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RESEARCH ARTICLE

# Characterization of extracellular redox enzyme concentrations in response to exercise in humans

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**Wadley AJ, Keane G, Cullen T, James L, Vautrinot J, Davies M, Hussey B, Hunter DJ, Mastana S, Holliday A, Petersen SV, Bishop NC, Lindley MR, Coles SJ.** Characterization of extracellular redox enzyme concentrations in response to exercise in humans. *J Appl Physiol* 127: 858–866, 2019. First published June 27, 2019; doi:10.1152/japplphysiol.00340.2019.—Redox enzymes modulate intracellular redox balance and are secreted in response to cellular oxidative stress, potentially modulating systemic inflammation. Both aerobic and resistance exercise are known to cause acute systemic oxidative stress and inflammation; however, how redox enzyme concentrations alter in extracellular fluids following bouts of either type of exercise is unknown. Recreationally active men ($n$ = 26, mean ± SD: age 28 ± 8 yr) took part in either: 1) two separate energy-matched cycling bouts: one of moderate intensity (MOD) and a bout of high intensity interval exercise (HIIE) or 2) an eccentric-based resistance exercise protocol (RES). Alterations in plasma (study 1) and serum (study 2) peroxiredoxin (PRDX)-2, PRDX-4, superoxide dismutase-3 (SOD3), thioredoxin (TRX-1), TRX-reductase and interleukin (IL)-6 were assessed before and at various timepoints after exercise. There was a significant increase in SOD3 (+1.5 ng/mL) and PRDX-4 (+5.9 ng/mL) concentration following HIIE only, peaking at 30- and 60-min post-exercise respectively. TRX-R decreased immediately and 60 min following HIIE (−7.3 ng/mL) and MOD (−8.6 ng/mL), respectively. In non-resistance trained men, no significant changes in redox enzyme concentrations were observed up to 48 h following RES, despite significant muscle damage. IL-6 concentration increased in response to all trials, however there was no significant relationship between absolute or exercise-induced changes in redox enzyme concentrations. These results collectively suggest that HIIE, but not MOD or RES increase the extracellular concentration of PRDX-4 and SOD3. Exercise-induced changes in redox enzyme concentrations do not appear to directly relate to systemic changes in IL-6 concentration.

**NEW & NOTEWORTHY** Two studies were conducted to characterize changes in redox enzyme concentrations after single bouts of exercise to investigate the emerging association between extracellular redox enzymes and inflammation. We provide evidence that SOD3 and PRDX-4 concentration increased following high-intensity aerobic but not eccentric-based resistance exercise. Changes were not associated with IL-6. The results provide a platform to investigate the utility of SOD3 and PRDX-4 as biomarkers of oxidative stress following exercise.

antioxidant; oxidative stress; peroxiredoxin; reactive oxygen species; redoxkine

## INTRODUCTION

It is well documented that acute exercise perturbs cellular reduction/oxidation (redox) balance through the increased production of reactive oxygen species (ROS) within actively contracting skeletal muscle (34) as well as other infiltrating cell types (35). Evidence suggests that ROS such as hydrogen peroxide ($H_2O_2$) and peroxynitrite ($ONOO^-$) have important roles in facilitating muscle contractile activity (25) and regulating the expression of genes involved with metabolism and endogenous antioxidant protection (14, 39). Conversely, heightened levels of exercise-induced $H_2O_2$ at the expense of antioxidant defense systems can elicit oxidative stress, which may limit contractile function and promote fatigue (33). Given this biphasic relationship, studies have previously evaluated alterations in redox balance in response to both aerobic and resistance-type exercise. These studies have focused primarily on the quantification of distal markers in extracellular fluids, such as the oxidation biomolecules and/or activity of antioxidant enzymes in plasma (48), serum (31), saliva (11), and urine (41), highlighting exercise duration (3), intensity (17), and muscle-damage (4) as factors governing greater increases. However, criticisms are commonly made with regard to the direct relationship of these markers with the redox state of active tissues during exercise (9). Recent evidence has highlighted that intracellular redox enzymes such as peroxiredoxin (PRDX) can be secreted from skeletal muscle myocytes (28) and immune cells (40) in response to increasing concentrations

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of $H_2O_2$ in vitro. Human studies are also beginning to provide evidence that plasma/serum PRDX-2 and PRDX-4 concentrations could serve as important biomarkers of the intracellular redox state in the context of acute and chronic inflammatory conditions (27, 40).

