High-dose antioxidant vitamin C supplementation does not prevent acute exercise-induced increases in markers of skeletal muscle mitochondrial biogenesis in rats

G. D. Wadley\textsuperscript{1,2} and G. K. McConell\textsuperscript{1,3}

\textsuperscript{1}Department of Physiology, The University of Melbourne, Parkville, Victoria; and \textsuperscript{2}Centre for Physical Activity and Nutrition, School of Exercise and Nutrition Sciences, Deakin University, Burwood, Victoria; \textsuperscript{3}Institute of Sport, Exercise and Active Living and Biomedical and Health Sciences, Victoria University, Victoria, Australia

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Wadley GD, McConell GK. High-dose antioxidant vitamin C supplementation does not prevent acute exercise-induced increases in markers of skeletal muscle mitochondrial biogenesis in rats. \textit{J Appl Physiol} 108: 1719–1726, 2010. First published April 15, 2010; doi:10.1152/japplphysiol.00127.2010.—High doses of the antioxidant vitamin C prevent the increases in skeletal muscle mitochondrial biogenesis after exercise training. Since exercise training effects rely on the acute stimulus of each exercise bout, we examined whether vitamin C supplementation also attenuates the increases in skeletal muscle metabolic signaling and mitochondrial biogenesis in response to an acute exercise bout. Male Sprague-Dawley rats performed 60 min of treadmill running (27 m/min, 5% grade) or remained sedentary. For 7 days before this, one-half of the rats received water containing 500 mg/kg body wt vitamin C. Acute exercise significantly ($P < 0.05$) increased the phosphorylation of p38 MAPK, AMP-activated kinase-\(\alpha\), and activating transcription factor (ATF)-2 and the ratio of oxidized to total glutathione (GSSG/TGSH) in the gastrocnemius. However, vitamin C had no effect on these increases. Similarly, vitamin C did not prevent the exercise-induced increases in peroxisome proliferator-activated receptor-\(\gamma\) coactivator-1\(\alpha\), nuclear respiratory factor (NRF)-1, NRF-2, mitochondrial transcription factor A, glutathione peroxidase-1, MnSOD, extracellular SOD, or glucose transporter 4 ($P < 0.05$) mRNA after exercise. Surprisingly, vitamin C supplementation significantly increased the basal levels of GSSG/TGSH, NRF-1, and NRF-2 mRNA and basal ATF-2 phosphorylation. In summary, despite other studies in rats showing that vitamin C supplementation prevents increases in skeletal muscle mitochondrial biogenesis and antioxidant enzymes with exercise training, vitamin C had no affect on the acute exercise-induced increases of these markers.

contraction; reactive oxygen species; peroxisome proliferator-activated receptor-\(\gamma\) coactivator-1\(\alpha\)

UNDERSTANDING THE MOLECULAR MECHANISMS that regulate skeletal muscle mitochondrial biogenesis (synthesis) has important implications given the crucial role that mitochondria play in skeletal muscle metabolism. The ability of endurance exercise training to increase skeletal muscle mitochondrial volume has been known for over 40 yr (7, 9). Key signaling steps during exercise that appear to be involved with the activation of exercise-induced mitochondrial biogenesis [and increased glucose transporter (GLUT)4 expression] in skeletal muscle include increases in skeletal muscle cytosolic Ca\(^{2+}\) levels and the associated activation of calmodulin kinase, phosphorylation of p38 MAPK, and phosphorylation of AMP-activated protein kinase (AMPK) (17, 18, 36, 42). p38 MAPK-mediated phosphorylation of activating transcription factor-2 (ATF-2) plays a key role in inducing the expression of the putative master regulator of mitochondrial biogenesis peroxisome proliferator-activated receptor-\(\gamma\) coactivator-1\(\alpha\) (PGC-1\(\alpha\)) after exercise (1, 43). In the hours after acute exercise, skeletal muscle PGC-1\(\alpha\), nuclear respiratory factor (NRF)-1, and NRF-2 DNA binding are increased, as are NRF-1 and NRF-2 mRNA, which are also involved in coordinating the exercise training response (16, 39, 43). Furthermore, this activation of mitochondrial biogenesis is involved in the regulation of antioxidant enzymes, since PGC-1\(\alpha\) is required for the induction of SOD, glutathione peroxidase (GPx), and catalase (33).

