12-2011

Muscle-Fiber Type and Blood Oxidative Stress After Eccentric Exercise

John Quindry

Lindsey Miller

Graham McGinnis

Megan Irwin

Charles Dumke
University of Montana - Missoula, charles.dumke@umontana.edu

See next page for additional authors

International Journal of Sport Nutrition and Exercise Metabolism, 2011, 21, 462-470

Muscle-Fiber Type and Blood Oxidative Stress After Eccentric Exercise

John Quindry, Lindsey Miller, Graham McGinnis, Megan Irwin, Charles Dumke, Meir Magal, N. Travis Triplett, Jeffrey McBride, and Zea Urbiztondo

Acute strength exercise elicits a transient oxidative stress, but the factors underlying the magnitude of this response remain unknown. The purpose of this investigation was to determine whether muscle-fiber type relates to the magnitude of blood oxidative stress after eccentric muscle activity. Eleven college-age men performed 3 sets of 50 eccentric knee-extensions. Blood samples taken pre-, post-, and 24, 48, 72, and 96 hr postexercise were assayed for comparison of muscle damage and oxidative-stress biomarkers including protein carbonyls (PCs). Vastus lateralis muscle biopsies were assayed for relative percentage of slow- and fast-twitch muscle fibers. There was a mixed fiber composition (Type I = 39.6% ± 4.5%, Type IIa = 35.7% ± 3.5%, Type IIx = 24.8% ± 3.8%; p = .366). PCs were elevated 24, 48, and 72 hr (p = .032) postexercise, with a peak response of 126% (p = .012) above baseline, whereas other oxidative-stress biomarkers were unchanged. There are correlations between Type II muscle-fiber type and postexercise PC. Further study is needed to understand the mechanisms responsible for the observed fast-twitch muscle-fiber oxidative-stress relationship.

Keywords: antioxidant capacity, free radicals, protein carbonyls, reactive oxygen species

Acute muscle activity elicits numerous stimuli including redox perturbations that result in tissue and blood plasma oxidative stress (Allessio et al., 2000; Bloomer, Fry, Falvo, & Moore, 2006; Hudson et al., 2008; Quindry, Stone, King, & Broeder, 2003). Conventional understanding of exercise-generated oxidative stress holds that free-radical production is proportional to total oxygen flux, where a nominal percentage (2–5%) of molecular oxygen is incompletely reduced to form superoxide (Boveris & Chance, 1973; Loschen, Azzi, Richter, & Flohe, 1974). Unquenched or dismutated superoxide then initiates a reaction cascade involving reactive oxygen species (ROS), resulting in damage to intracellular and extracellular constituents. Although early findings on the topic demonstrated that elevated biomarkers for oxidative damage were associated with acute aerobic exercise (Hessel, Haberland, Muller, Lerche, & Schimke, 2000; Liu et al., 1999; Mastaloudis, Leonard, & Traber, 2001; McAnulty et al., 2007), this classic understanding has evolved with the discovery that far less than 1% of oxygen is converted to superoxide during tightly coupled State 3 mitochondrial respiration (Anderson, Yamazaki, & Neufer, 2007). More recent findings from human studies by Alessio et al. (2000) and Quindry et al. (2003) support this latter understanding that exercise-generated oxidative stress does not necessarily accrue solely as a function of total aerobic metabolism (Allessio et al., 2000; Quindry et al., 2003).

An alternative explanation is that blood oxidative stress can be elicited by participation in high-intensity aerobic (Allessio et al., 2000; Bergholm et al., 1999; Quindry et al., 2003) and resistance exercise (Allessio et al., 2000; Bloomer, Goldfarb, Wideman, McKenzie, & Consitt, 2005; Hudson et al., 2008) independent of oxidative metabolism. Relative to high-intensity resistance exercise in which total oxidative metabolism is low, recent investigations employing the most sensitive biomarkers of oxidative damage demonstrate that a transient blood oxidative stress occurs in the initial minutes and hours of postexercise recovery (Bloomer, Falvo, et al., 2006; Bloomer, Fry, et al., 2006; Bloomer et al., 2005; Hudson et al., 2008). Despite this understanding, much about the acute oxidative-stress response to resistance exercise remains unknown. In human-based applied physiology studies, a direct relationship between blood indices of oxidative stress and contracting skeletal muscle has yet to be identified. Fundamental to this topic is the need to understand whether the oxidative-stress response to resistance exercise is affected by muscle-fiber type. Recent work in isolated muscle-fiber bundles indicates that compared with the more oxidative Type I fibers, Type II fibers have intrinsic properties that favor ROS production

Quindry, Miller, and McGinnis are with the Dept. of Kinesiology, Auburn University, Auburn AL. Irwin, Triplett, McBride, and Urbiztondo are with the Dept. of Health, Leisure, and Exercise Science, Appalachian State University, Boone, NC. Dumke is with the Dept. of Health and Human Performance, University of Montana, Missoula, MT. Magal is with the Div. of Mathematics and Science, North Carolina Wesleyan College, Rocky Mount, NC.

(Anderson & Neufer, 2006). Given this understanding, there is reason to suspect that resistance exercise may elicit a more pronounced oxidative-stress response from individuals with a higher percentage of fast-twitch muscle fibers. To date, the relationship between muscle-fiber type and oxidative stress after strength-type exercise remains unknown.

Based on this rationale, we undertook the current study to examine the relationship between muscle-fiber type and blood oxidative-stress magnitude in the days after muscle-damaging eccentric resistance exercise. We hypothesized that individuals with a higher percentage of fast-twitch muscle fibers would experience greater oxidative stress after eccentric resistance exercise. To ensure the likelihood that a single bout of eccentric exercise would elicit blood oxidative stress, an oxidative-stress biomarker panel was chosen to better understand the dynamic oxidative-stress process that includes both antioxidant depletion and oxidant damage to proteins and lipids (Buettner, 1993).

