Modification of Blood Redox Homeostasis by High-Intensity Interval Training

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ABSTRACT | Sprint interval training, a high-intensity interval training (HIIT) modality, is associated with improved plasma antioxidant capacity, which suggests that sprint interval training may have potential positive effects on health promotion. However, because its strenuous nature, sprint interval training may intimidate sedentary individuals. This study investigated the acute and chronic effects of HIIT on blood redox homeostasis, using a training protocol based on a higher number of bouts, of increased duration, performed at 90–110% of aerobic peak power. Seventeen healthy physically active young men performed a single HIIT session, in cycle ergometer, composed of eight bouts of 1 min at 90% of peak power, intercalated with 75 s-intervals of active recovery at 30 W. The plasma total antioxidant capacity was lower immediately after exercise (p = 0.05), an indicative of increased generation of reactive species in response to HIIT, and erythrocyte superoxide dismutase activity was increased (p = 0.03). No effect of acute HIIT session on blood thiobarbituric acid reactive substances (TBARS) content was observed. Nine of the initial seventeen volunteers engaged in a four-week progressive HIIT program, 3 days per week, totaling 12 training sessions. Each training session involved 8–12 cycling exercise bouts, at 90–110% peak power for 60 s with active recovery intervals of 75 s at 30 W. The plasma total antioxidant capacity as well as erythrocyte catalase activity were increased in response to training (p < 0.01 and p = 0.03, respectively), which may have contributed to the reduced TBARS content observed with erythrocytes (p = 0.05). Because HIIT improves blood redox homeostasis, it may have a potential therapeutic effect on the treatment of diseases related to redox imbalance, besides its health promotion effects.

KEYWORDS | Acute exercise; Aerobic training; Catalase; Erythrocyte; Ferric reducing ability of plasma; Oxidative stress; Superoxide dismutase

ABBREVIATIONS | CAT, catalase; FRAP, ferric reducing ability of plasma; GPx, glutathione peroxidase; HIIT, high-intensity interval training; MDA, malondialdehyde; RONS, reactive oxygen and nitrogen species;
1. INTRODUCTION

Reactive oxygen and nitrogen species (RONS) are continuously produced in our body as a consequence of normal cellular physiological processes [1, 2] and they regulate several key biological processes, including proliferation and differentiation [3, 4]. However, excess RONS can damage macromolecules, modify the cellular redox status, and alter cell functions [5]. The oxidative action of RONS is counteracted by an elaborate network of antioxidants, composed of enzymes, such as catalase (CAT) and superoxide dismutase (SOD), and non-enzymatic antioxidant compounds (e.g., vitamins C and E, uric acid, and glutathione, among others), that neutralizes RONS and maintains an adequate cellular redox status. An imbalance between RONS production and antioxidant system action can result in disruption of the cellular redox signaling and control and/or molecular damage, a condition known as oxidative stress [6].

Changes in redox homeostasis—a shift in the level of reactive species, oxidant biomarkers, antioxidants and/or redox active molecules [7]—are induced by many physiological and non-physiological stimuli, including acute aerobic and anaerobic exercises (reviewed in [8]). Acute exercise effect on human redox homeostasis was first suggested by Dillard et al. who reported a 1.8-fold increase in expired pentane, a biomarker of lipid peroxidation, by subjects submitted to a 60-min cycling at 50% of maximal oxygen consumption (VO\textsubscript{2} max) [9]. Later, Ashton et al., using electron spin resonance spectroscopic, directly demonstrated a three-fold increase in plasma free radical concentration after a maximum exercise [10]. This was accompanied by higher plasma malondialdehyde (MDA) and hydroperoxides levels, thus indicating that changes in redox homeostasis induced by exercise promote oxidative damage to lipids. Since then a series of studies have demonstrated that acute exercise, of different duration, intensity, and mode, results in alteration of the redox homeostasis [11–14]. It has been debated regarding the consequences of exercise-induced redox homeostasis alterations. While some studies have indicated that alteration of redox homeostasis may have detrimental effects on muscle force production and fatigue [15, 16], numerous other studies suggest that RONS produced during exercise also serve as a signal that regulates the molecular machinery controlling the expression of genes that elicits the appropriate adaptive response to exercise training [17–19]. The adaptive responses triggered by RONS include, amongst others, mitochondrial biogenesis [20, 21], hypertrophy [22], and upregulation of the antioxidant defense system [19] that copes muscle with an improved capacity for decreasing the adverse effects of increased ROS production. This upregulation in endogenous antioxidant defense is often associated with lower levels of oxidative stress biomarkers in trained individuals [18].

