Lymphocyte enzymatic antioxidant responses to oxidative stress following high-intensity interval exercise

G. Fisher,1 D. D. Schwartz,2 J. Quindry,1 M. D. Barberio,1 E. B. Foster,1 K. W. Jones,3 and D. D. Pascoe1

1Department of Kinesiology, Auburn University, Auburn; 2Department of Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine, Auburn University, Auburn; and 3Clinical Laboratory Sciences, Auburn University at Montgomery, Montgomery, Alabama

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Fisher G, Schwartz DD, Quindry J, Barberio MD, Foster EB, Jones KW, Pascoe DD. Lymphocyte enzymatic antioxidant responses to oxidative stress following high-intensity interval training (HIIT). 1) determine changes in lymphocyte enzymatic antioxidant activity in lymphocytes following HIIT; and 2) assess pre-HIIT, 3-h post-HIIT, and 24-h post-HIIT lymphocyte viability following oxidative stress exposure in vitro. Eight recreationally active males completed three identical HIIT protocols. Blood samples were obtained at preexercise, immediately postexercise, 3 h postexercise, and 24 h postexercise. Total number of circulating leukocytes, lymphocytes, and neutrophils, as well as lymphocyte antioxidant enzyme activities, gene expression, cell viability (CV), and plasma thiobarbituric acid-reactive substance (TBARS) levels, were measured. Analyses were compared using a three (day) × four (time) ANOVA with repeated measures on both day and time. The a priori significance level for all analyses was P < 0.05. Significant increases in superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) activities were observed in lymphocytes following HIIT. No significant increases in lymphocyte SOD, CAT, or GPX gene expression were found. A significant increase in TBARS was found immediately post-HIIT on days 1 and 2. Lymphocyte CV in vitro significantly increased on days 2 and 3 compared with day 1. Additionally, there was a significant decrease in CV at 3 h compared with pre- and 24 h postexercise. These findings indicate lymphocytes respond to oxidative stress by increasing antioxidant enzyme activity. Additionally, HIIT causes oxidative stress but did not induce a significant postexercise lymphocytopenia. Analyses in vitro suggest that lymphocytes may become more resistant to subsequent episodes of oxidative stress. Furthermore, the analysis in vitro confirms that lymphocytes are more vulnerable to cytotoxic molecules during recovery from exercise.

oxidative stress; antioxidant enzymes; high-intensity interval training; lymphocytes; immunosuppression

Routine moderately intense exercise stimulates the immune system and enhances resistance to infectious diseases (27, 28). However, during prolonged/strenuous exercise or a heavy schedule of training and competition, there is often immunosuppression during the recovery period (23). The postexercise immune response is similar to that seen in both infection and inflammation, consisting of both neutrophilia and lymphocytopenia (35, 37). The mechanisms associated with these exercise-induced immune changes include oxidative stress, as well as neuroendocrine factors such as catecholamines, growth hormone, and cortisol (28). While there is substantial evidence linking prolonged/strenuous exercise to oxidative stress and immunosuppression, comprehensive investigation of postexercise inflammation, endogenous antioxidant response interactions is lacking.

Oxidative stress occurs when reactive oxygen species (ROS)/reactive nitrogen species (RNS) production and antioxidant defenses become imbalanced. Redox regulation via ROS/RNS and the antioxidant defenses represents a tightly controlled system that can have both deleterious and beneficial effects within the cellular environment. For instance, low concentrations of ROS upregulate the expression of superoxide dismutase (SOD) and glutathione peroxidase (GPX) in skeletal muscle (15). Catalase (CAT), SOD, and GPX present in mononuclear cells scavenge both exogenous and endogenous ROS. Additionally, lymphocyte SOD and GPX activities increase in response to acute oxidant exposure in vitro and following exhaustive endurance exercise, while neutrophils exhibit decreased antioxidant enzyme activities following intense exercise (3, 35–37). Considering that acute exercise increases ROS production (7, 18) and immune cells express antioxidants, it is important to understand the integrated dynamics between oxidative stress and immune cell function. Increases in oxidative stress during exercise and the subsequent immune response during recovery from exercise provide a unique opportunity to examine the relationship between stress and immunity. Therefore, using exercise as a model to induce stress that yields both an increase in ROS and an acute immune response may facilitate a better understanding of relationships between these two systems.