Methods

Participants

Study approval was granted by the Appalachian State University institutional review board before initiation of this investigation. Eleven untrained college-age male participants were recruited from the Appalachian State University population and local community. Once informed consent was obtained, medical histories were collected to confirm participant suitability for the study. To avoid potential confounding effects on oxidative-stress outcomes, participants were instructed to abstain from dietary supplements and anti-inflammatory drugs 2 days before and 1 week after the eccentric-exercise protocol for oxidative-stress assessment.

Study Design

Participants reported to the human performance laboratory, unfasted, nine times during the study. Sessions 1 and 2 included baseline anthropometric, cardiorespiratory-fitness, and body-composition measurements; squat exercise; and a series of jump-performance tests. Session 3 consisted of a Wingate test, Sessions 4–8 included the eccentric-exercise bout and subsequent blood sampling for oxidative-stress biomarkers (24, 48, 72, and 96 hr after muscle-damaging exercise), and Session 9 included a muscle biopsy. The study design is outlined in Figure 1. Subjects were instructed to maintain their normal diet for the duration of study participation and to avoid large-dose vitamin/mineral supplements (above 100% RDA), anti-inflammatory agents, and herbs during this investigation.

Anthropometric and Performance Trials

Body composition was assessed with dual-energy X-ray absorptiometry (DEXA; Hologic, Inc., Bedford, MA). Peak oxygen uptake (VO_{2peak}) tests (MedGraphics Cardiorespiratory Diagnostics, St. Paul, MN) were performed on a motor-driven treadmill (Trackmaster, Full Vision, Inc., Newton, KS) and employed the Bruce protocol to the point of volitional exhaustion. Muscle strength and power were measured using isometric-squat, static-jump, countermovement-jump, drop-jump, and Wingate-cycle tests. The 30-s Wingate-cycle test was performed on a cycle ergometer (model 868, Monark-Crescent AB, Varberg, Sweden) against a prescribed load (body mass [kg] × 0.095). The isometric-squat and jump protocols consisted of three trials of each action with a 1-min rest in between, performed on command on a force plate (BP6001200, AMTI, Watertown, MA). For the isometric squat, a knee angle of 90° was measured, and proper form during the 3-s contraction was ensured through visual inspection. Total force production and rate of force development were measured and recorded for each trial (LabView v. 7.1, National Instruments, Austin, TX). Participants performed the static jump, countermovement jump, and drop jumps while holding an unweighted plastic bar connected to linear position transducers (PT5A-150; Celesco Transducer Products, Chatsworth, CA) to measure velocity and height traveled.

Eccentric-Exercise Trial

Blood oxidative stress was assessed before and after an eccentric-exercise trial designed to induce muscle damage. Participants were instructed to abstain from all formal exercise and strenuous activity for a time window beginning 48 hr before and ending 96 hr after the eccentric-exercise protocol. Based on an existing protocol, the eccentric-exercise session consisted of three sets of 50 eccentric contractions by the left leg using a Kin-Com isokinetic dynamometer (Isokinetic International, Harrison, TN) at 30°/s (with an arc of motion of approximately 85°)

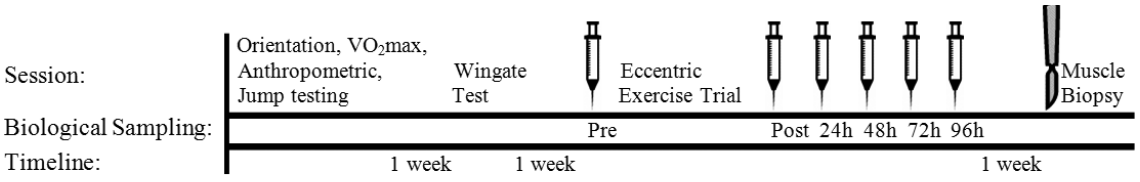

Figure 1 — The study design presenting laboratory sessions, biological-sample collection, and data-collection time course. Syringe icons represent blood draws; scalpel icon indicates muscle biopsy.

with 3-min rests between sets (MacIntyre, Reid, Lyster, & McKenzie, 2000). Subjects were instructed to relax their quadriceps muscle as the machine passively extended the leg to the start angle (~180°; Byrne, Eston, & Edwards, 2001) and to resist the Kin-Com as strongly as possible during the eccentric phase (Eston, Finney, Baker, & Baltzopoulos, 1996). Each repetition consisted of a passive leg extension followed by eccentric resistance to leg flexion. Maximal concentric muscle strength was assessed in the left leg at baseline, immediately after eccentric exercise, and at all follow-up blood draws.

Blood and Tissue Sampling

Blood samples were collected before any muscle contractions, immediately after, and 24, 48, 72, and 96 hr after the eccentric-exercise bout. Blood samples were drawn from the antecubital vein and collected in heparinized Vacutainer tubes. The blood plasma was separated and frozen at –80 °C for subsequent biochemical analyses. Blood analysis for creatine kinase (CK) was assayed as previously described (Matthews, Altman, Campbell, & Royston, 1990). Blood analysis of lactate dehydrogenase was performed by automated Coulter STKS analyzer at the Watauga Medical Center in Boone, NC. Additional aliquots were assayed for protein carbonyls and lipid hydroperoxides. Plasma antioxidant capacity was measured by plasma Trolox-equivalent antioxidant capacity (TEAC), and uric acid and total plasma antioxidant potential were determined by ferric-reducing antioxidant potential (FRAP).

At the end of the study protocol, approximately 50–100 mg of muscle tissue was extracted from the vastus lateralis by percutaneous needle biopsy (Bergstrom & Hultman, 1969). Local anesthetic (2% xylocaine) was injected subcutaneously and intramuscularly before incision. A small incision (~0.5 cm) was used to facilitate percutaneous needle biopsy of the vastus lateralis under the influence of suction (Evans, Phinney, & Young, 1982). Muscle-tissue samples were immediately coated with mounting medium (Negative 50 frozen-section medium, Richard-Allan Scientific, Kalamazoo, MI) and cooled to storage temperature in liquid-nitrogen-chilled isopentane. Samples were stored at –80 °C until histochemical analysis.

