The influence of acute resistance exercise on cyclooxygenase-1 and -2 activity and protein levels in human skeletal muscle

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The influence of acute resistance exercise on cyclooxygenase-1 and -2 activity and protein levels in human skeletal muscle. Am J Physiol Regul Integr Comp Physiol 305: R24–R30, 2013. First published May 1, 2013; doi:10.1152/ajpregu.00593.2012.—This study evaluated the activity and content of cyclooxygenase (COX)-1 and -2 in response to acute resistance exercise (RE) in human skeletal muscle. Previous work suggests that COX-1, but not COX-2, is the primary COX isof orm elevated with resistance exercise in human skeletal muscle. COX activity, however, has not been assessed after resistance exercise in humans. It was hypothesized that RE would increase COX-1 but not COX-2 activity. Muscle biopsies were taken from the vastus lateralis of nine young men (25 ± 1 yr) at baseline (preexercise), 4, and 24 h after a single bout of knee extensor RE (three sets of 10 repetitions at 70% of maximum). Tissue lysate was assayed for COX-1 and COX-2 activity. COX-1 and COX-2 protein levels were measured via Western blot analysis. COX-1 activity increased at 4 h (P < 0.05) compared with preexercise, but returned to baseline at 24 h (PRE: 60 ± 10, 4 h: 106 ± 22, 24 h: 72 ± 8 nmol PGH2·g total protein⁻¹·min⁻¹). COX-2 activity was elevated at 4 and 24 h after RE (P < 0.05, PRE: 51 ± 7, 4 h: 100 ± 19, 24 h: 98 ± 14 nmol PGH2·g total protein⁻¹·min⁻¹). The protein level of COX-1 was not altered (P > 0.05) with acute RE. In contrast, COX-2 protein levels were nearly 3-fold greater (P > 0.05) at 4 h and 5-fold greater (P = 0.06) at 24 h, compared with preexercise. In conclusion, COX-1 activity increases transiently with exercise independent of COX-1 protein levels. In contrast, both COX-2 activity and protein levels were elevated with exercise, and this elevation persisted to at least 24 h after RE.

skeletal muscle; cyclooxygenase; human; prostaglandins; exercise

Prostaglandins are a product of cyclooxygenase activity and elicit an array of tissue-specific functions (9). In skeletal muscle, prostaglandins regulate protein synthesis and degradation after exercise (5, 20, 23, 25) and are necessary for skeletal muscle injury recovery (2, 28, 29). Prostaglandins are derived from arachidonic acid (AA) via a reaction catalyzed by cyclooxygenase (COX), a ubiquitous enzyme that is expressed in many cell types, including skeletal muscle (31). In this reaction, AA is first converted to hydroperoxy endoperoxide (PGG2) and then is further reduced to PGH2, which is the precursor to prostaglandins, thromboxanes, and prostacyclins (4). At least two isoforms of COX have been identified, COX-1 and COX-2. Additionally, two variants of COX-1 (COX-1v1 and COX-1v2) have been detected in skeletal muscle (5, 39). COX-1 is traditionally viewed as a constitutively expressed protein that primarily has homeostatic functions, while COX-2 is induced by an array of stimuli, including tissue injury (38). There is likely some overlap in the function of these enzymes (16, 24, 41). Skeletal muscle has been shown to express both isoforms of COX (5, 31, 32, 35, 39), and both isoforms appear to have an important role in regulating skeletal muscle protein synthesis and degradation (25, 26, 37). For example, consumption of the COX-inhibiting drugs ibuprofen or acetaminophen inhibits acute exercise-induced increases in skeletal muscle protein synthesis and PGF2α in humans (36, 37). In contrast, when consumed chronically during resistance training these same COX inhibitors enhance muscle hypertrophy (35). Additionally, several animal and cell culture studies suggest that COX-2 is essential for skeletal muscle protein synthesis, growth, and tissue repair following injury or pathologies (2, 3, 16, 17, 19, 28). More recent studies (5, 35), however, link the postexercise protein synthetic response to COX-1. Specifically, consumption of a selective COX-2 inhibitor did not inhibit the increase in skeletal muscle protein synthesis (5) after a bout of acute eccentric resistance exercise. Additionally, this same acute exercise bout, without COX-2 inhibitor consumption, resulted in an increase in the mRNA expression of COX-1 but not COX-2 (5). Furthermore, chronic resistance exercise results in an increase in COX-1 protein content and mRNA expression with no change in COX-2 protein content (35). Together, these data support a hypothesis that COX-1 is the predominant COX enzyme involved in the skeletal muscle response to acute resistance exercise. However, in these previous studies (5, 35, 39), the activity of the COX enzymes after resistance exercise was not evaluated. Therefore, the purpose of this study was to evaluate skeletal muscle COX enzyme activity after a single bout of resistance exercise. We hypothesized that acute resistance exercise would increase the activity of COX-1, while COX-2 activity would remain unchanged. To our knowledge, no studies have directly assessed COX enzyme activity levels in human skeletal muscle after acute resistance exercise, especially a bout commonly used to induce skeletal muscle hypertrophy when performed chronically (35, 40).