Traditional endurance training (ET) [55%-65% maximal oxygen consumption (V̇O2max), 60–120 min] induces a variety of beneficial adaptations, while large doses impose deleterious oxidative stress and immunosuppressive responses (34, 41). While ET yields long-term adaptations to improve health and longevity, it has also been shown to be a double-edged sword: when performed for a prolonged duration or at a strenuous intensity, it can cause oxidative stress and suppress the immune system (18, 23). High-intensity interval training (HIIT), by contrast, involves brief intermittent bouts of exercise (usually ≥90% of V̇O2max) separated by a few minutes of rest or low-intensity exercise during the recovery period (14). Recent investigations demonstrate that HIIT stimulates muscular and vascular adaptations previously associated with ET (4, 5, 13, 32). Specifically, HIIT has been shown to stimulate mitochondrial biogenesis and oxidative enzyme activity (13, 14, 19). HIIT also improves peripheral arterial stiffness, GLUT4 expression, and insulin sensitivity (19, 32). Perhaps the most

Address for reprint requests and other correspondence: G. Fisher, Dept. of Nutrition Sciences, Univ. of Alabama at Birmingham, 1675 University Blvd., WEBB 413, Birmingham, AL 35294 (e-mail: grdnfs@uab.edu).
unique aspect of these HIIT findings is the fact that they transpire in response to lower weekly training time and volume compared with ET (12). Very little information exists regarding the postexercise inflammation response to HIIT. Therefore, the purposes of this study were to investigate the acute immune and oxidative stress response during 1 wk of HIIT. A further objective was to ascertain whether changes in lymphocyte antioxidant gene expression and enzyme activity occurred following HIIT, and if these changes augment lymphocyte cell viability in vitro.

METHODS AND PROCEDURES

Participants

Male volunteers aged 19–35 yr meeting the following inclusion criteria were invited to enroll in the study: 1) peak oxygen consumption (VO\textsubscript{2peak}) between 35 and 55 ml·kg\textsuperscript{-1}·min\textsuperscript{-1}, 2) healthy, as determined by Medical Par Q Screening; 3) currently engaging in no more than 3 days/wk of moderate strength and/or endurance training (self-reported); and 4) currently not taking any antioxidant supplementation or nonsteroidal anti-inflammatory drugs (NSAIDs).

Preliminary Procedures and Assessments

Qualifying volunteers were scheduled for an initial visit to the Auburn University Thermal Lab for further screening. On arrival, volunteers were provided with and asked to sign an institutionally approved consent document before any screening processes. Volunteers meeting all of the inclusion criteria then continued with anthropometric measurements and preliminary physiological assessments.

Physiological Assessment

Anthropometric measurements including height and weight were obtained. Height was determined to the nearest 0.25 in. with a stadiometer, and weight was measured to the nearest 0.25 kg using a calibrated scale (Michelli Scales, Harahan, LA). Three-site skinfold assessment was obtained using skinfold calipers (Lange Skinfold Caliper, Beta Technology, Cambridge, MD), and body density was calculated. Participants performed a 30-s Wingate maximal anaerobic power test on a cycle ergometer with resistance determined by body weight (0.075 kp/kg body wt) during the first visit and then performed an incremental VO\textsubscript{2peak} test on an electronically braked cycle ergometer (Quinton Excalibur, Quinton Instrument). Participants performed a HIIT protocol, consisting of three exercise sessions separated by 24 h postexercise each day.

