Resistance exercise and cyclooxygenase (COX) expression in human skeletal muscle: implications for COX-inhibiting drugs and protein synthesis


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Weinheimer EM, Jemiolo B, Carroll CC, Harber MP, Haus JM, Burd NA, LeMoine JK, Trappe SW, Trappe TA. Resistance exercise and cyclooxygenase (COX) expression in human skeletal muscle: implications for COX-inhibiting drugs and protein synthesis. Am J Physiol Regul Integr Comp Physiol 292: 2241–2248, 2007. First published February 22, 2007; doi:10.1152/ajpregu.00718.2006.—We have shown that ibuprofen and acetaminophen block cyclooxygenase (COX) synthesis of prostaglandin PGF\textsubscript{2\alpha}, and the muscle protein synthesis increase following resistance exercise. Confusingly, these two drugs are purported to work through different mechanisms, with acetaminophen apparently unable to block COX and ibuprofen able to nonspecifically block COX-1 and COX-2. A recently discovered intron-retaining COX, now known to have three variants, has been shown to be sensitive to both drugs. We measured the expression patterns and levels of the intron 1-retaining COX-1 variants (-1b\textsubscript{1}, -1b\textsubscript{2}, and -1b\textsubscript{3}), COX-1, and COX-2 at rest and following resistance exercise to help elucidate the COX through which PGF\textsubscript{2\alpha}, ibuprofen, and acetaminophen regulate muscle protein synthesis. Skeletal muscle biopsy samples were taken from 16 individuals (8M, 8F) before, 4, and 24 h after a bout of resistance exercise and analyzed using real-time RT-PCR. Relatively few individuals expressed the intron 1-retaining COX-1b variants (COX-1b\textsubscript{1}, -1b\textsubscript{2}, and -1b\textsubscript{3}) at any time point, and when expressed, these variants were in very low abundance. COX-1 was the most abundant COX mRNA before exercise and remained unchanged (P > 0.05) following exercise. COX-2 was not expressed before exercise, but increased significantly (P < 0.05) at 4 and 24 h after exercise. The inconsistent and low levels of expression of the intron 1-retaining COX-1 variants suggest that these variants are not likely responsible for the inhibition of PGF\textsubscript{2\alpha} production and skeletal muscle protein synthesis after resistance exercise by ibuprofen and acetaminophen. Skeletal muscle-specific inhibition of COX-1 or COX-2 by these drugs should be considered.

We have previously shown that the normal increase in muscle protein synthesis following resistance exercise is blocked by over-the-counter doses of either ibuprofen (1,200 mg/day) or acetaminophen (4,000 mg/day) (37, 38). Further, in the same individuals, both of these drugs were able to block cyclooxygenase (COX) production of PGF\textsubscript{2\alpha} (37, 38). These findings suggest two things: First, muscle protein synthesis after acute resistance exercise, which causes muscle hypertrophy when performed chronically, is regulated by the COX enzyme and its product PGF\textsubscript{2\alpha}. This suggestion is in accordance with studies in animals and isolated cells, which show PGF\textsubscript{2\alpha} to be a potent regulator of skeletal muscle protein synthesis (14, 23, 27, 39). Second, both ibuprofen and acetaminophen are able to block the COX enzyme that produces PGF\textsubscript{2\alpha}, and stimulates muscle protein synthesis following resistance exercise. The difficulty with these findings is that ibuprofen and acetaminophen are purported to not work through the same mechanism (i.e., COX enzyme). That is, it is generally understood that ibuprofen acts through the nonselective inhibition of the two commonly known isofoms of cyclooxygenase, COX-1 and COX-2, (18, 40, 41, 43). Conversely, both COX-1 and COX-2 have been shown to be fairly insensitive to acetaminophen at therapeutic levels in vitro (5, 7).

Shortly after the discovery of two COX isoforms (COX-1 and COX-2) (18, 43) in the early 1990s, it was also determined that two variants of COX-1 exist, COX-1 variant 1 (COX-1v1) and COX-1 variant 2 (COX-1v2) (11, 29). Almost all previous investigations examining COX-1 provided no distinction between COX-1v1 or COX-1v2; thus the precise variant that has been studied and provides the basis of our COX-1 knowledge at both the mRNA and protein levels remains unclear.