PRDXs are a major family of ubiquitous redox proteins, which modulate intracellular redox balance through a highly reactive cysteine thiolate group. The reaction rate of this cysteine is markedly greater than any other thiol-containing protein (50), allowing rapid regulation of cellular $H_2O_2$, with some evidence to suggest that this may facilitate muscle contraction (26). Therefore, PRDXs are reliable footprints of intracellular redox state, with heightened oxidation of the PRDX cysteine indicative of oxidative stress (37). In addition, upon secretion from immune cells, PRDX can directly bind to Toll-like receptor (TLR)-4 to initiate inflammatory cytokine production [e.g., interleukin (IL)-6] (38), providing some support for the association between PRDX and inflammation (27, 40). Recent work has begun to explore changes in the PRDX catalytic cycle in blood cells isolated from humans before and after acute exercise (6, 46, 47). In parallel with increases in soluble markers of inflammation (e.g., IL-6 and C-reactive protein), an increase in the oxidation of PRDX (i.e., dimer and overoxidized states) has been reported following intensive cycling and running exercise (46, 47). To our knowledge, changes in PRDX have yet to be assessed in the context of exercise in humans and represent a potentially unexplored area of exercise and redox biology. Interestingly, PRDX-2 can be secreted in tandem with its enzymatic reducing partners thioredoxin (TRX-1) and thioredoxin reductase (TRX-R) (20, 40). TRX-1 and TRX-R are cysteine and selenium based-antioxidant enzymes, respectively, with higher reduction potentials than PRDX, thus contributing toward maintaining the antioxidant function of PRDX. In addition, the enzyme superoxide dismutase 3 (SOD3) is an extracellular antioxidant released upon cellular stimulation, providing an immediate change in extracellular antioxidant capacity (15, 20). Given the emerging body of literature supporting a relationship between intracellular oxidative stress, redox enzyme secretion, and soluble inflammatory markers, the quantification of PRDX-2, PRDX-4, TRX-1, TRX-R, and SOD3 in extracellular fluids offers the potential for accurate assessment of changes in oxidative stress and inflammation after different types of exercise.

Based upon existing knowledge of the factors that can impact acute changes in exercise-induced oxidative stress, we sought to perform two experiments to understand how novel markers such as PRDX-2, PRDX-4, TRX-1, TRX-R and SOD3 respond to acute exercise and whether relationships exist between changes in inflammation. Specifically, we aimed to characterize how these markers would be impacted by aerobic exercise intensity and eccentric-based resistance exercise. We tested the hypothesis that both protocols would elicit an increase in the concentrations of redox enzymes within plasma/serum after exercise, with higher exercise intensity causing a larger increase following aerobic exercise.

## METHODS

### *Participants*

Healthy, untrained participants were recruited for two independent studies (Table 1) Participants in both studies completed the International Physical Activity Questionnaire, which addresses habitual levels of weekly physical activity. Participants gave their informed, written consent, and all studies were approved by the local ethics review committee in accordance with the Declaration of Helsinki, 2008. Participants were all nonsmokers and had not taken any antioxidant vitamin supplements or anti-inflammatory drugs for 8 wk before the laboratory visits. All participants were required to refrain from any strenuous physical activity, consumption of alcoholic beverages, or caffeine for ≥2days before the experimental sessions.

### *Experimental Sessions*

The full workflow for this project is detailed in Fig. 1. Experimental sessions took place in the morning (7 to 8 AM start time) under stable climatic conditions (18–20°C and humidity between 45 and 55%) and following at least a 10-h fast. After a period of rest, height (Seca Alpha, Hamburg, Germany) and mass (Tanita, Tokyo, Japan) were determined.

In *study 1*, participants first visited the laboratory for an assessment of cardiorespiratory fitness ($\dot{V}O_{2max}$) using a ramp test to exhaustion on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, The Netherlands). The protocol involved commencing pedalling at 100 W, followed by fixed 30-W increments every 4 min. Oxygen uptake was assessed continuously using a breath-by-breath system (Oxycon Pro, Jaeger, Wuerzberg, Germany) and heart rate monitored using a Polar Vantage heart rate monitor (Polar, Kempele, Finland). The test ended when the participant reached volitional exhaustion or when a plateau in oxygen consumption was observed with an increase in workload (49). A final obtained value of rate of oxygen consumption was accepted as $\dot{V}O_{2max}$ and expressed relative to body weight ($mL \cdot kg^{-1} \cdot min^{-1}$). At least 1 wk later, participants then undertook the first of two energy and time-matched cycling trials in a randomized order ≥1 wk apart: a continuous bout of moderate-intensity cycling at ~60% $\dot{V}O_2$ ($\%_{MAX}$) for 58 min (MOD) and a bout of high-intensity interval exercise (HIIE) consisting of 10 × 4-min intervals at 85% $\dot{V}O_2$ ($\%_{MAX}$), with 2-min rest intervals. In both trials, oxygen uptake was assessed continuously and power output was adjusted where necessary to maintain target and $\dot{V}O_2$ equal energy expenditure between MOD and HIIE (*study 1*). Rating of perceived exertion (RPE) was monitored every 5 min throughout the trials (5).

Table 1. *Demographics for participants in studies 1 and 2*

| | Energy-Matched Trials (*Study 1*) | Eccentric-Based Resistance Exercise (*Study 2*) | Statistical Analysis |
|---|---|---|---|
| No. of Participants | 9 | 16 | |
| Age, yr | 29 ± 5 | 25 ± 9 | $P$ = NS |
| Body mass index, kg/m$^2$ | 24.2 ± 3.4 | 25.3 ± 4.1 | $P$ = NS |
| IPAQ (METs-min/wk) | 6,683 ± 3,835* | 2,540 ± 2,022 | *$P$ = 0.004 |
| Watt maximum (Watt/kg) | 3.4 ± 0.5 | | |
| $\dot{V}O_{2max}$, $mL \cdot kg^{-1} \cdot min^{-1}$ | 44.5 ± 6.4 | | |

Values are means ± SE. Blank cells indicate missing data. IPAQ, International Physical Activity Questionnaire; NS, not significant. *Significant difference in comparison with *study 2*; $P < 0.05$.

