Effects of Acute Aerobic and Anaerobic Exercise on Blood Markers of Oxidative Stress

Richard J. Bloomer,[1,2] **Allan H. Goldfarb,**[1] **Laurie Wideman,**[1] **Michael J. McKenzie,**[1] **and Leslie A. Consitt**[1]

[1]Department of Exercise and Sport Science, University of North Carolina at Greensboro, Greensboro, North Carolina 27402; [2]Department of Health and Sport Sciences, The University of Memphis, Memphis, Tennessee 38152.

Abstract. Bloomer, R.J., A.H. Goldfarb, L. Wideman, M.J. McKenzie, and L.A. Consitt. Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress. *J. Strength Cond. Res.* 19(2):276–285. 2005.—The purpose of this study was to compare oxidative modification of blood proteins, lipids, DNA, and glutathione in the 24 hours following aerobic and anaerobic exercise using similar muscle groups. Ten cross-trained men (24.3 ± 3.8 years, [mean ± *SEM*]) performed in random order 30 minutes of continuous cycling at 70% of $\dot{V}O_2$max and intermittent dumbbell squatting at 70% of 1 repetition maximum (1RM), separated by 1–2 weeks, in a crossover design. Blood samples taken before, and immediately, 1, 6, and 24 hours postexercise were analyzed for plasma protein carbonyls (PC), plasma malondialdehyde (MDA), and whole-blood total (TGSH), oxidized (GSSG), and reduced (GSH) glutathione. Blood samples taken before and 24 hours postexercise were analyzed for serum 8-hydroxy-2′-deoxyguanosine (8-OHdG). PC values were greater at 6 and 24 hours postexercise compared with pre-exercise for squatting, with greater PC values at 24 hours postexercise for squatting compared with cycling (0.634 ± 0.053 vs. 0.359 ± 0.018 nM·mg protein^{-1}). There was no significant interaction or main effects for MDA or 8-OHdG. GSSG experienced a short-lived increase and GSH a transient decrease immediately following both exercise modes. These data suggest that 30 minutes of aerobic and anaerobic exercise performed by young, cross-trained men (a) can increase certain biomarkers of oxidative stress in blood, (b) differentially affect oxidative stress biomarkers, and (c) result in a different magnitude of oxidation based on the macromolecule studied. Practical applications: While protein and glutathione oxidation was increased following acute exercise as performed in this study, future research may investigate methods of reducing macromolecule oxidation, possibly through the use of antioxidant therapy.

Key Words. lipid peroxidation, protein carbonyls, reactive oxygen species

Introduction

Oxidative stress is a condition in which the cellular production of prooxidants exceeds the physiologic capacity of the system to render these inactive. This occurs by way of the body's endogenous antioxidant defense system, in conjunction with exogenous antioxidants consumed through dietary sources (14). The generation of reactive oxygen species (ROS), such as singlet oxygen (·O), superoxide radical (O$_2^{·-}$), and hydroxyl radical (·OH), occur as a consequence of normal cellular metabolism and appear to be increased under conditions of both psychological and physical stress (49). While regular exercise training is associated with numerous health benefits, it can be viewed as an intense physical stressor leading to increased oxidative cellular damage, likely due to enhanced production of ROS (23, 49). Cellular damage is often represented by modifications to macromolecules, including proteins, lipids, and nucleic acids, and can occur as a result of high intensity or moderate- to long-duration exercise (40).

A single bout of exercise can result in activation of several distinct systems of radical generation (21) and may be separated into both primary (e.g., electron leakage through the mitochondria during aerobic respiration, prostanoid metabolism, catecholamines, and the enzymes xanthine oxidase [perhaps via ischemia-reperfusion conditions] and nicotinamide adenine dinucleotide phosphate [NADPH] oxidase), as well as secondary sources (e.g., phagocytic cells, disruption of iron containing proteins, and excessive calcium accumulation). While it is fairly well accepted that ROS production and subsequent tissue damage resulting from aerobic exercise is largely due to an increased flux in electron transport leading to an increased leakage of superoxide radicals, the generation of ROS during and following anaerobic exercise may be mediated through a variety of other pathways (21). These include xanthine and NADPH oxidase production, ischemia reperfusion, prostanoid metabolism, phagocytic respiratory bursts, disruption of iron containing proteins, and excessive calcium accumulation, often resulting from the performance of muscle-damaging isotonic or eccentric biased muscle actions, which commonly produce muscle injury (35).

Due to the myriad of potential mechanisms by which an acute exercise bout could induce ROS production and subsequent macromolecule damage, it is possible that aerobic and anaerobic exercise bouts have differing effects on the generation of ROS. Unfortunately, data addressing this issue are scarce at the present time. In fact, only 5 studies have compared aerobic and anaerobic exercise bouts with regards to oxidative tissue damage (2, 3, 18, 32, 46). Further, with the exceptions of Sahlin et al. (46) and Alessio et al. (3), no investigation has made an attempt to compare exercise modes matched for total time. Probably equally important, no investigation to date has directly compared aerobic and anaerobic work bouts matched for muscle mass recruited, exercise intensity, or total work. Therefore, the purpose of this study was to compare oxidative modification to proteins, lipids, DNA, and glutathione in the 24-hour period following aerobic

TABLE 1. Characteristics of 10 cross-trained men.

Variable	Mean $\pm$ SD
Age	24.3 $\pm$ 3.8
Height (cm)	180.8 $\pm$ 5.5
Weight (kg)	89.1 $\pm$ 10.9
Percent body fat	15.1 $\pm$ 4.7
Cycle $\dot{V}O_2$max (ml·kg^{-1}·min^{-1})	44.7 $\pm$ 7.6
Aerobic exercise per wk (h)	2.8 $\pm$ 2.2
Cycling exercise per wk (h)	1.1 $\pm$ 1.7
Years aerobic exercise training	4.5 $\pm$ 2.1
Dumbbell squat 1 repetition maximum (kg)	152.5 $\pm$ 35.8
Resistance exercise per wk (h)	3.8 $\pm$ 1.8
Squatting exercise per wk (h)	1.4 $\pm$ 0.49
Years resistance exercise training	5.7 $\pm$ 2.7

and anaerobic exercise bouts matched for similar muscle groups recruited, time of exercise session, and percentage of maximal aerobic capacity and maximal muscle strength in cross-trained men.

METHODS

Experimental Approach to the Problem

To compare the extent and time course of macromolecule oxidation following acute bouts of aerobic and anaerobic exercise, markers of protein, lipid, DNA, and glutathione oxidation were assessed using a repeated measures crossover design, with attempts made to control for exercise duration, relative intensity, and muscle-group recruitment.

Subjects

Ten apparently healthy, cross-trained men between the ages of 18 and 35 years were recruited to participate in this study (Table 1). Sample size was determined to observe a statistically detected effect given 80% statistical power based on the power calculation using oxidized glutathione:total glutathione (GSSG:TGSH) as the dependent variable.

Eligible subjects were currently participating in both aerobic (e.g., including regular cycling) and anaerobic (e.g., resistance training, including regular squatting) exercise no less than 3 days per week for a minimum of 30 minutes per session. The subjects' exercise sessions required a minimum rating of 15 (i.e., hard) on the Borg rating of perceived exertion (RPE) scale, and subjects needed to be involved in these activities consecutively for a minimum of 6 months. In addition to the above, criteria for subject enrollment included a cycling maximal oxygen consumption ($\dot{V}O_2$max) of $\geq$40 ml·kg^{-1}·min^{-1} and a 1 repetition maximum (1RM) in the bent knee squat exercise of $\geq$1.5 times their body weight. Furthermore, no subject was a smoker or used tobacco products or anti-inflammatory drugs. Subjects completed a health history, drug usage, and physical activity questionnaires to determine eligibility. All subject recruitment and data collection was completed over a 3-month period of similar environmental conditions (e.g., temperature, ozone), as these variables may have altered oxidative stress biomarkers. Prior to participation, each subject was informed of all procedures, potential risks, and benefits associated with the study through both verbal and written form in accordance with the procedures approved by the University Institutional Review Board for Human Subjects Research and

signed an informed consent form prior to being admitted as a subject.

