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ORIGINAL ARTICLE

Intramuscular adaptations to eccentric exercise and antioxidant supplementation

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Abstract Prophylactic supplementation of *N*-acetyl-cysteine (NAC) and epigallocatechin gallate (EGCG) was studied for physiological and cellular changes in skeletal muscle after eccentric muscle contractions. Thirty healthy, active males (20.0 ± 1.8 years, 160 ± 7.1 cm, 76.1 ± 17.0 kg) ingested for 14 days either 1,800 mg of NAC, 1,800 mg of EGCG, or 1,000 mg of fiber (glucomannan) placebo (PLC) in a double blind, prophylactic fashion. Subjects completed one eccentric exercise bout (100 repetitions at 30°/s) using the dominant knee extensors. Strength and soreness were assessed, and blood and muscle samples obtained before and 6, 24, 48, and 72 h with no muscle sample being collected at 72 h. Separate mixed factorial repeated measures ANOVA ($P < 0.05$) were used for all statistical analysis. All groups experienced significantly reduced peak torque production after 6 and 24 h, increased soreness at all time points from baseline [with even greater soreness levels 24 h after exercise in PLC when compared to EGCG and NAC ($P < 0.05$)], increased lactate dehydrogenase at 6 h, and increased creatine kinase 6, 24 and 48 h after exercise. No significant group × time interaction effects were found for serum cortisol, neutrophil counts, and the neutrophil:lymphocyte ratio; although, all values experienced significant changes 6 h after exercise ($P < 0.05$), but at no other time points. At 48 h after the exercise bout the Neu:Lym ratio in EGCG was significantly less than NAC ($P < 0.05$), whereas there was a trend ($P = 0.08$) for the EGCG values to be less when compared to PLC at this time point. Markers of intramuscular mitochondrial and cytosolic apoptosis were assessed (e.g., bax, bcl-2, cytochrome C, caspase-3 content/enzyme activity, and total DNA content). Significant increases ($P < 0.05$) in muscle levels of bax and bcl-2 were observed in all groups with no significant differences between groups, whereas no changes ($P > 0.05$) were reported for cytochrome C, caspase-3 content, caspase-3 enzyme activity, and total DNA. Caspase-3 enzyme activity was significantly greater in all groups 48 h after exercise when compared to baseline ($P < 0.05$) and 6 h ($P < 0.05$) after exercise. An eccentric bout of muscle contractions appears to significantly increase muscle damage, markers of mitochondrial apoptosis, apoptotic enzyme activity, and whole-blood cell markers of inflammation with no changes in oxidative stress. While soreness ratings were blunted in the two supplementation groups 24 h after exercise when compared to PLC values, more research is needed to determine the potential impact of EGCG and NAC supplementation on changes related to oxidative stress, apoptosis, and eccentric exercise.

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Introduction

An integrated intracellular antioxidant system works to prevent free radical production and subsequent damage to amino acids, nucleic acids, cell membranes, and genetic material (Sen 1995). In low-stress states, free radical levels are maintained (Sen 1995; Sen and Packer 2000). These systems, however, are overwhelmed during more stressful states (e.g., radiation, eccentric exercise, disease, etc.), which facilitates skeletal muscle fatigue and damage to intracellular structures (Sen 1995, 2001). Eccentric muscle contractions result in myofibrillar disruption, proteolytic breakdown, mitochondrial apoptosis, and inflammation (Kerksick et al. 2008; Stupka et al. 2001; Willoughby et al. 2003a, b) while also increasing free radical accumulation and subsequent oxidative stress markers in the blood (Alessio 1993; Kanter 1998; Nikolaidis et al. 2007; Paschalis et al. 2007). In addition and when compared to traditional concentric concentrations, increases in soreness and loss of force production are greater after one and two similar bouts of eccentric-only contractions (Vissing et al. 2008).

With mixed results, prolonged and repeated exercise bouts can impact endogenous levels of antioxidants (Evans 2000; Kanter 1998). It is purported that increasing the endogenous antioxidant capacity of skeletal muscle may offset fatigue and accumulation of free radical production to facilitate greater recovery from demanding exercise. For this reason, exogenous consumption of various antioxidants [e.g., *N*-acetyl-cysteine (NAC), green tea catechins (epigallocatechin gallate, EGCG), vitamin A, vitamin C, vitamin E, etc.] are purported to increase the antioxidant potential (Evans 2000; Sen 1995), but these findings are not universal (Kanter 1998).

N-acetyl-cysteine is an antioxidant tightly associated with glutathione production. Cellular turnover of glutathione from its oxidized (GSSG) and reduced (GSH) forms is a key intracellular process that works to remove hydrogen peroxide from the cell structure and potential oxidative damage. In this capacity, NAC has been shown to decrease free radical production and oxidative stress at rest (Cotgreave 1997; Reid et al. 1994) and during prolonged exercise (Medved et al. 2003). For example, infusion of NAC in conjunction with a 90-min exhaustive treadmill run reduced markers of oxidative stress and apoptosis (Quadrilatero and Hoffman-Goetz 2004, 2005), but studies involving resistance-based models are less common and have provided mixed results. Childs et al. (2001) initially reported that damaging eccentric exercise can increase muscle damage and oxidative stress markers, while a combination of vitamin C (12 mg/kg body weight) and NAC (10 mg/kg body weight) provided immediately after exercise resulted in significantly higher levels of lactate dehydrogenase (LDH), creatine kinase and lipid hydroperoxides suggesting that the supplementation results in pro-oxidant status (Childs et al. 2001). In contrast, Bloomer et al. (2007) reported no impact of prophylactic vitamin C (378 mg) + vitamin E (1,000 mg of mixed tocopherols/tocotrienols) supplementation on levels of muscle damage (LDH, CK, soreness) and oxidative stress (protein carbonyls and peroxides) markers before a single bout of eccentric exercise. Similarly, Silva et al. (2008) supplemented participants in a prophylactic fashion with NAC at a dosage of 10 mg/kg body weight for 14 days before completing an eccentric exercise protocol with the elbow flexors. Eccentric exercise increased soreness, malondialdehyde, and carbonyl levels after exercise. Serum levels of interleukin-10 (an anti-inflammatory cytokine) increased in all groups 4 days after exercise, but were only maintained at these levels on day 7 in the NAC group suggesting NAC supplementation may impact soreness and inflammation (Silva et al. 2008).

Epigallocatechin gallate, a catechin and polyphenol with high antioxidant activity is found in high concentrations of green tea. EGCG is purported to impact many aspects of health (Kazi et al. 2002) and has been linked to increasing endurance capacity and lipid oxidation during exercise (Murase et al. 2005). Additionally, laboratory rats fed a diet fortified with EGCG for 14 days had lower levels of intramuscular oxidative stress and higher endogenous levels of circulating antioxidants after electrical stimulation (Nagasawa et al. 2000), while tea polyphenols (including EGCG) have been shown to inhibit the large multi-catalytic 20S proteasome associated with the ubiquitin proteolytic pathway (Tisdale 2005). Furthermore, in a cell culture model to investigate the role of EGCG on proteasome inhibition, cancer prevention and subsequent inhibition of tumor growth, various proteasomes in EGCG-cultured cells were inhibited causing a reduction of several markers of tumor cell growth and proliferation (Nam et al. 2001) providing preliminary evidence that EGCG administration may impact cellular turnover. In this study, we hypothesized that prophylactic NAC and EGCG supplementation would yield improvements in markers of muscle damage and cellular adaptations associated with oxidative stress, inflammation and apoptosis when compared to placebo (PLC) supplementation. Therefore, the purpose of this study was to investigate the impact of 14 days of prophylactic NAC and EGCG supplementation after a single damaging bout of eccentric muscle contractions for changes in muscle damage as well as serum and intramuscular markers of muscle damage, oxidative stress, and mitochondrial apoptosis.