Muscle-Fiber Typing

Muscle-biopsy samples were serially sectioned (10 μm), mounted on glass slides, and fixed with Accustain 10% formalin solution (Sigma-Aldrich, St. Louis, MO). Muscle-fiber-type properties were identified with the use of myofibrillar ATPase assay to distinguish between fast- (Type IIa, IIx) and slow-twitch (Type I) muscle fibers (Brooke & Kaiser, 1970). Specifically, muscle sections were preincubated with acidic (pH = 4.3) or basic (pH = 10.3) solutions to inhibit myosin ATPase activity in Type II and Type I fibers, respectively. Computerized images of histochemical sections were taken with a high-definition video microscopy system (MHC799S40MX, Sony Corporation, Tokyo, Japan). Fiber types were expressed as percentages of total fiber counts, which constituted ~150–200 fibers per muscle section.

Oxidative-Stress Biomarker Analysis

Plasma antioxidant capacity was measured by the plasma TEAC technique, whereby a radical cation of the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) reaction is quenched by sample-specific antioxidant fortifications (Cao & Prior, 1998). Total plasma antioxidant potential was determined by the FRAP assay according to the methodology of Benzie and Strain (1996). Both TEAC and FRAP produce colorimetric solutions that are quantified spectrophotometrically. Lipid peroxidation was determined by the ferrous oxidation-xylenol orange assay of Nourooz-Zadeh, Tajaddini-Sarmadi, and Wolff (1994), in which ferrous ions are oxidized by lipid hydroperoxides to ferric ions and subsequently react with the ferrous-sensitive dye containing xylenol orange. In the presence of lipid hydroperoxides, this reaction forms a spectrophotometrically quantifiable complex. Protein carbonyls were analyzed using a commercially available ELISA kit (Zentech Technology, Dunedin, New Zealand). All assays were performed in triplicate and exhibited within-sample coefficients of variation of 2%–5%. Before analysis, all plasma samples were assayed in quadruplicate for protein concentration based on the methods of Bradford (1976) and adjusted to 4 mg/ml protein using a phosphate buffer (Bradford, 1976). Plasma uric acid was determined by the spectrophotometric assay (Kovar, el Bolkiny, Rink, & Hamid, 1990).

Statistical Analysis

A one-way repeated-measures analysis of variance (ANOVA) was used to analyze muscle damage, muscle soreness, eccentric-exercise performance, and oxidative-stress biomarkers. Where appropriate, Tukey's post hoc tests were used to examine time effects. Pearson's product–moment correlation coefficients were used to examine correlations between oxidative-stress outcomes, muscle-fiber type, and anthropometric and exercise-performance data. For this investigation, statistical significance was set at $p < .05$ a priori. Statistical analyses were performed using a statistical software package (SPSS, Version 17.0, SPSS, Inc., Chicago, IL).

Results

Participant physical characteristic and performance data are presented in Table 1. The college-age participants had an average of 16% body fat. Based on vastus lateralis biopsy, the group did not predominate toward either slow- or fast-twitch muscle-fiber type ($p = .333$). Fitness-test performance data are presented in Table 2. Aerobic-fitness, Wingate, and jump-test performance data were suggestive of recreationally fit individuals. At the completion of the eccentric-exercise trial, a significant loss in concentric muscle strength was observed. Mean eccentric- and concentric-strength values returned to

Table 1 Participant Physical Characteristics, *M* ± *SEM*

Characteristic	Value
Age (years)	21.1 ± 1.3
Height (cm)	179.0 ± 1.4
Body mass (kg)	77.7 ± 3.4
Lean body mass (kg)	65.2 ± 3.4
Body fat (%)	16.1 ± 1.0
Type I muscle fibers (%)	39.6 ± 4.5
Type II muscle fibers (%)	60.4 ± 4.5
Type IIa muscle fibers (%)	35.7 ± 3.5
Type IIx muscle fibers (%)	24.7 ± 3.8

Table 2 Fitness-Test Performance Data, *M* ± *SEM*

Fitness test	
VO_{2peak} (ml · kg^{-1} · min^{-1})	46.6 ± 1.5
Wingate test	
average power (W)	714 ± 39
total work (J)	21,956 ± 1,227
peak power (W)	988 ± 54
fatigue rate (W/s)	–20.9 ± 1.5
Jump tests	
isometric-squat force (N)	2,009 ± 130
static-jump force (N)	1,778 ± 65
drop-jump force (N)	2,209 ± 222
countermovement-jump force (N)	1,880 ± 124
Eccentric-exercise trial	
pretrial concentric force (N)	526 ± 39
immediately posttrial concentric force (N)	387 ± 36*
24 hr posttrial concentric force (N)	515 ± 35
48 hr posttrial concentric force (N)	530 ± 39
72 hr posttrial concentric force (N)	537 ± 42
96 hr posttrial concentric force (N)	568 ± 38

*Significant difference from pretrial concentric force, $p \leq .05$.

baseline 24 hr postexercise and remained similar to pretrial values for the duration of the study.