Anthropometric Measurements

Subjects' height and weight were measured using a Seca stadiometer and electronic scale (Hanover, MD), respectively. Body fat percentage was estimated using a 7-site (chest, triceps, midaxillary, suprailium, abdominal, subscapular, thigh) skinfold test using Harpenden calipers (Creative Health Products, Ann Arbor, MI) as previously described (20).

Maximal Exercise Testing

Prior to each test, both the flow meter and the gas analyzers on the SensorMedics Vmax 229 metabolic system (SensorMedics, Yorba Linda, CA) were calibrated. In addition, the environmental conditions in the laboratory were measured. A maximal graded-exercise test on a Lode Excalibur electronically braked cycle ergometer (Seattle, WA) was performed for determination of $\dot{V}O_2$max. The highest mean 1-minute $\dot{V}O_2$ value obtained during testing (whether peak or max $\dot{V}O_2$) was used to calculate the workload to be used during the submaximal aerobic-exercise protocol. On a separate occasion, subjects performed a 1RM test for the bent-knee squat exercise using free weights. Each subject was instructed as to the proper performance of this movement, observed demonstrations, and was given several attempts (6–8) as they worked toward their 1RM, with 3 minutes between each attempt. The maximum amount of weight that could be lifted 1 time in perfect form (using a 2-second eccentric/concentric tempo) was recorded as the 1RM and was used to calculate the weight to be used during the submaximal anaerobic exercise protocol.

Submaximal Exercise Protocols

Within 2 weeks of the initial assessment, subjects returned to the exercise physiology laboratory (between 7 and 10 AM) following an overnight fast (8–12 hours). Subjects were instructed not to perform any exercise for the 48-hour period preceding the 2 submaximal exercise days and were also instructed to complete a food diary as described below. The weight of each subject was then obtained to determine if any change occurred since the day of maximal exercise testing. After a 10-minute rest period, an initial blood sample (pre-exercise) was taken from an antecubital vein by a trained phlebotomist. Approximately 10 ml of blood was obtained by vacutainer, with the same sterile procedures used for all blood samples taken throughout this study.

In random order, and separated by 1–2 weeks, subjects performed both aerobic exercise (cycling at 70% of $\dot{V}O_2$max for 30 minutes on a cycle ergometer) and anaerobic exercise (squatting at 70% of 1RM for 30 minutes—total time including both work and rest) in a crossed design. The cycling exercise was continuous, while the squat routine was intermittent (i.e., subjects performed sets to a point of momentary muscular failure [i.e., 5–12 repetitions] and rested for 90–120 seconds between each set).

For the submaximal cycling protocol, subjects first warmed up for a 6-minute period, with 2 minutes spent at workloads equal to 40, 50, and 60% of $\dot{V}O_2$max values as assessed during $\dot{V}O_2$max testing. The workload was then increased to that which correlated with 70% $\dot{V}O_2$max and was adjusted every 5 minutes if necessary in order

to maintain 70% $\dot{V}O_2$max for the entire 30-minute exercise session. Subjects were allowed to consume water during the exercise as needed. At the conclusion of the 30-minute exercise protocol, a blood sample was immediately taken (0 hours post).

For the submaximal squat protocol, subjects were also fitted with a facemask for collection of expired gases and the mask remained on for the entire test period. Subjects were then asked to warm up by performing 5 minutes of light stationary cycling and performed 3 warm-up sets in the squat exercise for 5–6 reps each, with 90–120 seconds of rest between each, at a weight equal to 40, 50, and 60% of 1RM values as assessed during 1RM testing. The weight was then increased to 70% 1RM and sets were performed at this weight (or a weight 10% less than this weight if subjects could not complete at least 5 reps in proper form) for the entire 30-minute exercise session. Each set was performed to a point of momentary muscular failure, which allowed for the performance of 5–12 reps (mean $\pm$ *SEM* = 7.16 $\pm$ 0.53 reps per set), depending on the subject and the degree of fatigue as the protocol progressed. Subjects rested for 90–120 seconds between each set. The total time of the session was 30 minutes, including both work and rest time. That is, a watch was started and subjects performed their first set and rested, performed another set and rested, and so on until 30 minutes of time elapsed. Therefore, the protocol was not based on the total number of sets performed but rather on the total time of the session. This allowed for some subjects to complete 1 to 2 more sets than other subjects, depending on the total duration of each of their sets (mean $\pm$ *SEM* = 13.10 $\pm$ 0.28 sets per 30 minutes). Subjects were allowed to consume water during the exercise as needed. When subjects neared the end of the 30-minute period, the final set was carried to failure and they immediately sat in a chair for blood collection (0 hours post).

Following both exercise modes, subjects remained in the laboratory until 1-hour postexercise, at which time a third (1 hour post) blood sample was obtained. Subjects returned to the laboratory at 6 hours postexercise for a fourth (6 hour post) blood sample. Subjects also returned the following morning (between 7 and 10 AM), again in a fasted state, for a fifth (24 hour post) blood sample. Procedures were similar for all subjects, and during both the cycling and squatting sessions, $\dot{V}O_2$ was monitored continuously and recorded.

Dietary Records

All subjects were instructed to maintain their normal diet during the study period and to complete daily food records to allow for nutrient intake assessment between the days surrounding each protocol. The same researcher provided instruction regarding portion sizes and recording of foods and beverages consumed. Records were kept for the 3 days prior to each submaximal exercise protocol, in addition to the day of testing. Upon completion of the first protocol of assignment, diet records were analyzed and results returned to subjects so that they could attempt to consume a similar diet during the days surrounding the performance of the second protocol. Records were analyzed for total calories, protein, carbohydrate, fat, vitamin C, vitamin E, and vitamin A intake (Nutritionist IV, The Hearst Corporation San, Bruno, CA).

Handling and Analysis of Blood Samples

Blood samples for analysis of plasma proteins, protein carbonyls (PC), malondialdehyde (MDA), and glutathione status were collected into 10-ml vacutainer tubes containing 0.117 ml of 15% ethylenediaminetetraacetic acid (EDTA) solution and immediately processed. Three milliliters of whole blood were immediately removed, treated, and stored at $-80°$ C (Revco, Ashville, NC) until analyzed for glutathione status. The remaining blood was immediately centrifuged at 3,000 rpm for 15 minutes at 4° C in a Beckman (J2-21) centrifuge (Fullerton, CA) to obtain plasma. The plasma was stored at $-80°$ C until analyzed for the dependent variables. Additionally, at the pre- and 24-hour postexercise collection periods, 5 ml of blood was collected into serum collection vacutainer tubes for analysis of 8-hydroxy-2'-deoxyguanosine (8-OHdG). Blood was allowed to clot at room temperature for 30 minutes and then serum was separated by centrifugation at 3,000 rpm for 15 minutes at 4° C in a Beckman (J2-21) centrifuge. The serum was then stored at $-80°$ C until analyzed for 8-OHdG as described below. All assay procedures were performed in duplicate. Intra- and interassay variability was less than 6%.

Plasma Protein

Plasma protein was determined by the method of Lowry et al. (31), comparing values to known standards. Values were then adjusted to 4 mg·ml^{-1} protein using phosphate buffer (100 mM potassium phosphate + 100uM EDTA) for the assay of plasma protein carbonyls as described below.

Protein Carbonyls

Plasma was adjusted to 4 mg·ml^{-1} protein using a phosphate buffer. Protein carbonyls were determined using the 2,4-dinitrophenolhydrazine (DNPH) spectrophotometric method as described by Levine et al. (28). Briefly, samples containing either 2 N HCL or DNPH were passed through columns containing Sephadex G 10 and rinsed with 2 N HCl. The effluent was collected, mixed with guanidine HCL, and the absorbance determined at 360 nm on a Shimadzu UV-1601 spectrophotometer (Shimadzu, Columbia, MD). For all samples, the change in absorbance with and without DNPH was calculated. Values are expressed as molar quantities using the extinction coefficient 22,000 M^{-1}·cm^{-1} in nM·mg protein^{-1}.