High-intensity interval training (HIIT) is a low volume, high-intensity exercise mode that involves...
alternating short bursts of high-intensity exercise with recovery periods of light exercise or rest. A HIIT session is characterized by a relatively brief intermittent burst of vigorous exercise (80–95% of VO2 peak or > 90% of maximum heart rate) interspersed with periods of low-intensity exercise or rest [23]. The total duration of a HIIT session tends to be short (30 min approximately) which reduces the total weekly exercise and training time compared to the current public health guidelines [24]. HIIT improves fitness and induces beneficial metabolic adaptations [24, 25], and also improves the cardiorespiratory fitness in a range of populations, including those with coronary artery disease or congestive heart failure, as well as those with obesity [26, 27]. Thus, HIIT can be a time-efficient intervention to improve metabolic health and reduce the risk of chronic diseases [28].

Despite the growing popularity of HIIT as a training modality for both non-athletes and patients, its effects on redox homeostasis were only marginally investigated. Until now there are only two studies that reported acute and chronic effects of sprint interval training (SIT) on redox homeostasis [29, 30]. Both studies demonstrated increased TBARS levels after a single exercise session, a response that was attenuated after the training period. SIT also resulted in increased activity of antioxidant enzymes in the plasma [30], which showed an adaptive antioxidant protective response to repeated sprint interval training sessions. More recently, Tossige-Gomes et al. [11], using a different HIIT protocol (eight bouts of 1 min at 90–100% of aerobic peak power intercalated with 75 s-intervals of active recovery at 30 W), reported lymphocyte redox imbalance in response to a single exercise session, suggesting that the redox homeostasis response to HIIT is dependent on the sample (the plasma or leukocytes) examined and/or the exercise protocol.

SIT is a high-intensity interval exercise modality that entails four to six 30 s maximal sprint bouts interspersed with four min of rest periods [31]. The characteristic strenuous nature of sprint interval training may intimidate sedentary individuals. It was recently demonstrated that SIT increases the experience of negative emotions and exertion in sedentary middle-age men [32] and that the rate of perceived exertion is higher during SIT compared to HIIT [33]. Evidence also suggests that HIIT elicits different metabolic responses compared to SIT [33], which can directly affect the exercise effect on the redox homeostasis. Therefore, this study investigated the acute and chronic effects of HIIT on blood redox homeostasis, using a training protocol based on a higher number of bouts (8–12) of increased duration (60 s) performed at 90–110% of the aerobic peak power. We hypothesized that acutely HIIT would disrupt blood redox homeostasis thus resulting in an adaptive response of the blood antioxidant system to the training.

2. METHODS

2.1. Subjects

Seventeen non-smoker young health male subjects were selected to participate in this study. Volunteers were recruited from the local community, based on the following criteria: not been engaged in a regular exercise program and not been in use of anti-inflammatory medications and antioxidant supplements. None of the subjects self-reported neuromuscular or musculoskeletal injuries, heart, or lung diseases. Volunteers were tested for glucose and cholesterol levels, and the physical activity readiness questionnaire (PAR-Q) was used to exclude individuals at risk for performing high-intensity exercise. The study was approved by the Internal Review Board of the Universidade Federal dos Vales do Jequitinhonha e Mucuri, and all the participants provided written informed consent.