Plasma CK values were modestly but significantly elevated 24 hr ($p < .001$) after the eccentric-exercise trial and remained elevated 72 hr after eccentric exercise (Figure 2). Plasma biomarkers for aqueous-phase antioxidant capacity are presented for TEAC, FRAP, and uric acid in Figure 3. No alterations in these biomarkers of plasma antioxidant capacity were noted at any time after the eccentric-exercise trial (TEAC, $p = .348$; FRAP, $p = .558$; uric acid, $p = .245$). Mean plasma lipid

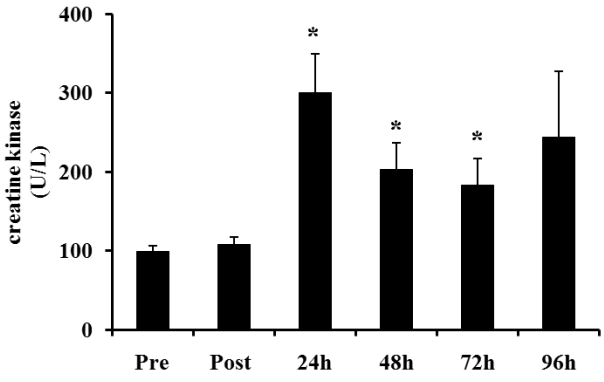

Figure 2 — Plasma creatine kinase levels before and after an eccentric-resistance-exercise trial, *M* ± *SEM*, $n = 11$/measure. *Significantly different than Pre values; $p \leq .05$.

hydroperoxide and protein carbonyl values are displayed in Figure 4. Lipid hydroperoxides ($p = .378$) were not affected by the eccentric-exercise trial. In contrast, mean protein carbonyl levels ($p = .032$) were increased 24 hr after the oxidative-stress/eccentric-exercise trial and remained elevated through 72 hr after eccentric exercise. The mean increase in protein carbonyls 96 hr after eccentric exercise was not significantly different than pretrial values ($p = .056$).

In light of the fact that protein carbonyls were the only oxidative-stress biomarker to yield significant differences after eccentric exercise, and because the peak rise for individual subjects occurred between 24 hr and 72 hr postexercise ($n = 3$ at 24 hr, $n = 3$ at 48 hr, and $n = 5$ at 72 hr), correlation analyses were performed for peak protein carbonyl values and other study variables. Correlation results are presented in Table 3. The peak rise in protein carbonyls did not correlate with Type I ($r = .326$, $p = .218$), IIa ($r = .277$, $p = .300$), and IIx ($r = .253$, $p = .345$) muscle-fiber percentages, whereas Type II (IIa and IIx combined) did ($r = .643$, $p = .045$). No statistical relationship was observed between plasma CK, muscle soreness, or aerobic fitness and peak elevation in protein carbonyls. In contrast, Wingate-test performance parameters and jump-test performance outcomes were significantly correlated with the measured rise in protein carbonyls. Finally, during the eccentric-exercise trial, the rise in plasma protein carbonyl content also correlated positively with maximal concentric-force production. Plasma protein carbonyl elevations did not correlate with concentric force immediately after resistance exercise.

Discussion

Muscle-damaging eccentric resistance-type leg exercise was used for this blood oxidative-stress investigation with the intent of examining the relationship to muscle-fiber type. The major novel outcome of this study is that the peak rise in plasma protein carbonyl concentration after relatively low-intensity eccentric leg exercise correlates positively with Type II muscle fiber (Types IIa and IIx

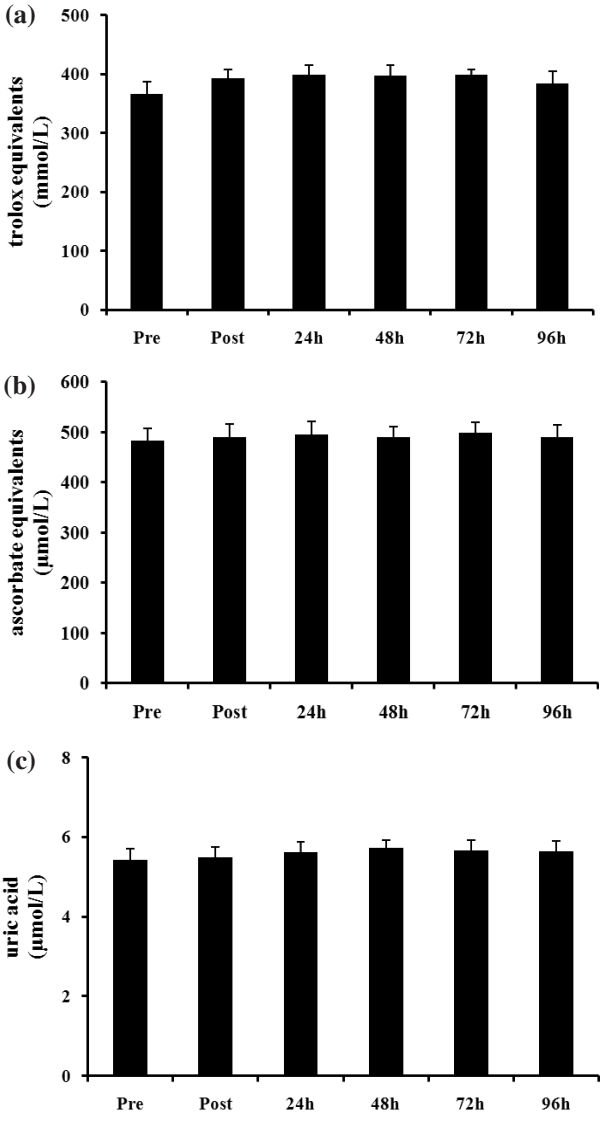

Figure 3 — Biomarkers of plasma antioxidant capacity, $M \pm SEM$, $n = 11$/measure. (a) Plasma Trolox-equivalent antioxidant capacity, (b) ferric-reducing ability of blood plasma, and (c) plasma uric acid concentrations before and after an eccentric-resistance-exercise trial.