There is also now a growing body of evidence supporting a role for ROS in the regulation of skeletal muscle mitochondrial biogenesis (5, 11, 13, 22, 25, 31, 33, 34). However, most of the data relates to the increased mitochondrial biogenesis after an exposure to exogenous oxidative stress (11, 33, 34) or in response to oxidant injury (22) and may therefore represent an adaptive response to a pathological state when ROS levels are chronically elevated. However, levels of ROS also increase during contraction in rodent skeletal muscle (3, 15, 19, 37), and these small, temporary, and, therefore, physiological increases in ROS may therefore be involved in the increased mitochondrial biogenesis after exercise (5, 25). Vitamin C supplementation (a nonspecific antioxidant) during 8 wk of exercise training in rats completely abolishes increases in several markers of exercise-induced skeletal muscle mitochondrial biogenesis and prevents increases in the expression of antioxidant enzymes, including GPx-1 and the mitochondrial manganese form of SOD (MnSOD) (5). Accordingly, vitamin C supplementation prevented the improvements in exercise capacity during exercise training (5). Similarly, recent compelling data in healthy humans have reported that a combination of vitamin E and vitamin C supplementation during 4 wk of exercise training prevents increases in several markers of mitochondrial biogenesis, antioxidant enzymes, and insulin sensitivity (25). Therefore, it appears that antioxidants may negate some of the major beneficial effects of exercise. Thus, these findings have important implications for people who take antioxidant supplements and exercise regularly in the belief they are getting substantial health benefits from this combination.

The adaptations to exercise training are considered to largely reflect an accumulation of a series of acute exercise bouts (8). Therefore, it is necessary to understand the molecular signals after an acute exercise bout that, when added together with

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\textsuperscript{1}Address for reprint requests and other correspondence: G. D. Wadley, School of Exercise and Nutrition Sciences, Deakin Univ., 221 Burwood Highway, Burwood, Victoria 3125, Australia (e-mail: glenn.wadley@deakin.edu.au).  
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other acute exercise bouts, will stimulate increases in mitochondrial volume and antioxidant enzymes. Indeed, each bout of exercise switches on gene expression for a multitude of proteins, including SODs, GPx-1, GLUT4, and regulators of mitochondrial volume (1, 4, 6, 43). However, an accumulation of acute bouts is required before substantial changes in protein expression and morphological changes become apparent. Furthermore, the recent results using antioxidants and exercise training have implied that antioxidants may be having effects on exercise signaling during acute exercise, which translate into the attenuated activation of gene expression of mitochondrial biogenesis markers and antioxidant enzymes after the acute exercise bout. Therefore, the aim of the present study was to determine whether supplementation with the nonspecific antioxidant vitamin C attenuates increases in skeletal muscle metabolic signaling and activation of the mitochondrial biogenesis pathway and antioxidant enzymes after acute exercise. We hypothesized that vitamin C supplementation would attenuate the activation of key metabolic enzymes (p38 MAPK, and ATF-2) during acute exercise and prevent increases in the expression of mitochondrial biogenesis and antioxidant enzymes after acute treadmill running in rats.

MATERIALS AND METHODS

Animal care and dietary treatment. Male Sprague-Dawley rats aged ~6 wk and weighing 227 ± 2 g were obtained from The University of Melbourne Pharmacology and Physiology Animal House. Animals were housed in an environmentally controlled laboratory (temperature: 22°C) with a 12:12-h light-dark cycle. One week before experimentation, animals were familiarized to treadmill running on 2 separate days for 15 min each day at speeds progressing up to 27 m/min at a 5% incline. The University of Melbourne Animal Experimentation Ethics Committee approved all experimental procedures.