Blood Sampling Procedures

Participants reported to the lab on testing days after an overnight fast. A venous butterfly needle (23 ga × 3/4; Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) was inserted into a superficial vein in the antecubital region of the arm and then capped with an intermittent injection port (Kawasumi Laboratories, Tampa, FL). Venous blood samples were collected with suitable vacutainers with EDTA as the anticoagulant. Immediately following blood draw, fresh whole blood was used to quantify leukocytes, lymphocytes, neutrophils, hemoglobin, and hematocrit using an automated analyzer system (Coulter LH750, Hematology Analyzer). Hemoglobin and hematocrit concentrations from whole blood samples were used to estimate plasma volume shifts resulting from the exercise sessions via the method of Dill and Costill (8).

Lymphocyte Isolation

Blood (8 ml) was collected in EDTA tubes mixed with an equal volume of phosphate-buffered saline and carefully layered onto a lymphocyte separation solution (8 ml) and centrifuged for 20 min at 400g at 22°C for 30 min. The lymphocyte layer was removed and the slurry was washed twice with phosphate-buffered saline and centrifuged for 10 min at 260g at 22°C. It is important to note this isolation procedure yields 20–25% of monocytes and 75–80% of lymphocytes (we will use the general term lymphocytes throughout the manuscript). The isolated lymphocytes were 1) used for gene expression studies, 2) used for antioxidant enzyme activity assays, or 3) cultured in RPMI 1640 medium/10% fetal bovine serum and 2% phytohemagglutinin (PHA-GIBCO) for cell viability studies.

Gene Expression in Lymphocytes

Real-time PCR analyses of CuZn-SOD, Mn-SOD, GPX, and CAT were performed in lymphocyte RNA samples. Total lymphocyte RNA yields 20–25% of monocytes and 75–80% of lymphocytes (we will use the general term lymphocytes throughout the manuscript).

Table 1. Baseline physiological characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>22 ± 2</td>
<td>21</td>
<td>28</td>
</tr>
<tr>
<td>Height, cm</td>
<td>181 ± 4.8</td>
<td>177</td>
<td>187</td>
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<tr>
<td>Weight, kg</td>
<td>83.0 ± 13.6</td>
<td>68</td>
<td>112</td>
</tr>
<tr>
<td>Body fat, %</td>
<td>12 ± 6</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>VO\textsubscript{2peak}, ml·kg\textsuperscript{-1}·min\textsuperscript{-1}</td>
<td>44.6 ± 8.2</td>
<td>32.0</td>
<td>55.0</td>
</tr>
<tr>
<td>Wingate, W</td>
<td>871 ± 134</td>
<td>670</td>
<td>1,066</td>
</tr>
</tbody>
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All values are presented as mean ± SD and minimum and maximum values. VO\textsubscript{2peak} peak oxygen consumption.

On arrival to the lab, participants provided a urine sample for determination of hydration status. Samples were analyzed for specific gravity using a refractometer (American Optical, Keene, NH), and the participant was cleared to exercise if urine specific gravity was <1.020 g/ml. Venous blood samples were taken from the antecubital vein before exercise, immediately postexercise, 3 h postexercise, and 24 h postexercise each day.

Exercise Conditions

Participants performed a HIIT protocol on an electrically braked cycle ergometer (Quinton Excalibur, Quinton Instrument). Participants performed a 20-min protocol, consisting of 4 min of cycling at 15% of maximum anaerobic power (Max-AP) followed by 30 s at 90% of Max-AP. These workloads were based on pretrial Wingate tests. This cycle was repeated four times within each protocol, ending with 2 min at 15% of Max-AP. It should be noted that the cycle ergometer did not transition wattages in a square-wave fashion. Wattage increased/decreased at each power transition at a rate of 150 W/s.