2.2. Study Design

The acute and chronic effects of HIIT on blood redox homeostasis of non-trained men were evaluated on this study (Figure 1). Volunteers were initially submitted to preliminary evaluations that included anthropometric measurements, VO2 peak determination, and aerobic performance assessment. Then days after the initial evaluations, volunteers performed an acute HIIT session, and blood samples were collected before (pre-ex) and immediately after (post-ex) the exercise session. Then, volunteers initiated a 4-week HIIT program, and blood samples were collected 48 h after the last training session (post-HIIT). Samples collected before the acute HIIT session were used to evaluate the pre-training status (pre-HIIT). VO2 peak determination and aerobic performance assessment were repeated 72 h after the last training session.
2.3. Pre-HIIT Evaluations

Volunteers underwent anthropometric measurements (height and body mass) and had their body fat percentage estimated using dual X-ray absorptiometry (Lunar iDXA, GE, USA). Peak oxygen consumption (VO₂ peak) and maximum power output were assessed during a graded exercise test. Maximum exercise test was conducted on an electronically braked cycle ergometer (Excalibur Sport, Lode, USA) using the ramp protocol [34], as previously described [35]. The power was increased every 60 s at an individual rate based on the subjects’ exercise history questionnaire (Veterans Specific Activity Questionnaire) [36] to induce fatigue within 8 to 12 min. The test was completed when subjects could no longer maintain the required power, despite verbal encouragement. The greatest power value obtained by each subject was registered, and the VO₂ peak was estimated using the ACSM equation [37]:

\[
\text{VO}_2 \text{ peak (O}_2 \text{ ml}^{-1} \times \text{ min}^{-1}) = 10.8 \times \left[\text{work rate (kg} \times \text{min}^{-1}\right] / \text{body mass (kg)} + 7
\]

Forty-eight h after the maximum exercise test, the volunteers’ aerobic performance was evaluated by a 15-km cycling time trial [38]. The volunteers were instructed to cover 15 km in the shortest possible time, on a mechanically braked cycle ergometer (Ideal, Maxx, Brazil), with an initial load (in kg) corresponding to 50% of the maximum load (pedaling at 50 rpm) achieved in the VO₂ max test. During the time trial, the volunteers were free to change the cadence and, consequently, the power output. Every 3 km, the subject was informed of the distance covered, but no information was provided about test duration. Volunteers were not allowed to drink water during the test, and the time to cover every km and 15 km were registered. The tests were conducted in an environmental chamber controlled at 22°C dry bulb temperature and 60% relative humidity. Volunteers
performed familiarization sessions 48 h before VO₂ peak and aerobic performance tests. Both tests were repeated 72 h after the last HIIT session.

2.4. The Acute HIIT Session

Ten days after the last pre-HIIT evaluation, volunteers (n = 17) performed an acute HIIT session (between 7:30 AM and 8:30 AM) in an environmental chamber (22°C, 60% relative air humidity). Exercise was performed on a cycloergometer (Excalibur Sport, Lode, USA) and consisted of eight bouts of 1 min at 90% of peak power, intercalated with 75 s-intervals of active recovery at 30 W. Volunteers cycled for 2 min at 30 W before and after the exercise session. Before (pre-ex) and immediately after (post-ex) exercise, blood samples (approximately 10 ml) were collected from the antecubital vein and transferred to vacuum tubes containing ethylenediaminetetraacetic acid (EDTA) (Vacutainer, Becton Dickinson, USA) for separation of the plasma and erythrocytes and further analysis of blood redox homeostasis.

Volunteers were asked to refrain from strenuous physical activity for 24 h, caffeine consumption for 48 h, alcoholic beverages for 24 h, topical corticosteroid or aspirin use for 48 h, and systemic antihistamines or corticosteroid use for 1 week prior to the exercise session. Participants were also asked to sleep 8 h the night before the exercise session. In the morning of the tests, volunteers were asked to eat a standardized breakfast based on the American College of Sports Medicine [39], which recommends the intake of 200 to 300 g of carbohydrates before vigorous exercise. The intake of 500 ml of water 2 h before the test was also recommended [39].