Malondialdehyde

Total malondialdehyde was measured as previously described by Gerard-Monnier et al. (13). Samples were mixed with 500 mM butylated hydroxytoluene in acetonitrile and 12 N HCl and allowed to incubate at 60° C for 80 minutes. Following this, samples were cooled to room temperature and working reagent (i.e., 1 volume of 100% methanol with 3 volumes of 10.3 mM N-methyl-2-phenylindole in acetonitrile) was added to each sample tube, mixed, and centrifuged (Eppendorf 5415C, Brinkman Instruments, Westbury, NY) at 13,000 rpm for 5 minutes. The clear supernatants were transferred to microcentrifuge tubes, 12 N HCl was added, and tubes were centrifuged at 13,000 rpm for 5 minutes. The supernatants were transferred to cuvettes, the absorbance measured at 586 nm on a Shimadzu UV-1601 spectrophotometer and

TABLE 2. Dietary variables of 10 cross-trained men during the 3 days prior to and 1 day following cycling and squatting exercise.*

Variable	Cycling mean $\pm$ SD	Squatting mean $\pm$ SD	Statistical significance
Calories	2,887 $\pm$ 788	2,802 $\pm$ 646	$F(1, 18) = 0.0698, p = 0.7946$
Protein (g)	150 $\pm$ 51	131 $\pm$ 42	$F(1, 18) = 0.8804, p = 0.3605$
Protein (%)	21 $\pm$ 1.6	19 $\pm$ 1.6	$F(1, 18) = 0.8445, p = 0.3703$
Carbohydrate (g)	397 $\pm$ 126	406 $\pm$ 112	$F(1, 18) = 0.0300, p = 0.8645$
Carbohydrate (%)	54 $\pm$ 9.6	58 $\pm$ 7.8	$F(1, 18) = 0.9334, p = 0.3468$
Fat (g)	78 $\pm$ 28	73 $\pm$ 35	$F(1, 18) = 0.1796, p = 0.6767$
Fat (%)	25 $\pm$ 8.7	23 $\pm$ 7.7	$F(1, 18) = 0.2866, p = 0.5990$
Vitamin C (mg)	243.7 $\pm$ 161.1	240.1 $\pm$ 185.9	$F(1, 18) = 0.0021, p = 0.9636$
Vitamin E (mg)	15.3 $\pm$ 4.1	14.9 $\pm$ 3.3	$F(1, 18) = 0.0569, p = 0.8141$
Vitamin A (RE)	1,127.2 $\pm$ 480.5	1,159.3 $\pm$ 456.2	$F(1, 18) = 0.0235, p = 0.8799$

* Gram quantities for each macronutrient are provided, with corresponding percentages below. Vitamin C and vitamin E values are provided in mg; vitamin A values are provided in retinol equivalents, 1 RE = 1 μg retinol or 12 μg β-carotene.

values compared with those of known standards. Values are expressed in nM·mg protein^{-1}.

8-Hydroxy-2′-Deoxyguanosine

Measurement of 8-OHdG was performed using an enzyme-linked immunosorbent assay kit purchased from OXIS Health Products, Inc. (Portland, OR), and the assay procedures followed those of the manufacturer. Briefly, 50 μl of ultrafiltrated serum or standard was added per microtiter plate well, in addition to 50 μl of reconstituted primary antibody. The plate was sealed tightly and allowed to incubate at 37° C for 1 hour. The wells were washed thoroughly, 100 μl of reconstituted secondary antibody was added per well, the plate was sealed tightly and then allowed to incubate at 37° C for 1 hour. The chromogen was diluted with 100 volumes of chromogen dilution buffer, the plate washed again, and 100 μl of the diluted chromogen added per well. The plate incubated at room temperature in the dark for 15 minutes. Following this, 100 μl of stop solution was added to each well, and the absorbance was read at 450 nm on a microplate reader (Molecular Devices, Sunnyvale, CA). All values are expressed in ng·ml^{-1}.

Glutathione Status

Whole blood was used for determination of glutathione status, using the method described by Anderson (5). Both TGSH and GSSG were analyzed using 5,5′-diothiobis-2 nitrobenzoic acid (DTNB) to combine with reduced glutathione (GSH) to form 5-thio-2-nitrobenzoic acid (TNB). GSSG was reduced back to GSH by glutathione reductase in the presence of NADPH. The rate of TNB formation determined at 412 nm using a Shimadzu UV-1601 spectrophotometer was used. GSH was computed by subtracting 2 times the value obtained for GSSG from TGSH (GSH = TGSH − 2[GSSG]).

Statistical Analyses

The data obtained for PC, MDA, and glutathione status (TGSH, GSH, GSSG, and GSSG/TGSH) were analyzed using a 2 × 5 repeated-measures analysis of variance (ANOVA). The data obtained for 8-OHdG were analyzed using a 2 × 2 repeated-measures ANOVA. Significant interactions and main effects were analyzed using Tukey's post hoc tests. Dietary variables were compared using a 1-way ANOVA. All analyses were performed using JMP statistical software version 4.0 (SAS Institute, Cary, NC). Statistical significance was set at $p \leq 0.05$. The data are presented as mean $\pm$ SEM, except for subject character-

istics and dietary variables, which are presented as mean $\pm$ SD.

RESULTS

Submaximal Cycling and Squatting Data

All subjects completed all aspects of the submaximal exercise sessions with 100% compliance. The mean percent of $\dot{V}O_2max$ (73.06 $\pm$ 0.50) and 1RM (61.81 $\pm$ 1.58) maintained during the cycling and squatting sessions, respectively, deviated slightly from the initially planned fixed values of 70% $\dot{V}O_2max$ and 1RM. As expected, subjects consumed more total oxygen during the cycling protocol than during squatting (87.51 $\pm$ 3.39 vs. 53.31 $\pm$ 2.61 L, $p \leq 0.0001$).

Dietary Data

The mean daily calories, protein, carbohydrate, fat, vitamin C, vitamin E, and vitamin A intake during the 3 days preceding and the 1 day following the submaximal exercise sessions are presented in Table 2. Subjects consumed an average diet comprised of sufficient calories, macronutrients, and vitamins (C, E, and A). No statistically significant differences were noted between exercise modes for any measured dietary variable.

Protein Carbonyls

Plasma protein carbonyl data before and following the submaximal cycling and squatting sessions are presented in Figure 1. There was a significant exercise mode × time interaction for plasma protein carbonyls, with values statistically greater at 6 and 24 hours postexercise compared with pre-exercise for squatting ($p \leq 0.05$). Additionally, values were statistically greater at 24 hours postexercise for squatting compared with cycling ($p \leq 0.05$).

Malondialdehyde

Plasma malondialdehyde data before and following the submaximal cycling and squatting sessions are presented in Figure 2. There was no significant interaction or main effects for plasma malondialdehyde.

