. Although scarce, biomonitoring data of HIIT practitioners demonstrated greater DNA damage after a typical training session [41]. Other studies also reported that intense physical exercise training increase DNA strand breaks with resulting compromised stability [42,43].

4. Materials and Methods

The experimental protocol was approved by the host University ethical committee (CEFADE 21—2019) and followed the Declaration of Helsinki of the World Medical Association for research with humans. Before participation, subjects signed an informed consent and were familiarized with the WOD training session and corresponding experimental protocol. Subjects were instructed not to change their normal nutritional habits, to refrain from additional vitamin or antioxidant dietary supplementation and to abstain from exhaustive exercise for three weeks before data collection and during the experimental protocol period.

Ten subjects (6 men and 4 women), with more than two years of CrossFit® training experience, volunteered to take part in the current study (Table 1).

One week before the experiments, body composition was evaluated using Dual Energy X-Ray absorptiometry (Hologic, Model Discovery WI, Hamburg, Germany). Thereafter, the participants performed an incremental treadmill (Quasar-Med, Nussdorf, Germany) test until voluntary exhaustion to determine maximal oxygen uptake (VO_2max) and HRmax. Expired respiratory gas fractions were measured using an open circuit breath-by-breath (K4b^2 , Cosmed, Rome, Italy).

Immediately before and after the CrossFit® session, 5 mL venous blood samples were collected from the antecubital vein in anticoagulant tubes (EDTA-K3 GV0414, Iberlab, Porto, Portugal). For medium-throughput alkaline Comet assay [18], an aliquot of blood samples was cryopreserved an equal amount of 1:4 (*v/v*) mixture of DMSO and RPMI 1640, then stored in aliquots (200 μL each) at -80°C until the day of analyze. Plasma was separated by centrifugation (CENCE L500, Hunan, China) at $3000 \times g$ for 10 min at 4°C , aliquoted and frozen at -80°C for later biochemical analysis.

Plasma AOC was spectrophotometrically measured using a commercial kit (Bio-QuoChem, KF-01-003, Madrid, Spain) and plasma content of total thiol groups were spectrophotometrically assessed at 412 nm [44]. Capillary blood samples were also collected (at the earlobe) before and immediately after the CrossFit® workout, and at 3, 5, and 7 min of the recovery period, for $[\text{La}^-]$ analysis (Lactate Pro2 analyzer, Arkay, Inc, Kyoto, Japan).

The CrossFit® WOD lasted 40 min and was divided into four blocks, each one including typical exercises performed in CrossFit® competitions (Table 2). Respiratory and pulmonary gas exchange data were measured breath-by-breath during the WOD session using a telemetric portable gas analyzer (K4b^2 , Cosmed, Rome, Italy) that was previously calibrated following the manufacturer instructions with gases of known concentrations (16% O_2 and 5% CO_2). HR was also continuously monitored during the entire WOD (Polar, Model FT1, Kempele, Finland).

One week after the WOD, the same participants went to the laboratory to perform a TR session. In this protocol, each subject was instructed to run for the time (40 min) and at a velocity equivalent to the mean oxygen consumption obtained during the CrossFit® WOD session. To authenticate running intensity, oxygen consumption was also continuously monitored during the running protocol, using the same metabolic equipment described above. We use the % VO_2max range (e.g., 91~100% VO_2max : Maximal, 64~90% VO_2max : Vigorous, 46~63% VO_2max : Moderate, 37~45% VO_2max : Light) to categorize exercise intensity. Moreover, HR was continuously recorded, and maximal blood lactate concentration was assessed in the end of the running period, as described above. In accordance with the methodology used in the WOD protocol, blood samples were collected immediately before and after the treadmill running session.

The studied variables are presented as mean \pm SD, with data normality evaluated by the Shapiro–Wilk test. As all the variables presented a normal distribution, an unpaired *t*-test was used comparing male and female characteristics. Repeated-measures ANOVA was used to establish whether total AOC, total thiols, and DNA damage data obtained after both sessions were significantly different from baseline results. Differences between WOD and TR regarding the mean VO_2 , HR, HRmax and $[\text{La}^-]$ values were assessed using a Student's paired *t*-test. All tests were performed using GraphPad Prism 6 and the significance level was set at 5%.

5. Conclusions

In summary, our results suggest that a WOD session induces an increased condition of oxidative injury and affects the antioxidant capacity in experienced CrossFit® performers. We can also speculate that mitochondrial electron transport chain and the overall oxidative metabolism could not be considered the preferred source of ROS production during a

CrossFit® WOD, being other mechanisms/sources, which may include XDH/XO, more plausible as relevant contributors to the observed redox alterations.

Practical Applications

Knowing the altered redox homeostasis in experimented CrossFit® performers after each WOD and given the central physiological role of ROS as signaling molecules essential for cellular adaptation to stress stimuli, including exercise training, caution should be recommended when complementary strategies, such as antioxidant supplementation, possibly masking training-induced adaptive redox-related alterations that ultimately lead to enhanced CrossFit® practitioners' physical condition, are considered.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee of CEFAD 21—2019.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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