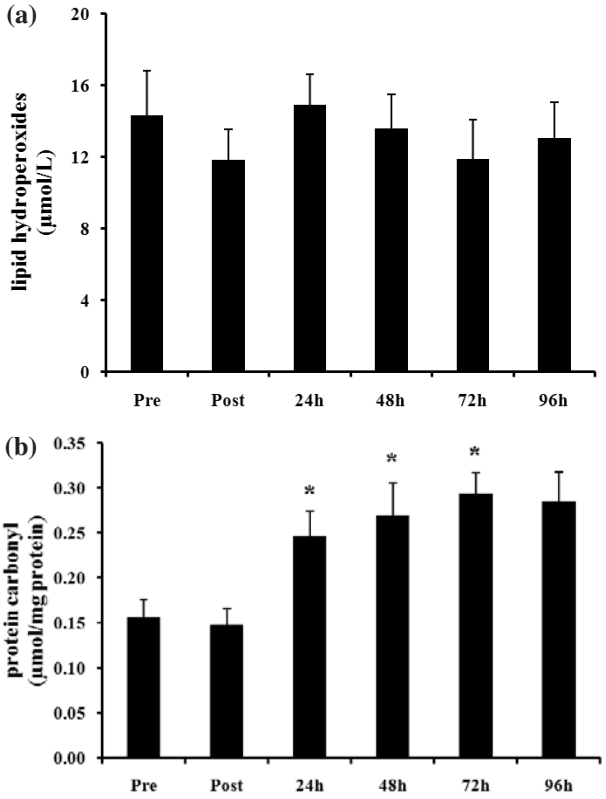

Figure 4 — Biomarkers of plasma oxidative damage, $M \pm SEM$, $n = 11$/measure. (a) Plasma lipid hydroperoxide content and (b) plasma protein carbonyl content before and after an eccentric-resistance-exercise trial. *Significantly different than Pre values; $p \leq .05$.

combined). In contrast, this measure of oxidative stress was not related to Type I, Type IIa, or Type IIx isoforms. Given this relationship, the peak oxidative-stress response after eccentric resistance exercise also correlated significantly with higher lean body mass, Wingate cycle-test power, and jump-test performance, indicating that the capacity for force production is proportional to the postexercise rise in circulating protein carbonyls. In total, these data demonstrate for the first time that blood oxidative stress after low-intensity eccentric resistance exercise is related to fast-twitch muscle-fiber predominance, lean body mass, and anaerobic exercise performance.

Acute Exercise and Blood Oxidative Stress

Conventional understanding of the magnitude of exercise-induced blood oxidative stress is based on mitochondrial production of superoxide proportional to energy expenditure (Boveris & Chance, 1973; Loschen et al., 1974). Reid, Shoji, Moody, and Entman (1992) were the first to identify skeletal-muscle-to-extracellular oxidative-stress transmission; however, their findings did not provide direct support for the notion that free-radical production was proportional to total oxygen flux at the mitochondrial level. Relative to the topic of fiber type and oxidative stress, recent evidence derived from experiments conducted in permeabilized human skeletal-muscle fibers indicates that Type II fibers may be more prone to ROS production than Type I fibers. Underscoring the short half-life of the superoxide parent molecule, and the more physiologically relevant H_2O_2, this previous work indicates that basal and maximal rates of mitochondrial ROS production are disproportionately higher in Type IIb fibers (Anderson & Neufer, 2006). You et al. (2005) examined muscle and blood oxidative stress in response to

Table 3 Correlation-Analysis Results Between the Peak Rise in Plasma Protein Carbonyls After Eccentric Exercise and Muscle-Fiber Type, Anthropometric, and Performance Relationships

Independent variable	*r*	*p*
Muscle and anthropometric		
muscle-fiber Type I	.326	.218
muscle-fiber Type II	.643	.045
muscle-fiber Type IIa	.277	.300
muscle-fiber Type IIx	.253	.345
lean body mass	.806	.005
Damage and soreness		
peak delayed-onset muscle soreness	.059	.871
peak creatine kinase rise	.339	.337
peak lactate dehydrogenase rise	.172	.635
VO_{2peak}	.129	.723
Wingate test		
peak power	.714	.020
fatigue rate	.641	.046
average power	.758	.011
total work	.756	.011
Jump tests		
isometric-squat force	.869	.001
static-jump force	.678	.031
drop-jump force	.746	.013
countermovement-jump force	.859	.001
Oxidative-stress trial		
pretrial concentric force (N)	.750	.012
immediately posttrial concentric force (N)	.490	.151
24 hr posttrial concentric force (N)	.775	.008
48 hr posttrial concentric force (N)	.804	.005
72 hr posttrial concentric force (N)	.787	.007
96 hr posttrial concentric force (N)	.680	.031

eccentric treadmill running in rats. Findings revealed that both muscle and plasma oxidative-damage biomarkers were elevated after exercise, although the oxidative-stress magnitude was most prominent in muscle, implicating diffusing of oxidative stress from the site of release.

More recent work suggests that exercise intensity, independent of total energy expenditure, also contributes to the magnitude of postexercise oxidative stress. Alessio et al. (2000) found that plasma protein carbonyl elevations after resistance-type handgrip exercise were similar to that with VO_{2max} treadmill exercise. Given the differences in total metabolic costs between handgrip and VO_{2max} exercise, Alessio et al.'s findings were attributable to ratings of perceived exertion, which were similar for the two trials. The disconnect between aerobic metabolism and postexercise oxidative-stress magnitude was further advanced by Quindry et al. (2003) in a study demonstrating that aerobic exercise intensity, and not total caloric expenditure, was associated with the most dramatic elevations in oxidant-damage biomarkers. A subsequent series of investigations demonstrated that resistance exercise also elicits blood oxidative stress (Bloomer, Falvo, et al., 2006; Bloomer, Fry, et al., 2006; Bloomer et al., 2005; Hudson et al., 2008). Indeed, Bloomer, Fry, et al. (2006) demonstrated that blood biomarkers for oxidative stress were identifiable after a single set of high-intensity resistance exercise. The current study extends those findings by demonstrating an elevation in blood oxidative stress for several days after three sets of muscle-damaging eccentric isokinetic contractions by the quadriceps of a single leg. In comparison with previous investigation of blood oxidative stress after high-intensity exercise, it is notable that these findings occurred after a bout of muscle-damaging eccentric exercise.