Experimental Procedures

Participants performed a HIIT protocol, consisting of three exercise sessions separated by 48 h, over the course of 1 wk. Participants were asked to refrain from other physical activity during the course of the study and to continue similar dietary practices. Participants performed each exercise session at the same time of day. Participants arrived to the laboratory for exercise following an overnight fast. They refrained from food and beverage consumption (with the exception of water) until the 3-h postexercise blood sampling period.
was extracted using the RNeasy Plus Kit according to the manufacturer’s instructions (Qiagen, Valencia, CA). Complementary cDNA was synthesized using iScript first-stand synthesis kit (Bio-Rad) containing 200 ng total RNA. Real-time PCR was performed on a myiQ real-time PCR detection instrument using SYBR green. The gene-specific primers used for real-time PCR were as follows: superoxide dismutase 1 Cu/Zn-SOD (accession no. AY450286), forward 5′-AGGCTCTCGACATCTCGAA, reverse 5′-CTACAGGTACCTTAAAGACACTT; superoxide dismutase 2 Mn-SOD (accession no. BC035422.1), forward 5′-GGCCTGATTATCTAAAAGC, reverse 5′-CGATCGTGGTTTACTTTTTGCA; GPX (accession no. NM_002084), forward 5′-TTCGCCGTGCAAC-CAGTTTG, reverse 5′-TTACCTCGCACCCTCTCGAA; and catalase (accession no. NM_001752), forward 5′-TTTTCCCCAGGAGATGCTTGAC, reverse 5′-ACCTTGGTGAGATCGAATGG. The thermocycler was programmed to an initial denaturation at 94°C for 10 min, followed by 35 cycles of 94°C for 15 s, 60°C for 30 s and 68°C for 1 min. Melt curve analysis showed the presence of 1 amplon, and no primer dimerization. PCR efficiency was determined by 10-fold cDNA dilutions followed by real-time PCR. Data were normalized to two housekeeping genes: glyceraldehyde 3-phosphate dehydrogenase, forward 5′-GCAAAATTCATGCGACCGCT, reverse 5′-GCCCCACCTGATTTTGGAGG; and acidic ribosomal phosphoprotein Po (Arhp) (accession no. X03025), forward 5′-CG-GCTACCAACATCCAAAGGAA, reverse 5′-GCTGGAATTCCGG-GCTGCA. Data were analyzed using a modification of the ΔΔCt method as described by Vandesompele et al. (39), and gene expression was expressed as a fraction of that occurring preexercise.

Lymphocyte Antioxidant Enzyme Activities

The activities of SOD, CAT, and GPX were measured in lymphocytes. All activities were determined with a SmartSpec plus spectrophotometer (Bio-Rad, Hercules, CA). Cell numbers were calculated for each sample. Cell samples were ruptured via the addition of 500 µl of deionized H2O per 2 × 10^6 cells. All assays were performed in duplicate.

Total SOD activity was measured using the method of McCord and Fridovich (21). This assay utilizes the xanthine/xanthine oxidase method. Cytosol (20 µl) of the solution was reduced in the presence of O2^- and was monitored at an absorbance of 550 nm. CAT activity was measured by the spectrophotometric method of Aebe (1) based on the decomposition of hydrogen peroxide (H2O2). This reaction depends on the concentration of H^+ donor and the steady-state concentration or rate of production of H2O2 in the solution. The active catalase-H2O2 complex I is formed first. In the presence of O2·− decomposition of H2O2 was read at an absorbance of 240 nm. GPX and the steady-state concentration or rate of production of H2O2 in the decomposition of H2O2, in which a second molecule of H2O2 serves as the H+/H2O2

Lymphocyte Cell Viability Studies

Lymphocyte samples from preexercise, 3 h postexercise, and 24 h postexercise for each exercise session were cultured in RPMI 1640 medium containing 10% fetal bovine serum and 2% phytohemagglutinin. Lymphocytes (1 × 10^6 cells/ml) were cultured and exposed to H2O2 (200 µM–1 mM) or its vehicle for 4 h. Cell viability was determined by the Trypan blue exclusion assay. Following the 4-h incubation period, the culture medium was removed from the culture plate and placed into 6-ml centrifuge tubes. Cells were centrifuged at 500 g for 10 min at 22°C. The supernatant was aspirated and the cell pellet was resuspended in 1 ml PBS. Ten microliters of cell suspension was mixed with 10 µl of Trypan blue dye, and the numbers of live and dead cells were assessed using a hemocytometer (on average 200 cells were counted). Cell viability was calculated using the following equation: viable cell % = (total number of viable cells per ml of aliquot)/(total number of cells per ml of aliquot) × 100.