Recent evidence suggests a third isoform exists, provisionally named COX-3, that is sensitive to both acetaminophen and ibuprofen (7). Because COX-3 is derived from the COX-1 gene and is distinctive because of the retention of intron 1 in the mRNA, some researchers prefer to use the COX-1b nomenclature (20, 24). The retention of intron 1 results in the addition of 94 base pairs (7), and it has been argued that this would result in a reading frame shift yielding a prematurely terminated COX-inactive protein (12, 30). However, studies have suggested the existence of mRNA containing intron 1 of COX-1 in human, rat, and mouse tissues (7, 20, 22, 24). Furthermore, it has recently been reported that three splice variants of COX-1b exist: COX-1b\textsubscript{1}, COX-1b\textsubscript{2}, and COX-1b\textsubscript{3} (24). COX-1b\textsubscript{1} retains the full intron 1 (94 bp), while COX-1b\textsubscript{2} and COX-1b\textsubscript{3} are missing 1 nucleotide from intron 1 (93 bp) (24).

In light of our previous findings that showed both ibuprofen and acetaminophen blocked muscle protein synthesis after resistance exercise and the recent evidence of newly discovered COX-1 variants that may be sensitive to both of these drugs, we sought to examine the expression of the known COX isoforms and variants in response to a single bout of resistance exercise. We hypothesized that the intron-retaining COX-1b variants would be induced with resistance exercise. The presence of a COX enzyme that has previously been shown to be sensitive to both ibuprofen and acetaminophen would explain how these two commonly consumed drugs interfere with the
anabolic response to resistance exercise and give us insight into the COX enzyme(s) that regulate muscle protein metabolism and growth.

MATERIALS AND METHODS

Subjects

Sixteen recreationally active individuals (8 males: 26 ± 1 yr; 181 ± 3 cm; 82 ± 5 kg; 8 females: 24 ± 1 yr, 169 ± 2 cm, 69 ± 3 kg) were recruited for this investigation. To control for potential influences of the menstrual cycle, all females were studied between 1 and 5 days following cessation of menstruation. Females taking oral contraceptives were accepted in the investigation. All subjects included were nonobese (BMI ≤ 30 kg/m²), nonsmokers, and did not consume any analgesic or anti-inflammatory drug(s), prescription, or nonprescription, chronically or for the duration of the study. All of the procedures, risks, and benefits associated with the study were explained to the subjects before they signed a consent form adhering to the guidelines of the Institutional Review Board at Ball State University.

Experimental Design

On the evening before the preexercise muscle biopsy, subjects were provided with and consumed their evening meal. The preexercise muscle biopsy was preceded by a 12-h fast and was performed after resting quietly for 30 min in the supine position. The exercise trial immediately followed, and upon completion, the subjects rested quietly in the laboratory until their 4-h postexercise muscle biopsy. The subjects then received their evening meal, identical to the previous evening, to consume at home during a specified time. The following morning, the subjects arrived following a 12-h fast, and the 24-h postexercise muscle biopsy was performed.

Dietary and Activity Control

The evening meals were supplied before the resting and 24-h postexercise muscle biopsies in liquid form (Ensure Plus, Ross, Columbus, OH; 53% carbohydrate, 15% protein, and 32% fat), and provided 50% of the subjects estimated caloric need [1.5 times the subjects predicted resting metabolic rate (16)]. This level of dietary control standardized the composition, amount, and timing (i.e., duration of fast) of the final meal consumed prior to the morning skeletal muscle biopsies and has been used previously by us and others in similar studies of protein metabolism (6, 36, 42). In addition, subjects were asked to refrain from physical activity or exercise training three days before the start of the study and for the duration of the study.