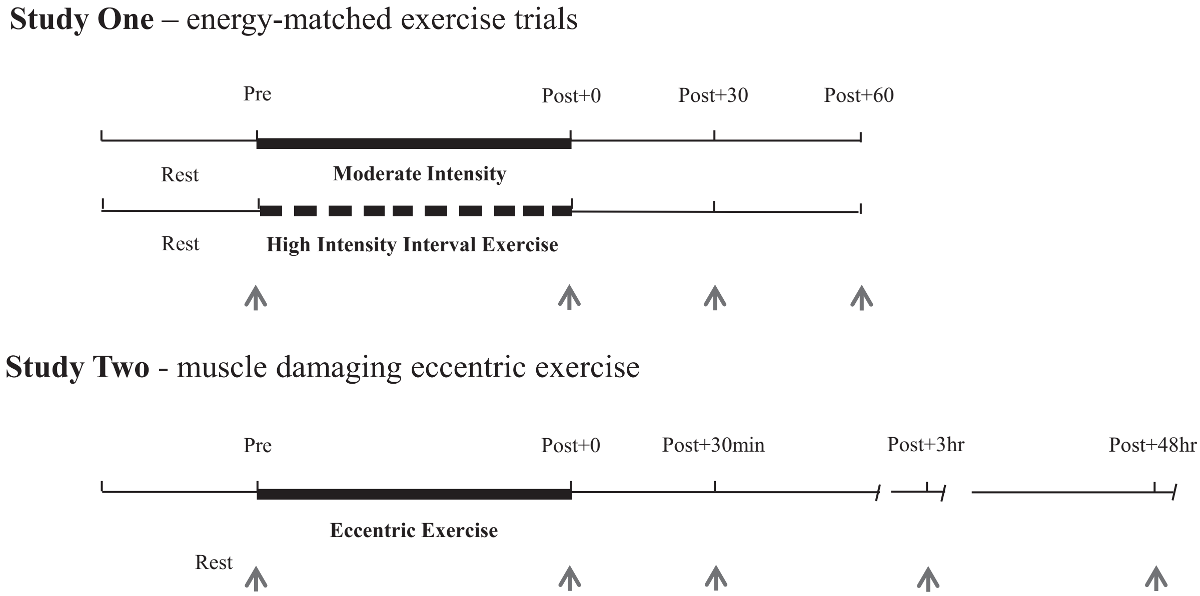


Fig. 1. Schematic of the 2 exercise studies. Thick lines represent the exercise session, with thin lines indicating pre- and postexercise resting periods. Gaps between dark lines indicate the rest periods during the high-intensity interval exercise trial. Blood samples taken for each study are indicated as arrows.

In *study 2* ($n$ = 16), nonresistance-trained men undertook an eccentric-based resistance exercise protocol adapted from a previous study by Alemany et al. (1). This muscle-damaging protocol was performed on a Humac Norm dynamometer (CSMI). The dynamometer lever arm was programmed to flex the participant's knee from a start position of 10° of flexion to 90° of flexion, thus allowing a range of motion of 80°. The participants began with their leg at the start position and were asked to maximally contract their quadriceps against a resistance while the lever arm moved to the finish position (90° knee flexion). Once at the finish position, they were advised to relax their leg, and the dynamometer moved them back to the start position to avoid a concentric contraction being performed. The lever arm moved at a set speed of 60°/s. The bout consisted of 20 sets of 10 repetitions, with each set being separated by 1 min of rest. Visual feedback and verbal encouragement were provided to all participants to maximize torque output for each contraction.

### *Blood sampling and Plasma Isolation*

For both studies, a catheter (Appleton Woods, Birmingham, UK) was inserted into the antecubital vein of the arm before exercise to obtain a baseline blood sample after 30 min of rest (Pre). The catheter was continually kept clear with isotonic saline solution (0.9% sodium chloride). As indicated in Fig. 1, blood samples were then taken immediately, 30 min, and 60 min after both HIIE and MOD (*study 1*: Pre, Post + 0, Post + 30, and Post + 60) and immediately, 30 min, 3 h, and 48 h following the muscle damage protocol (*study 2*: Pre, Post + 0, Post + 30min, Post +3 h, and Post + 48 h). The post + 48 h (*study 2*) blood sample was taken via venepuncture. At each time point, 12 mL of blood was drawn into vacutainer tubes containing either potassium ethylene diaminetetraacetic acid in *study 1* (Becton, Dickson & Company, Oxford, UK) or no anticoagulant in *study 2*. In *study 1*, whole blood was centrifuged at 1, 525 *g* for 15 min at room temperature. In *study 2*, whole blood was allowed to clot at room temperature for 20 min and then centrifuged at 1,500 *g* for 15 min. The resulting plasma (*study 1*) and serum (*study 2*) were aliquoted and frozen at −80°C for future analysis of redox enzymes, IL-6, creatine kinase (CK), and lactate dehydrogenase (LDH). Capillary blood samples were obtained from the earlobe after 4 min of exercise and then every 6 min thereafter (i.e., end of each HIIE interval) in *study 1*. These samples were used for analysis of blood glucose and lactate concentrations to verify intensity-dependent differences between each protocol.