Statistical Analysis

A three (day) × four (time) ANOVA with repeated measures on both day and time was used to compare superoxide dismutase, catalase, glutathione peroxidase (enzyme activity), TBARS, total leukocyte number, neutrophil number, lymphocyte number, and cell viability for each day. Gene expression of antioxidant enzymes was compared with day 1 preexercise expression. Gene expression was also assessed within days using preexercise expression as control. Significant differences were further explored utilizing a Tukey post hoc analysis. The significance level for this study was set at P < 0.05.

RESULTS

Participants

In total, nine male volunteers met the study inclusion criteria and agreed to participate in the study. One participant withdrew from the study because of time commitment; therefore eight participants completed the entire study protocol. Participant baseline physiological characteristics are presented in Table 1.

Exercise Intervention

All participants were able to complete the three exercise trials. Each exercise session lasted ~20 min and was identical in power settings and cycle ergometer settings for each participant. Participants completed the exercise protocol following an overnight fast. Exercise was performed during the same time of day and was separated by 48 h. Percentage of VO2peak was based on each participant’s power output at VO2peak.

Effect of Exercise on Leukocyte, Neutrophil, and Lymphocyte Counts

Leukocyte, neutrophil, and lymphocyte cell numbers for pre-, post-, 3 h post-, and 24 h postexercise for each of the three exercise days are depicted in Fig. 1. There was no significant difference in cell number between exercise days for any of the cell types. Circulating leukocytes increased significantly immediately post- and 3 h postexercise compared with preexercise and returned to baseline by 24 h postexercise on days 1 and 2, while day 3 only showed a significant increase immediately postexercise. Neutrophil number increased significantly immediately post- and 3 h postexercise compared with preexercise on days 1 and 2, while day 3 was only significantly increased
Lymphocyte number increased significantly immediately postexercise compared with preexercise and returned to preexercise values by 3 h postexercise on days 1, 2, and 3. A significant decrease in lymphocytes from preexercise values was observed 24 h postexercise on day 2.

Plasma Oxidative Stress Determinant Following Exercise

Oxidative stress was measured indirectly using a TBARS assay kit. Plasma TBARS levels were determined preexercise, postexercise, and 3 h postexercise for each day of exercise (Fig. 2). TBARS levels were significantly elevated immediately postexercise compared with preexercise on days 1 and 2. A significant decrease from preexercise on day 2 was observed 24 h postexercise on day 2.

Lymphocyte Antioxidant Gene Expression Following Exercise

Antioxidant gene expression for CuZn-SOD, Mn-SOD, CAT, and GPX were assessed in lymphocytes following HIIT. Gene expression was assessed between days and within days. All changes in gene expression between days were compared with preexercise on day 1 (control value). To determine gene expression changes within days, all 3-h postexercise values were compared with preexercise expression. No significant differences in antioxidant gene expression were observed at any of the sampling times. Antioxidant gene expression comparing each day and time point with preexercise day 1 is shown in Fig. 3.

Changes in Lymphocyte Antioxidant Enzyme Activities Following Exercise

SOD, GPX, and CAT enzyme activities were determined in lymphocytes following three HIIT sessions (Table 2). Enzyme activities were determined preexercise, postexercise, 3 h postexercise, and 24 h postexercise.

CAT. CAT activity was significantly elevated from pre- to postexercise on day 1 (95.4 ± 89%) and day 2 (66 ± 76%) (P < 0.05). No significant differences in CAT activity were found at any other sampling times.

SOD. There were no significant differences in activity between days 1, 2, or 3 at any sample time for SOD. SOD activity was significantly elevated postexercise on day 1 (45.6 ± 23%), day 2 (41 ± 83%), and day 3 (50 ± 34%), and 3 h postexercise day 1 (44 ± 11%), day 2 (33 ± 88%), and day 3 (36 ± 22%)

Fig. 1. Leukocyte, neutrophil, and lymphocyte changes following high-intensity interval training (HIIT). Values are reported as means ± SE. *Significant increase from preexercise. **Significant increase from preexercise on days 1 and 2. #Significant increase from preexercise. ##Significant decrease from preexercise on day 2. †Significant increase from preexercise on days 1 and 2. ‡Significant increase from preexercise.