METHODS

Subjects. The study was approved by the Midwestern University Institutional Review Board, and written informed consent was obtained from all participants. Nine young, recreationally active men (age: 25 ± 1, weight: 81.0 ± 2.3, height: 178 ± 2, body mass index: 26 ± 1) were recruited from the Glendale, AZ, area. Subjects completed a detailed health and exercise history questionnaire and were excluded if they had any condition in which resistance exercise would be contraindicated, if they were chronically consuming any prescription or nonprescription COX-inhibiting drugs, and if they were diabetic or used tobacco products (5, 35, 39).
Exercise bout. Prior to the resistance exercise bout, each individual’s single leg, concentric one repetition maximum (1RM) was evaluated on two separate occasions on a leg extension device (VR3 Leg Extension; Cybex International, Medway, MA). Approximately 2 wk after the strength assessments, subjects returned to the laboratory. After 5 min of light cycling (Ergomedic 828E; Monark, Vansbro, Sweden), subjects performed two sets of leg extension with light warm-up loads followed by three sets of 10 repetitions of unilateral knee extension. Exercise intensity was set at 70% of 1RM (6, 35), and each set was interspersed by a 2-min rest period. Each lift was completed through a full range of motion in a controlled manner (1–2 s up and 1–2 s down).

Skeletal muscle biopsy. A muscle biopsy was taken from the vastus lateralis, one immediately prior to the resistance exercise bout and again at 4 and 24 h after the resistance exercise bout. Tissue was obtained following local anesthetic (lidocaine HCl 1%) using a 5-mm Bergstrom needle with suction (1). Muscle was cleansed of excess blood and stored in liquid nitrogen (−190°C). Each muscle biopsy was taken from a separate incision distal to the previous biopsy.

Diet and activity control. Because of the known influence of increased activity on skeletal muscle protein metabolism, subjects were asked to refrain from any activity outside of their normal daily activities during the study. In addition, for 3 days before the muscle biopsies and exercise bout, subjects were asked to abstain from structured exercise.

Subjects were required to consume a standardized evening meal (Ensure Plus; Ross, Columbus, OH; 53% carbohydrate, 15% protein, and 32% fat) the evening before the preexercise and 4-h biopsies and the evening prior to the 24-h biopsy. Subjects arrived at the laboratory for the exercise bout and 24-h biopsy after a 12-h fast. The meal contained 50% of the subject’s estimated caloric need (1.5 times the subject’s predicted resting metabolic rate (10)). This type of diet control has been used previously in similar studies of skeletal muscle metabolism (5, 7, 39).

Cyclooxygenase activity. At least 20 mg of muscle was homogenized (2 ml tissue grinder, 440410, Radnoti Glass Technology, Monrovia, CA) in 20 volumes of buffer [100 mM Tris·HCl with proteinase inhibitors (Roche Diagnostics, Chicago, IL)]. After homogenization, samples were centrifuged at 10,000 H11002 g for 10 min, and the supernatant was retained for assessing COX activity and protein content. COX activity was then assessed using a cyclooxygenase supernatant was retained for assessing COX activity and protein content was determined using a bicinchoninic acid protein assay kit.