In the current study, circulating protein carbonyls more than doubled in the days after three sets of eccentric single-leg exercise. The current observation of elevated plasma protein carbonyls confirms similar findings of elevated plasma carbonyls in the days after an eccentric-exercise challenge (Lee et al., 2002). The current study extends on previous findings in that the peak rise in plasma protein carbonyls appears to be linked to lean muscle mass, power performance, and Type II muscle-fiber type. The peak protein carbonyl rise was significantly correlated with eccentric-exercise force production in addition to squat, jump-test, and Wingate cycle-test power. The consistent, proportional relationship between peak protein carbonyls and muscle strength (squat exercise), muscle power (Wingate and jump testing), body composition, and eccentric resistance exercise suggests that those with a higher percentage of Type II muscle fibers will show a greater exercise-induced oxidative stress in response to exhausting resistance exercise.

The mechanistic source responsible for this free-radical production and the chain of events resulting in elevated plasma protein carbonyls cannot be specified at this time. From the logistical perspective of cellular/subcellular compartmentalization, oxidative stress represents a cumulative outcome in a diverse redox milieu. As such, we cannot ascertain whether the carbonylated proteins in postexercise plasma samples are native to plasma, muscle, endothelial cells, and so on. Drawing on the collective understanding, the blood oxidative-stress magnitude is influenced by exercise intensity as it applies to both aerobic (%VO_{2max}) and resistance-type (%1RM) exercise (Allessio et al., 2000; Bloomer, Falvo, et al., 2006). This notion would broadly support both the "classic" understanding of free-radical generation through high intensity as a %VO_{2max} (Bergholm et al., 1999) and/or long-duration endurance-type activity (Hessel et al., 2000; Liu et al., 1999; Mastaloudis et al., 2001; McAnulty

et al., 2007). Moreover, during the resistance-type-exercise scenarios of the current investigation, decreased ATP levels and cytosolic calcium overload can result in rapid free-radical production through activation of the enzymes xanthine oxidase and NADPH oxidase (Houston, Chumley, Radi, Rubbo, & Freeman, 1998; Kondo, Nakagaki, Sasaki, Hori, & Itokawa, 1993).

Speculation on the source of the post-eccentric-exercise rise in protein carbonyls is complex. Although not confirmed currently, it is plausible that enzymatic free-radical generation may explain the observed oxidative-stress response. Elevated purine metabolism of AMP by xanthine oxidase would catalyze superoxide radical formation and produce a telltale rise in circulating uric acid as previously observed (Houston et al., 1998; Kondo et al., 1993). In the current study, however, neither uric acid levels nor a concomitant increase in FRAP (Hudson et al., 2008; Mikami, Yoshino, & Ito, 2000) was observed. An alternative source of ROS, notably independent of mitochondrial superoxide generation, in intact contracting skeletal-muscle fibers could be NADPH oxidase (Michaelson, Shi, Ward, & Rodney, 2010). Alternative explanations could include inflammation-derived oxidative stress subsequent to eccentric exercise (Quindry et al., 2003). Accordingly, one might expect the peak rise in protein carbonyls to correlate proportionally to a rise in circulating muscle-damage markers. Although modestly elevated plasma CK and protein carbonyls were observed after a similar time course, significant correlations were not present between these variables. As such, the free-radical source responsible for the oxidative-stress outcome observed in the current study cannot be specified. Future examination of blood oxidative stress after exercise protocols that elicit more dramatic CK responses may better reveal muscle-damage–oxidative-stress relationships not present after the current protocol.

Technical Considerations of Blood Biomarkers for Exercise-Induced Oxidative Stress

The current blood oxidative-stress biomarker panel encompassed potential alterations in antioxidant capacity and oxidative damage. The complex redox milieu of blood plasma (Buettner, 1993) may account for equivocal results when these and other biomarkers are applied to a variety of exercise stimuli (Allessio et al., 2000; Bloomer, Falvo, et al., 2006; Bloomer, Fry, et al., 2006; McAnulty et al., 2005). Inconsistencies across investigations almost certainly relate to fundamental differences in study design relative to exercise type, intensity, sample handling, and oxidative-stress biomarker selection. Although it has been shown previously that TEAC, FRAP, and uric acid are sensitive measures of plasma antioxidant capacity in resistance-exercise studies (McAnulty et al., 2005), none of these measures were appreciably influenced in the current study. From the multimethod approach of the current study, it should be acknowledged that nonspecific radical-quenching assays such as TEAC may not be sensitive to oxidation of single components within the redox cascade. A prime example of this is the ratio of oxidized to reduced glutathione (GSSG:GSH) where antioxidant perturbations are not reflected in the final biomarker.

Lipid hydroperoxides and protein carbonyls were chosen as biomarkers for oxidative damage. Despite an apparent lipid hydroperoxide increase 24 hr after eccentric exercise, values were statistically similar to baseline. This outcome may result from biomarker lability; the relatively long half-life of even the "gold standard" F_2-isoprostanes is less than 1 hr. Alternatively, the observation that lipid hydroperoxides were not elevated in the immediately postexercise blood sample may be attributable to sensitivity limits in that particular biomarker. In contrast to lipid hydroperoxides, plasma protein carbonyls were elevated at 24, 48, and 72 hr after eccentric exercise. The current outcome supports existing data that suggest that antibody-derived assessment of plasma protein carbonyls is a sensitive oxidative biomarker for resistance-exercise applications (Bloomer, Falvo, et al., 2006; Hudson et al., 2008). Notably, the peak response, rather than the mean value, 24, 48, or 72 hr postexercise appears to be most important to capture the relationship between oxidative stress and Type II muscle-fiber composition.