Fig. 2. Thiobarbituric acid reactive substances (TBARS) following HIIT. Values are reported as means ± SE. *Significant increases from preexercise levels. MDA, malondialdehyde.

Fig. 3. Effect of exercise on antioxidant gene expression in lymphocytes. Values are reported as means ± SD. Gene expression was measured using RT-PCR. Pre-day 1 exercise was arbitrarily referred to as 1. All changes in gene expression were compared with Pre-day 1. No significant increases in gene expression were observed. CAT, catalase; SOD, superoxide dismutase; GPX, glutathione peroxidase.
compared with preexercise levels ($P < 0.05$). SOD activity returned to preexercise levels by 24 h.

GPX. GPX activity was significantly elevated from pre- to postexercise on day 1 (67 ± 18%) and day 3 (36 ± 24%) ($P < 0.05$). No significant differences were found pre- to postexercise on day 2. Additionally, postexercise GPX activity was significantly elevated from 3 h and 24 h postexercise on day 1, day 2, and day 3 ($P < 0.05$). GPX activity returned to preexercise levels by 3 h postexercise.

Effect of Exercise on Lymphocyte Viability Following H$_2$O$_2$ Treatment In Vitro

Each concentration of H$_2$O$_2$ treatment was analyzed independently to determine if cell viability improved between days 1, 2, and 3 following treatment. A significant increase in cell viability was observed at day 2 and day 3 compared with day 1 during the 3-h postexercise period for the 1 mM H$_2$O$_2$ challenge ($P < 0.05$). Cell viability data 3 h postexercise are presented in Fig. 4. There were no significant differences observed between the other three concentrations.

To determine if cells were less resistant to an oxidative insult at a particular time period pre- and postexercise, we used the mean cell viability following the 1 mM H$_2$O$_2$ challenge for all 3 days at preexercise, 3 h postexercise, and 24 h postexercise. Cell viability was significantly reduced at 3 h postexercise compared with preexercise and 24 h postexercise (P < 0.05). Cell viability at each time period is presented in Fig. 5.

DISCUSSION

This is the first study to examine postexercise oxidative stress to high-intensity cycle exercise and subsequent antioxidant fortifications within the immune system. Specifically, the purposes of this study were to examine the acute immune and oxidative stress responses following 1 wk of HIIT. The key finding of this study is that within the context of acute post-HITT exercise inflammation and oxidative stress, changes in antioxidant enzyme activity and cell viability were observed in lymphocytes. Specific study findings indicate 1) HIIT did not induce a postexercise lymphocytopenia as seen following traditional ET; 2) HIIT did elicit oxidative stress; 3) lymphocyte adaptations to HIIT include increases in SOD, GPX, and CAT activity, but not increased gene expression; and 4) analysis of cell viability in vitro revealed lymphocytes were more resistant to H$_2$O$_2$ on days 2 and 3 of exercise compared with day 1, and lymphocytes were least resistant to the H$_2$O$_2$ challenge during the postexercise recovery period. A cornerstone of the current experimental design was to examine the oxidative stress and immune system responses to a serial regimen of HITT exercise trials. Given the many beneficial adaptations observed with HITT exercise, we sought to understand whether this approach would also avoid the well-established immunosuppressive effects of ET (23, 24, 27, 36, 37). By extension, the current post-HITT findings are consistent with previous investigations and demonstrated a 44% increase in circulating leukocytes immediately postexercise, a 79% increase in circulating neutrophils 3 h postexercise, and a 72% increase in circulating lymphocytes immediately postexercise. However, the lymphocytopenia was not observed during the recovery period, as lymphocytes returned to preexercise values at 3 h post-HIIT. This significant finding, as recently shown, demonstrates that HIIT induces short-term increases in both aerobic and anaerobic conditioning (4, 5, 13, 14). Thus HIIT may be an ideal mode of training for improving health, with less immunosuppression compared with ET. It is also worth noting in the current exercise protocol that the 48-h recovery between HITT trials provided an avenue to achieve a HIIT stimulus without overtraining effects. However, the authors acknowledge that the acute immune responses following 1 wk of HIIT may not represent the immune response following...
chronic HIIT. Therefore, further work is needed to determine the long-term effects of HIIT on immunity.