8-Hydroxy-2′-Deoxyguanosine

Serum 8-OHdG data before and following the submaximal cycling and squatting sessions are presented in Figure 3. There was no significant interaction or main effects for serum 8-OHdG. However, the main effect for time approached statistical significance ($p = 0.0612$).
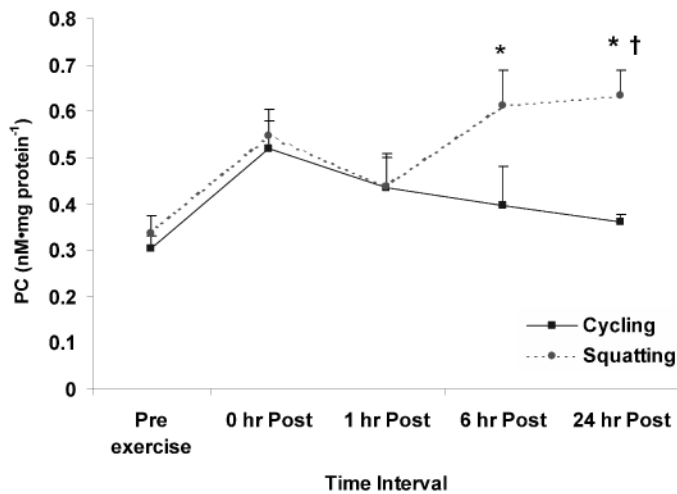
FIGURE 1. Exercise mode by time mean $\pm$ *SEM* for plasma protein carbonyls. * Different from pre-exercise for squatting mode ($p < 0.05$). † Difference between cycling and squatting modes ($p < 0.05$).
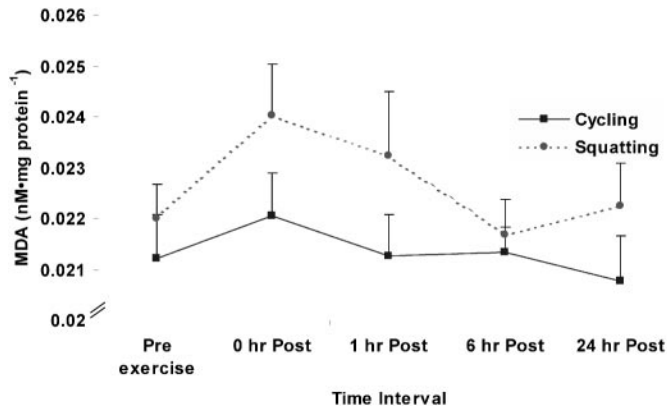

FIGURE 2. Exercise mode by time mean $\pm$ *SEM* for plasma malondialdehyde (MDA).
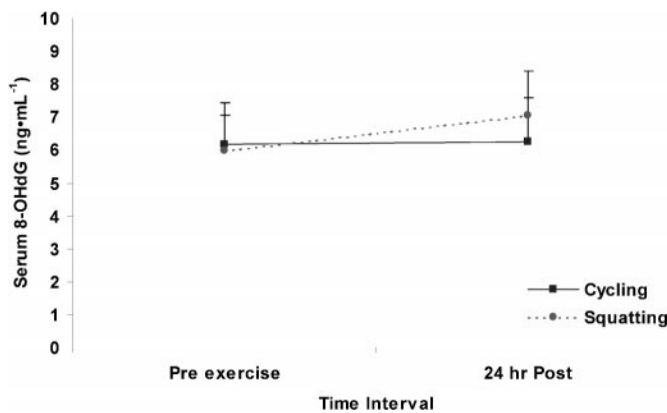

FIGURE 3. Exercise mode by time mean $\pm$ *SEM* for serum 8-OHdG.

Glutathione Status

There was no significant exercise mode or time main effects or exercise mode $\times$ time interaction effect for whole-blood TGSH. There was no significant difference between exercise modes for GSSG. However, during cycling, GSSG
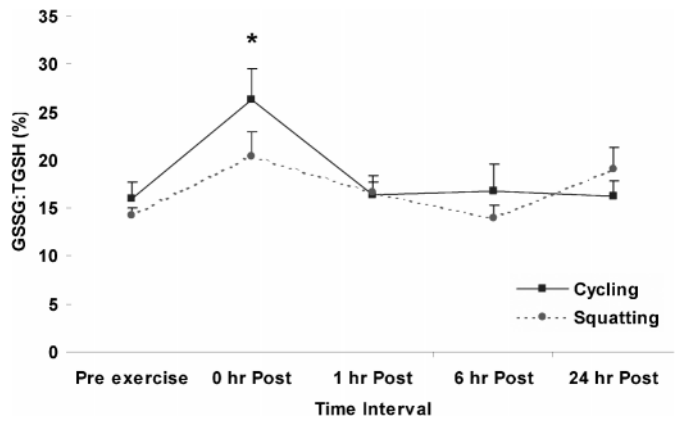

FIGURE 4. Exercise mode by time mean $\pm$ *SEM* for whole-blood glutathione:total glutathione (GSSG:TGSH). * Time main effect. Zero hour post different from all other time points ($p < 0.05$).

was greater immediately postexercise compared with all time points ($p \leq 0.05$). Because this pattern was not observed for squatting, a significant exercise mode-by-time interaction occurred. There was no exercise mode $\times$ time interaction or exercise mode main effect for whole-blood GSH. A main effect for time was noted for whole-blood GSH. Values were lower immediately postexercise compared with all other time points ($p \leq 0.05$). There was no significant interaction or exercise mode main effect for whole-blood GSSG:TGSH. However, a time main effect was noted, with whole-blood GSSG:TGSH values statistically greater immediately postexercise compared with all other time points ($p \leq 0.05$), reflecting both the decrease in TGSH and GSH and the increase in GSSG concentrations immediately postexercise (Figure 4).

DISCUSSION

The present study compared oxidative modification of blood proteins, lipids, DNA, and glutathione during the 24-hour period following both aerobic and anaerobic exercise bouts performed by cross-trained men. To our knowledge, this is the first investigation to compare oxidative stress with these popular modes of exercise while attempting to match for muscle groups recruited, total time of the exercise bout, and relative exercise intensity. The main findings of this investigation were (a) protein oxidation based on PC was increased after anaerobic exercise compared with pre-exercise and was 1.8 times higher at 24 hours postexercise for anaerobic compared with aerobic exercise; (b) lipid peroxidation based on MDA revealed an increasing trend immediately postexercise following both aerobic and anaerobic exercise but failed to reach statistical significance; (c) DNA oxidation based on 8-OHdG tended to increase from pre-exercise to 24 hours postexercise for anaerobic exercise, but the increase failed to reach statistical significance; and (d) glutathione oxidation increased immediately postaerobic exercise as evidenced by a statistically significant increase in GSSG and a decrease in GSH immediately postexercise.

All subjects appeared to consume adequate dietary energy, macronutrients, and vitamins (C, E, and A) during the days surrounding both the cycling and squatting sessions. In fact, subjects' mean intake of vitamin C was al-

most 3 times the current recommended dietary allowance (RDA) of 90 mg·d^{-1}. While such an intake exceeds current recommendations, it is far less than the 500–3,000 mg amounts of vitamin C that have been used previously in an attempt to attenuate oxidative stress after exercise (1, 7). Therefore, it is not believed that subjects' vitamin C intake impacted the results. Furthermore, with the exception of vitamins E and A (approximately consumed at the recommended intake of 15 mg·d^{-1} and 900 retinol equivalents·d^{-1}, respectively), there exists no evidence suggesting that any dietary variables analyzed influenced the markers of oxidative stress. While it is possible that long-term intake at values slightly greater than the RDA could potentially impact the biomarkers studied here, conclusive evidence in regard to this is unavailable. Therefore, we have no reason to suspect that the slight differences in dietary intake impacted the results obtained for the dependent variables in this investigation.

Relatively few data are currently available in relation to PC following exercise, especially in reference to human subjects. Pertaining to aerobic exercise, Alessio et al. (3) reported increased PC immediately postexercise following a graded treadmill protocol, with values approaching baseline 1 hour after exercise. These findings were supported by Patrick et al. (personal communication), who noted an approximate fourfold increase in PC immediately following 30 minutes of treadmill running at 75% $\dot{V}o_2$max. While PC in the present study was highest immediately postexercise following 30 minutes of cycling at approximately 73% $\dot{V}o_2$max, the elevation from baseline did not reach statistical significance (0.3020 ± 0.0279 to 0.5180 ± 0.0609 nM·mg protein^{-1}, mean ± *SEM*). It is possible that aerobic exercise of a greater intensity or duration could have produced PC at greater concentrations after exercise, as shown previously by Goldfarb et al. (15). It is interesting to note that the mean PC values for subjects in the present investigation were lower (0.518 nM·mg protein^{-1}) than those reported by Goldfarb et al. (15) in subjects following the 60% (0.60 nM·mg protein^{-1}) and 80% (1.20 nM·mg protein^{-1}) $\dot{V}o_2$max bouts. Davis and coworkers (10) recently demonstrated an exercise duration effect on PC elevation following cycling exercise in trained men. The PC increase was observed within 1 hour after exercise, with the greatest elevation following a 120-minute ride (0.78 nM·mg protein^{-1}), compared with either a 30-minute (0.51 nM·mg protein^{-1}) or 60-minute (0.58 nM·mg protein^{-1}) ride. Additionally, under all conditions, PC levels approached baseline by 1 hour postexercise. Based on these findings, which were obtained under similar cycling exercise conditions, it appears that the training status of the subjects could have impacted blood protein oxidation. That is, subjects in the present study, in addition to those in the Davis et al. (10) investigation, were aerobically trained and experienced an attenuated increase in PC compared with untrained subjects in the Goldfarb et al. (15) study.