Methods

Subjects

Thirty healthy (20.0 ± 1.8 years, 160 ± 7.1 cm, 76.1 ± 17.0 kg), non resistance-trained men defined as less than one workout per month over the last 6 months participated in this study. All participants were classified as low risk for cardiovascular disease with no contraindications to exercise according to American College of Sports Medicine criteria (ACSM 2000). No nutritional supplements (including multi-vitamins) were consumed at least 3 months prior to the study.

Study design

One familiarization and five identical testing sessions were completed after the supplementation period (baseline) and 6, 24, 48, and 72 h after exercise occurred. Interested participants first signed IRB-approved consent forms before completing medical and physical activity questionnaires in accordance with the Declaration of Helsinki and Baylor University. During familiarization, participants were explained the study protocol and completed practice trials on all testing equipment. Strength, soreness, fasting blood and muscle samples were taken at baseline and 6, 24, 48, and 72 h after completion of the exercise bout. No muscle sample was collected 72 h post exercise. Prior to all scheduled tests, participants observed a 10 h fast while refraining from exercise for at least 48 h prior to the exercise bout and for the entire data collection period.

Supplementation protocol

In a double-blind, prophylactic and parallel fashion, all participants were matched in clusters according to age and body weight for assignment into one of three groups [PLC (glucomannan), NAC (Puritan's Pride, Inc., Oakdale, NY, USA) or EGCG (Nutrition for Optimum Wellness, Bloomingdale, IL, USA). Each daily dose provided 1,000 mg/day of PLC, 1,800 mg/day of NAC, and 1,800 mg/day of EGCG (98% total polyphenols, 80% total catechins, and 50% EGCG) throughout the 14 day prophylactic supplementation period. Participants were given all capsules in which they were instructed to ingest according to the supplementation regimen. Participants were instructed to take each dose first thing in the morning on an empty stomach and to ingest all capsules according to the supplementation regimen before their next scheduled visit. Compliance to this regimen was monitored with regular reminder phone calls and recording what time of day they ingested each dose in addition to bringing back their empty bottles to their next scheduled testing session. All participants completed a 48 h dietary recall prior to and after completing the supplementation protocol and were instructed to not alter their diet during the supplementation or testing period. At the conclusion of the supplementation period, participants recorded the incidence and severity of any adverse events as a result of supplementation.

Eccentric exercise session

After supplementation, an eccentrically-based isokinetic protocol was completed by all participants. According to previous guidelines (Stupka et al. 2001), the exercise protocol involved 10 sets of 10 repetitions (100 total repetitions) at an isokinetic eccentric speed of 60°/s on a Biodex System 3 isokinetic dynamometer (Shirley, NY, USA). During familiarization, body position was standardized and all repetitions were conducted in a (80°) range of motion which began at (0°) of knee flexion and continued through (80°) of knee flexion. For all repetitions, no concentric effort was exerted and a torque threshold of 300 ft-lbs of torque was established to maximize and standardize eccentric effort. Immediate changes in peak and average torque were collected after each set (ICC: 0.982, $P < 0.001$). No more than 10 s rest was given between each repetition and 1 min of rest was given after each set. The entire exercise bout spanned approximately 25 min.

Soreness assessment

Perceived soreness was assessed along a 10 cm scale (0 cm = no soreness, 10 cm = extreme soreness) at all time points by drawing a line perpendicular to the continuum line extending from 0 to 10 cm (ICC = 0.943, $P < 0.001$). According to previously published guidelines, the distance of each mark was measured from 0 and rounded up to the nearest one-tenth (Sorichter et al. 1997; Willoughby et al. 2003d).

Muscle strength testing

Using a Biodex System-3 isokinetic dynamometer (Biodex Medical Systems, Inc., NY, USA), static and dynamic muscle strength was assessed at all time points. Prior to testing, participants warmed-up for 10 min on a cycle ergometer (Monark 828E, Varberg, Sweden) at a standardized work rate of 360 kg m/min in addition to completing five repetitions at isokinetic speeds of 60°, 180°, and 300°/s first with 50% and followed with 75% of the perceived maximal effort. Changes in dynamic strength of the knee extensors was then assessed by having participants complete ten maximal repetitions at each respective speed in a concentric–concentric fashion. Each repetition was completed in a consecutive fashion with 1 min of rest

between each set (Brown and Whitehurst 2003). After determination of maximal dynamic strength, a rest period of 5 min was provided prior to determination of peak isometric torque production. Using the dominant knee extensors, a total of three maximal voluntary contractions over 5 s duration were completed with 60 s of rest between each repetition. Participants were verbally encouraged to produce a maximal effort throughout the entire 5 s period. The peak torque exerted throughout all three repetitions was regarded as peak isometric torque and normalized to body weight in kilograms. All isometric repetitions were completed at an angle of 90° of flexion, where 180° is full extension (± 1%, ± 1°/s). Test–retest reliability of these tests has yielded intraclass coefficients ranging from 0.840 to 0.967, $P < 0.001$.

Blood and muscle sampling

Fasting blood and muscle samples were collected at all time points with no muscle being collected 72 h after exercise. Venous blood samples were obtained from an antecubital vein and collected into a 5-ml EDTA and two 10-ml serum separation Vacutainer™ tubes. After being inverted five consecutive times, blood samples remained at room temperature for 10 min before centrifugation at 15,000 *g* for 15 min in a standard bench top centrifuge (Cole Palmer, Vernon Hills, IL, USA; Model # 17250-10). Serum was subsequently removed and frozen at −80°C for later analysis.

Under local anesthetic (1% lidocaine), using a Tru Core 16-gauge biopsy needle (AngioTech, Gainesville, FL, USA) and the fine needle aspiration technique, percutaneous muscle biopsies were obtained from the middle portion of the vastus lateralis at the midpoint between the patella and the greater trochanter of the femur at a depth between 4 and 5 cm. Using these procedures, extracted muscle tissue averaged 15.22 ± 2.33 mg for all participants. All subsequent biopsy samples were collected from the same approximate location as the initial biopsy by previous biopsy markings on the skin and depth markings on the needle in a medial to lateral fashion. Adipose tissue was trimmed and each muscle specimen was immediately frozen in liquid nitrogen and then stored at −80°C for later analysis.