Resistance Exercise Protocol

Each subject completed a bout of isotonic unilateral high-intensity eccentric exercise with each leg on a Cybex Eagle knee extension machine (Cybex, Medway, MA). This protocol was identical to that used in our previous study that examined the influence of ibuprofen and acetaminophen on postexercise prostaglandin production and muscle metabolism (6, 36, 42). In addition, subjects predicted resting metabolic rate (16). This level of dietary and activity control standardized the composition, amount, and timing (i.e., duration of fast) of the final meal consumed prior to the morning skeletal muscle biopsies and has been used previously by us and others in similar studies of protein metabolism (6, 36, 42). In addition, subjects were asked to refrain from physical activity or exercise training three days before the start of the study and for the duration of the study.

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Muscle Biopsy

A muscle biopsy was taken from the vastus lateralis of each subject prior to exercise, 4, and 24 h after the resistance exercise. The preexercise biopsy was taken from the dominant leg, while the two postexercise biopsies were taken from the nondominant leg. The second and third biopsies were taken through separate incisions, with the third being 3 to 5 cm proximal to the second. Tissue was obtained following local anesthetic (Lidocaine HCl 1%) using a 5-mm Bergstrom needle with suction (1). A portion of the muscle was divided and immediately stored in 0.5 ml RNAalater (Ambion, Austin, TX) at 4°C for a 24-h incubation period and then placed at −20°C until analysis of COX mRNA (described below). The remaining tissue was cleansed of excess blood and fat and immediately frozen and stored in liquid nitrogen (−190°C) until analysis of muscle COX-2 protein and myosin heavy chain composition (described below).

Real-Time RT-PCR Analysis

The goal of the real-time RT-PCR analysis was to determine the mRNAs levels of the purified ibuprofen and acetaminophen-sensitive intron 1 retaining COX-1b variants (−1b1, −1b2, and −1b3) (Fig. 1), as well as COX-1 and COX-2. During the primer development for this investigation, it came to our attention that two variants of COX-1 exist: COX-1 variant 1 (COX-1v1) and COX-1 variant 2 (COX-1v2) (Fig. 2) (11, 29). Although almost no studies that have investigated COX-1 provide any distinction between COX-1v1 and COX-1v2, we felt it may add to our understanding if both variants were measured in this investigation.

Total RNA extraction and RNA quality check. Each muscle sample was removed from the RNAalater and placed in a mixture of 0.8 ml of RNA isolation reagent, TRI reagent, and 4 ml of polyacryl carrier (Molecular Research Center, Cincinnati, OH). The tissue was homogenized, and total RNA was extracted according to the manufacturer’s protocol. The RNA pellet was dissolved in 30 ml of nuclease free water, incubated at 55°C for 10 min, and then stored at −80°C.

One microliter of each total RNA extract was analyzed using the RNA 6000 Nano LabChip kit on an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). This system reported detailed information about quantity and quality (integrity and purity) of the RNA samples. Each RNA sample was electrophoretically separated into two peaks of 18S and 28S ribosomal RNA. Data were displayed as a gel-like image and an electropherogram (19, 26). Sample analyses were performed as described by the manufacturer. The quality of RNA was confirmed by the presence of ribosomal peaks with no additional signals (DNA contamination or total RNA degradation) below the ribosomal bands and no shifts to lower fragments. On average, the yield of total RNA from ~10 mg of muscle tissue from young women was 84.19 ± 11.87 ng/μl and from young males was 81.55 ± 8.13 ng/μl.

Reverse transcription. Oligo (dT) primed first-strand cDNA was synthesized using SuperScript II RT (Invitrogen, Carlsbad, CA). This system was optimized for sensitive RT-PCR on low amounts of RNA. A first reaction mix of 12 μl for each sample, consisting of 2.5 μl of RNA extract (50 ng), 1 μl of 10 mM dNTP, 1 μl of Oligo (dT) 12–18 (0.5 μg/μl), and 7.5 μl of DNase- and RNase-free water, was incubated at 65°C for 5 min, and then placed on ice for 1 min. A second reaction mix of 7 μl, consisting of 4 μl of 5× first-strand buffer, 2 μl of 0.1 M DTT, and 1 μl of RNaseOUT recombinant RNase inhibitor, was then added to the first reaction mix and incubated at 42°C for 2 min. Finally, 1 μl (50 U) of SuperScript II RT was added to each tube (giving a total volume of 20 μl), incubated at 42°C for 50 min, then 70°C for 15 min to terminate the reaction, and chilled to −4°C thereafter. Produced cDNA samples were diluted with water to a final volume of 60 μl. All thermal incubations and chilling were done in a Peltier Thermal Cycler with dual-block DNA engine (MJ Research, Waltham, MA) to provide temperature homogeneity and identical temperature ramping for all samples.