One-half of the animals were fed vitamin C (500 mg·kg body wt−1·day−1, Sigma, St. Louis, MO) (5) ad libitum in the drinking water for 7 days or had ad libitum access to water. During the 7 days of treatment, fluid ingestion and body weight were measured daily. The total amount of food consumed during this period was also recorded. To ensure that rats ingested the correct daily dose of vitamin C, it was added fresh to drinking water on a daily basis into containers stored at 80°C until analysis. Blood samples were obtained via cardiac puncture and placed in tubes containing lithium-heparin.

Blood was spun, and the plasma was immediately stored at ~80°C until analysis. Plasma vitamin C levels were measured using HPLC.

Gene expression. RNA was isolated from frozen rat gastrocnemius muscles using the Micro-to-Midi Total RNA Purification System kit with TRIzol and DNase on-column digestion (Invitrogen, Melbourne, Australia). RNA integrity was verified, and the concentration was determined on an Experion Automated Electrophoresis System (Bio-Rad Laboratories, New South Wales, Australia). First-strand cDNA was generated from 0.5 µg RNA using avian myeloblastosis virus reverse transcriptase (Promega, Madison, WI) as previously described (41). After reverse transcription, the remaining RNA was degraded by treatment with RNase H (Invitrogen), which was incubated in the dark at 80°C for 5 min before the measurement of fluorescence (24).

NRF-1 (catalog no. Rn01455954_m1) was assessed using pre-designed/prevalidated FAM-labeled Assays-on-Demand from Applied Biosystems (Foster City, CA). Primer sequences were obtained from gene sequences from GenBank AB014089, GLUT4: NM_012751.1, GPx-1: NM_030826.3, MnSOD: NM_017051.2, cytosolic copper/zinc-containing (CuZn) SOD: NM_017050.1, and extracellular (ec)SOD: NM_012880.1. Primer sequences were as follows: PGC-1α, 5′-ACCCACAGATGACAAACC-3′ and 5′-GACAAATGTGCTTCTGGTATTGCG-3′; NRF-2, 5′-CCCCATTGTCACATTTCTC-3′ and 5′-CTTGGTGA-ACCATTCTCCT-3′; mtTFA, 5′-AGGCAATGGAGAGGACCTT-3′ and 5′-TTGTCATACCCCTTCCACTGCTT-3′; PPARγ, 5′-CGACATGACCGGATATAG-3′ and 5′-GGACCATGTTGGTGAGGGG-3′; MnSOD, 5′-GGGAGAGCTTGTGGGTGTTTCTGTTTT-3′ and 5′-ATGTTTAGGTTGTTGAGGG-3′. Sample amplification was determined by real-time PCR using SYBR green chemistry as previously described (40) using sequence detector software (Rotor-Gene version 6, Corbett Research, Sydney, Australia). Samples were subjected to a heat dissociation protocol after the final cycle of PCR to ensure that only one product was detected. The mRNA of each gene was normalized to the cDNA content in each sample using the OligGreen assay as described above. This has previously been shown to be a robust and suitable method of normalization that avoids the many problems associated with “housekeeping genes” (14, 23, 24).

Immunoblot analysis. Frozen muscle (10 µl buffer/mg muscle) was homogenized in freshly prepared ice-cold buffer [50 mM Tris (pH 7.5) containing 1 mM EDTA, 10% (vol/vol) glycerol, 1% (vol/vol) Triton X-100, 50 mM NaF, 5 mM Na4P2O7, 1 mM TTT, 1 mM PMSF, and 5 µl/m protease inhibitor cocktail (P8340, Sigma)]. Tissue lysates were incubated on ice for 20 min and then spun at 16,000 g for 20 min at 4°C. Protein concentration was determined using a BCA protein assay (Pierce, Rockford, IL) with BSA as the standard. Equal amounts of proteins were separated by SDS-PAGE and electrotransfer of proteins from the gel to polyvinylidene difluoride membranes [25 mmol/l Tris (pH 8.3), 152 mmol/l glycine, and 20% (vol/vol) methanol] was performed for 90 min at 95 V (constant). Blots were probed with anti-phosphop38 MAPK (Thr180/Tyr182) rabbit polyclonal antibody (Cell Signaling Technology, Hertsfordshire, UK), anti-AMPK-α rabbit polyclonal antibody, and anti-phospho-ATF-2 (Thr71) rabbit polyclonal antibodies (Cell Signaling Technology). Binding was detected with IRDye goat anti-rabbit IgG IRDye 800-conjugated anti-rabbit IgG (Molecular Probes, Eugene, OR) fluorescent secondary antibodies. As a loading control, blots were reprobed with anti-α-tubulin mouse monoclonal antibody (Sigma). Data
were expressed as ratios of integrated intensity after infrared detection (Odyssey Imaging system, LI-COR Biosciences, Lincoln, NE). For p38 MAPK and AMPK-α signaling, membranes were then stripped (2% SDS (wt/vol) in 25 mM glycine (pH 2.0)), and successful stripping was verified by an incubation with anti-rabbit IgG fluorescent secondary antibody followed by infrared detection. Stripped membranes were then reprobed with anti-p38 MAPK rabbit polyclonal antibody (Cell Signaling) or anti-phospho-AMPK-α (Thr172) rabbit polyclonal antibody (Upstate Biotechnology, New York, NY), and phosphorylation was expressed relative to p38 MAPK or AMPK-α protein abundance, respectively.