The present study is the first that we are aware of that has extended the time course of measurement of PC beyond 60 minutes postaerobic exercise. Similar to previous work using aerobic exercise, the greatest rise in PC occurred immediately postexercise, with a progressive decline toward baseline values in the hours after exercise. The transient rise in PC following aerobic exercise is likely due to increased oxygen flux through the mitochondrial electron transport chain because oxygen uptake during exercise may increase approximately 10-fold but returns rapidly to baseline levels postexercise.

Four published reports related to PC following anaerobic exercise currently exist, with 2 studies investigating PC in human blood samples (3, 26), 1 in human skeletal muscle (47), and 1 in rat lung tissue (41). The present study showed an initial, but statistically insignificant, increase in PC immediately postexercise, with a secondary rise at 6 and 24 hours postexercise that reached statistical significance. This study adds to the current literature by showing that isotonic resistance exercise can increase plasma PC at 6 hours postexercise, earlier than previously reported following pure eccentric exercise (26).

This secondary rise in PC following resistance exercise may be attributed mechanistically to invasion of phagocytic cells into damaged muscle tissue. This typically occurs several hours postexercise and can generate a substantial amount of oxygen radical species (21, 23) while simultaneously increasing inflammation and subsequent muscle soreness. Subjects in the present study reported significant muscle soreness in the hours following squatting, which reached a mean peak rating of 6.35 (on a 10-cm visual analog scale) by 24 hours postexercise. While muscle soreness is considered an indirect marker of muscle injury and may not directly relate to loss of structural or contractile muscle protein, it does appear to have a moderate degree of correlation with PC ($r = 0.498$, $p < 0.0003$) as reported by Lee et al. (26). Concordantly, analysis of the present data also revealed a correlation between PC and muscle soreness for the squatting protocol ($r = 0.468$, $p = 0.0006$), suggesting that muscle soreness may be a reasonable predictor of protein oxidation following resistance-type exercise. Additionally, despite the moderate to high levels of anaerobic training performed by subjects, the increase in PC and muscle soreness still occurred. Previous studies have suggested that chronic exercise training can impart a protective mechanism against muscle damage (35) and oxidative stress by way of an upregulation in antioxidant enzymes and thiols, reduction of radical leakage during oxidative phosphorylation, and/or reduced production of oxidants during and following exercise (22). However, based on data from the present study, such protection does not appear adequate to totally eliminate muscle soreness or protein oxidation, provided the workload is of sufficient intensity.

Besides the potential increase in protein oxidation due to invasion of phagocytic cells, the disruption of iron-containing proteins, such as erythrocytes, can lead to an increase in free iron, which is known to catalyze radical reactions (21). Therefore, exercise that creates a significant degree of trauma (e.g., high-force eccentric and concentric muscle actions) may lead to destruction of these heme proteins, allowing for increased free-iron availability to aid in the production of ROS. An imbalance in calcium homeostasis, which is believed to be a major contributor to muscle injury resulting from resistance type exercise (12), may also be associated with ROS generation through activation of phospholipase and proteolytic enzymes (21). Furthermore, the enzyme xanthine oxidase has been noted to be a radical species generator (21, 23) and may be mediated by high-intensity exercise conditions where muscle is metabolically compromised and perhaps damaged (i.e., adenosine triphosphate degradation is greater than generation and calcium homeostasis is lost), as was purported in the squatting protocol. Thus,

these factors could help to explain the elevation in protein oxidation observed following squatting in the present study. The increase in ROS production resulting from any of the these sources could lead to oxidation of amino acid side chains and fragmentation of polypeptides, as all amino acids are susceptible to metal-catalyzed oxidation (40).

Only Alessio et al. (3) has compared PC following aerobic and anaerobic exercise, and noted greater values following a graded-exercise treadmill test compared with intermittent isometric handgrip exercise. While these findings are in opposition to those of the present study, the exercise protocols are vastly different. Unlike the current investigation, Alessio et al. (3) did not attempt to match the amount/size of muscle mass recruited nor the intensity of activity between the aerobic and anaerobic conditions. To our knowledge, the current investigation is the first to compare aerobic and anaerobic exercise bouts matched for muscle groups recruited, time of exercise session, and relative exercise intensity, in relation to PC.

Results from the present study indicate that lipid peroxidation, as measured by total plasma MDA (free and protein-bound Schiff base conjugates), was not significantly elevated at any time point postexercise following either cycling or squatting. Sacheck and colleagues (45) and Alessio et al. (1) reported increases in MDA and thiobarbituric acid reactive substances (TBARS), respectively, immediately postaerobic exercise. Both of these studies used treadmill running instead of cycling, with subjects working at slightly higher percentages of their $\dot{V}O_2$max (75–80% vs. 73% in the current study), a factor that has been reported to affect the degree of lipid peroxidation (25, 30). Thus, it is possible that the intensity and/or the duration of cycling performed in the present study was not great enough to produce a significant increase in MDA. Values returned to baseline by 1 hour postexercise and, as previously suggested, this short-lived rise in MDA is likely due to increased catabolism, excretion, or redistribution throughout the body (16, 25). This hypothesis is underscored by the fact that MDA-reactive substances turn over within minutes and not many hours to days as is the case for PC (9). Other investigations utilizing protocols of similar intensity and duration to that used in the present study corroborate our findings of little to no rise in MDA (33, 44). Therefore, higher intensity aerobic protocols (>80% $\dot{V}O_2$max) appear to be necessary to increase MDA significantly.

While lipid peroxidation appears to be increased in an intensity-dependent manner following aerobic exercise, it is unclear what is required mechanistically to increase lipid peroxidation during anaerobic exercise. Similar to the current study, several investigators have reported no change in either MDA or TBARS following anaerobic isotonic or eccentric exercise (6, 8, 17, 27, 47, 53), while some have reported an increase following isometric (11, 50) and isotonic exercise (34). Others have reported an increase in lipid hydroperoxides exclusively without an increase in MDA following handgrip exercise (3), suggesting the biomarker chosen may be important.

Only 3 studies have used isotonic resistance exercise involving large-muscle groups in the study of lipid peroxidation, with 2 agreeing with the findings of the present study (6, 53). In the present study, we did not observe an increase in MDA during the 24-hour period following squatting, findings that corroborate all other studies of eccentric and isotonic resistance exercise, with the excep-

tion of 1 (34). It is possible that elevations in MDA due to ROS production are more dependent on increased oxygen uptake or transient periods of ischemia/reperfusion, as may be likely during isometric handgrip exercise, rather than infiltration of phagocytic cells and a potential loss in calcium homeostasis secondary to muscle injury, as may be the case for PC. More research is needed to fully understand the impact of anaerobic exercise, in particular moderate-intensity resistance exercise, in relation to MDA.

Of the 3 studies that have made comparisons between aerobic and anaerobic exercise in relation to lipid peroxidation, all have reported either similar responses between the 2 modes or a more pronounced increase following anaerobic exercise (2, 3, 32). While these results suggest that anaerobic exercise can increase lipid peroxidation to a greater extent than aerobic exercise, the protocols used in these investigations differed greatly from those used in the current study (i.e., isometric or sprint protocols compared with isotonic full-body squatting), which noted no difference in MDA between exercise modes. Additionally, because subjects in the present study were cross-trained, it is possible that the lack of increase in MDA was due to greater cellular protection against ROS-mediated oxidation, as previously reported (40).