Study Implications, Conclusions, and Considerations for Future Research

Data from the current study support our hypothesis that the magnitude of oxidative stress after eccentric resistance exercise is influenced by Type II muscle-fiber composition. This finding adds to growing evidence that resistance exercise elicits a significant transient oxidative-stress response in the blood (Bloomer, Falvo, et al., 2006; Bloomer, Fry, et al., 2006; Bloomer et al., 2005; Hudson et al., 2008). Understanding the link between cellular and extracellular oxidative stress during resistance exercise is important with respect to athletic performance, training status, long-term health, and nutrition. A recent paradigm shift for understanding oxidative stress holds that acute fluctuations in redox control, during routine nonpathological physiologic stimuli such as exercise, may be a necessary stimulus for adaptation (reviewed in Jones, 2006). Accordingly, we concur with existing positions that antioxidant supplementation to prevent exercise-induced oxidative stress may be shortsighted (Powers, DeRuisseau, Quindry, & Hamilton, 2004). Despite these uncertainties, the current findings provide methodological insight for better understanding the relationship between both oxidative stress and muscularity and resistance exercise. Undeniably, participants recruited for the current study were homogeneous with respect to fiber-type distribution. Further research performed on a more diverse population including individuals with Type I, Type IIa, and Type IIx fiber predominance may better reveal relationships between plasma oxidative stress and muscle-fiber type after resistance exercise. In addition, larger sample sizes are recommended for future studies in an attempt to better understand why only combined Type

II fiber percentages were correlated with the postexercise rise in protein carbonyls. We recommend that future studies of this topic examine the relationship between oxidative stress and work performed during the exercise challenge. Future investigation should also examine oxidative stress in muscle samples before and after exercise for a clearer understanding of the muscle–plasma oxidative-stress relationship. The additional contribution of dietary factors that may affect oxidative stress (e.g., antioxidant supplements, alcohol, or caffeine consumption, or other redox-related compounds) should also be considered in future investigations of this topic. Caution is warranted in the investigation of antioxidant supplementation, however, because the stimulus of moderate-intensity exercise promotes numerous redox advantages (Gomez-Cabrera, Domenech, & Vina, 2008). Moreover, new evidence suggests that exogenous use of dietary antioxidants will attenuate many of these exercise-derived adaptations (Ristow et al., 2009).

Acknowledgments

The authors thank Dr. L. Bruce Gladden for his invaluable insight in manuscript preparation.

References

Allessio, H.M., Hagerman, A., Fulkerson, B., Ambrose, J., Rice, R., & Wiley, L. (2000). Generation of reactive oxygen species after exhaustive and isometric exercise. *Medicine and Science in Sports and Exercise, 32*(9), 1576–1581.

Anderson, E.J., & Neufer, P.D. (2006). Type II skeletal myofibers possess unique properties that potentiate mitochondrial H(2)O(2) generation. *American Journal of Physiology. Cell Physiology, 290*(3), C844–C851.

Anderson, E.J., Yamazaki, H., & Neufer, P.D. (2007). Induction of endogenous uncoupling protein 3 suppresses mitochondrial oxidant emission during fatty acid-supported respiration. *The Journal of Biological Chemistry, 282*(43), 31257–31266.

Benzie, I.F., & Strain, J.J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Analytical Biochemistry, 239*(1), 70–76.

Bergholm, R., Makimattila, S., Valkonen, M., Liu, M-l., Lahdenpera, S., & Taskinen, M-R. (1999). Intense physical training decreases circulating antioxidants and endothelium-dependent vasodilation. *Atherosclerosis, 145,* 341–349.

Bergstrom, J., & Hultman, E. (1969). Glycogen content of skeletal muscle in patients with renal failure. *Acta Medica Scandinavica, 186*(3), 177–181.

Bloomer, R.J., Falvo, M.J., Fry, A.C., Schilling, B.K., Smith, W.A., & Moore, C.A. (2006). Oxidative stress response in trained men following repeated squats or sprints. *Medicine and Science in Sports and Exercise, 38*(8), 1436–1442.

Bloomer, R.J., Fry, A.C., Falvo, M.J., & Moore, C.A. (2006). Protein carbonyls are acutely elevated following single set anaerobic exercise in resistance trained men. *Journal of Science and Medicine in Sport, 10*(6), 411–417.

Bloomer, R.J., Goldfarb, A.H., Wideman, L., McKenzie, M.J., & Consitt, L.A. (2005). Effects of acute aerobic and anaerobic exercise on blood markers of oxidative stress. *Journal of Strength and Conditioning Research, 19*(2), 276–285.

Boveris, A., & Chance, B. (1973). The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. *The Biochemical Journal, 134*(3), 707–716.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72,* 248–254.

Brooke, M.H., & Kaiser, K.K. (1970). Three "myosin adenosine triphosphatase" systems: The nature of their pH lability and sulfhydryl dependence. *The Journal of Histochemistry and Cytochemistry, 18*(9), 670–672.

Buettner, G.R. (1993). The pecking order of free radicals and antioxidants: Lipid peroxidation, alpha-tocopherol, and ascorbate. *Archives of Biochemistry and Biophysics, 300*(2), 535–543.

Byrne, C., Eston, R.G., & Edwards, R.H. (2001). Characteristics of isometric and dynamic strength loss following eccentric exercise-induced muscle damage. *Scandinavian Journal of Medicine & Science in Sports, 11*(3), 134–140.

Cao, G., & Prior, R.L. (1998). Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clinical Chemistry, 44*(6 Pt 1), 1309–1315.

Eston, R.G., Finney, S., Baker, S., & Baltzopoulos, V. (1996). Muscle tenderness and peak torque changes after downhill running following a prior bout of isokinetic eccentric exercise. *Journal of Sports Sciences, 14*(4), 291–299.