The supernatant was used to determine the levels of COX-1 and COX-2 protein was also examined using standard immunoblotting methods (18). Human COX-1 antibody specificity was verified using human coronary vascular smooth muscle lysate (Santa Cruz, CA) in a previous study (18). COX-1 antibody specificity was verified with LPS-stimulated RAW 264.7 mouse macrophage cell lysate (Santa Cruz Biotechnology, Santa Cruz, CA) in a previous study (18). COX-1 antibody specificity was verified using human coronary vascular smooth muscle lysate (Fig. 3B; cells were purchased from ScienCell (Carlsbad, CA)). All data were analyzed using Image Studio 2.0 software (LI-COR). Reagents for Western blot analysis were purchased from Sigma-Aldrich Chemical (St. Louis, MO) unless otherwise noted.

Statistics and analysis. COX-1 and COX-2 enzyme activity was evaluated together using a balanced two-way repeated-measures ANOVA with time [preexercise (PRE), 4 h, and 24 h] and enzyme (COX-1 or COX-2) as factors. Total activity and COX protein content were evaluated with a one-way repeated-measures ANOVA.

For data from Western blot analysis, samples from each subject, which included preexercise, 4-h, and 24-h samples, were run on the same blot for direct comparison. Optical densities for the top bands for both COX-1 and COX-2 were normalized to GAPDH (loading control). Data are represented as the intensity ratio (COX-2 or COX-1 activity/protein separation). A Mini PROTEAN Tetra electrophoresis system (Bio-Rad Laboratories, Hercules, CA) was used for protein separation. Proteins were transferred to nitrocellulose membranes, and membranes were blocked by incubation at room temperature for 1 h in PBS containing 1% Tween PBS (TPBS) and 3% dry milk (Kroger Brand, Cincinnati, OH). After blocking, membranes were incubated in a monoclonal anti-COX-1 (1:500 dilution, cat. no. 160110; Cayman Chemical) or polyclonal anti-COX-2 (1:500 dilution, cat. no. 160126; Cayman Chemical) TPBS solution overnight at 4°C. After TPBS washes (5 × 5 min), the membranes were incubated in a fluorescent goat anti-rabbit IR 800 secondary antibody for detection of COX-2 and a fluorescent goat anti-mouse IR 800 secondary antibody for COX-1 detection. Membranes were once again washed in TPBS (5 × 5 min), and bands were visualized using an Odyssey infrared imager (LI-COR). For all COX-1 membranes, the blots were lightly stripped using NaOH (0.2 M) for 2 min and then washed with TPBS and reblocked for 15 min. Blots were then rinsed and exposed to a TPBS/anti-GAPDH (1:15,000 dilution, cat. no. MAB374; Millipore, Temecula CA) incubation solution, incubated overnight at 4°C. The next day, membranes were washed, exposed to a fluorescent donkey anti-mouse IR680 secondary antibody, and imaged. GAPDH was used for verification of equal loading of protein. COX-2 membranes were not stripped between anti-COX-2 and GAPDH incubations. COX-2 antibody specificity was verified with LPS-stimulated RAW 264.7 mouse macrophage cell lysate (Santa Cruz Biotechnology, Santa Cruz, CA) in a previous study (18).

RESULTS

COX enzyme activity. When normalized to tissue wet weight, total COX activity tended to increase at 4 but not 24 h postexercise (PRE: 12.3 ± 1.4, 4 h: 15.1 ± 2.0, and 24 h: 13.8 ± 1.2 nmol PGH2·g muscle−1·min−1; P = 0.10, Fig. 1A). Similarly, when total activity was expressed relative to protein, total activity increased at 4 but not 24 h postexercise (PRE: 190.3 ± 18.1, 4 h: 283.4 ± 49.7, and 24 h: 234.8 ± 22.2 nmol PGH2·g−1 protein·min−1; P < 0.05, Fig. 1B).