Serum and whole blood quantitation

Serum levels of creatine kinase and LDH were determined photometrically using a Dade Dimension RXL automated clinical chemistry analyzer (Dade-Behring, Inc., Newark, DE, USA; CV: 1.8–2.95%). Using an Abbott Cell Dyne 3500 (Abbott Park, IL, USA; CV: 2–4%) hematology analyzer, whole blood samples were analyzed for changes in leukocyte counts throughout the testing protocol. Manufacturer provided quality control and calibration procedures for these devices were completed each day prior to any analysis. Using either enzyme-linked immunosorbent assays or enzyme immunosorbent assays (EIA), serum concentrations of superoxide dismutase (Cayman Chemical, Ann Arbor, MI, USA; CV: 3.2 ± 1.6%), 8-isoprostane (Cayman Chemical, Ann Arbor, MI, USA; CV: 3.0 ± 1.4%), cortisol (DSLabs, Webster, TX, USA; Catalog # DSL-10-2000; CV: 2.0 ± 1.2%), and tumor necrosis factor-α (TNF-α; Ray Biotech, Norcross, GA, USA; Catalog # ELH-TNFalpha-001; CV: < 10%) were determined in duplicate with a Wallac Victor-1420 microplate reader (Perkin-Elmer Life Sciences, Boston, MA, USA). All assays were performed using the manufacturer recommended wavelength against a known standard curve depending on the specifications of the protocol.

Total DNA and muscle protein isolation

All muscle samples were first separated and weighed prior to being isolated for total DNA and protein using the Tri-Reagent (Sigma Chemical Co., St Louis, MO, USA) procedure according to Chomczynski and Sacchi (1987). Total DNA content was isolated with 100% ethanol, 0.1 M sodium citrate, and 8 mM sodium hydroxide. The DNA concentration was determined at OD_{260} with concentration expressed relative to muscle wet-weight where 1 OD being equivalent to 50 μg/ml of DNA (Ausubel et al. 1999). Total protein was isolated with isopropanol, ethanol, and 0.3 M guanidine hydrochloride. Myofibrillar protein was further isolated with 0.1% SDS. Protein content was then determined using a fluorometric spectrophotometric (Pro-Stain®, Active Motif; Carlsbad, CA, USA; CV: ± 10%) protocol at an excitation wavelength of 485 nm and an emission wavelength of 635 nm using a Wallac Victor 1420 (Perkin Elmer, Boston, MA, USA) microplate reader. Protein samples were then diluted to a standard concentration of 50 μg of protein for later use. Due to an inadequate amount of protein in two of the samples, one sample was subsequently diluted to 30 μg of protein while the other was diluted to 40 μg of protein.

Muscle quantitation procedures

Using commercial EIA kits (Assay Designs, Ann Arbor, MI, USA), the muscle protein content of bax (Catalog # 900-138; CV: 4.9–6.3%), bcl-2 (Catalog # 900-133; CV: 2.4–4.2%), and cytochrome C (Catalog # 900-141; CV: 2.7–4.2%) was determined in duplicate with a Wallac Victor-1420 microplate reader (Perkin-Elmer Life Sciences, Boston, MA, USA). All assays were performed using the manufacturer recommended wavelength against a known standard curve. Caspase-3 content (pmol) and caspase-3 enzyme specific activity (pmol AMC liberated/min/μg protein) were

determined with the CaspACE^{TM} fluorometric assay system (Promega; Madison, WI, USA) based on the manufacturer's guidelines using standard 96-well plates at an excitation wavelength of 355 nm and an emission wavelength of 460 nm (Willoughby et al. 2003b).

Dietary records

Subjects recorded their daily food and fluid intake for 48 h prior to beginning and ending the supplementation protocol. Participants were instructed to provide detailed information regarding their dietary habits and were provided informational handouts with pictorial examples of food quantities to help increase the accuracy of their reporting. Further, participants were provided a list of foods known to be naturally high in both cysteine-residue antioxidants as well as constituents of green tea were provided and were asked to refrain from uncustomary consumption of these foods. Individuals who self-reported high intakes of these foods during familiarization were excluded from the study. All dietary records were analyzed using the ESHA Food Processor program, version 8.6 (Salem, OR, USA) to establish mean caloric and macronutrient intake normalized to body mass in kilograms in addition to assessing dietary intake of antioxidants.

Statistical analysis

Relative peak isometric torque, soreness and serum measures were analyzed by separate 3 × 5 [group (EGCG, PLA, NAC) × Test (baseline, 6, 24, 48, and 72 h post exercise)] mixed factorial repeated measures ANOVA. Similarly, intramuscular changes were analyzed with separate 3 × 4 (group × test) repeated measures ANOVA. When the sphericity assumption was not met, the conservative Huynh-Feldt epsilon correction was utilized. Confidence interval adjustments were made using Bonferroni corrections and LSD pairwise post hoc comparisons were used to further investigate any significant interaction or main effects for time. When necessary, single one-way ANOVA with LSD post hoc procedures were used to assess group difference at specific time intervals. All statistical decisions were made using a probability of 0.05 with all data in figures being presented as mean ± SEM with all other data being presented as mean ± SD.

Results

Compliance and adverse outcomes

Thirty out of 33 participants (91%) completed all testing. Two participants withdrew after their pre-supplementation biopsy while a third participant was excluded due to non-compliance. Compliance to the supplementation protocol was 99.3% (417 consumed doses/420 possible doses) after three participants reported missing one dose. No adverse outcomes were reported to the supplementation protocol.

Dietary intake

All participants completed a 2-day food record at the beginning and end of supplementation. No significant main effects or interaction effects were found for energy, carbohydrate, protein or fat intake normalized to kilograms of body mass throughout the study (Table 1).

Torque production

Peak isometric torque was normalized to body weight and assessed at five time points throughout the protocol. No significant group × test interaction effects were found ($P = 0.66$). A significant main effect for time ($P < 0.001$) was found whereby torque production at 6 h (2.12 ± 0.52 ft-lbs/kg; $P < 0.01$) and 24 h (2.03 ± 0.46 ft-lbs/kg; $P < 0.001$) post-exercise were significantly lower than baseline levels while these values only approached significance after 48 h (2.10 ± 0.50 ft-lbs/kg; $P = 0.08$), with no difference ($P > 0.05$) at 72 h post-damage (Fig. 1). Fatigue index calculations (EGCG: 48.9 ± 16.9%, PLA: 52.6 ± 21.1%, NAC: 54.6 ± 17.0%; $P = 0.78$) revealed no significant difference among groups.

Soreness

Perceived levels of soreness were measured at all time points (Fig. 2). Significance values associated with group × test interactions approached significance thresholds ($P = 0.08$), while significant main effects for time were found ($P < 0.001$). When compared to baseline (0.4 ± 0.7), soreness was significantly increased at 6 h (3.4 ± 2.1; $P < 0.001$), 24 h (4.1 ± 2.2; $P < 0.001$), 48 h (3.4 ± 2.1; $P < 0.001$), and 72 h (2.1 ± 1.9; $P < 0.001$) after the exercise bout. Post hoc tests revealed that the PLA (5.55 ± 1.64) group had significantly higher levels of muscle soreness 24 h after the exercise bout (EGCG: 3.30 ± 2.08, PLA: 5.55 ± 1.64, NAC: 3.49 ± 2.15; $P = 0.03$) when compared to EGCG and NAC.