Real-time PCR. Quantification of mRNA transcription (in duplicate) was performed in a 72-well Rotor-Gene 3000 centrifugal real-
time cycler (Corbett Research, Mortlake, New South Wales, Australia). GAPDH was used as a housekeeping gene for internal control. The reaction mix consisted of 12.5 μl of SYBR Green JumpStart Taq Ready Mix (Sigma-Aldrich, St. Louis, MO), 0.5 μl of 10 μM each for forward and reverse primers, 2.5 μl of cDNA, and RNase-free water to a final volume of 25 μl. All primers used in this study were mRNA-specific and designed for gene expression real-time PCR analysis using Vector NTI Advance 9 software (Invitrogen) (Table 1).

In the case of COX-1β, two sets of primers were designed to detect COX-1β1,2 (COX-1β1 and COX-1β2) and COX-1β1,3 (COX-1β1 and COX-1β3), due to difficulties in detecting the one base difference within the three individual variants. It was difficult to design primers with real-time PCR conditions, for the short and G-C rich intron 1 sequence of the COX-1β variants, which will not produce primer-dimers or partial amplicon.

The PCR parameters were as follows: initial denaturing at 95°C for 2 min to activate the Taq DNA polymerase followed by 45 cycles of 20 s at 95°C, 20 s at 59°C or 60°C (Table 1), and 20 s at 72°C with fluorescence reading. A melting curve analysis was generated by the Rotor-Gene software following the end of the final cycle for each sample, by continuously monitoring the SYBR Green fluorescence throughout the temperature ramp from 72°C to 99°C in one-degree increments and 5 s hold at each degree. A single melt peak observed for each sample was used to validate that only a single product was present.

Relative quantification of real-time PCR assay. The gene expression was evaluated by $2^{-ΔC_t}$ relative quantification method. This method is based on the fact that the difference in threshold cycles ($ΔC_t$) between the gene of interest (GOI) and the housekeeping gene (GAPDH) is proportional to the relative expression level of the GOI. The validation of the GAPDH was performed to ensure that its expression was unaffected by experimental treatment and sex. The relative amount of GAPDH was calculated by using the $2^{-ΔC_t}$ equation, where $ΔC_t = C_{t \text{time X}} - C_{t \text{time 0}}$, and $C_{t \text{time 0}}$ represents $C_t$ at any time point (including Pre/time 0) and $C_{t \text{time 0}}$ represents the mean $C_t$ for preexperimental condition (21, 28). The fold change for GAPDH for all subjects at postexercise compared with preexercise time was 1.11 ± 0.17 and 1.07 ± 0.14 for 4 and 24 h, respectively. The preexercise level of GAPDH from the young males and young
females was also evaluated by amplifying cDNA produced on a known amount (50 ng) of total RNA. The CT values of GAPDH for males and females were 14.80 ± 0.31 and 14.42 ± 0.40, respectively.

A serial dilution (1, 0.5, 0.250, 0.125, 0.062, 0.031) curve of cDNA for each gene was also amplified by real-time PCR using gene-specific primers to evaluate reaction efficiencies. To make the dilution curve for GAPDH, the cDNA from RNA of tested subjects was used; to make the dilution curve for GOI, the cDNA of human skeletal muscle total RNA (1 μg) (Ambion, Austin, TX) was used. The amplification calculated by the Rotor-Gene software was specific and highly efficient (efficiency = 0.99 ± 0.008; R² = 0.98 ± 0.004; slope = 3.36 ± 0.018) for all groups with intra-assay %CV of 3.85 ± 0.55 (2.18 to 6.91%).

**COX-2 Western Blot Analysis**

Because of the COX-2 mRNA results, we also completed Western blot analysis to determine the levels of the COX-2 protein preexercise, and 24 h after exercise. No attempt was made to quantify any of the blot to control for intra-assay variability.