2.5. The HIIT Program

The HIIT protocol was performed as described before [38]. Nine of the seventeen volunteers who performed the acute HIIT session trained in a cycle ergometer (Movement, BM2800PRO) for 4 weeks, 3 days per week, totaling 12 training sessions. Each HIIT session consisted of 8–12 cycling exercise bouts with intensity between 90 and 110% of the peak power for 60 s followed by active recovery of 75 s at 30W. Both cycling bouts (8–12) and exercise intensity (90–110% of the peak power) were increased progressively during the training period. During every HIIT session, heart rate and rating of perceived exertion were measured by the end of each bout and after active recovery. Forty-eight h after the last training session (post-HIIT), blood samples (approximately 10 ml) were collected as described before. Blood samples collected before the acute HIIT session (pre-HIIT) were used to evaluate blood redox status before training.

2.6. Blood Redox Homeostasis

Both the plasma and erythrocytes lysates were used to evaluate blood redox status. Blood was centrifuged (1000 g, 10 min) and the plasma was removed and stored at −80°C until assay. The leukocyte buffy coat was removed and discharged, and the erythrocyte lysate was prepared as previously described [40]. Thiobarbituric acid reactive substances (TBARS) content and ferric reducing ability of plasma (FRAP) were evaluated as previously described [41]. Briefly, TBARS formation during an acid-heating reaction [42] was determined at 532 nm, based on a standard curve with known concentrations of malondialdehyde (MDA) (1,1,3,3-tetramethoxypropane) (Sigma, MO, USA). The amount of MDA produced was interpreted as the TBARS levels. The results are expressed as MDA equivalents per mg protein (MDA/mg). Plasma capacity to reduce the ferric-tripyrtdyltriazine [Fe(III)-TPTZ] complex to ferrous tripyridyltriazine [Fe(II)-TPTZ] was measured at 550 nm [43]. A standard curve of known concentrations of FeSO₄ was used to estimate FRAP. The results are expressed as Fe(II) equivalents per mg protein.

Erythrocyte TBARS content and SOD and CAT activities were determined as described before [44]. SOD activity was determined using the assay based on the inhibition of pyrogallol autoxidation, monitored spectrophotometrically at 420 nm for 4 min [45]. SOD activity was calculated as units per mg protein (U/mg), and one unit of enzyme was considered as being the amount that caused 50% inhibition of pyrogallol autoxidation. CAT activity was estimated by monitoring the H₂O₂ decomposition, during 1 min, at 240 nm (ΔE), using a spectrophotometer [46]. Catalase activity was expressed as the relative amount of H₂O₂ decomposed per min per mg protein (ΔE/min/mg).

The protein concentration of samples was determined by the Bradford method [47] using bovine serum albumin (BSA) (1 mg/ml) as a standard. All measurements were performed in triplicate.
2.7. Statistical Methods

The GraphPad Prism (version 6.00 for Mac OS X, GraphPad Software, San Diego, CA, USA) was used for statistical analysis. Data are reported as the mean ± SD. The Shapiro–Wilk test was used to assess the normality of the data. Paired Student’s t-test (two-tailed) was performed to test the acute and chronic HIIT effects on blood redox status. A significance level of p ≤ 0.05 was used.

3. RESULTS

Seventeen young non-trained health men performed a single HIIT session (Table 1) to evaluate the acute effect on blood redox homeostasis (Figure 2). The acute HIIT session had no effect on plasma TBARS content, but the plasma total antioxidant capacity was reduced (p = 0.05) immediately after exercise (Figure 2A and 2B). The SOD activity of erythrocytes was increased (p = 0.04) after a single HIIT session (Figure 2D). The acute HIIT session had no effect on either erythrocyte TBARS content (Figure 2C) or CAT activity (Figure 2E).

Nine of the 17 volunteers who performed the acute HIIT session underwent 4 weeks of HIIT (12 training sessions) in order to evaluate whether it would induce adaptations on blood redox homeostasis. Physical and physiological characteristics of the 9 volunteers who trained were not different from the initial group of 17 volunteers who performed the acute HIIT session (data not shown). As shown in Table 2, after the HIIT, the time spent to complete the 15-km time trial was reduced, which demonstrated an improvement in the aerobic performance of the volunteers in response to the training program. Also, VO₂ peak after the HIIT program was higher and mean peak HR in the last training session was reduced, indicating cardiovascular adaptations to HIIT.