Serum markers of muscle damage

No significant group × time interaction for either creatine kinase ($P = 0.79$) or LDH ($P = 0.43$) were found (Table 2). A significant main effect for time was found for creatine kinase ($P < 0.05$) and lactate dehydrogenase ($P < 0.05$). Creatine kinase levels increased from baseline

Table 1 Nutritional intake before and after supplementation protocol

Variable	Group	Day 0	Day 14	*P* value	
				Within group	G × T
Raw data normalized to body mass (kg)					
Energy intake (kcal/kg/day)	EGCG	32.7 ± 14.6	40.6 ± 9.5	0.09	0.65
	PLA	41.8 ± 11.6	43.0 ± 20.3	0.98	
	NAC	40.0 ± 14.8	40.2 ± 12.0	0.85	
Carbohydrate intake (g/kg/day)	EGCG	4.6 ± 2.5	5.2 ± 1.9	0.46	0.92
	PLA	6.0 ± 2.1	6.2 ± 4.3	0.92	
	NAC	5.6 ± 3.6	5.4 ± 2.2	0.92	
Protein intake (g/kg/day)	EGCG	1.1 ± 0.4	1.4 ± 0.4	0.14	0.72
	PLA	1.4 ± 0.5	1.5 ± 0.4	0.72	
	NAC	1.5 ± 0.5	1.6 ± 0.6	0.39	
Fat intake (g/kg/day)	EGCG	1.1 ± 0.5	1.5 ± 0.3*	<0.005	0.32
	PLA	1.4 ± 0.5	1.4 ± 0.6	0.36	
	NAC	1.3 ± 0.4	1.4 ± 0.5	0.92	
Raw data					
Energy intake (kcal/day)	EGCG	2,588 ± 1,332	3,155 ± 830	0.07	0.54
	PLA	3,078 ± 2,016	2,878 ± 857	0.75	
	NAC	2,961 ± 1,194	3,259 ± 1,688	0.94	
Carbohydrate intake (g/day)	EGCG	363 ± 218	401 ± 142	0.51	0.84
	PLA	449 ± 470	389 ± 169	0.69	
	NAC	477 ± 261	474 ± 350	0.99	
Protein intake (g/day)	EGCG	86 ± 28	108 ± 30	0.13	0.36
	PLA	108 ± 33	110 ± 38	0.79	
	NAC	105 ± 35	114 ± 29	0.29	
Fat intake (g/day)	EGCG	85 ± 45	118 ± 28*	<0.005	0.022
	PLA	96 ± 33	103 ± 33	0.41	
	NAC	103 ± 31	104 ± 42	0.84	

Data presented in top section is normalized to body mass in kilograms and the bottom section provides the raw data. All data is presented as mean ± SD

* Significantly different from day 0 ($P < 0.05$)

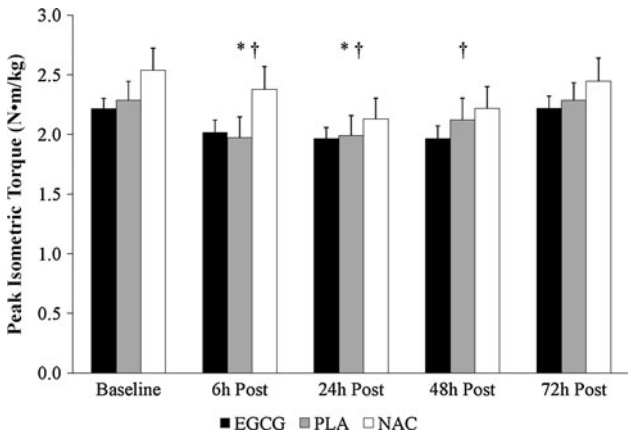

Fig. 1 Mean values for relative peak isometric torque (N m/kg) represented as mean ± SEM for all time points. All groups significantly different from day 0 (*$P < 0.05$). All groups significantly different from 72 h post (†$P < 0.05$)

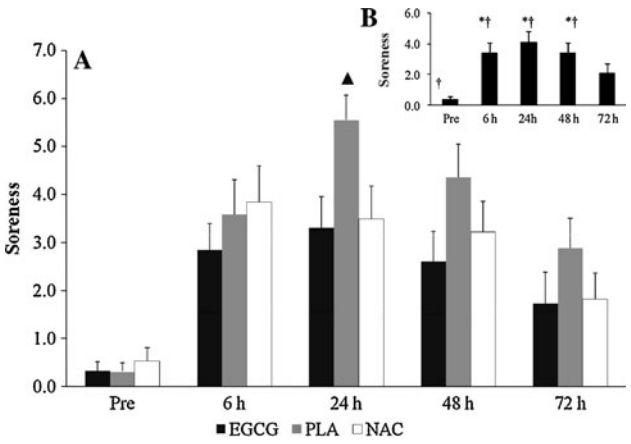

Fig. 2 Perceived soreness ratings across time for all groups (*graph* **a**). Significantly different value from PLA (▲$P < 0.05$). Collapsed group changes across time (*inset graph* **b**). Significantly different from day 0 (*$P < 0.05$). Significantly different from 72 h post (†$P < 0.05$). Data is represented as mean ± SEM for all time points

Table 2 Serum measures of muscle damage, oxidative stress and inflammation

Variable	Group	Baseline	6 h Post	24 h Post	48 h Post	72 h Post	*P* value	
							Within group	G × T
Creatine kinase (U/l)	EGCG	85 ± 33	189 ± 50*	207 ± 81*	201 ± 124	193 ± 203	0.17	0.79
	PLA	137 ± 113	201 ± 85	236 ± 133	212 ± 179	220 ± 191	0.36	
	NAC	162 ± 118	275 ± 179	372 ± 253	306 ± 232	382 ± 455	0.18	
Lactate dehydrogenase (U/l)	EGCG	113 ± 18	151 ± 39	110 ± 15	124 ± 24	138 ± 72	0.12	0.43
	PLA	124 ± 39	159 ± 53	134 ± 34	123 ± 30	134 ± 26	<0.05	
	NAC	130 ± 27	137 ± 37	140 ± 37	128 ± 41	136 ± 41	0.79	
Neutrophils (cells per million)	EGCG	3.6 ± 1.1	5.2 ± 1.6	3.4 ± 1.0‡	2.7 ± 1.0‡	2.9 ± 0.7‡	<0.001	<0.05
	PLA	3.0 ± 1.0	4.1 ± 1.4*	2.9 ± 0.8	3.6 ± 1.2	3.0 ± 1.2	<0.001	
	NAC	2.7 ± 0.9‡	4.2 ± 1.9	2.9 ± 1.3‡	3.2 ± 1.2	2.4 ± 0.9‡	<0.01	
Neutrophil:lymphocyte ratio	EGCG	1.9 ± 0.6	2.4 ± 0.7	2.1 ± 0.5	1.5 ± 0.5†	1.6 ± 0.5	<0.005	0.14
	PLA	1.6 ± 0.7	2.4 ± 1.2	1.9 ± 0.9‡	2.0 ± 0.9	1.8 ± 0.8	<0.005	
	NAC	1.6 ± 0.6	2.2 ± 0.6	1.6 ± 0.5‡	1.9 ± 0.9	1.9 ± 1.3	0.36	
Superoxide dismutase (U/l)	EGCG	0.14 ± 0.07	0.14 ± 0.07	0.15 ± 0.07	0.13 ± 0.08	0.15 ± 0.07	0.15	0.27
	PLA	0.15 ± 0.07	0.12 ± 0.05	0.15 ± 0.07	0.14 ± 0.04	0.16 ± 0.07	0.06	
	NAC	0.13 ± 0.02	0.11 ± 0.03	0.12 ± 0.03	0.12 ± 0.03	0.12 ± 0.04	0.81	
8-Isoprostane (pg/ml)	EGCG	37.3 ± 27.4	32.9 ± 18.2	33.3 ± 24.5	26.8 ± 17.7	28.6 ± 12.7	0.31	0.44
	PLA	35.4 ± 18.0	35.0 ± 15.5	37.8 ± 33.3	47.6 ± 56.6	31.3 ± 20.6	0.53	
	NAC	23.0 ± 9.2	21.2 ± 15.4	28.7 ± 25.4	20.0 ± 7.0	23.9 ± 10.6	0.36	
Cortisol (mg/dl)	EGCG	24.2 ± 6.0‡	14.1 ± 6.2	22.3 ± 6.1‡	21.6 ± 6.6	22.8 ± 6.8‡	<0.001	0.96
	PLA	25.9 ± 6.3‡	16.6 ± 4.5	25.0 ± 6.3‡	24.8 ± 4.8	25.5 ± 6.6	<0.005	
	NAC	23.2 ± 6.6	16.1 ± 5.0	20.4 ± 5.1	21.5 ± 6.7	21.9 ± 6.6	<0.05	
TNF-α (pg/ml)	EGCG	1.8 ± 0.6	1.7 ± 0.6	1.8 ± 0.7	1.8 ± 0.9	1.7 ± 0.7	0.47	0.48
	PLA	2.0 ± 0.6	2.0 ± 0.7	1.9 ± 0.6	2.0 ± 0.7	1.9 ± 0.7	0.12	
	NAC	2.3 ± 0.9	2.2 ± 0.9	2.2 ± 0.9	2.1 ± 0.6	2.1 ± 0.7	0.44	