COX-1 activity was present in nonexercised skeletal muscle and was not upregulated with exercise when normalized to tissue wet weight (P > 0.05; PRE: 4.0 ± 0.8; 4 h: 5.7 ± 1.0; 24 h: 4.3 ± 0.5 nmol PGH2·g muscle−1·min−1) but was 97% greater than preexercise at 4 (P < 0.05) but not 24 h (PRE: 60.1 ± 10.5; 4 h: 106.2 ± 21.9; 24 h: 71.7 ± 8.0 nmol PGH2·g−1 protein−1·min−1) after the exercise bout when normalized to tissue protein content (Fig. 2).
COX-2 activity was also present in nonexercised muscle (Fig. 2). In contrast to COX-1 activity, COX-2 activity was elevated ($P < 0.05$) at 4 and 24 h postexercise when normalized to tissue wet weight (PRE: $3.4 \pm 0.5; 4$ h: $5.4 \pm 0.9; 24$ h: $5.7 \pm 0.7$ nmol PGH$_2$/g muscle$^{-1}$·min$^{-1}$) and protein content (PRE: $51.3 \pm 6.6; 4$ h: $100.0 \pm 18.8; 24$ h: $97.7 \pm 13.6$ nmol PGH$_2$/g protein$^{-1}$·min$^{-1}$). There was no difference between COX-1 and COX-2 activity at rest and 4 h after resistance exercise ($P > 0.05$). COX-2 activity was greater than COX-1 activity when compared at 24 h postexercise ($P < 0.05$). The percentage of total activity attributable to COX-1 was not different than COX-2, both at rest and 4 h postexercise ($P > 0.05$; Table 1). However, the percentage of activity attributable to COX-2 was greater than COX-1 24 h postexercise ($P < 0.05$). The total percentage of COX activity due to COX-2 also increased with exercise at both 4 ($P = 0.10$) and 24 h ($P < 0.05$, Table 1). Together, COX-1 and COX-2 accounted for only $60–73\%$ of total COX activity at any time point (Table 1).

Western blot analysis. COX-1 protein levels were detected in human vastus lateralis at preexercise and at all time points postexercise in all samples tested (representative blot, Fig. 3A). Compared with human coronary vascular smooth muscle lysate, detection of bands using anti-COX-1 in human skeletal muscle were not as robust as in the vascular smooth muscle sample. COX-1 protein levels were not elevated ($P < 0.05$) with exercise at any time point (Fig. 3B). Although COX-1 appeared to increase slightly with exercise (Fig. 3B), this was largely due to one outlier subject, whose exercise response was $4$-fold higher than the mean due to a very low COX-1 protein content preexercise. This outlier is included in Fig. 3B. Removing this outlier subject did not alter the statistical interpretation of the data (data not shown).

COX-2 protein levels were also detected in human vastus lateralis samples at preexercise and at all time points postexercise in all samples tested (representative blot, Fig. 4A). In contrast to COX-1, COX-2 protein levels were nearly threefold greater ($P > 0.05$) at 4 h and five-fold greater ($P = 0.06$).