HIIT induced adaptations on blood redox homeostasis (Figure 3). The plasma TBARS content was not different (p = 0.06), but the plasma total antioxidant capacity was elevated (p < 0.05) after HIIT (Figure 3A and 3B). The TBARS concentration in erythrocytes was reduced (p = 0.05) after training and, in contrast, CAT activity was increased (p = 0.03) (Figure 3C and 3E). Different from the response to the acute session, HIIT did not modify erythrocyte SOD activity (Figure 3D).

4. DISCUSSION

We investigated whether blood redox homeostasis is acutely and chronically affected by HIIT. After a single HIIT session, plasma TAC as well as erythrocyte TBARS content were reduced, whereas erythrocyte SOD activity was increased. In response to the training, plasma TAC and erythrocyte CAT activity were increased and erythrocyte TBARS content was reduced. Our results thus demonstrate an acute modification and an adaptive response of blood redox homeostasis to HIIT.

Others have previously investigated the acute and chronic effects of low volume high-intensity exercise on plasma redox homeostasis. Different from our findings, using a SIT protocol, Fisher et al. [29] and Bogdanis et al. [30] reported increased plasma TBARS content after a single exercise session. On the other hand, Bloomer et al. [48] and Farney et al. [49] reported no alteration in plasma MDA or protein carbonyl content of trained men after 5–6 maximal sprints. We also did not observe modification of plasma TBARS, but plasma TAC was reduced immediately after the acute HIIT session.

Reduction of plasma TAC can be interpreted as an indication of redox homeostasis modification by the exercise session. This assay measures the ferric reducing ability of the sample, which is mainly attributed to the plasma content of uric acid, ascorbic acid, alpha-tocopherol, proteins, and bilirubin [43]. Because the neutralization reaction performed by many of the antioxidants present in the plasma involves the reduction of the RONS, these antioxidants may be temporarily unavailable to reduce the ferric-TPTZ complex to its ferrous form [Fe(II)-TPTZ] if RONS have been produced during HIIT, thus resulting in reduced antioxidant capacity. Neutralization of RONS produced during exercise by plasma antioxidants, as suggested by our results, may have protected plasma components, including lipids, from oxidative injuries, which may explain why we did not observe modification of plasma TBARS content in response to the acute HIIT session. Also, differences between our data and others previously published [29, 30] can be due to different exercise protocols employed. As recently demonstrated, HIIT and SIT evoke different metabolic responses, including post-exercise blood lactate response [33], which may contribute to the exercise effect on the redox homeostasis response.
We also investigated the effect of a single HIIT session on erythrocyte redox homeostasis because these cells are significantly susceptible to oxidative damage due to the high amount of membrane polyunsaturated fatty acids and the high concentrations of oxygen and hemoglobin. To our knowledge, no other study has investigated how erythrocyte redox homeostasis is affected by HIIT, both acutely and chronically. We found that erythrocyte SOD activity was increased after a single HIIT session and the exercise session had no effect on erythrocyte TBARS content. Others reported no effect or increased TBARS content in erythrocytes in response to continuous high-intensity exercises [50]. We hypothesize that both reduced plasma TAC and increased SOD activity after HIIT may protect erythrocytes from oxidative damage, which may account for the TBARS result observed. Thus, we showed that although acutely HIIT had increased RONS generation, the blood antioxidant system of untrained physically active men

| TABLE 1. Characterization of the volunteers who performed the acute HIIT session |
|---------------------------------|-----------|
| Age (years)                     | 23.5 ± 3.1|
| Weight (kg)                     | 68.7 ± 9.2|
| Height (cm)                     | 170.6 ± 7.0|
| Body fat (%)                    | 23.5 ± 5.2|
| BMI (kg/m²)                     | 23.4 ± 2.1|
| Blood glucose (mg/dl)           | 87.0 ± 8.1|
| Blood cholesterol (mg/dl)       | 184.2 ± 30.1|
| VO2peak (ml·kg⁻¹·min⁻¹)         | 38.3 ± 5.1|
| Maximum power output (watts)    | 257.5 ± 59.8|

Note: Data are expressed as mean ± SD (n=17). BMI, body mass index.