**MHC Isoform Distribution Analysis**

For each biopsy sample, a piece of muscle ~10 mg was weighed on a precision microbalance at −35°C. Each sample was homogenized in 40 volumes of cold homogenizing buffer (250 mM sucrose, 100 mM potassium chloride, 20 mM imidazole, and 5 mM EDTA; pH 6.8) in a ground-glass homogenizer (Radnoti Glass Technology, Monrovia, CA) (8). Samples were then centrifuged at 20,000 g for 30 min at 4°C. The supernatant (i.e., sarcoplasmic protein fraction) was discarded, and the pellet was resuspended in 40 volumes of cold homogenizing buffer and taken as the myofibrillar protein fraction (33, 35). MHC isoform distribution of each sample (~400 ng) was determined in triplicate by SDS-PAGE. Aliquots of the myofibrillar protein fraction were diluted in SDS sample buffer and heated to 100°C for 5 min. MHC protein isoforms were separated with a 3.5% stacking gel and 5% separating gel. Electrophoresis was then performed at 150 V for ~15 h in a Tris-glycine buffer at 4°C (Hoeffer SE 600, Amersham Pharmacia Biotech, Piscataway, NJ). The separating gels were silver stained (15), digitally photographed (FluorChem SP, Alpha Innotech), and densitometry was completed to determine the percent contribution of each isoform of total (100%). The average density of each isoform from the three lanes loaded was taken as the MHC distribution for that sample.

**Statistical Analysis**

The main objective of the statistical analysis was to determine the influence of acute resistance exercise on the mRNA expression of each of the COX isoforms and variants measured. A two-way (sex and time) ANOVA with repeated measures on the time factor was conducted for each isoform and variant, comparing the expression levels across the two groups and three time points. The same two-way ANOVA was used to compare the COX-2 protein levels. A r-test was used to detect significance between MHC isoform distribution and sex. The alpha-level for these primary comparisons was set to 0.05. A one-way repeated-measures ANOVA and Tukey’s HSD post hoc test were conducted for those variables (time factor) in which the two-way ANOVA was significant to identify the source of the differences. All data are presented as means ± SE.
RESULTS

The mRNA levels for all of the isoforms and variants measured preexercise, 4, and 24 h after exercise are presented in Figs. 3 and 4. The number of subjects that expressed each gene at each time point is summarized in Table 2.

Two primer sets were used to detect COX-1b variants. The first set detected COX-1b1 and COX-1b2 (COX-1b1,2), while the second set detected COX-1b1 and COX-1b3 (COX-1b1,3). Expression of COX-1b1,2 and COX-1b1,3 was unchanged (P > 0.05) from preexercise following the exercise bout. In general, expression of these variants was low and sporadic. Two individuals expressed COX-1b1,2 preexercise, and 4 individuals had expression at 4 and 24 h postexercise, respectively. No men expressed COX-1b1,3 at any time point, and no women had expression before exercise. Four hours following exercise, 4 women expressed COX-1b1,3, and 2 women had expression at 24-h postexercise.

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COX-1v1 and COX-1v2 were the most abundant COX mRNA levels before exercise (2.04 ± 0.26 AU and 3.21 ± 0.46 AU, respectively) and remained unchanged (P > 0.05) following exercise. COX-1v2 levels were significantly higher (P < 0.05) in the men (3.85 ± 1.26 AU) compared with the women (2.54 ± 0.25 AU).

The COX-2 mRNA and protein levels preexercise, and the response following exercise, was similar (P > 0.05) between men and women. Before exercise, no subjects expressed any detectable amount of COX-2 mRNA. COX-2 mRNA expression was significantly increased (P < 0.05) from rest at 4 (0.85 ± 0.26 AU) and 24 (0.82 ± 0.28 AU) h postexercise in both men and women. Before exercise, as well as 4 and 24 h after exercise, all subjects expressed detectable amounts of COX-2 protein (Fig. 5). The levels of COX-2 protein were unaltered (P > 0.05) at 4 and 24 h after exercise.