### *Analytical Procedures*

*PRDX-2, PRDX-4, TRX-1, TRX-R, and SOD3 ELISAs.* ELISAs for the detection of PRDX-2, PRDX-4, TRX, TRX-R, and SOD3 were developed in-house. Commercially available antigens and antibodies (i.e., PRDX-2, PRDX-4, TRX, and TRX-R) were purchased from either Abcam (Cambridge, UK; ab) or Sigma Aldrich (Dorset, UK; SRP). The human SOD3 antigen and rabbit antiserum directed against human SOD3 were developed as previously described (16, 20). Plasma or serum and standards (100 μL) were loaded onto individual wells of an ELISA plate (Thermo Scientific F8 polysorp immune wells), and protein was left to bind overnight at 4°C. Wells were then prewashed with PBS wash buffer, supplemented with 0.1% casein (PBSwC, 200 μL), and then blocked with 1% casein in PBS (200 μL) for 30 min at room temperature with gentle agitation. Anti-human rabbit antibodies for PRDX-2 (ab133481, 1:2,000), PRDX-4 (ab59542, 1:2,000), and SOD3 (in-house, 1:2,000) and anti-human mouse antibodies for TRX-1 (ab16965, 1:8,000) and TRX-R (ab16847, 1:1,000) were then added to each well and diluted in PBSwC for 45 min at room temperature. Following this, 100 μL of anti-rabbit (1:5,000) or anti-mouse (1:500) IgG biotin antibodies in PBSwC and streptavidin-horseradish peroxidase (1:2,000 in PBSwC) were added separately to each well, both for 45 min, with gentle agitation. Between all stages, all wells were washed three times with PBSwC. Finally, 100 μL of 3,3′,5,5′-tetramethylbenzidine (10 μg) was added per well, and the plate was left to develop in the dark for 15–25 min. Stop solution (1.5 mM $H_2SO_4$, 50 μL) was then added to each well and absorption at 450 nm subsequently evaluated by using a plate reader (Multiskan Ascent, Thermo Labsystems). Concentration of each antigen was then determined by comparing absorbance values of recombinant PRDX-2 (ab167977; Abcam), PRDX-4 (ab93947; Abcam), TRX-1 (ab51064; Abcam), TRX-R (SRP6081; Sigma-Aldrich), and SOD3 (in-house) proteins (0–50 ng/mL). ELISA validation experiments showed no cross-reactivity of the PRDX-2, PRDX-4, TRX-1, TRX-R, or SOD3 antibodies with the respective antigens, nor with serum albumin. All values were adjusted for plasma volume according to previous methods (12).

Table 2. *Physiological response to aerobic-based exercise (study 1)*

| Trial | Energy-matched Cycling Trials (*Study 1*) Continuous cycling for 58 min, predicted 60% $\dot{V}O_{2max}$ (MOD) | 10 × 4-Min cycling intervals, predicted 85% $\dot{V}O_{2max}$ (2 min rest intervals; total time = 58 min; HIIE) | Statistical Analysis |
|---|---|---|---|
| Mean $\dot{V}O_{2max}$, % | 56.5 ± 2.6 | 58.9 ± 4.3 | $P$ = NS |
| Energy expenditure, kJ | 2,077 ± 340 | 2,072 ± 339 | $P$ = NS |
| Average RPE | 12 ± 1 | 16 ± 1* | *$P < 0.0001$ |
| Mean blood lactate, mmol/L | 1.9 ± 0.6 | 6.8 ± 1.4* | *$P < 0.0001$ |
| Mean blood glucose, mmol/L | 3.9 ± 0.3 | 4.5 ± 0.6 | $P$ = NS |

Values are means ± SE. MOD, moderate-intensity exercise; HIIE, high-intensity interval exercise; NS, not significant; RPE, rating of perceived exertion. *$P <$ 0.0001, significant difference between MOD and HIIE.NS, $P > 0.05$.

*Other analyses.* In both studies, a cytometric bead array was used to quantify plasma (*study 1*) and serum (*study 2*) IL-6 concentrations on a BD C6 Accuri Flow Cytometer (BD Biosciences). In *study 1*, blood lactate and glucose concentrations were determined immediately following collection using an automated lactate and glucose analyzer (Biosen C-Line Clinic, EKF-Diagnostic, Barleben, Germany). In *study 2*, serum CK and LDH concentrations were determined to monitor muscle damage using an automated ABX Pentra 400 system (Horiba UK). Haematocrit and hemoglobin concentrations were used to ascertain plasma volume changes and make appropriate adjustments in plasma redox enzyme and IL-6 concentrations (Beckman Coulter, London, UK).

### *Statistical Analysis*

The Shapiro-Wilk test was used to test for normality in scale data at all time points. Differences between participant characteristics and the physiological responses to exercise in both studies were assessed using unpaired-samples *t*-tests or nonparametric Mann-Whitney *U*-tests. The influence of exercise on plasma/serum PRDX-2, PRDX-4, SOD3, TRX-1, TRX-R, and IL-6 concentration was assessed over time by repeated-measures analysis of variance (ANOVA) or nonparametric Wilcoxon signed-rank tests, depending on variable normality. Post hoc analysis of any significant effect of time or interaction effect (*study 1*: group × time) was performed by a test of simple effects by pairwise comparisons with Bonferroni correction. Effect sizes for main effects and interaction effects of ANOVA are presented as partial eta$^2$ ($\eta^2_p$), using Cohen's definition of $\eta^2_p$ of 0.01, 0.06, and 0.14 for "small," "medium," and "large" effects, respectively (10). Pearson correlation and Spearman rank were used to assess the relationship between parametric and nonparametric data, respectively. All values are presented as means ± SD or error (indicated throughout this article). Statistical significance was accepted at the $P < 0.05$ level. Statistical analyses were performed using SPSS (PASW Statistics, release 23.0; SPSS, Chicago, IL).