Table 1. Isoform-specific COX activity as a percentage of total activity

<table>
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<tr>
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<th>COX-1</th>
<th>COX-2</th>
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<tr>
<td>PRE</td>
<td>$31.8 \pm 5.0$</td>
<td>$27.9 \pm 3.9$</td>
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<tr>
<td>4</td>
<td>$37.0 \pm 3.9$</td>
<td>$36.5 \pm 3.9^*$</td>
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<td>24</td>
<td>$31.8 \pm 3.6$</td>
<td>$41.2 \pm 3.0^\dagger$</td>
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Values are expressed as means ± SE. COX, cyclooxygenase; PRE, preexercise. $^*P = 0.10$, PRE vs. 4 h; $^\dagger P < 0.05$, PRE vs. 24 h; $^\ddagger P < 0.05$, COX-2 vs. COX-1.
primary COX needed for muscle hypertrophy (5, 35). Burd et al. (5) have clearly shown that inhibition of COX-2 does not inhibit induction of skeletal muscle protein synthesis after resistance exercise. In contrast, administration of a nonspecific COX inhibitor blocks the induction of protein synthesis after resistance exercise (37). Interestingly, and consistent with previous work (5), we did not detect an increase in COX-1 protein levels with acute resistance exercise. Chronic resistance exercise, however, does increase COX-1 protein levels (35). Therefore, it is possible that the brief increase in COX-1 activity with acute resistance exercise is sufficient to drive skeletal muscle protein synthesis, even out to 24 h postexercise (5), and the repeated need to increase protein synthesis with chronic training leads to an increase in COX-1 protein. In contrast, our findings suggest that COX-2 likely contributes to skeletal muscle prostaglandin production after acute resistance exercise. The rapid elevation of COX-2 protein content compared with COX-1 and the prolonged increase in COX-2 enzyme activity may have a greater role in regulating postexercise muscle protein synthesis than previously thought. It is, however, difficult to resolve the fact that administration of an oral COX-2 inhibitor did not block postexercise muscle protein synthesis (5). It is possible that the dose of Celebrex given in

DISCUSSION

To our knowledge, this is the first study evaluating the influence of acute resistance exercise on isoform-specific COX activity in human skeletal muscle. Our data support previous studies (5, 31, 32, 35, 39), which have indicated that COX-1 and COX-2 are expressed in resting skeletal muscle. As with previous work (5, 39) in human skeletal muscle, COX-1 protein content was not elevated with acute resistance exercise. In contrast, COX-2 protein was detected in all samples evaluated and was increased nearly five-fold 24-h postexercise.

Contrary to our hypothesis, both COX-1 and COX-2 enzyme activity increased with acute resistance exercise; however, only COX-2 enzyme activity remained elevated at 24 h postexercise. The increase in COX-1 activity did not require an increase in protein levels, and changes in COX-2 activity did not correlate with changes in protein levels.

Although COX-1 activity was only upregulated for a short time period, our data do not refute the idea that COX-1 is the

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**Fig. 3.** Cyclooxygenase-1 (COX-1) protein levels from human skeletal muscle at preexercise (PRE), 4 h, and 24 h postexercise. A: representative blot of human vastus lateralis muscle lysate probed with anti-COX-1 and anti-GAPDH from two human subjects. GAPDH served as a loading control. Human coronary vascular smooth muscle (HCVSM) lysate was used as a positive control. B: Western blot analysis of COX-1 protein levels. Data are represented as intensity ratio (COX-1 optical density/GAPDH optical density) of the 4-h postexercise and 24-h time points for each subject normalized to its corresponding preexercise intensity ratio. A standard (Std) served as a marker for band migration assessment. Values are reported as means ± SE (n = 7).

At 24 h, compared with preexercise (Fig. 4B). In general, taking into consideration equal amounts of protein loading for each blot (30 μg), the band density for COX-1 protein levels appears lighter compared with COX-2 in human skeletal muscle. COX-1 protein levels were also much lower than levels detected in human coronary vascular smooth muscle, which was used as a positive control (Fig. 3A).