The myosin heavy-chain isoform distribution was not different between men (type I: 31 ± 3%; type IIa: 50 ± 2%; type IIx: 19 ± 2%) and women (type I: 36 ± 4%; type IIa: 49 ± 2%; type IIx: 15 ± 3%).

DISCUSSION

The present study is the first investigation to examine in human tissue the in vivo expression of all of the known COX isoforms and variants. It was our goal to examine these isoforms and variants at rest and following an exercise stimulus to

Table 2. Number of the sixteen subjects studied expressing each gene for the three time points

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gain a better understanding of the PG-mediated regulation of muscle protein synthesis. The main findings of this investigation were 1) Relatively few individuals expressed the intron 1-retaining COX-1 variants (COX-1b1, COX-1b2, COX-1b3) at any time point, and when expressed, these variants were in very low abundance. 2) COX-1 (v1 and v2) mRNA was constitutively expressed in human skeletal muscle. 3) COX-2 mRNA was not present at rest but was significantly induced at 4 and 24 h postexercise. 4) COX-2 protein was present at rest and at 4 and 24 h postexercise in all individuals.

The goal of this investigation was to further expand on our previous findings that demonstrated ibuprofen and acetaminophen block the normal increase in PGF2α and muscle protein synthesis following resistance exercise (37, 38). To allow for comparison between studies, we used the same resistance exercise protocol, dietary and activity control, and subject inclusion criteria. In addition, we expanded the study population to include women. It is likely that PGF2α stimulation, as well as ibuprofen and acetaminophen inhibition of skeletal muscle protein synthesis work through the same COX isoform/variant. We hypothesized this regulation would be through one of the recently discovered intron 1 retaining COX-1b variants. This hypothesis was based on evidence that the enzyme produced by an intron 1 retaining COX-1b variant was sensitive to acetaminophen and ibuprofen at therapeutic levels (7, 31). The COX-1b variant results in the current investigation showed that very few individuals expressed this isoform at any time point (Table 2). By comparing the real-time RT-PCR results from the two COX-1b primers (COX-1b1.2 and COX-1b1.3) that were designed to detect two of the three variants, we can deduce which variant was expressed at the different time points. No men expressed the COX-1b1 and COX-1b3 variants before or after exercise; thus the only message that was produced at any time point (3 men at 4 h and 4 men at 24 h postexercise) was the COX-1b2 variant. Similarly, the only variant produced by the women preexercise (n = 2) was COX-1b2. After exercise, a few of the women produced one or more of the three variants. Overall, the expression of the COX-1b variants in human skeletal muscle at rest and following a resistance exercise stimulus was sporadic and in very low amounts. It appears that this recently discovered variant is not likely responsible for the ibuprofen and acetaminophen-induced PGF2α and protein synthesis blockade in human skeletal muscle.

The role of the COX-1b variants is still unclear, although evidence suggests these variants may be involved in relevant physiological processes (7, 10, 17, 20, 22, 24, 32). COX-1b2 is believed to encode a full-length protein, and functional studies revealed that it was able to catalyze the synthesis of PGF2α from arachidonic acid in vitro (24). COX-1b3 is also believed to encode a full-length and COX active protein, but the ability of this variant to synthesize PGs has not been examined. Because of the full retention of intron 1, which causes a reading frame shift and insertion of a stop codon at bp 249, COX-1b1 is reported to encode a truncated and likely inactive COX protein (24). Recent evidence also suggests that the intron 1 retaining COX-1b variants likely do not encode functional proteins in humans, but the mRNA of these variants may play a role in the regulation of the exon only COX-1 variants (COX-1v1 and COX-1v2) (22).