As with proteins and lipids, DNA appears to undergo oxidation following aerobic exercise in an intensity- and duration-dependent fashion (4, 36, 37, 42). Shorter duration trials similar to those performed by subjects in the current study have failed to result in an increase in DNA oxidation measured by 8-OHdG (45, 51, 52). However, at least 1 study has shown a slight increase in DNA oxidation in white blood cells following a relatively shorter duration (e.g., 90 minutes) treadmill running (54). In this investigation, animals were forced to run downhill at 24 m·min^{-1}, a protocol that may have induced muscle damage and the generation of ROS. Furthermore, the exercise duration was 3 times that of the present study, making appropriate comparisons difficult. Taken together with the few studies in the literature utilizing more widely practiced aerobic exercise bouts (e.g., 30 minutes, 60–70% $\dot{V}O_2$max), the evidence from the current investigation reinforces previous data suggesting that moderate duration and intensity aerobic exercise does not increase DNA oxidation as measured by 8-OHdG. It should be noted that this is the first study that we are aware of that has measured serum 8-OHdG in relation to exercise. Therefore, collectively with the current literature, it appears that neither urinary nor serum 8-OHdG values are altered as a result of moderate-duration and -intensity aerobic exercise. This, however, does not exclude the possibility that changes in 8-OHdG could be present in the active skeletal muscle, as both urinary and blood measurements of this biomarker may be diluted in comparison with the actual site of generation (e.g., the active skeletal muscle).

Pertaining to anaerobic exercise, only 2 studies are currently available in regard to DNA oxidation, reporting an increase in skeletal-muscle 8-OHdG (43) and the number of micronuclei in 3,000 binucleated blood lymphocytes (48) following eccentric and exhaustive sprint exercise, respectively. These findings differ with those of the current study that did not find any statistically significant increase in serum 8-OHdG from pre- to 24 hours postexercise, despite an increase in 9 of 10 subjects. A post hoc

power analysis indicated that 28 subjects would have been needed in order to observe a statistically significant effect. Differences in findings may be attributed to the dissimilarity in exercise protocols, the type of sample analyzed, in addition to the fact that subjects in the present study were anaerobically well trained and possibly better protected against oxidative modifications to DNA. It was shown by Radak and colleagues (39) that regular aerobic exercise training in rats can increase the activity of 8-OHdG repair enzymes, attenuating 8-OHdG in both middle-aged (20-month-old) and aged (30-month-old) animals. Radak et al. (38) extended these findings recently by demonstrating that a single bout of exercise can increase the activity of a specific DNA repair enzyme in human skeletal muscle (human 8-oxoG DNA glycosylase) that functions to curb the elevation in 8-OHdG. While these findings were in aerobically trained subjects following a marathon run, it is possible that such adaptations could also be present in strength-trained and cross-trained individuals following an acute bout of resistance exercise, as performed in the present investigation. This hypothesis would help to explain the minimal elevation in 8-OHdG following the squatting protocol. It is possible that the lack of a statistically detected increase in this oxidative stress marker could be due to either the timing of sample obtainment (24 hours postexercise), the lack of a sufficient intensity and/or duration of stimulus, the type of sample analyzed (serum vs. skeletal muscle), or an upregulation of the protective mechanisms against oxidative DNA damage as described by Radak et al. (38, 39). The 24-hour postexercise time point was chosen because, in previous studies, the greatest degree of DNA oxidation was reported to occur at this time (48). If measures were obtained at time points closer to the end of exercise, perhaps elevations in this marker might have been observed. Future work with the inclusion of more frequent sampling following resistance exercise may provide more insight into the extent and possible time course of DNA oxidation following this form of exercise. Additionally, it should be noted that, while only a small percentage (~10%) of total DNA oxidation is represented by 8-OHdG (29), the lack of statistical elevations in this marker does not exclude the possibility that DNA oxidation of other base pairs could have occurred. This was the first study that we are aware of that has compared aerobic and anaerobic exercise bouts in relation to DNA oxidation.

Results from the present study indicate that whole-blood GSSG was statistically greater immediately postexercise than at all other time points for cycling, with no statistically detected increase observed for squatting. Additionally, GSH was found to be decreased immediately following both cycling (32.6%) and squatting (20.9%), while TGSH remained relatively unaffected by exercise. As expected, subjects consumed more total oxygen during cycling compared with squatting (87.51 ± 3.39 vs. 53.31 ± 2.61 L, respectively), which may have enhanced ROS production and subsequent glutathione oxidation as previously described (22). In a similar manner as lipid peroxidation, it is probable that the oxidation of glutathione is most dependent on oxygen utilization.

Our findings pertaining to aerobic exercise appear well supported by previous literature (19, 24, 55), suggesting an acute oxidation of glutathione following aerobic exercise. It is believed that such transient changes in glutathione are due to the rapid conversion of GSSG back to GSH, through the activity of glutathione reductase. The reduction in GSH may be explained by its consumption either to regenerate ascorbic acid and alpha tocopherol or to scavenge certain ROS, such as superoxide anion and singlet oxygen.

Glutathione status has also been studied following anaerobic exercise, with some investigators reporting a postexercise increase in glutathione oxidation (16, 18, 50), with others reporting no change (46). When compared with previous studies assessing glutathione oxidation following anaerobic exercise, the findings from the present study in relation to the magnitude of change are in agreement. That is, while GSSG increased approximately 22% and 25% from pre- to immediately post and 24 hours postexercise, respectively, following squatting. While these changes failed to reach statistical significance, the magnitude of change is similar to that observed in other studies. Perhaps a larger sample size would have allowed for detection of a statistically significant effect.

Only 2 studies have compared glutathione oxidation following acute bouts of aerobic and anaerobic exercise. Sahlin et al. (46) found similar increases in TGSH during 80 minutes of intermittent isometric handgrip exercise and cycling exercise performed at 60% $\dot{V}O_2$max. Inal et al. (18) compared bouts of 800- and 100-m swimming in trained swimmers on blood GSH and noted similar changes following both intensities of exercise, with GSH decreasing during the 40 minutes after exercise. Results from the present study suggest little change for TGSH following either exercise mode, with a decrease in GSH mainly following cycling. Because neither of the previous studies measured GSSG, it is not known whether or not this marker was affected by such protocols. Furthermore, the anaerobic exercise bouts described above are far different from the squatting protocol used in the present study. Therefore, making comparisons between these investigations is difficult.

Results indicate that (a) 30 minutes of aerobic and anaerobic exercise performed by young, cross-trained men can increase protein and glutathione oxidation in human blood while having little impact on lipid or DNA oxidation; (b) protein oxidation appears to be more greatly affected by anaerobic exercise and the magnitude of protein oxidation is greater following anaerobic compared with aerobic exercise; and (c) glutathione oxidation appears to be more greatly affected by aerobic exercise.

Based on these findings, it appears that ROS-mediated oxidation of proteins, lipids, nucleic acids, and glutathione is not solely dependent on oxygen flux through the mitochondria, as oxygen uptake differed drastically between exercise modes. Rather, multiple factors, including xanthine oxidase, disruption of iron-containing proteins, calcium imbalance secondary to muscle injury, and inflammatory-mediated production of ROS and subsequent oxidation of macromolecules following acute bouts of aerobic and anaerobic exercise, may be involved. Furthermore, the oxidizing potential of these factors may differ based on the macromolecule studied.

In the present investigation, an attempt was made to equate the muscle mass activated, the relative exercise intensity, and the duration of the exercise protocols. Despite these attempts, cycling exercise resulted in a greater total oxygen uptake (87.51 ± 3.39 vs. 53.31 ± 2.61 L). Based on these findings, it does not seem possible to equate all variables (e.g., $\dot{V}O_2$, muscle mass, exercise in-

tensity, exercise time) when attempting to match aerobic and anaerobic exercise modes. Therefore, future studies comparing the degree of oxidative stress resulting from the performance of acute aerobic and anaerobic exercise bouts should consider the marker of investigation prior to designing the study. That is, if the markers of interest are those that appear to be more greatly affected by total oxygen consumption (i.e., MDA and GSSG), attempts should be made to equate $\dot{V}O_2$ between exercise modes. Likewise, if a marker such as PC is the dependent variable, it may be best to equate the degree of muscle activation, perhaps by using electromyography techniques.