**Fig. 4.** Cyclooxygenase-2 (COX-2) protein levels from human skeletal muscle at preexercise (Pre), 4 h, and 24 h postexercise. A: representative blot of human vastus lateralis muscle lysate probed with anti-COX-2 and anti-GAPDH. Molecular weight markers (Std from LI-COR and Kaleidoscope MW from Invitrogen) were loaded in first two lanes. B: Western blot analysis of COX-2 protein levels. GAPDH served as a loading control. Data are represented as the intensity ratio (COX-2 optical density/GAPDH optical density) of the 4-h and 24-h time point for each subject normalized to its corresponding preexercise value. Values are reported as means ± SE. *P = 0.06, increase with exercise; n = 8.
the study by Burd et al. (5) was not sufficient to suppress prostaglandin production to an extent that would limit muscle protein synthesis in humans, especially after such an intense bout of eccentric exercise. It is also possible that the increase in COX-2 activity results in the production of PGE$_2$, which could then contribute to an increase in muscle protein degradation (26). The intense exercise bout used in the study by Burd et al. (5) may also convolute comparisons to the current investigation. Regardless, our findings at minimum, suggest that COX-2 may have a greater role in regulating postexercise adaptations in skeletal muscle and possibly protein synthesis. The fact that even a modest bout of exercise results in a rapid induction of both COX-2 enzyme activity and COX-2 protein synthesis demonstrates that further studies are needed to delineate the role of COX-2 in skeletal muscle exercise adaptations.

Our study is the first to report an increase in COX-2 protein content and activity after acute resistance exercise. COX-2 activity was elevated to a similar extent as COX-1 activity but continued to be elevated at 24 h postexercise. COX-2 protein levels also increased after only 24 h. The difference in induction of COX-1 and COX-2 protein is not surprising, given the reported different roles of COX-1 and COX-2 (9). Given that inhibition of COX-2 does not prevent an increase in skeletal muscle protein synthesis, it is not clear what role the prolonged increase in COX-2 activity has in the postexercise response. It could be that the increase in COX-2 with resistance exercise is related to an inflammatory response. Prostaglandins produced during inflammation are important for muscle regeneration (22), and there are several studies that indicate COX-2 is necessary for muscle regeneration after tissue injury (2, 28–30). This, however, assumes that our exercise bout was sufficient to induce tissue damage or that an inflammatory response occurred without obvious muscle damage. Similar exercise bouts have been shown to result in an inflammatory response (8), and Pizza et al. (21) have shown that moderate nondamaging exercise can induce an inflammatory response in skeletal muscle of rats. Thus, the increase in COX-2 could be related to an inflammatory response, and not necessary for stimulation of muscle protein synthesis. It is also possible that, similar to tendon (14), COX-2 is producing PGE$_2$ in response to exercise to contribute to increases in blood flow or protein degradation. However, PGE$_2$ is only transiently elevated in skeletal muscle after one-legged cycling exercise (12) and not significantly elevated 24 h after a bout of resistance exercise (36). COX-2 also seems to be necessary for muscle hypertrophy in certain animal models of muscle overload (3). However, as previously discussed (5), these models of hypertrophy far exceed the amount of muscle growth achieved with resistance training in humans (35).

Although we did not have sufficient tissue to measure prostaglandin levels after exercise, Trappe et al. (34) have demonstrated using microdialysis that a resistance exercise bout similar to the current study increases skeletal muscle PGF$_{2\alpha}$ at 5 or 6 and 8 or 9 h, but not 24 h after exercise in older subjects. A much more intense bout of resistance exercise (10 sets of 10 eccentric knee extensions) can increase PGF$_{2\alpha}$ in young individuals at 24 h postexercise. On the basis of our data, it seems likely that both COX-1 and COX-2 may be contributing to prostaglandin production 4 h after exercise, but COX-2 is contributing to the majority of prostaglandin production at later time points.

Also of interest is the observation that enzyme activity did not necessarily correlate with changes in protein content. For example, COX-1 activity increased without any detectable change in protein concentration. Several studies have suggested that COX activity does not always correlate with protein content (11). Salvemini et al. (27) have recently published an excellent review highlighting the cross-talk between nitric oxide and cyclooxygenase pathways. They discuss several examples of COX activity being altered without an associated change in protein content. The lack of correlation between COX protein content and activity after exercise suggests that the regulation of COX activity with resistance exercise likely involves unknown factors that require further study and that the exercise response is more complicated than simply changing COX protein content. COX-2 protein expression is regulated by several cytokines and growth factors and multiple signaling pathways, including MAPK pathways (13), which are also altered with exercise. Thus, many candidate molecules could be evaluated in future studies.