Skeletal muscle GSSG levels. GSSG and total glutathione (TGSH) were determined in freeze-dried skeletal muscle spectrophotometrically using a commercially available kit (Bioxideytech GSH/GSSG-412, Oxis Health Products) as previously described (28). The ratio of GSSG to TGSH (GSSG/TGSH) is expressed in glutathione equivalents since one molecule of glutathione yields two molecules of GSSG (i.e., GSSG/TGSH = 2 × GSSG + TGSH).

Skeletal muscle protein carbonyl content. Skeletal muscle was homogenized in ice-cold phosphate buffer (pH 6.7) containing 1 mM EDTA (10 µl buffer/mg muscle) and then spun at 10,000 g for 15 min at 4°C. The total protein carbonyl content (a common marker of protein oxidation) in the supernatant was determined spectrophotometrically using a commercially available kit according to the manufacturer’s instructions (Cayman Chemical).

Statistical analyses. For the comparison of plasma vitamin C levels, results were analyzed using an unpaired t-test. Results were analyzed using two-factor ANOVA (with/without vitamin C and with/without exercise) with repeated measures where appropriate (i.e., daily fluid intake and body weight). If this analysis revealed a significant interaction, specific differences between mean values were located using the Fisher’s least-significance-difference test. All data are presented as means ± SE. The level of significance was set at P < 0.05.

RESULTS

The amount of vitamin C consumed by each rat was 524 ± 7 mg·kg body wt·day⁻¹. Fluid intake was significantly lower in vitamin C-supplemented rats (~15%) due largely to 20% less fluid ingested during the first few days. However, importantly, the rats became accustomed to the vitamin C-supplemented water, so that by day 7 fluid intake was not significantly different between the groups. Overall, vitamin C supplementation was well tolerated by the animals, since total food intake and increases in body weight were not significantly different between supplemented and nonsupplemented rats. Furthermore, there were no observable differences in the rats’ ability to run on the treadmill between vitamin C-supplemented and nonsupplemented rats.

Effect of vitamin C supplementation on plasma vitamin C and markers of skeletal muscle ROS. Seven days of vitamin C supplementation significantly increased plasma vitamin C levels by 33%, from 37.4 ± 1.7 µmol/l in basal (nonexercised) rats to 49.9 ± 2.6 µmol/l in supplemented rats (P < 0.05, n = 7 rats/group). In skeletal muscle, 7 days of vitamin C supplementation resulted in significantly higher GSSG/TGSH (P < 0.05, main effect for vitamin C; Fig. 1A). Immediately after exercise, GSSG/TGSH was significantly increased (P < 0.05, main effect for exercise; Fig. 1A). The effects of vitamin C supplementation and exercise on GSSG/TGSH were due to significantly higher levels of GSSG (P < 0.05, main effect for exercise, and P < 0.05 main effect for vitamin C; Fig. 1B). There were no differences in TGSH levels between groups (P > 0.05; Fig. 1C). Importantly, these GSSG and TGSH values correspond with what have been reported in the literature in skeletal muscle using this assay when normalized to total protein content (2, 38). Also, the ~60% increase in GSSG levels after exercise (Fig. 1B) is similar to the increase in GSSG in mouse skeletal muscle after in vitro contraction (28).