PRACTICAL APPLICATIONS

Based on the findings of the present study, it appears as though both protein and glutathione can undergo oxidation following short-duration, moderate- to high-intensity anaerobic and aerobic exercise, respectively. It has been reported that supplementation with antioxidant vitamins and minerals may provide protection against exercise-induced oxidative stress in some, but certainly not all, investigations. Therefore, athletes and individuals performing regular structured exercise may consider the use of a complete antioxidant supplement as a component of their overall training and nutrition plan in an attempt to attenuate any potential increase in oxidative stress. However, it should be noted that the data in the present investigation alone do not lend support to the use of antioxidant supplementation in individuals who are already well trained. Rather, these data provide grounds for further research in this field aimed at investigating the effects of such antioxidant therapy as related to suppression of oxidative stress following aerobic and anaerobic exercise bouts. Most important, it seems prudent that all individuals perform regular exercise training in an effort to upregulate endogenous antioxidant systems, as this activity has been shown repeatedly to prove effective.

REFERENCES

1. ALESSIO, H.M., A.H. GOLDFARB, AND G. CAO. Exercise-induced oxidative stress before and after vitamin C supplementation. *Int. J. Sport Nutr.* 7(1):1–9. 1997.
2. ALESSIO, H.M., A.H. GOLDFARB, AND R.G. CUTLER. MDA content increases in fast-and slow-twitch skeletal muscle with intensity of exercise in a rat. *Am. J. Physiol.* 255:C874–C877. 1988.
3. ALESSIO, H.M., A.E. HAGERMAN, B.K. FULKERSON, J. AMBROSE, R.E. RICE, AND R.J. WILEY. Generation of reactive oxygen species after exhaustive aerobic and isometric exercise. *Med. Sci. Sports Exerc.* 32:1576–1581. 2000.
4. ALMAR, M., J.G. VILLA, M.J. CUEVAS, J.A. RODRIGUEZ-MARROYO, C. AVILLA, AND J. GONZALEZ-GALLEGO. Urinary levels of 8-hydroxydeoxyguanosine as a marker of oxidative damage in road cycling. *Free Radic. Res.* 36:247–253. 2002.
5. ANDERSON, M.E. Determination of glutathione and glutathione disulfide in biological samples. *Methods Enzymol.* 113:548–555. 1985.
6. BOYER, B.T., A.H. GOLDFARB, AND A.Z. JAMURTAS. Relationship of prostaglandin E2, leukotriene B4, creatine kinase, lactic acid, and DOMS. *Med. Sci. Sports Exerc.* 28:S154. 1996.
7. BRYER, S., AND A.H. GOLDFARB. The effect of vitamin C supplementation on blood glutathione status, DOMS, and creatine kinase. *Med. Sci. Sports Exerc.* 33:S122. 2001.
8. CHILD, R., S. BROWN, S. DAY, A. DONNELLY, H. ROPER, AND J. SAXTON. Changes in indices of antioxidant status, lipid peroxidation and inflammation in human skeletal muscle after eccentric muscle actions. *Clin. Sci.* 96:105–115. 1999.
9. DAVIES, K.L., AND A.L. GOLDBERG. Oxygen radicals stimulate intracellular proteolysis and lipid peroxidation by independent mechanisms in erythrocytes. *J. Biol. Chem.* 262:8220–8226. 1987.
10. DAVIS, P.G., R.J. BLOOMER, L. WIDEMAN, L.A. CONSITT, A.H. GOLDFARB, T. YOU, AND R.A. WEAVER. Effect of exercise duration on plasma protein carbonyls in male cyclists. *Med. Sci. Sports Exerc.* 35:S344. 2003.
11. DOUSETT, E., J.G. STEINBERG, M. FAUCHER, AND Y. JAMMES. Acute hypoxemia does not increase the oxidative stress in resting and contracting muscle in humans. *Free Radic. Res.* 36:701–704. 2002.
12. FRIDEN, J., AND R.L. LIEBER. Eccentric exercise-induced injuries to contractile and cytoskeletal muscle fibre components. *Acta. Physiol. Scand.* 171:321–326. 2001.
13. GERARD-MONNIER, D., I. ERDELMEIER, K. REGNARD, N. MOZE-HENRY, J.C. YADAN, AND J. CHAUDIERE. Reactions of 1-methyl-2-phenylindole with malondialdehyde and 4-hydroxyalkenals. Analytical applications to a colorimetric assay of lipid peroxidation. *Chem. Res. Toxicol.* 11:1176–1183. 1998.
14. GOLDFARB, A.H. Nutritional antioxidants as therapeutic and preventive modalities in exercise-induced muscle damage. *Can. J. Appl. Physiol.* 24(3):249–266. 1999.
15. GOLDFARB, A.H., T. YOU, R.J. BLOOMER, S. LANDES, AND C. MURPHY. Blood oxidative stress markers in response to aerobic exercise: Effect of gender. *Med. Sci. Sports Exerc.* 34:S249. 2002.
16. GROUSSARD, C., F. RANNOU-BEKONO, G. MACHEFER, M. CHEVANNE, S. VINCENT, O. SERGENT, J. CILLARD, AND A. GRATAS-DELAMARCHE. Changes in blood lipid peroxidation markers and antioxidants after a single sprint anaerobic exercise. *Eur. J. Appl. Physiol.* 89:14–20. 2003.
17. HELLSTEN, Y., U. FRANDSEN, N. ORTHENBLAD, B. SJODIN, AND E.A. RICHETER. Xanthine oxidase in human skeletal muscle following eccentric exercise: A role of inflammation. *J. Physiol.* 498:239–248. 1997.
18. INAL, M., F. AKYUZ, A. TURGUT, AND W.M. GETSFRID. Effect of aerobic and anaerobic metabolism on free radical generation swimmers. *Med. Sci. Sports Exerc.* 33:564–567. 2001.
19. INAYAMA, T., J. OKA, M. KASHIBA, M. SAITO, M. HIGUCHI, K. UMEGAKI, Y. YAMAMOTO, AND M. MATSUDA. Moderate physical exercise induces the oxidation of human blood protein thiols. *Life Sci.* 70:2039–2046. 2002.
20. JACKSON, A.S., AND M.L. POLLOCK. Generalized equations for predicting body density of men. *Br. J. Nutr.* 40:497–504. 1978.
21. JACKSON, M.J. Exercise and oxygen radical production by muscle. In: *Handbook of Oxidants and Antioxidants in Exercise.* C.K. Sen, L. Packer, O. Hanninen, eds. Amsterdam: Elsevier Science, 2000. pp. 57–68.
22. JI, L.L. Exercise-induced modulation of antioxidant defense. *Ann. N. Y. Acad. Sci.* 959:82–92. 2002.
23. KNIGHT, J.A. *Free Radicals, Antioxidants, Aging, and Disease.* Washington, DC: American Association for Clinical Chemistry Press, 1999.
24. LAAKSONEN, D.E., M. ATALAY, L. NISKANEN, M. UUSITUPA, O. HANNINEN, AND C.K. SEN. Blood glutathione homeostasis as a determinant of resting and exercise-induced oxidative stress in young men. *Redox. Rep.* 4(1–2):53–59. 1999.
25. LEAF, D.A., M.T. KLEINMAN, M. HAMILTON, AND T.J. BARSTOW. The effect of exercise intensity on lipid peroxidation. *Med. Sci. Sports. Exerc.* 29:1036–1039. 1997.
26. LEE, J., A.H. GOLDFARB, M.H. RESCINO, S. HEDGE, S. PATRICK, AND K. APPERSON. Eccentric exercise effect on blood oxidative-stress markers and delayed onset of muscle soreness. *Med. Sci. Sports Exerc.* 34:443–448. 2002.
27. LENN, J., T. UHL, C. MATTOCOLA, G. BOISSONNEAULT, J. YATES, W. IBRAHIM, AND G. BRUCKNER. The effects of fish oil and isoflavones on delayed onset muscle soreness. *Med. Sci. Sports Exerc.* 34:1605–1613. 2002.