Data presented as mean ± SD

$G \times T$ = group × test interaction

* Significantly different from baseline ($P < 0.05$)

‡ Significantly different from 6 h post ($P < 0.05$)

† Significantly different from 48 h post ($P < 0.05$)

(129 ± 99 U/l) and peaked at 24 h post resulting in significantly greater levels at 6 h (223 ± 123 U/l; $P < 0.001$), 24 h (275 ± 185 U/l; $P < 0.005$) and 48 h (242 ± 185 U/l; $P < 0.05$). When compared to baseline levels (122 ± 28.7 U/l), LDH levels experienced a sharp increase after 6 h (148.7 ± 42 U/l; $P < 0.05$), while all other time points were not significantly different ($P > 0.05$) from baseline levels.

Serum markers of oxidative stress

Oxidative stress levels were determined at all time points by assessing the serum concentrations of 8-isoprostane and superoxide dismutase (Table 2). No significant group × time interaction was found for either variable (8-isoprostane: $P = 0.44$ and superoxide dismutase: $P = 0.27$). No main effect for time was found for 8-isoprostane ($P = 0.53$), while superoxide dismutase levels tended to increase ($P = 0.07$).

Serum markers of inflammation

To assess changes in inflammation, blood levels of cortisol, TNF-α, neutrophil count, and the neutrophil:lymphocyte ratio were determined at all time points. No significant group × time interaction effects were found for cortisol ($P = 0.96$), TNF-α ($P = 0.53$) and the neutrophil:lymphocyte ratio ($P = 0.14$), while a significant interaction was found for neutrophil count ($P < 0.05$). Follow-up between group analysis using one-way ANOVA with delta values revealed that neutrophil counts for EGCG at 48 h were significantly lower than NAC ($P < 0.05$) and tended to be lower than PLA ($P = 0.07$). No significant time effect was found for TNF-α levels ($P = 0.32$), while

significant time effects for cortisol ($P < 0.001$), neutrophil count ($P < 0.001$), and neutrophil:lymphocyte ratio ($P < 0.001$) were found. Serum cortisol reached nadir levels at 6 h post (15.5 ± 5.3 mg/dl) and were significantly different from baseline (24.4 ± 6.1; $P < 0.001$), 24 h (22.6 ± 5.9; $P < 0.001$), 48 h (22.6 ± 6.1; $P < 0.001$), and 72 h (23.4 ± 6.6 mg/dl; $P < 0.001$) levels. Neutrophil levels were significantly greater at 6 h (4.5 ± 1.7 cells/million) when compared to baseline (3.1 ± 1.0; $P < 0.001$), 24 h (3.1 ± 1.0; $P < 0.001$), 48 h (3.1 ± 1.2; $P < 0.001$), and 72 h (2.8 ± 1.0; $P < 0.001$). Similarly the neutrophil:lymphocyte ratio peaked at 6 h (2.3 ± 0.8) and was significantly greater when compared to baseline (1.7 ± 0.6; $P < 0.001$), 24 h (1.8 ± 0.7; $P < 0.001$), 48 h (18 ± 0.8; $P < 0.001$), and 72 h (1.7 ± 0.9; $P < 0.001$).

Intramuscular markers of apoptosis

Intramuscular mitochondrial protein levels of bax, bcl-2, bax:bcl-2, cytochrome c, caspase-3 content, caspase-3 enzyme activity, and total DNA were determined as markers of cytosolic and mitochondrial apoptosis. No significant group × time interaction was found for bcl-2 ($P = 0.27$), cytochrome c ($P = 0.37$), caspase-3 content ($P = 0.36$), caspase-3 enzyme activity ($P = 0.80$), and total DNA ($P = 0.20$). Group × time interaction values approached significance for bax ($P = 0.06$) and the bax:bcl-2 ratio ($P = 0.08$). Significant main effects for time were found for bax ($P < 0.001$) and bcl-2 ($P < 0.001$), while the bax:bcl-2 ratio tended ($P = 0.06$) to change. Bax and bcl-2 levels significantly increased in all groups ($P < 0.05$ at all respective combinations for bax and bcl-2) with peak levels occurring at 48 h (Bax: 30.0 ± 19.7 pg/mg; Bcl-2: 5.4 ± 3.3 pg/mg) when compared to baseline (Bax: 16.3 ± 10.4; Bcl-2: 2.9 ± 2.1), 6 h (Bax: 18.1 ± 10.9; Bcl-2: 3.8 ± 3.3), and 24 h (Bax: 19.6 ± 7.2; Bcl-2: 3.9 ± 3.1 pg/mg). No main effects for time were found for cytochrome c ($P = 0.35$), caspase-3 content ($P = 0.30$), caspase-3 enzyme activity ($P = 0.10$), and total DNA ($P = 0.66$). Although, follow-up analysis did reveal significantly greater levels of caspase-3 enzyme activity 48 h after the exercise bout in all groups when compared to those values at baseline and 6 h after exercise ($P < 0.05$; Table 3).