## RESULTS

There was no significant difference in age or BMI between the participants taking part in the two studies. Participants in *study 1* ($P$ = 0.004) had significantly higher self-reported physical activity than those in *study 2*.

### *Acute Physiological Responses to HIIE and MOD*

For *study 1*, the physiological responses during each exercise bout are reported in Table 2. Peak $\dot{V}O_2$ and RPE were significantly greater in HIIE compared with MOD ($P <$ 0.00001), but there were no statistically significant differences in mean $\dot{V}O_2$ and energy expenditure. Whole blood lactate and glucose data are reported in Table 2. Mean lactate concentration was significantly higher during HIIE than MOD ($P <$ 0.0001), but there was no significant difference in average glucose concentration between trials.

### *Effects of Eccentric-Based Resistance Exercise on Muscle Damage Markers*

Changes in markers of muscle damage are reported in Table 3. A stepwise increase (Post + 48 h > Post + 3 h > Post + 30 min > Post + 0 > Pre) in serum CK concentration was observed over time, peaking above Pre at Post + 48 h ($P <$ 0.001). Serum LDH concentration was elevated above Pre at all postexercise time points ($P < 0.05$), also increasing Post + 3 h and Post + 48 h, relative to Post + 30 min ($P < 0.05$).

### *Effects of Aerobic and Eccentric-Based Resistance Exercise on IL-6 Concentration*

IL-6 data are presented in Fig. 3. In *study 1*, plasma IL-6 increased in both trials [time effect: $F(3) = 15.5$, $P < 0.0001$, $\eta^2 = 0.66$], being elevated above resting values, both immediately ($P = 0.004$) and Post + 30 ($P = 0.002$), but not Post + 60 (Fig. 3A). The magnitude of this increase was significantly greater Post-Ex in HIIE ($P = 0.031$) than in MOD [time × condition effect: $F(3) = 7.0$, $P < 0.001$, $\eta^2 = 0.47$]. IL-6 concentration decreased Post + 30 ($P = 0.004$) and Post + 60 ($P = 0.007$) relative to Post + 0 and Post + 60 and relative to Post + 30 ($P =$ 0.026) in HIIE only. In *study 2* (Fig. 3B), IL-6 concentration was significantly higher at all time points ≤3 h, but not 48 h after exercise, relative to Pre [time effect: $F(4) = 14.3$, $P < 0.0001$, $\eta^2 = 0.30$].

Table 3. *Changes in markers or muscle damage following eccentric-based resistance exercise (study 2)*

| | Pre | Post + 0 | Post + 30 min | Post + 3 h | Post + 48 h |
|---|---|---|---|---|---|
| Creatine kinase, U/L | 147.6 ± 27.1 | 236.1 ± 65.5* | 289.9 ± 86.0*† | 560.8 ± 273.5**†# | 575.9 ± 290.8**†#‡ |
| Lactate dehydrogenase, U/L | 254.9 ± 130.6 | 282.7 ± 70.9* | 274.1 ± 77.1* | 290.3 ± 77.8*† | 299.9 ± 165.2*† |

Values are means ± SD. *$P < 0.05$ and **$P < 0.001$, significant difference in comparison to Pre. †Significant difference in comparison to Post + 0; $P$ <0.05. #Significant difference in comparison to Post + 30 min, $P < 0.05$. ‡Significant difference in comparison to Post + 3 h, $P < 0.05$.

### *Effects of Aerobic Exercise on PRDX-2, PRDX-4, TRX-1, TRX-R, and SOD3 Concentration*

No differences were observed in resting concentrations of PRDX-2, PRDX-4, TRX-1, TRX-R, or SOD3 when quantified in plasma and serum across all trials. Changes in plasma PRDX-2, PRDX-4, TRX-1, TRX-R, and SOD3 in response to MOD and HIIE are reported in Fig. 2*A*. There was a significant increase in plasma SOD3 [trial × time effect: $F(3,1) = 5.3$, $P = 0.028$, $\eta^2 = 0.31$] and PRDX-4 following HIIE only (nonparametric tests: all $P < 0.05$). SOD3 concentration was elevated above pre-exercise values at all post-HIIE time points, peaking at Post + 0 ($P = 0.015$) and Post + 30 ($P = 0.013$), but only significantly higher than MOD at Post + 30 ($P = 0.05$). Plasma SOD3 concentration decreased relative to Post + 30 at Post + 60 ($P = 0.013$). Relative to Pre, PRDX-4 concentration increased at Post + 30 ($P = 0.015$) and Post + 60 ($P = 0.008$) following HIIE, with PRDX-4 concentration higher at all postexercise time points compared with MOD ($P < 0.038$). There was a significant decrease in plasma TRX-R concentration in both MOD and HIIE. Relative to Pre, TRX-R significantly decreased at Post + 0 in HIIE only ($P = 0.021$), with values significantly less than MOD ($P = 0.011$). Following MOD, TRX-R was significantly lower at Post + 60, relative to all time points (all $P < 0.038$). There were no statistically significant changes in PRDX-2 and TRX-1 concentration over time in either trial; however, TRX-1 concentration was significantly higher in HIIE than MOD Post + 60 only ($P = 0.021$).