The postexercise oxidative stress response is less well defined compared with postexercise immune function. However, recent well-controlled studies have demonstrated that high-intensity exercise is associated with a marked rise in oxidative stress biomarkers (2, 31). While we did not measure rate of perceived exertion (RPE) directly in the current study, our protocol elicited maximal efforts during all three HIIT trials. In response significant increases in plasma TBARS levels were observed immediately postexercise on days 1 and 2 and at the 3-h time point postexercise on day 3 (Fig. 2). TBARS were not elevated in response to day 3 HIIT exercise. This is among the first investigations to examine the acute blood oxidative stress response to serial exercise sessions. Current findings indicate a transient oxidative stress response to HIIT; thus future oxidative stress research should incorporate protocols that use multiple exercise sessions and additional sampling periods to confirm findings observed following a single session.

The fundamental link between the immune and oxidative stress responses to prolonged endurance or high-intensity exercise are undeniable (31, 35). Elevated ROS production following exercise permeates the nucleus and induces DNA cell damage in lymphocytes, implicating exercise-induced oxidative stress may contribute to previous observations of postexercise lymphocyte apoptosis (20, 40). We sought to determine if the exercise-associated oxidative stress altered lymphocyte antioxidant enzyme gene expression and/or activity. No changes in CuZn-SOD, Mn-SOD, CAT, or GPX mRNA expression occurred following HIIT (Fig. 3). However, even within a relatively small sample size, CAT, GPX, and most consistently, SOD enzyme activity did increase following 3 days of HIIT exercise (Table 2). Lymphocyte enzymatic antioxidant defenses have shown the ability to adapt to exercise-induced oxidative stress. Previous investigations have reported increases in CuZn-SOD, Mn-SOD, and heme oxygenase-1 (HO-1) mRNA expression in lymphocytes following various forms of exercise (6, 38). A significant downregulation in CuZn-SOD, Mn-SOD, and GPX mRNA levels have previously been reported in skeletal muscle of untrained rats, yet to our knowledge, this has not been observed in lymphocytes (17, 26). These data suggest that changes in antioxidant enzyme activity following HIIT are products of posttranscriptional modifications, increased mRNA stability, or decreased activity of regulatory modulators, opposed to pretranscriptional regulation.

Prior investigations (6, 37) of lymphocyte antioxidant enzyme activities have yielded equivocal results. Tauler et al. (35) examined antioxidant enzyme activities following both maximal and submaximal cycle ergometer tests. They found no significant changes in antioxidant enzyme activities following the maximal test, while GPX activity increased following submaximal exercise (35, 36). A later study by the same group examined the immune and oxidative stress responses following a mountain cycling stage and reported both an increase in oxidative stress and lymphocytopenia (37). They observed significant increases in lymphocyte GPX and SOD activities, yet this increased antioxidant activity was unable to prevent lymphocyte oxidative damage. Our data showed significant increase in each antioxidant enzyme measured; differences in CAT and GPX activity were observed between HIIT sessions, whereas SOD activity responded similarly to each HIIT session (Table 2). It is difficult to compare results between studies, as different modes of exercise were used and the aforementioned studies mentioned used only a single sampling period. Lymphocytes appear to respond to oxidative stress challenges by increasing antioxidant defenses, although the activation of these antioxidant enzymes may be unique to exercise mode and duration.