Discussion

This study examined the effects that a 14-day prophylactic supplementation period of NAC and EGCG would have on

Table 3 Intramuscular measures of apoptosis

Variable	Group	Baseline	6 h Post	24 h Post	48 h Post	*P* value	
						Within group	G × T
Bax (pg/mg)	EGCG	21.6 ± 14.2§	23.1 ± 16.8§	22.7 ± 7.5§	44.4 ± 26.7	<0.05	0.06
	PLA	13.6 ± 8.9	13.8 ± 4.2	16.6 ± 6.0	20.0 ± 8.1*	<0.05	
	NAC	13.7 ± 4.7	17.3 ± 5.7*	19.5 ± 7.2*	22.8 ± 8.1	<0.05	
Bcl-2 (pg/mg)	EGCG	3.4 ± 1.5	4.3 ± 3.3	4.4 ± 2.9	7.2 ± 3.0*	<0.01	0.27
	PLA	4.4 ± 2.3	5.5 ± 3.8	5.7 ± 3.5	6.1 ± 3.7	0.15	
	NAC	1.1 ± 0.7§	1.5 ± 0.9§	1.6 ± 1.0	2.9 ± 1.4	<0.001	
Bax:Bcl-2	EGCG	6.6 ± 3.2	6.3 ± 2.8	6.4 ± 3.1	6.3 ± 2.8	0.97	0.08
	PLA	4.5 ± 4.0	4.3 ± 3.5	4.4 ± 3.4	4.3 ± 2.9	0.94	
	NAC	14.9 ± 6.5	18.2 ± 17.9	15.3 ± 7.5	8.4 ± 1.8*	0.08	
Cytochrome C (pg/mg)	EGCG	7.6 ± 6.0	6.1 ± 3.8	5.4 ± 1.7	8.3 ± 5.2	0.63	0.37
	PLA	4.2 ± 1.0	4.2 ± 1.0	3.8 ± 1.0	3.8 ± 1.1	0.12	
	NAC	4.4 ± 0.8	5.5 ± 2.3	4.0 ± 0.8	4.4 ± 1.8	0.40	
Caspase-3 content (pmol/mg)	EGCG	18.5 ± 10.4	15.8 ± 8.7	14.2 ± 5.7	21.6 ± 13.3	0.35	0.36
	PLA	13.7 ± 3.4	13.2 ± 3.3	12.2 ± 3.5	12.1 ± 4.0	0.53	
	NAC	15.5 ± 3.7	17.2 ± 3.9	14.0 ± 3.2	15.1 ± 4.6	0.23	
Total DNA (μg/mg)	EGCG	0.08 ± 0.03	0.08 ± 0.04	0.06 ± 0.03	0.05 ± 0.03	<0.05	0.20
	PLA	0.08 ± 0.05	0.07 ± 0.06	0.06 ± 0.06	0.05 ± 0.06	0.57	
	NAC	0.08 ± 0.04	0.08 ± 0.05	0.05 ± 0.03	0.04 ± 0.04	0.59	

Data presented as mean ± SD and normalized to tissue mass

$G \times T$ = group × test interaction

* Significantly different from baseline ($P < 0.05$)

§ Significantly different from 48 h post ($P < 0.05$)

changes in relative peak torque production, perceived muscle soreness, and markers of muscle damage, oxidative stress, inflammation, and apoptosis after a single bout of eccentric muscle contractions. In accordance with previous reports, the primary findings from this study are that a single bout of eccentric contractions sufficiently induces muscle damage (Warren et al. 2002; Willoughby et al. 2003a). Other previously reported findings were the increases in intramuscular markers of cytosolic and mitochondrial apoptosis (Kerksick et al. 2008; Willoughby et al. 2003b) and increases in inflammation (Finaud et al. 2006; Hirose et al. 2004), while the lack of change in oxidative stress markers does conflict with previous studies (Bloomer et al. 2005, 2006; Michailidis et al. 2007). Additionally, prophylactic supplementation of NAC and EGCG appeared to invoke little changes over these outcomes.

Markers of muscle damage

As seen previously (Willoughby et al. 2003a), significant reductions in relative peak torque production occurred in the present study (Fig. 1), likely due to decreased motor unit recruitment, EMG activity, and failure of the excitation–contraction uncoupling process (Warren et al. 2002). In addition, significant increases in soreness were reported 6 h after completion of exercise and remained elevated through all remaining timepoints (Willoughby et al. 2003a, c), with significantly higher ($P < 0.05$) levels reported in the PLC group 24 h after exercise (Fig. 2). As mechanical disruption ensues, various proteins (e.g., creatine kinase, troponin-I, LDH, myoglobin) can be quantified in the bloodstream and are used as another marker of myofibrillar damage. Significant increases in LDH and creatine kinase were found 6 h after the exercise bout and remained elevated until 48 h after the exercise bout, a result found in previous studies as well (Sorichter et al. 1997; Willoughby et al. 2003a, c). These responses, however, did not appear to be impacted by NAC and EGCG supplementation. While no studies have reported on supplementation with NAC and EGCG, one study (Bloomer 2004) used prophylactic supplementation of vitamin C, vitamin E, and selenium before eccentric contractions to find an attenuation of peak creatine kinase and soreness levels.

Indicators of oxidative stress and antioxidant activity

Previously published studies have reported significant modulations in the development of oxidative stress as well as endogenous antioxidant systems after aerobic exercise (Finaud et al. 2006; Inal et al. 2001), various modes of anaerobic activity (Bloomer 2004; Mahoney et al. 2003), and after damaging and/or pro-inflammatory situations (Finaud et al. 2006). Findings from the present study, however, did not support these conclusions as no changes in serum concentrations of 8-isoprostane and superoxide dismutase were seen at any time point throughout the study. However, several available markers of lipid peroxidation and oxidative stress are available (e.g., malondialdehyde, protein carbonyls, catalase, reduced glutathione, oxidized glutathione, etc.) and each marker exhibits different responses to exercise mode, intensity, volume, training status, and chosen sampling time points (Fisher-Wellman and Bloomer 2009; Michailidis et al. 2007; Nikolaidis et al. 2008). Isoprostanes are specific end products of ROS-catalyzed oxidation of arachidonic acid and their quantification in plasma or urine is considered an ideal marker of lipid peroxidation, while other indirect markers such as malondialdehyde and its reactive associates (e.g., thiobarbituric acids reactive substances, i.e., TBARS) are also used (Nikolaidis et al. 2008). While our findings do contrast with other previous reports showing increases in 8-isoprostane after a 50 km ultramarathon (Mastaloudis et al. 2004), the likely reason is the quantifiable difference in metabolic and mechanical damage that is likely to occur over an ultramarathon and one bout of eccentric contractions as was completed in this present study. In this light, studies using similar modes of exercise as the present study have provided mixed results. For example, two studies have reported no change in serum levels of 8-isoprostane (Child et al. 1999; Kerksick et al. 2008). Of particular interest, an additional study (Lee et al. 2002) used a similar exercise protocol and time points to collect blood samples and found no change in oxidative glutathione and total glutathione, two key constituents of the endogenous glutathione antioxidant system, while also finding similar changes in soreness and creatine kinase activity as in the present study. Other studies, however, have reported increases in TBARS, an indirect marker of MDA activity and lipid peroxidation, after one (Paschalis et al. 2007) and two bouts (Nikolaidis et al. 2007) of eccentric muscle contractions in addition to significant increases in reduced glutathione, oxidized glutathione, protein carbonyls, catalase, uric acid, and total antioxidant capacity (all markers of oxidative stress). These mixed findings call into discussion the possibility that oxidative stress accumulation may be highly variable among individuals and that people may be able to withstand a fairly large amount of eccentric overload on a muscle group without large increases in oxidative stress. Regardless, findings from the present study suggest that eccentric exercise did not have any impact on serum levels of 8-isoprostane, an indirect marker of lipid peroxidation. Similarly, supplementation with NAC and EGCG appeared to not have any influence over serum levels of lipid peroxidation. While published studies are completely void

using these particular antioxidants, one previous study using vitamins C and E did report improved levels of lipid peroxidation after 3 weeks of supplementation when measured 2 and 6 h after prolonged endurance activity, but the inherent differences between these antioxidant and exercise modes (e.g., ultramarathon) make comparisons difficult (Mastaloudis et al. 2004).