Skeletal muscle protein carbonylation levels were not altered by vitamin C supplementation or exercise (6.4 ± 0.5 vs. 5.9 ± 0.7 vs. 4.9 ± 0.1 vs. 5.6 ± 0.3 nmol/ml for basal without...
vitamin C vs. exercise without vitamin C vs. basal with vitamin C vs. exercise with vitamin C, respectively, \( P > 0.05, n = 4 \) rats/group).

**Effect of vitamin C supplementation on exercise-induced mitochondrial biogenesis signaling.** Immediately after 60 min of treadmill running, phosphorylation of p38 MAPK (Thr\(^{180}\)Tyr\(^{182}\)), AMPK-\(\alpha\) (Thr\(^{172}\)), and ATF-2 (Thr\(^{71}\)) increased similarly either with or without vitamin C supplementation (\( P < 0.05\), main effect for exercise; Fig. 2, A–C, respectively). Although phosphorylation of p38 MAPK (Thr\(^{180}\)Tyr\(^{182}\)) appeared higher after vitamin C supplementation, this was not significant (\( P = 0.16\), main effect for vitamin C; Fig. 2A). The protein abundance of p38 MAPK, AMPK-\(\alpha\), and \(\alpha\)-tubulin was not significantly different between groups (data not shown). Although two-way ANOVA revealed no significant effect of vitamin C supplementation on the phosphorylation of ATF-2 (Thr\(^{71}\)), the basal phosphorylation was \(\sim 50\%\) higher after vitamin C supplementation. This lack of statistical effect with two-way ANOVA may have been masked by the very high exercise-induced increase (\(\sim 500\%\)) in ATF-2 (Thr\(^{71}\)) phosphorylation. Indeed, the basal phosphorylation levels of ATF-2 (Thr\(^{71}\)) were significantly higher (\( P < 0.05\)) in vitamin C-supplemented (0.11 \(\pm\) 0.02 integrated intensity) compared with non-supplemented (0.07 \(\pm\) 0.01 integrated intensity) rats after unpaired \(t\)-test analysis. Therefore, although the basal phosphorylation of ATF-2 was not significantly increased statistically in the strictest sense (i.e., by two-way ANOVA), there was likely a biological effect.

**Effect of vitamin C supplementation on mitochondrial biogenesis markers and GLUT4 4 h after exercise.** Four hours after 60 min of treadmill running, PGC-1\(\alpha\), mtTFA, NRF-1, and NRF-2 mRNA were significantly increased, by \(\sim 500\%, 43\%, 31\%,\) and 24\%, respectively (\( P < 0.05\), main effect for exercise; Fig. 3, A–D, respectively). Vitamin C supplementation also significantly increased NRF-1 and NRF-2 mRNA (\( P < 0.05\), main effect for vitamin C; Fig. 3, A–D). GLUT4 mRNA was increased by 56\% 4 h after exercise, with no significant effect of vitamin C (\( P < 0.05\), main effect for exercise; 1.7 \(\pm\) 0.1 vs. 2.3 \(\pm\) 0.1 vs. 1.7 \(\pm\) 0.1 vs. 2.7 \(\pm\) 0.5 arbitrary units for basal without vitamin C vs. exercise without vitamin C vs. basal with vitamin C vs. exercise with vitamin C, respectively). Levels of single-stranded DNA, as measured by OliGreen, were not significantly different between any group (\( P > 0.05\); data not shown).

**Effect of vitamin C supplementation and exercise on antioxidant enzymes.** Four hours after 60 min of treadmill running, GPx-1, MnSOD, and ecSOD mRNA were significantly increased (\( P < 0.05\), main effect for exercise; Fig. 4, A–C, respectively). There was a tendency for MnSOD and CuZn-SOD mRNA to be higher in the vitamin C-supplemented groups (\( P = 0.08\), main effect for vitamin C; Fig. 4, B and D, respectively).