The question remains as to which COX isoform(s) is responsible for the production of PGF2α and subsequent increase in muscle protein synthesis following resistance exercise. Recent data from animal and cell culture studies, most of which were published after the start of the current investigation, suggest that muscle growth may be regulated through the COX-2 isoform. Sollow et al. (34) demonstrated that the nonspecific inhibitor ibuprofen inhibited plantaris hypertrophy by ∼50% following 14 days of chronic overload in rats. In addition, COX-2 mRNA expression increased 20-fold at 14 days of chronic overload. This COX-2 hypothesis is also supported by the work of Bondesen et al. (3), who reported that treatment with the COX-2 inhibitor SC-236 attenuated myofiber growth after hindlimb suspension-induced atrophy in both the soleus and plantaris muscles in mice. COX-2 selective inhibition (SC-236) has also been shown to attenuate the increase in cross-sectional area of regenerating muscle fibers, whereas COX-1 selective inhibition (SC-560) did not (2). These data suggest that COX-2 may play a common regulatory role during various types of muscle growth.

In this investigation, we report the presence of the COX-2 protein at rest and at 4 and 24 h after exercise, as well as the induction of the COX-2 mRNA following exercise in men and women. The reason for this discrepancy between the message and the protein responses following exercise is unclear but may be related to the efficiency and rate of COX-2 protein translation and/or the degradation rate of the COX-2 protein in human skeletal muscle. There are very few previous data from human skeletal muscle; however, the COX-2 protein has been reported to be significantly higher in rectus abdominis and vastus lateralis muscles of septic patients compared with healthy controls (25). Nonetheless, the data from the current study suggest that the COX-2 enzyme is available for the production of PGF2α and subsequent stimulation of muscle protein synthesis following resistance exercise. Thus, COX-2 could be inhibited by nonspecific and specific COX-inhibiting drugs after exercise. If COX-2 is the isoform through which PGF2α is produced to regulate muscle protein synthesis in humans and considering our previous work (37, 38), COX-2 in skeletal muscle must be sensitive to both acetaminophen and ibuprofen at over-the-counter doses.
Although the results from the current study and the existing animal and cell culture data may lead to the speculation that COX-2 is involved in PG-mediated regulation of muscle protein synthesis, we cannot exclude COX-1 (i.e., COX-1v1 or -1v2). COX-1 has been shown to be constitutively expressed in various human tissues (41, 43) but has not been examined in human skeletal muscle. Despite the large number of reports that have examined COX-1 in other tissues and the central role that this enzyme plays in the pharmaceutical and health care industry, only one recent report has distinguished between these two variants (29). Given the relatively large abundance of these two variants (Fig. 3), further clarification of which variant is involved in the different physiological processes linked to COX-1 is needed. In general, COX-1 inhibition via paracetamol (acetaminophen) has been shown to have anti-cancer action in humans with a significant inverse association between paracetamol use and ovarian cancer, which overproduces COX-1 (4, 9, 13). The sensitivity of COX-1 to acetaminophen in these studies suggests a tissue-specific analgesic-COX interaction and provides some evidence that one of the COX-1 variants may be involved in the acetaminophen and ibuprofen blockade of the increase in PGF$_{2\alpha}$ and muscle protein synthesis following resistance exercise.

There were no differences in the myosin heavy chain isoform distribution data, which suggests that muscle fiber type does not explain the differences in the COX-1 variant expression between the men and the women. Rodemann and Goldberg (27) first observed in rats that slow muscle (i.e., type I) was most responsive to increases in muscle protein synthesis induced by arachidonic acid. More recently, Bondesen et al. (3) reported that after 2 wk of hindlimb suspension in mice, myofiber growth was attenuated in both the soleus and plantaris muscles when a COX-2-selective inhibitor (SC-236) was consumed. From the currently available data, it appears that fiber type does not influence COX expression, and thus we might expect nonspecific COX-inhibiting drugs to influence muscle protein synthesis in all human muscles to a similar extent.

In summary, this was the first investigation to examine the in vivo mRNA expression of the known COX isoforms and variants in human skeletal muscle. These results suggest that the intron 1-retaining COX-1b variants are likely not the COX through which PGF$_{2\alpha}$ is produced to stimulate skeletal muscle protein synthesis. Likely, ibuprofen and acetaminophen inhibit muscle protein synthesis through inhibition of COX-1 or COX-2 in a tissue-specific manner. With 60 million people consumed. From the currently available data, it appears that ibuprofen and acetaminophen inhibit muscle protein synthesis in all human muscles to a similar extent.

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GRANTS

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REFERENCES