Evans, W.J., Phinney, S.D., & Young, V.R. (1982). Suction applied to a muscle biopsy maximizes sample size. *Medicine and Science in Sports and Exercise, 14*(1), 101–102.

Gomez-Cabrera, M.C., Domenech, E., & Vina, J. (2008). Moderate exercise is an antioxidant: upregulation of antioxidant genes by training. *Free Radical Biology & Medicine, 44*(2), 126–131.

Hessel, E., Haberland, A., Muller, M., Lerche, D., & Schimke, I. (2000). Oxygen radical generation of neutrophils: A reason for oxidative stress during marathon running? *Clinica Chimica Acta, 298*(1-2), 145–156.

Houston, M., Chumley, P., Radi, R., Rubbo, H., & Freeman, B. (1998). Xanthine oxidase reaction with nitric oxide and peroxynitrite. *Archives of Biochemistry and Biophysics, 355*(1), 1–8.

Hudson, M.B., Hosick, P.A., McCaulley, G.O., Schrieber, L., Wrieden, J., & McAnulty, S.R. (2008). The effect of resistance exercise on humoral markers of oxidative stress. *Medicine and Science in Sports and Exercise, 40*(3), 542–548.

Jones, D.P. (2006). Disruption of mitochondrial redox circuitry in oxidative stress. *Chemico-Biological Interactions, 163*(1-2), 38–53.

Kondo, H., Nakagaki, I., Sasaki, S., Hori, S., & Itokawa, Y. (1993). Mechanism of oxidative stress in skeletal muscle atrophied by immobilization. *The American Journal of Physiology, 265*(6 Pt 1), E839–E844.

Kovar, K.A., el Bolkiny, M.N., Rink, R., & Hamid, M.A. (1990). An enzymatic assay for the colorimetric and fluorometric determination of uric acid in sera. *Archiv der Pharmazie, 323*(4), 235–237.

Lee, J., Goldfarb, A.H., Rescino, M.H., Hegde, S., Patrick, S., & Apperson, K. (2002). Eccentric exercise effect on blood oxidative-stress markers and delayed onset of muscle soreness. *Medicine and Science in Sports and Exercise, 34*(3), 443–448.

Liu, M-L., Bergholm, R., Makitmattila, S., Lahdenpera, S., Valkonen, M., & Hilden, H. (1999). A marathon run increase the susceptibility of LDL to oxidation in vitro and modifies plasma antioxidants. *The American Journal of Physiology, 276,* E1083–E1091.

Loschen, G., Azzi, A., Richter, C., & Flohe, L. (1974). Superoxide radicals as precursors of mitochondrial hydrogen peroxide. *FEBS Letters, 42*(1), 68–72.

MacIntyre, D.L., Reid, W.D., Lyster, D.M., & McKenzie, D.C. (2000). Different effects of strenuous eccentric exercise on the accumulation of neutrophils in muscle in women and men. *European Journal of Applied Physiology, 81*(1-2), 47–53.

Mastaloudis, A., Leonard, S.W., & Traber, M.G. (2001). Oxidative stress in athletes during extreme endurance exercise. *Free Radical Biology & Medicine, 31*(7), 911–922.

Matthews, J.N., Altman, D.G., Campbell, M.J., & Royston, P. (1990). Analysis of serial measurements in medical research. *British Medical Journal, 300,* 230–235.

McAnulty, S.R., McAnulty, L.S., Nieman, D.C., Morrow, J.D., Utter, A.C., & Dumke, C.L. (2005). Effect of resistance exercise and carbohydrate ingestion on oxidative stress. *Free Radical Research, 39*(11), 1219–1224.

McAnulty, S.R., Owens, J.T., McAnulty, L.S., Nieman, D.C., Morrow, J.D., & Dumke, C.L. (2007). Ibuprofen use during extreme exercise: Effects on oxidative stress and PGE2. *Medicine and Science in Sports and Exercise, 39*(7), 1075–1079.

Michaelson, L.P., Shi, G., Ward, C.W., & Rodney, G.G. (2010). Mitochondrial redox potential during contraction in single intact muscle fibers. *Muscle & Nerve, 42*(4), 522–529.

Mikami, T., Yoshino, Y., & Ito, A. (2000). Does a relationship exist between the urate pool in the body and lipid peroxidation during exercise? *Free Radical Research, 32,* 31–39.

Nourooz-Zadeh, J., Tajaddini-Sarmadi, J., & Wolff, S.P. (1994). Measurement of plasma hydroperoxide concentrations by the ferrous oxidation-xylenol orange assay in conjunction with triphenylphosphine. *Analytical Biochemistry, 220*(2), 403–409.

Powers, S.K., DeRuisseau, K.C., Quindry, J., & Hamilton, K.L. (2004). Dietary antioxidants and exercise. *Journal of Sports Sciences, 22*(1), 81–94.

Quindry, J.C., Stone, W.L., King, J., & Broeder, C.E. (2003). The effects of acute exercise on neutrophils and plasma oxidative stress. *Medicine and Science in Sports and Exercise, 35*(7), 1139–1145.

Reid, M.B., Shoji, T., Moody, M.R., & Entman, M.L. (1992). Reactive oxygen in skeletal muscle. II. Extra cellular release of free radicals. *Journal of Applied Physiology, 73*(5), 1805–1809.

Ristow, M., Zarse, K., Oberbach, A., Kloting, N., Birringer, M., & Kiehntopf, M. (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. *Proceedings of the National Academy of Sciences of the United States of America, 106*(21), 8665–8670.

You, T., Goldfarb, A.H., Bloomer, R.J., Nguyen, L., Sha, X., & McKenzie, M.J. (2005). Oxidative stress response in normal and antioxidant supplemented rats to a downhill run: Changes in blood and skeletal muscles. *Canadian Journal of Applied Physiology, 30*(6), 677–689.