Interestingly, not all PGH$_2$ production was inhibited in our experiments (Table 1). This suggests that either there may be an additional source of prostaglandin production other than COX-1 or COX-2 or that the concentration of inhibitor was not sufficient to inhibit all prostaglandin production. Testa et al. (32) found similar inhibition of prostaglandin production in skeletal muscle with inhibitor concentrations ranging from 0.1 μM to 1 μM. As suggested by Testa et al. (32), with further increases in concentration, the selectivity of the COX inhibitors could be lost. The lack of complete inhibition of COX activity does not negate the validity or interpretation of our results. The effect of exercise on COX activity was a within-subjects comparison, and the same amount of tissue and inhibitor was used at each time point. Data were also normalized to total protein content. Thus, the increase in COX activity represents a true increase and not a limitation of the assay or inhibitor concentration used.

Two previous studies (5, 35) of human skeletal muscle have been unable to detect a band migrating with known COX-2 standards (72 kDa). As previously suggested (15), we have included molecular weight standards and a positive control and detected bands at the projected migration distance of COX-2 (72 kDa). The primary difference in methodology in the current study was our use of a COX-2 (mouse) polyclonal antibody raised against amino acids 580–598 of murine COX-2 (cat. no. 160126; Cayman Chemical) compared with a COX-2 monoclonal antibody raised against amino acids 580–599 of human COX-2 (cat. no. 160112; Cayman Chemical). The manufacturer does suggest that mouse COX-2 antibody may display cross-reactivity with human COX-2. It appears that the antibody raised to murine COX-2 may be more effective at detecting COX-2 in human skeletal muscle. Interestingly, we did detect at least one faint band migrating below the COX-2-positive control, as previously reported (5, 35).

The primary limitation to the interpretation of our findings, as is the case with most enzyme activity assays, is our use of an in vitro assay to determine COX activity. This enzyme assay functions by providing each sample with an abundance of AA, the precursor to prostaglandins, and the substrate for COX. Therefore, we cannot rule out in vivo regulation of AA availability and other factors that may influence the activity of COX-1 or COX-2 after exercise. Our findings provide an idea...
of the potential for COX-1 and COX-2 to produce prostaglandins after resistance exercise.

**Perspectives and Significance**

In summary, we demonstrate that both COX-1 and COX-2 enzyme activity is increased with acute resistance exercise in human skeletal muscle. COX-2, but not COX-1, protein content increased with exercise, and enzyme activity did not correlate directly with changes in protein content. Our data, and that of Trappe et al. (35), suggest that the timing of the upregulation of COX-1 and COX-2 activity and protein content with resistance exercise may not be equivalent, and future work is needed to evaluate the time frame of induction of each protein. The rapid upregulation of COX-2 protein content and sustained increase in COX-2 activity compared with COX-1 activity suggest, that in contrast to previous work, COX-2 likely has an important role in regulating skeletal muscle adaptations to exercise. Future studies are clearly needed to delineate the role of COX-1 and COX-2 in regulating postexercise muscle protein synthesis and other adaptations to resistance exercise, especially given the importance of these enzymes as targets for various analgesic medications. Interestingly, the previous work completed by Trappe et al. (35), demonstrating an increase in COX-1 protein content with resistance training, was completed in older individuals (35). It would be interesting to determine whether age-related differences exist in the upregulation of COX-1 and COX-2 protein content with chronic resistance training. Although many questions remain regarding the role of COX enzymes in the regulation of skeletal muscle adaptations following exercise, our data further highlight the importance of these enzymes in the response to resistance exercise and suggest that regulation of COX enzyme activity in skeletal muscle is not simply dependent on COX protein content.

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**

Author contributions: C.C.C. conception and design of research; C.C.C., D.T.O., R.S., J.D.D.M., D.R.M., J.A.W., J.E.R., and R.J.G. analyzed data; C.C.C. and R.J.G. interpreted results; C.C.C. and R.J.G. performed experiments; C.C.C. and R.J.G. created figures; C.C.C. and R.J.G. wrote the paper.

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