FIGURE 2. The effect of an acute HIIT session on blood redox homeostasis of health young men. (A) plasma TBARS content; (B) plasma total antioxidant capacity; (C) erythrocyte TBARS content; (D) erythrocyte SOD activity; (E) erythrocyte CAT activity. n = 17. *p ≤ 0.05, compared to pre-ex, paired Student’s t-test. TBARS, thiobarbituric acid reactive substances; SOD, superoxide dismutase; CAT, catalase.
could cope with the exercise-induced redox stress, avoiding oxidative damage to both plasma and erythrocyte lipids. However, for a more comprehensive picture of the acute effect of HIIT on blood redox homeostasis, the analysis of additional biomarkers, such as protein carbonyl, is recommended.

Both anaerobic and continuous aerobic training induce adaptations in a variety of systems, including the antioxidant system [27, 51, 52]. Erythrocyte alpha-tocopherol content, for example, as well as other indexes of antioxidant capacity were shown to be greater in running individuals [53]. Also, the activity of SOD and glutathione peroxidase (GPx) in erythrocytes of young males were increased after 12 weeks of running at 80% of maximal heart rate, for 60 min, 5 days/week [18]. We showed, in this study, that four weeks of HIIT resulted in improved blood redox status of young healthy men, as indicated by increased plasma TAC and erythrocyte CAT activity. Bognnais et al. [30] have previously shown increased plasma total antioxidant capacity and CAT and GPx activity after 8 sessions of SIT, but to our knowledge our study is the first report to show that HIIT improves erythrocyte antioxidant capacity. It has been proposed that exercise training effect on antioxidant defense can protect cells/tissues against RONS oxidative effects and thus decrease the accumulation of oxidative damage [54]. The training effect on erythrocyte TBARS content is indicative that the adaptive response of the antioxidant defenses to HIIT contributed to the protection against oxidative stress. This protective response induced by HIIT may be physiologically relevant due to the greater susceptibility of erythrocytes to oxidative damage.
It has been suggested that exercise-induced adaptive response in antioxidant defenses can be explained by the hormesis theory, which proposes that chemicals and toxic substances, such as RONS generated during exercise, may have a low-dose stimulatory effect [55]. It is well known that RONS are initiators of many redox-sensitive pathways involved in the induction of antioxidant enzymes [27], and thus, RONS generate during HIIT, as shown by reduced plasma TAC after the single exercise session, can contribute to the later adaptive effect on the antioxidant system.

Our findings showed that low volume HIIT, as well as continuous endurance training, improved blood enzymatic antioxidant defense system and thus acts as an antioxidant modality. It will be important to evaluate whether HIIT also induces antioxidant adaptation and protection against oxidative damage in other tissues. As demonstrated by Veskoukis et al. [56], oxidative stress biomarkers measured in blood in response to exercise have a very strong correlation with the response in skeletal muscle. So, it is reasonable to hypothesize that HIIT will probably induce beneficial antioxidant adaptations on other sites, such as skeletal muscle. We have recently observed reduced TBARS and increased non-enzymatic antioxidant response in the brain of rats after 8 weeks of HIIT [57]. Oxidative stress is an important pathological component of many diseases, including cardiovascular disease and metabolic syndrome [58, 59]. HIIT is now being employed to improve health condition in these situations, and modulation of the antioxidant system can be an important contribution of HIIT to the management of these diseases.

5. CONCLUSION

A single session of high-intensity interval exercise modified blood redox homeostasis, resulting in adaptation of the blood antioxidant system in healthy young men after four weeks of HIIT.

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REFERENCES

10. Ashton T, Rowlands CC, Jones E, Young IS, Jackson SK, Davies B, et al. Electron spin resonance spectroscopic detection of oxygen-


27. Angadi SS, Mookadam F, Lee CD, Tucker WJ,


10.1371/journal.pone.0167593.