**DISCUSSION**

The major finding of this study was that high-dose supplementation with the antioxidant vitamin C did not attenuate the acute exercise-induced increases in skeletal muscle signaling of AMPK, p38 MAPK, or ATF-2 or the activation of gene expression of mitochondrial biogenesis markers (PGC-1\(\alpha\), mtTFA, NRF-1, and NRF-2) or GLUT4 after exercise. Therefore, the molecular mechanisms to explain how vitamin C supplementation has previously been observed to prevent increased mitochondrial biogenesis after exercise training in rats (5) remains unclear but does not appear to be due to vitamin C preventing the increase in skeletal muscle metabolic signaling and activation of the mitochondrial biogenesis pathway after acute exercise.

The vitamin C dose of \(\sim 500\) mg·kg body wt\(^{-1}\)·day\(^{-1}\) for 7 days that was used in the present study was chosen based on a previous study (29) that showed that such a dose prevented...
increases in blood ROS levels during acute exercise in rats. However, the present study is the first to actually measure whether this high dose of vitamin C attenuates the increase in markers of ROS after acute exercise in rat skeletal muscle. ROS have short half-lives, making direct detection in tissue from an exercising animal very difficult (for a review, see Ref. 21). Consistent with this, we found exercise did not increase skeletal muscle H2O2 levels in our homogenized samples (data not shown) using a commercially available Amplex red fluorometric assay (Invitrogen). Therefore, indirect biomarkers of ROS production are required for exercise studies. GSSG/TGSH is a biomarker of cellular redox balance, since glutathione, a major cellular antioxidant, becomes oxidized in the catalyzed reduction of H2O2 to water (21). Importantly, previous studies have shown GSSG/TGSH increases after in vitro skeletal muscle contraction (27, 28) and that this increase can be prevented with the nonspecific antioxidant N-acetylcysteine (28). Also, consistent with elevated intracellular ROS, the level of GSSG increases in rat skeletal muscle after exhaustive treadmill running (4, 12) and in human skeletal muscle after heavy (but not exhaustive) intensity cycling (44). However, there are very few other suitable biomarkers for the measurement of ROS levels in skeletal muscle during whole body exercise. Indeed, the present study found that protein carbonyl levels, another indirect biomarker of ROS, was not altered in skeletal muscle after exercise. Although biomarkers that indicate oxidized products such as protein carbonylation or lipid peroxidation [i.e., thiobarbituric acid reactive substances (TBARS)] are commonly used in free radical research, these products exist in small amounts (21). Therefore, these oxidation products may not be sensitive enough to detect small changes in skeletal muscle ROS during the nonexhaustive exercise in the present study. Only small changes in protein carbonylation are detected in rodent skeletal muscle after exhaustive exercise (4), when ROS production might be expected to be much higher than in the nonexhaustive exercise in the present study. TBARS measurements have also been widely criticized for lack of specificity, particularly when used for in vivo experiments (26). Nevertheless, a key feature of the present study was that the acute exercise was nonexhaustive and still of sufficient intensity to significantly elevate GSSG/TGSH in skeletal muscle. However, what is clear from the present study is that high-dose vitamin C supplementation for 7 days does not effectively inhibit the increase in skeletal muscle GSSG/TGSH levels during acute exercise.

A somewhat surprising finding of the present study was that vitamin C supplementation elevated basal levels of GSSG/TGSH, NRF-1, and NRF-2 mRNA and basal phosphorylation of ATF-2 and tended to increase MnSOD and CuZnSOD mRNA. Collectively, these findings are consistent with elevated (i.e., prooxidant), rather than reduced, basal ROS levels in skeletal muscle and a possible compensatory antioxidant response. Paolini et al. (20) previously showed that the ingestion of 500 mg/kg body wt of vitamin C for 4 days has prooxidant effects via increased ROS in the liver of rats, although the effects in rat skeletal muscle were not reported.