We hypothesized that prophylactic supplementation of the antioxidants, NAC and EGCG, would invoke changes in antioxidant activity resulting in altered physiological outcomes to the damaging bout of exercise. Although our hypothesis was not accepted, several factors must be considered in the context of other studies including age, gender, training and nutritional status, antioxidant intake, etc. when evaluating the outcomes from our study and others (Clarkson and Thompson 2000; Goldfarb 1999). For example, Bloomer et al. (2006) illustrated the potential impact of training status, gender, and exercise mode on oxidative stress changes while Sacheck et al. (2003) and Nikolaidis et al. (2007) illustrated that age and repeated bouts, respectively, may influence outcomes associated with an oxidative stress response. Using NAC, a small number of studies have reported improvements in cysteine metabolism and prevention of fatigue (Cotgreave 1997; Reid et al. 1994). Most of these studies, however, used much higher doses and/or administered the antioxidant intravenously, two factors likely impacting the differences in outcomes (Medved et al. 2004; Reid et al. 1994). A higher dosage would likely make more substrate available after first-pass hepatic metabolism to the skeletal muscle and similarly intravenous administration bypasses gastric metabolism altogether and immediately makes the NAC available for tissue uptake. To date, limited studies have investigated the impact of EGCG on changes in oxidative stress. Using laboratory rats fed an EGCG-fortified diet for 2 weeks, significantly lower levels of protein carbonyls were reported when compared to a control diet (Nagasawa et al. 2000). However, no studies have used EGCG as part of a muscle damaging protocol to investigate intramuscular and biochemical adaptations.

Indicators of inflammation

Previous research involving eccentric muscle contractions has suggested that acute inflammation can be characterized by soreness and limb circumference increases for 1–5 days after the exercise bout (Smith 1991). Similarly, increases in circulating neutrophils and other phagocytic cells are activated and converge on the initial damage site (Finaud et al. 2006). These particular cells produce superoxide $\left(O_2^-\right)$ through oxidative burst activity and are subsequently quenched to a less reactive molecule, hydrogen peroxide, by the enzyme superoxide dismutase. Significant increases in soreness and inflammation (i.e., cortisol, neutrophil counts, and the neutrophil:lymphocyte ratio) were seen in the present study 6 h after the damaging exercise (Fig. 2; Table 2). Serum levels of cortisol in the present study decreased in all groups 6 h after completion of the exercise bout. While somewhat counterintuitive to increases in neutrophils and other inflammation-mediated leukocytes, this response has been reported previously (Kazunori and Clarkson 1996), and is thought to primarily result from the natural circadian rhythm seen with cortisol concentrations. Finally, no changes were found to occur as a result of the exercise bout or NAC and EGCG supplementation in the serum levels of TNFα, a pro-inflammatory cytokine. Previous studies employing prolonged bouts of endurance exercise (Dufaux and Order 1989) or short bouts of downhill running (Cannon et al. 1991) have reported increases in TNFα. Unique differences, however, exist between the exercise modes utilized in these studies (e.g., muscle mass involved, intermittent nature of contractions, rest between contractions, etc.) making direct comparisons difficult. To this end, other reports using similar eccentric contractions also reported no changes in serum levels of TNFα (Hirose et al. 2004). Similar to the present study, antioxidant supplementation of vitamins C and E over a 6-week period before a 50-km ultramarathon did not influence serum levels of TNFα (Mastaloudis et al. 2004).

Apoptosis

Previous studies using various forms of exercise have reported an increase in mitochondrial apoptosis (Kerksick et al. 2008; Phaneuf and Leeuwenburgh 2001), while the impact of NAC and EGCG supplementation is still undetermined (Quadrilatero and Hoffman-Goetz 2004, 2005). Results from the present study suggest that antioxidant supplementation imparted no effect over apoptotic changes. The eccentric exercise bout, however, increased intramuscular levels of bax, bcl-2, and the bax:bcl-2 ratio (Figs. 3, 4, 5) at several time points while caspase-3 enzyme activity was increased 48 h after exercise. Alterations in the pro-apoptotic bax protein and the anti-apoptotic bcl-2 protein, and their ratio, are impacted by various external and internal cellular signals, including glucocorticoid secretion, increases in intracellular calcium, TNFα secretion, and production of reactive oxygen species (Phaneuf and Leeuwenburgh 2001). Apoptosis induction typically occurs by way of three mechanisms: (1) through ligand binding to the Fas receptor via TNFα, (2) cytochrome C translocation secondary to alterations in the balance between the B cell proteins resulting in subsequent activation of caspase-3, or (3) alterations in calcium homeostasis leading to activation of procaspase-9 and subsequent activation of caspase-3 (Phaneuf and Leeuwenburgh 2001). In

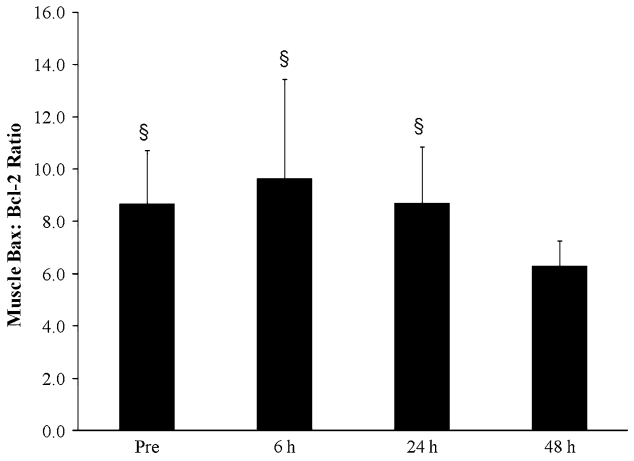

Fig. 3 Mean values for intramuscular levels of bax:bcl-2 ratio represented as mean ± SEM for all time points. Significantly different from 48 h post ($^{\S}P < 0.05$)

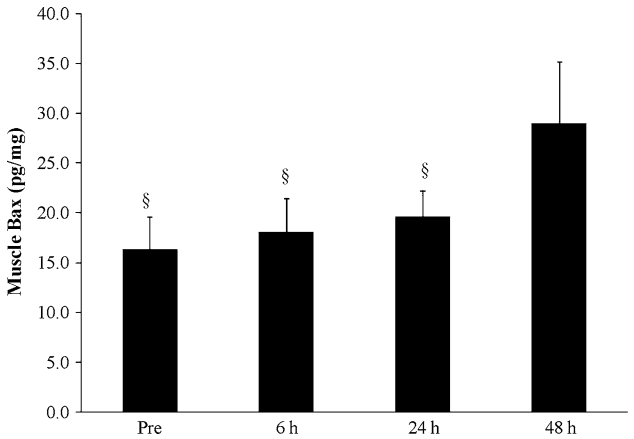

Fig. 4 Mean values for intramuscular levels of bax (pg/mg) represented as mean ± SEM for all time points. Significantly different from 48 h post ($^{\S}P < 0.05$)