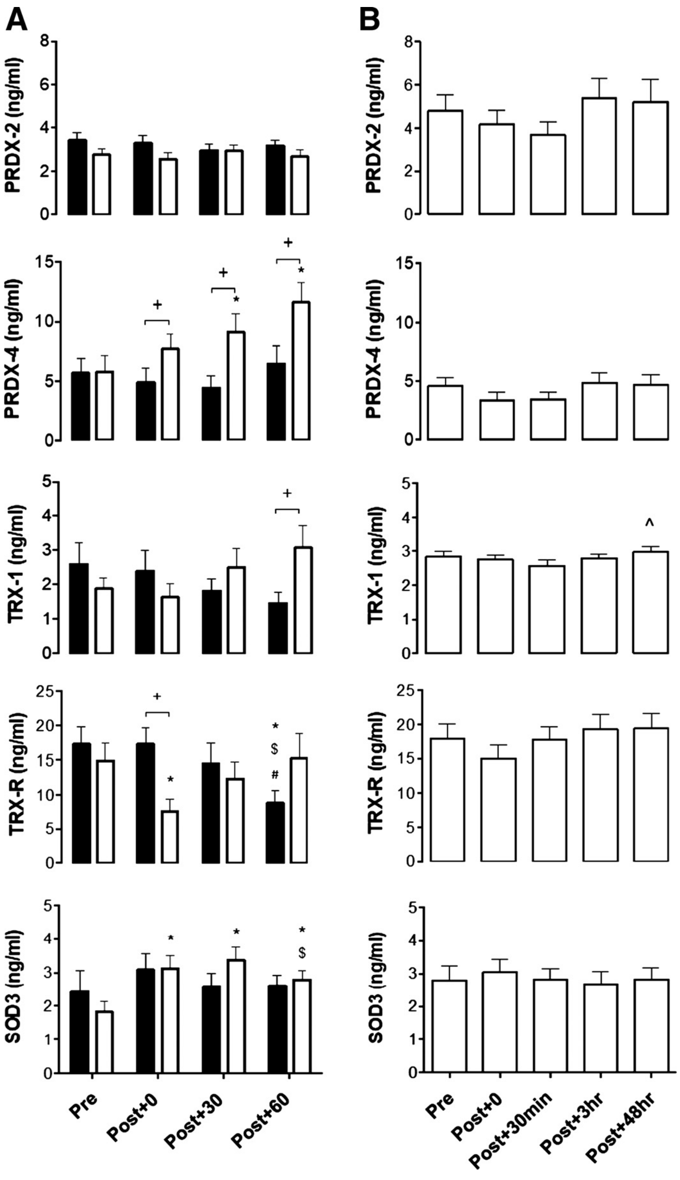


Fig. 2. Changes in redox enzyme concentration in response to 2 energy-matched cycling bouts. *A*: moderate steady-state (MOD; black bars) and high-intensity interval exercise (HIIE; open bars) and an eccentric-based resistance exercise protocol. *B*: peroxiredoxin (PRDX)-2, PRDX-4, thioredoxin-1 (TRX-1), thioredoxin-reductase (TRX-R), and superoxide dismutase 3 (SOD3). Values are means ± SE.*Significant differences relative to Pre, $P < 0.05$; #significant difference relative to Post + 0, $P < 0.05$; $significant difference relative to Post + 30, $P < 0.05$; $^+$significant difference between MOD and HIIE, $P < 0.05$; ^significant difference between Post + 30 min and Post + 48 h time points.

### *Effects of Eccentric-Based Resistance Exercise on PRDX-2, PRDX-4, TRX-1, TRX-R, and SOD3 Concentration*

Serum redox enzyme concentration changes in response to an eccentric-based resistance exercise protocol are presented in Fig. 2*B*. A trend was observed for a decrease in PRDX-2 concentration Post + 30min (−1.12 ng/mL); however, this did not reach statistical significance [time effect: $F(4) = 2.3$, $P = 0.065$, $\eta^2 = 0.13$]. Similarly, no significant changes were noted in PRDX-4, TRX-R, or SOD3 for ≤48 h following eccentric-based resistance exercise. A significant increase in TRX-1 was shown Post + 48 h, relative to Post + 30min ($P = 0.039$), but not Pre ($P = 0.309$).

## DISCUSSION

The current results have characterized the kinetic responses of endogenous redox enzymes within the extracellular environment after exercise for the first time. We highlight novel findings that high-intensity aerobic cycling induces a significant increase in SOD3 and PRDX-4 in healthy, untrained men. Similar responses were not observed following moderate-intensity cycling or muscle-damaging resistance exercise. In contrast, plasma TRX-R concentration decreased within 1 h following moderate- and high-intensity cycling exercise but not resistance exercise. Taken together, these findings provide novel insights into the regulation of extracellular redox enzymes in response to exercise.