28. LEVINE, R.L., D. GARLAND, C.N. OLIVER, A. AMICI, I. CLIMENT, A LENZ, B. AHN, S. SHALTIEL, AND E.R. STADTMAN. Determination of carbonyl content in oxidatively modified proteins. *Methods Enzymol.* 186:464–478. 1990.

29. LODOVICI, M., C. CASALINI, R. CARIAGGI, L. MICHELUCCI, AND P. DOLARA. Levels of 8-hydroxydeoxyguanosine as a marker of DNA damage in human leukocytes. *Free Radic. Biol. Med.* 28: 13–17. 2000.

30. LOVLIN, R., W. COTTLE, I. PYKE, M. KAVANAGH, AND A.N. BELCASTRO. Are indices of free radical damage related to exercise intensity. *Eur. J. Appl. Physiol. Occup. Physiol.* 56:313–316. 1987.

31. LOWRY, O.H., N.J. ROSENBROUGH, A.L. FARR, AND R.J. RANDALL. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193:265–275. 1951.

32. MARZATICO, F., O. PANSARASA, L. BERTORELLI, L. SOMENZINI, AND G. DELLA VALLE. Blood free radical antioxidant enzymes and lipid peroxides following long-distance and lactacidemic performances in highly trained aerobic and sprint athletes. *J. Sports Med. Phys. Fitness* 37:235–239. 1997.

33. MAXWELL, S.R., P. JAKEMAN, H. THOMASON, C. LEGUEN, AND G.H. THORPE. Changes in plasma antioxidant status during eccentric exercise and the effect of vitamin supplementation. *Free Radic. Res. Commun.* 19:191–202. 1993.

34. MCBRIDE, J.M., W.J. KRAEMER, T. TRIPLETT-MCBRIDE, AND W. SEBASTIANELLI. Effect of resistance exercise on free radical production. *Med. Sci. Sports. Exerc.* 30:67–72. 1998.

35. MCHUGH, M.P., D.A.J. CONNOLLY, R.G. ESTON, AND G.W. GLEIM. Exercise-induced muscle damage and potential mechanisms for the repeated bout effect. *Sports Med.* 27:157–170. 1999.

36. OKAMURA, K., T. DOI, K. HAMADA, M. SAKURAI, Y. YOSHIOKA, R. MITSUZONO, T. MIGITA, S. SUMIDA, AND Y. SUGAWA-KATAYAMA. Effect of repeated exercise on urinary 8-hydroxy-deoxyguanosine excretion in humans. *Free Radic. Res.* 26:507–514. 1997.

37. POULSEN, H.E., S. LOFT, H. PRIEME, K. VISTISEN, J. LYKKESFELDT, K. NYYSSONEN, AND J.T. SALONEN. Oxidative DNA damage in vivo: Relationship to age, plasma antioxidants, drug metabolism, glutathione-S-transferase activity and urinary creatinine excretion. *Free Radic. Res.* 29:565–571. 1998.

38. RADAK, Z., P. APOR, J. PUCSOK, I. BERKES, H. OGONOVSZKY, G. PAVLIK, H. NAKAMOTO, AND S. GOTO. Marathon running alters the DNA base excision repair in human skeletal muscle. *Life Sci.* 72:1627–1633. 2003.

39. RADAK, Z., H. NAITO, T. KANEKO, S. TAHARA, H. NAKAMOTO, R. TAKAHASHI, F. CARDOZO-PELAEZ, AND S. GOTO. Exercise training decreases DNA damage and increases DNA repair and resistance against oxidative stress of proteins in aged rat skeletal muscle. *Eur. J. Physiol.* 445:273–278. 2002.

40. RADAK, Z., A.W. TAYLOR, H. OHNO, AND S. GOTO. Adaptation to exercise-induced oxidative stress: From muscle to brain. *Exerc. Immun. Rev.* 7:90–107. 2001.

41. RADAK, Z., A. NAKAMURA, H. NAKAMOTO, K. ASANO, H. OHNO, AND S. GOTO. A period of anaerobic exercise increases the accumulation of reactive carbonyl derivatives in the lungs of rats. *Pflugers Arch. Eur. J. Physiol.* 435:439–441. 1998.

42. RADAK, Z., J. PUCSUK, S. BOROS, L. JOSFAI, AND A.W. TAYLOR. Changes in urine 8-hydroxydeoxyguanosine levels of super-marathon runners during a four-day race period. *Life Sci.* 66: 1763–1767. 2000.

43. RADAK, Z., J. PUCSOK, S. MECSEKI, T. CSONT, AND P. FERDINANDY. Muscle soreness-induced reduction in force generation is accompanied by increased nitric oxide content and DNA damage in human skeletal muscle. *Free Radic. Biol. Med.* 26: 1059–1063. 1999.

44. SACHECK, J.M., E.A. DECKER, AND P.M. CLARKSON. The effect of diet on vitamin E intake and oxidative stress in response to acute exercise in female athletes. *Eur. J. Appl. Physiol.* 83:40–46. 2000.

45. SACHECK, J.M., P.E. MILBURY, J.G. CANNON, R. ROUBEOFF, AND J.B. BLUMBERG. Effect of vitamin E and eccentric exercise on selected biomarkers of oxidative stress in young and elderly men. *Free Radic. Biol. Med.* 34:1575–1588. 2003.

46. SAHLIN, K., S. CIZINSKY, M. WARHOLM, AND J. HOBERG. Repetitive static muscle contractions in humans—A trigger of metabolic and oxidative stress? *Eur. J. Appl. Physiol.* 64:228–236. 1992.

47. SAXTON, J.M., A.E. DONNELLY, AND H.P. ROPER. Indices of free-radical-mediated damage following maximal voluntary eccentric and concentric muscular work. *Eur. J. Appl. Physiol.* 68: 189–193. 1994.

48. SCHIFFL, C., C. ZIERES, AND H. ZANKL. Exhaustive physical exercise increases frequency of micronuclei. *Mut. Res.* 389:243–246. 1997.

49. SEN, C.K., L. PACKER, AND O. HANNINEN, eds., *Exercise and Oxygen Toxicity.* Amsterdam: Elsevier Science, 1994.

50. STEINBERG, J., M. GAINNIER, M. FABRICE, M. FAUCHER, C. ARMAUD, AND Y. JAMMES. The post-exercise oxidative stress is depressed by acetylsalicylic acid. *Resp. Physiol. Neurobiol.* 130: 189–199. 2002.

51. SUMIDA, S., T. DOI, M. SAKURAI, Y. YOSHIOKA, AND K. OKAMURA. Effect of a single bout of exercise and beta-carotene supplementation on the urinary excretion of 8-hydroxy-deoxyguanosine in humans. *Free Radic. Res.* 27:607–618. 1997.

52. SUMIDA, S., K. OKAMURA, T. DOI, M. SAKURAI, Y. YOSHIOKA, AND Y. SUGAWA-KATAYAMA. No influence of a single bout of exercise on urinary excretion of 8-hydroxy-deoxyguanosine in humans. *Biochem. Mol. Biol. Int.* 42:601–609. 1997.

53. SURMEN-GUR, E., E. OZTURK, H. GUR, Z. PUNDUK, AND P. TUNCEL. Effect of vitamin E supplementation on post-exercise plasma lipid peroxidation and blood antioxidant status in smokers: With special reference to haemoconcentration effect. *Eur. J. Appl. Physiol.* 79:472–478. 1999.

54. UMEDAKI, K., P. DAOHUA, A. SUGISAWA, M. KIMURA, AND M. HIGUCHI. Influence of one bout of vigorous exercise on ascorbic acid in plasma and oxidative damage to DNA in blood cells and muscle in untrained rats. *J. Nutr. Biochem.* 11:401–407. 2000.

55. VIGUIE, C.A., B. FREI, M.K. SHIGENAGA, B.N. AMES, L. PACKER, AND G.A. BROOKS. Antioxidant status and indexes of oxidative stress during consecutive days of exercise. *J. Appl. Physiol.* 75: 566–572. 1993.

Acknowledgments