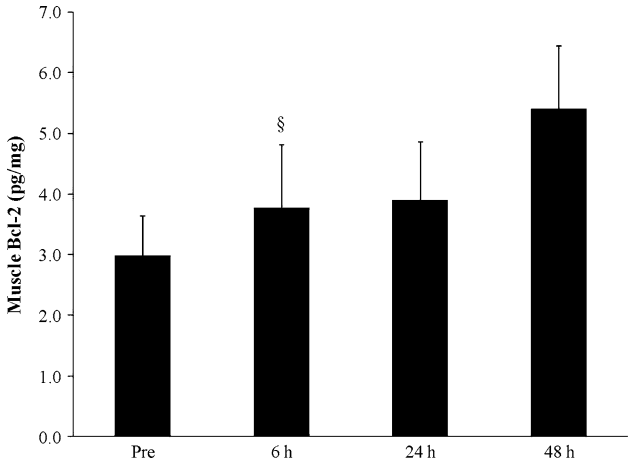

Fig. 5 Mean values for intramuscular levels of bcl-2 (pg/mg) represented as mean ± SEM for all time points. Significantly different from 48 h post ($^{\S}P < 0.05$)

this respect, the lack of change in cytochrome C would challenge the notion this pathway was involved in our caspase-3 activation; however, a tissue-specific response has been reported within skeletal muscle (Dirks and Leeuwenburgh 2002). The multinucleated state of skeletal muscle is thought to allow for only certain nuclei to be apoptotic targets as opposed to mononucleated cells, so it is possible the lack of change in cytochrome C did not adequately reflect the state of apoptosis throughout the myocytes. The lack of increases in serum cortisol and TNFα reduce the likelihood that the reported increases in caspase-3 enzyme activity were due to ligand binding and downstream activation of the Fas signaling pathway. Although muscle damage was not directly assessed, evidence of muscle damage existed (Warren et al. 2002). In this regard, the most likely mechanism by which caspase-3 activity increased in the present study without any changes in inflammation, oxidative stress, or cytochrome C relates to skeletal muscle damage that has been shown to occur after eccentric exercise. Damaging eccentric exercise can lead to an imbalance of calcium in and around the myocyte (Gissel 2006), and result in activation of the calcium-dependent calpains (Gissel 2006). Through this pathway, caspase-12 is activated which triggers a caspase cascade resulting in activation of caspase-3, independent of cytochrome C and Apaf-1 (Nakagawa and Yuan 2000). This suggestion is supported by the findings of Willoughby after he reported significant increases in the mRNA and protein levels of ubiquitin, E2 ubiquitin conjugating enzyme, and the 20S proteasome (all major constituents of the ubiquitin proteolytic pathway) after eccentric exercise, along with increases in caspase-3 enzyme activity (Willoughby et al. 2003c).

In this respect, previous research has indicated that NAC may serve an anti-apoptotic role by maintaining a greater balance of glutathione, controlling mitochondrial membrane depolarization, and promoting lower levels of caspase-3 and bcl-2 during repeated bouts of endurance exercise (Quadrilatero and Hoffman-Goetz 2004, 2005). Studies, however, using orally ingested doses and eccentric-only muscle contractions are lacking and these two factors are likely key reasons the apparent differences in our findings compared to previous research. As mentioned throughout, key factors such as dosing, training status, sampling time points, exercise mode, and number of contractions are all key considerations that make direct comparisons among the literature difficult. Relative to EGCG, the present study is the first study to investigate the impact of prophylactic EGCG supplementation during eccentric muscle contractions on intramuscular apoptotic changes. Cultures studies have reported an inhibition of proteasome activity (Nam et al. 2001); however, the relevance of this finding to apoptotic regulation remains to be determined. Nevertheless, results from the present study provide

preliminary support for an increase in apoptosis after eccentric exercise; however, supplementation with NAC or EGCG did not appear to have any further impact over the measured variables and need to continue to be researched due to the novelty of their use in this area.

Limitations do exist to our study design that warrants discussion. Much of the previous literature showing increases in oxidative stress and inflammatory cytokines have used long-duration endurance activity (Ironman triathlon or ultramarathon) or 30–60 min of downhill running. When comparing these exercise modes to isokinetic or other resistance-exercise based protocols (e.g., 100 repetitions at 120–150% 1RM), the latter invoke greater localized eccentric activity while the former incorporate greater aerobic or metabolic stress. Other factors including: (1) the amount of muscle mass recruited as part of these chosen modes (quadriceps muscle group in the present study vs. the entire lower body musculature), (2) the number of completed contractions (100 contractions in the present study vs. potentially thousands in a running protocol), and (3) the amount of recovery provided make the two eccentric protocols markedly different. Recent studies, however, have refuted this suggestion and have reported increases in oxidative stress after localized eccentric muscle contractions with (Childs et al. 2001) and without antioxidant supplementation (Nikolaidis et al. 2007). An additional point relates to the dosage provided and the timing of tissue and blood collection. Regarding dosing, few studies have utilized oral ingestion of NAC and EGCG and thus the manufacturer recommended dosages were utilized. Considering the higher dosages needed for NAC to invoke changes in fatigue (Quadrilatero and Hoffman-Goetz 2004; Reid et al. 1994), it is possible that our participants may have been under-dosed to yield our proposed study outcomes. In this regard, Reid et al. intravenously infused a single dose into human participants that was approximately tenfold higher than our dose and similarly Quadrilatero et al. infused into mice a dose that was almost 35-fold higher. Upon considering that each daily dose of NAC was 1.8 g in the present study, it is possible that the absolute dose could certainly be responsible for some of the differences seen between studies in addition to the mode of administration. In addition, providing supplementation until completion of data collection as performed previously by others may have impacted our outcomes (Bloomer 2004; Mastaloudis et al. 2004). Lastly, the chosen sampling times may have also attributed to our outcomes. Recently, Michailidis et al. (2007) investigated the impact of time course changes in oxidative stress markers in the blood after a bout of moderate intensity aerobic exercise and concluded that most markers returned to non-significant levels within 4 h of completing the exercise bout. Thus, it is possible that any serum or intramuscular perturbations in oxidative stress or lipid peroxidation returned to baseline before our 6 h time point. Currently, no data exists on any time course considerations of intramuscular adaptations related to oxidative stress and apoptosis. A recent study by Yang et al. (2005) did suggest that myogenic and metabolic gene expression may peak around 6–8 h post-exercise while Louis et al. (2007) reported that gene expression of selected inflammatory and proteolytic markers return to baseline 6 h after an exercise bout.

In conclusion, eccentric muscle contractions are widely accepted to invoke muscle damage resulting in additional physiological changes and were found to occur as part of this investigation. Downstream increases in oxidative stress were not found in the present study, but have been shown previously while indications of mitochondrial apoptosis are consistent with other findings in the literature. Relative to supplementation, more research needs to be conducted to effectively determine any impact NAC and EGCG supplementation may have on changes in muscle damage, oxidative stress, inflammation, and apoptosis after a single bout of eccentric muscle contractions. To further identify the relationship between muscle damage, inflammation, oxidative stress, and apoptosis it appears that alternative forms of exercise may be needed as our study and many others have reported little to no change in measures of oxidative stress and inflammation after eccentric exercise. Finally, these findings may help future researchers to identify new strategies in which proteolysis and apoptosis can be decreased in situations such as zero gravity, cancer cachexia, diabetes, spinal cord injuries, AIDS, sepsis, and aging.

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