The current data highlight modality and exercise intensity-specific increases in two abundant redox enzymes. In response to aerobic exercise, PRDX-4 but not PRDX-2 concentration increased 30 min following HIIE and remained elevated until Post + 60. The secretory pathways of PRDXs are isoform specific, with endoplasmic reticulum (ER, i.e., PRDX-4) and cytosolic (i.e., PRDX-2) resident isoforms released via classical and nonclassical secretory pathways, respectively (8). Therefore, the current data suggest that exercise may activate the ER-Golgi pathway to secrete PRDX-4 in an intensity-dependent manner. SOD3, which is also released via this pathway, increased more rapidly than PRDX-4 following HIIE (Post + 0), with levels tailing off Post + 60, relative to Post + 30. SOD3 is an antioxidant enzyme released directly from the cell membrane (15, 20), specifically secreted during exercise to

metabolize superoxide anions produced in the extracellular environment to $H_2O_2$ (30). The different peak concentrations of SOD3 (i.e., Post + 0) and PRDX-4 (i.e., Post + 30) following HIIE may be explained in part, by *1*) the membrane proximity of SOD3 compared with the ER location of PRDX-4 and *2*) the appearance of superoxide anions first in the extracellular space following exercise, before their metabolism to $H_2O_2$, which then induced PRDX-4 secretion. This may also be reflective of differential secretion rates of SOD3 and PRDX-4 from various tissues during and following exercise. Both proteins are expressed in skeletal muscle (19), a highly redox active tissue (36); however, PRDX-4 is primarily located in pancreas, liver, and heart (21), whereas SOD3 is expressed in the heart and vasculature tissue (42). The association with the vasculature may explain the more rapid increase in plasma SOD3 concentration following HIIE. Aside from these increases, a modest decrease was observed in plasma TRX-R after both MOD and HIIE (*study 1*), with this change being much more rapid in HIIE (Post + 0) compared with MOD (Post + 60). The mechanisms driving a decrease in TRX-R after exercise are unclear at present. The decrease may represent transient homeostatic fluctuations involving uptake of redox enzymes by neighboring cells and tissues, perhaps to regulate intracellular redox balance (23).

A finding that was in contrast to our hypothesis was that eccentric-based resistance exercise did not induce an increase in the extracellular concentrations of redox enzymes. The measurement of redox enzymes in plasma and serum is an emerging area of biomedical research, particularly in the context of acute (24) and chronic (13, 43) inflammatory conditions, where PRDXs and TRX-1 have been associated with enhanced cytokine and chemokine production (22, 38). The participants in both studies were relatively inactive, with participants in *study 2* in particular reporting significantly lower levels of habitual physical activity (Table 2) and being unaccustomed to eccentric-based resistance exercise. Unaccustomed eccentric exercise induces significant amounts of acute muscle damage and inflammation (7), as demonstrated by the stepwise increases in CK and LDH concentrations for ≤48 h following our protocol and IL-6 for ≤3 h postexercise (Fig. 3*B*). These data suggest that the increase in SOD3 and PRDX-4 observed in *study 1* is unlikely due to just a disruption to the plasma membrane, given that no changes were observed following a muscle-damaging bout of resistance exercise. It must be acknowledged that only selective time points were measured following the protocol, and perhaps the secretion of redox enzymes occurs between 3 and 48 h postexercise. Nevertheless, this study has highlighted for the first time that redox enzyme concentrations do not match that of established markers of muscle damage and inflammation when measured in serum samples following an eccentric-based resistance exercise bout. In response to aerobic-based exercise, we have recently demonstrated a positive association between intracellular peroxiredoxin (I–IV) overoxidation in immune cells and plasma IL-6 concentration (47). In the current study, IL-6 concentration increased in an intensity-dependent manner (HIIE > MOD) following aerobic exercise (Fig. 3*A*); however, there were no statistically significant relationships between absolute or exercise-induced changes in PRDX-4 and SOD3 with IL-6. Therefore, the observations across both studies suggest no relationship between IL-6 and redox enzymes after exercise. A larger sample size may be needed to adequately address these associations and support the previously documented relationship between plasma/serum redox enzymes and soluble inflammatory markers (27, 40).

The results of the current investigation demonstrate clear differences in the changes in SOD3, TRX-R, and PRDX-4 following aerobic versus eccentric-based resistance exercise. With regard to PRDX-2 and TRX-1, no changes were observed following aerobic or eccentric-based resistance exercise. Both PRDX-2 and TRX-1 are cytosolic redox enzymes that contain no $NH_2$-terminal signal peptide for secretion and thus are released via nonclassical pathways and are associated with extracellular vesicles (EVs) such as exosomes and nanopar-