The immunosuppression observed following exercise of a strenuous and prolonged nature has been linked to a decrease in circulating lymphocytes and a blunted natural killer cell activity (29). Oxidative stress, catecholamines, growth hormone, and cortisol have all been coupled to lymphocyte function. While cortisol has often been linked to postexercise immunosuppression (23, 25, 27), Peters et al. (30) assessed lymphocyte DNA damage and apoptosis in well-trained athletes and found that concentrations of apoptotic lymphocytes were not related to changes in serum cortisol concentrations (30). We did not measure hormonal markers in this study since our emphasis was the link between oxidative stress and immune function. Prior work has shown increases in lymphocyte antioxidant enzyme activities, yet this increase was not sufficient to prevent postexercise lymphocytopenia (37). The HIIT protocol used in the current study is quite different from traditional prolonged ET utilized in previous investigations. Although we did not measure growth hormone in this study, prior work in our lab (consisting of similar participants and the same HIIT protocol) revealed 60-fold increases in serum growth hormone concentrations from pre- to postexercise (Foster EB, Fisher G, Sartin JL, Elsasser TD, Wu G, Pascoe DD, unpublished observations). We find this particularly interesting as human growth hormone has been shown to diminish various forms of lymphocyte cell death in vitro by upregulating an essential apoptotic protein, Bcl-2 (16, 22). Therefore, it is possible that the increased growth hormone in conjunction with the increase in antioxidant enzyme activity following HIIT may be responsible for maintaining lymphocyte function. Future studies should be performed to determine if the increased growth hormone following HIIT is responsible for the preservation of circulating lymphocytes.

The lymphocyte concentration increase seen during exercise is thought to be due to recruitment of all lymphocyte subpopulations into circulation, specifically from tissue pools such as the spleen, lymph nodes, and the gastrointestinal tract (28). Additionally, animal models have shown a redistribution of lymphocytes from the circulation back into the tissue pools following exercise (33). Thus, examining changes in lymphocyte cell viability following an oxidative stress challenge in vitro may better illustrate lymphocyte ability to resist exercise-induced oxidative stress. In this study, lymphocytes were cultured from preexercise, 3 h postexercise, and 24 h post-HIIT blood samples and exposed to H2O2 for 4 h. Prior investigations found that H2O2 concentrations up to 200 μM induced changes in cytokines without affecting cell viability (9). Therefore, 200 μM, 500 μM, and 1 mM concentrations of H2O2 were used for the oxidative stress challenge. No significant changes in lymphocyte cell viability for control, 200 μM, and 500 μM H2O2 exposure were observed; thus cell viability does not appear to be affected at these concentrations. A unique finding from this study was the observation of a significant increase in lymphocyte cell viability at 3 h postexercise on day 2 (12%) and day 3 (13%) compared with day 1 of HIIT (Fig. 4). These data suggest that lymphocytes may adjust following exercise and become more resistant to subsequent episodes of oxidative stress. Additionally, a significant decrease in lymphocyte cell viability
was observed at 3 h postexercise compared with both pre- and 24 h postexercise (Fig. 5). These data support the postulate that lymphocytes may be more vulnerable to cytotoxic agents during recovery from exercise.

Strengths of this study included obtaining multiple samples over several exercise sessions, which enabled us to assess the transient immune, antioxidant, and oxidative stress responses to HIIT. Additional strengths include closely matching the age, body fat%, and fitness levels of the participants, and incorporating an in vitro experimental design with the in vivo experiments. A key limitation in this study is the measurement of only a single oxidative stress biomarker. Additionally, measuring oxidative stress within the lymphocytes would have strengthened our conclusions.

In conclusion, this is the first study to examine the acute oxidative stress and immune cell viability to high-intensity serial exercise sessions. Postexercise oxidative stress was associated with elevations in lymphocyte SOD, GPX, and CAT activity in a manner appearing consistent with posttranscriptional regulation. These findings were further associated with improved lymphocyte viability to an H2O2 challenge in vitro. In light of the fact that HIIT did not impair the immune system in vivo, this HIIT model may be an ideal form of exercise to improve health and performance without overstressing the immune system. Future research assessing these same variables, in addition to hormonal regulation, over longer training cycles should be conducted. Carefully designed experiments measuring both the immune and oxidative stress response over extended periods of time are needed. These types of studies would provide data necessary to better understand the link between oxidative stress and the immune system.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

REFERENCES