![Fig. 3. Peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α; A), mitochondrial transcription factor A (mtTFA; B), nuclear respiratory factor (NRF)-1 (C), and NRF-2 (D) mRNA in the gastrocnemius muscle of rats after 7 days of water or vitamin C ingestion under basal conditions and 4 h after 60 min of treadmill running (ex+recovery). Values are means ± SE; n = 8 rats/group. *P < 0.05, main effect for exercise; +P < 0.05, main effect for vitamin C.](http://jap.physiology.org/DownloadedFrom)
ATF-2 is phosphorylated in response to increased ROS levels in the liver (10) and endothelium (32), and the basal phosphorylation levels of ATF-2 are known to be elevated after age-related increases in oxidative stress (10). Furthermore, NRF-1 and NRF-2 are redox sensitive, and their mRNA is elevated by increased ROS levels (34, 35). MnSOD and CuZnSOD are major antioxidant enzymes in skeletal muscle, and the tendency for their gene expression to be higher after vitamin C supplementation is also consistent with a compensatory antioxidant response. On the other hand, skeletal muscle protein carbonylation was not significantly different for any group, although, as discussed earlier, this biomarker may not be a particularly sensitive measure of skeletal muscle ROS levels. Therefore, despite our efforts using common markers of skeletal muscle oxidative stress, it is not possible in the present study to make firm conclusions regarding the prooxidant effect of high-dose vitamin C supplementation on rat skeletal muscle. Further studies are now required to investigate the downstream implications for the elevated basal phosphorylation of ATF-2 and increased NRF-1 and 2 mRNA and antioxidant enzymes with high-dose vitamin C supplementation.

Consistent with previous findings, the present study observed robust increases in skeletal muscle metabolic signaling (phosphorylation of p38 MAPK, AMPK-α, and ATF-2), markers of mitochondrial biogenesis (PGC-1α, NRF-1, NRF-2, and mtTFA mRNA), and oxidative enzymes (GPx-1, MnSOD, and ecSOD mRNA) after acute exercise (1, 4, 6, 43). However, since high-dose vitamin C did not prevent or attenuate these adaptations, it is unclear how vitamin C prevents the exercise-induced increase in mitochondrial biogenesis and antioxidant enzymes observed after several weeks of training (5). It is possible that the effects of vitamin C supplementation on skeletal muscle mitochondrial biogenesis and antioxidant enzymes are different in the short term (several days) versus long term (several weeks). Studies that combine acute exercise and longer-term training with vitamin C supplementation are now needed to resolve this. Nevertheless, supplementation does not appear to cause any overt detrimental effects in the long term, since lifelong supplementation of vitamin C in mice (180 mg/kg body wt) has no effect on lifespan or markers of oxidative damage to the liver (30). However, lifelong vitamin C supplementation in mice does reduce the gene expression of several antioxidant enzymes in the liver (30), although the effects in skeletal muscle and the impact on mitochondrial biogenesis is unknown, as is the mechanism. Although speculative, an alternative explanation for the attenuated skeletal muscle mitochondrial biogenesis and antioxidant enzymes observed with training (5) could be due to an overall chronic downregulation of these pathways via an as-yet-unknown mechanism. Furthermore, since rodents produce endogenous vitamin C, future studies should also examine the consequences of several days of high-dose vitamin C supplementation in the skeletal muscle of higher-order primates, such as humans, that cannot produce endogenous vitamin C.

In summary, contrary to our hypothesis, supplementation with the antioxidant vitamin C did not prevent the increase in markers of skeletal muscle ROS levels, mitochondrial biogenesis, GLUT4, or antioxidant enzymes after acute exercise. Surprisingly, the present study found evidence for elevated basal levels of skeletal muscle GSSG/TGSH, NRF-1 and NRF-2 mRNA, and phosphorylation of ATF-2 and tendencies for higher antioxidant gene expression after vitamin C supple-
mentation. These observations are consistent with upregulated antioxidant defenses in response to elevated basal levels of ROS, and further work is required to investigate the downstream consequences of this. The molecular mechanism(s) to explain how vitamin C supplementation has previously been observed to prevent increased mitochondrial biogenesis after exercise training in rats (5) remains unclear but does not appear to be due to vitamin C preventing the increase in skeletal muscle metabolic signaling and activation of the mitochondrial biogenesis pathway after acute exercise.

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DISCLOSURES
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