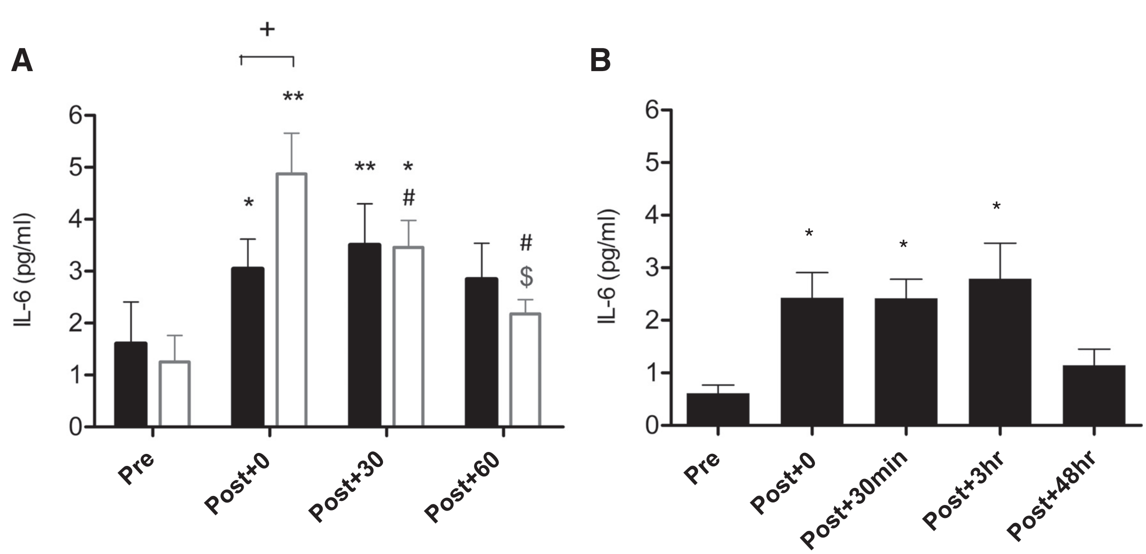


Fig. 3. Changes in plasma interleukin (IL)-6 in response to 2 energy-matched cycling bouts: moderate steady-state (MOD; black bars) and high-intensity interval exercise (HIIE; open bars) (*A*) and an eccentric-based resistance exercise protocol (*B*). Values are means ± SE. *Significant differences relative to Pre, $P < 0.05$; **$P < 0.001$; #significant difference relative to Post + 0, $P < 0.05$; $significant difference relative to Post + 30, $P < 0.05$; +significant difference between MOD and HIIE, $P < 0.05$.

ticles (26a). PRDX-2 and TRX-1 are detectable in plasma/serum samples through their association with the exofacial surface of the EV membrane (18, 44); however, their protein levels may be higher due to protein contained within the EVs. This protein would not be detectable by antibodies when enclosed within the lipid membrane during ELISA quantification, as previously shown (32). Indeed, recent evidence has highlighted that a series of leaderless redox enzymes (i,e, PRDX-1, PRDX-2, PRDX-5, PRDX-6, TRX-1, SOD1, and SOD2) are secreted in EVs via a nonclassical route following exposure to stress, with classically secreted SOD3, TRX-R, and PRDX-4 not detectable within EVs (2). This may explain why plasma/serum PRDX-2 and TRX-1 concentration did not significantly change following muscle-damaging or aerobic exercise. It must be noted that TRX-1 concentration was significantly higher 48 h after the eccentric-based resistance exercise protocol relative to Post + 0 (*study 2*) and also significantly higher at Post + 60 in HIIE compared with MOD (*study 1*). These findings again underpin intensity-dependent differences despite concentrations not being higher than pre-exercise values in both cases. In response to a far more extreme bout of exercise, Marumoto et al. (29) reported a marked increase in TRX-1 levels (17.9 ± 1.2 ng/mL at baseline to 70.1 ± 6.9 ng/mL) after a 2-day, 130-km ultra-endurance marathon; however, these exercise bouts were substantially different in nature and thus hard to directly compare. Although an ultramarathon is accompanied by significant amounts of muscle damage, given the findings of *study 2*, it is unlikely that muscle damage is the primary cause of TRX-1 secretion in this context. Further work is needed to clarify whether TRX-1 and PRDX-2 protein levels alter within EVs after conventional bouts (i.e., not ultra-endurance) of muscle-damaging and aerobic-based exercise.

This study has quantified the responses of antioxidant enzymes in the extracellular environment following acute exercise in age- and BMI-matched individuals from two independent exercise studies (Table 1). We must acknowledge that the studies would have benefited from a direct comparison between redox enzyme concentrations and other established biomarkers of oxidative stress (e.g., protein carbonyls and F2-isoprostanes). However, due to limited sample volume, this analysis was not feasible, and therefore, it should be prioritized as an area of future research. A second limitation is that the quantification of redox enzymes and IL-6 was undertaken in both plasma (*study 1*) and serum (*study 2*); however, there were no differences in any of these proteins when quantified in pre-exercise samples.

### *Conclusion*

The results of the present study have highlighted that plasma SOD3 and PRDX-4 concentration increased in response to acute exercise. Importantly, the secretion of these proteins appears to be intensity and modality dependent, with increases observed only in response to high-intensity aerobic cycling in untrained individuals. A decrease in TRX-R was also noted following different aerobic exercise bouts, with exercise intensity driving a more rapid decrease in TRX-R. Future research is required to pinpoint the precise mechanisms governing the secretion and uptake of redox enzymes and their role in regulating redox balance between tissues after exercise.


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## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

A.J.W., T.C., L.J., B.H., D.J.H., S.S.M., M.R.L., and S.J.C. conceived and designed research; A.J.W., G.K., T.C., L.J., J.V., M.D., B.H., D.J.H., and M.R.L. performed experiments; A.J.W., G.K., T.C., L.J., and J.V. analyzed data; A.J.W. interpreted results of experiments; A.J.W. and S.J.C. prepared figures; A.J.W. drafted manuscript; A.J.W., G.K., T.C., L.J., B.H., D.J.H., S.S.M., A.H., S.V.P., N.C.B., M.R.L., and S.J.C. edited and revised manuscript; A.J.W., G.K., T.C., L.J., J.V., M.D., B.H., D.J.H., S.S.M., A.H., S.V.P., N.C.B., M.R.L., and S.J.C. approved final version of manuscript.


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