Am J Physiol Regul Integr Comp Physiol 304: R198–R205, 2013.
First published December 5, 2012; doi:10.1152/ajpregu.00245.2012.

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Prostaglandin and myokine involvement in the cyclooxygenase-inhibiting drug enhancement of skeletal muscle adaptations to resistance exercise in older adults

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Submitted 29 May 2012; accepted in final form 3 December 2012

Trappe TA, Standley RA, Jemiolo B, Carroll CC, Trappe SW. Prostaglandin and myokine involvement in the cyclooxygenase-inhibiting drug enhancement of skeletal muscle adaptations to resistance exercise in older adults. *Am J Physiol Regul Integr Comp Physiol* 304: R198–R205, 2013. First published December 5, 2012; doi:10.1152/ajpregu.00245.2012.—Twelve weeks of resistance training (3 days/wk) combined with daily consumption of the cyclooxygenase-inhibiting drugs acetaminophen (4.0 g/day; $n = 11$, 64 ± 1 yr) or ibuprofen (1.2 g/day; $n = 13$, 64 ± 1 yr) unexpectedly promoted muscle mass and strength gains 25–50% above placebo ($n = 12$, 67 ± 2 yr). To investigate the mechanism of this adaptation, muscle biopsies obtained before and ~72 h after the last training bout were analyzed for mRNA levels of prostaglandin (PG)/cyclooxygenase pathway enzymes and receptors [arachidonic acid synthesis: cytosolic phospholipase A_2 ($cPLA_2$) and secreted phospholipase A_2 ($sPLA_2$); $PGF_{2\alpha}$ synthesis: $PGF_{2\alpha}$ synthase and PGE_2 to $PGF_{2\alpha}$ reductase; PGE_2 synthesis: PGE_2 synthase-1, -2, and -3; $PGF_{2\alpha}$ receptor and PGE_2 receptor-4], cytokines and myokines involved in skeletal muscle adaptation (TNF-α, IL-1β, IL-6, IL-8, IL-10), and regulators of muscle growth [myogenin, myogenic regulatory factor-4 (MRF4), myostatin] and atrophy [Forkhead box O3A (FOXO3A), atrogin-1, muscle RING finger protein 1 (MuRF-1), inhibitory κB kinase β (IKKβ)]. Training increased ($P < 0.05$) $cPLA_2$, $PGF_{2\alpha}$ synthase, PGE_2 to $PGF_{2\alpha}$ reductase, PGE_2 receptor-4, TNF-α, IL-1β, IL-8, and IKKβ. However, the $PGF_{2\alpha}$ receptor was upregulated ($P < 0.05$) only in the drug groups, and the placebo group upregulation ($P < 0.05$) of IL-6, IL-10, and MuRF-1 was eliminated in both drug groups. These results highlight prostaglandin and myokine involvement in the adaptive response to exercise in older individuals and suggest two mechanisms underlying the enhanced muscle mass gains in the drug groups: *1*) The drug-induced $PGF_{2\alpha}$ receptor upregulation helped offset the drug suppression of $PGF_{2\alpha}$-stimulated protein synthesis after each exercise bout and enhanced skeletal muscle sensitivity to this stimulation. *2*) The drug-induced suppression of intramuscular PGE_2 production increased net muscle protein balance after each exercise bout through a reduction in PGE_2-induced IL-6 and MuRF-1, both promoters of muscle loss.

acetaminophen; ibuprofen; sarcopenia

WE PREVIOUSLY REPORTED that daily consumption of over-the-counter doses of acetaminophen (4.0 g/day) or ibuprofen (1.2 g/day) promoted muscle mass and strength gains 25–50% above a placebo consuming group in 60- to 78-yr-old men and women completing strength training 3 days per wk for 12 wk (10, 59). These results were surprising given that consumption of the same drugs and doses blocked the normal muscle protein synthesis response to a single bout of resistance exercise (61) through a cyclooxygenase (COX)-derived $PGF_{2\alpha}$-mediated mechanism (60, 61). Additional studies (7, 69), along with analysis of muscle biopsies taken before and after the 12 wk of training (59) have concluded COX-1 is the specific isoform likely involved with the prostaglandin production after exercise in healthy humans and is sensitive to both acetaminophen and ibuprofen in human skeletal muscle. Furthermore, the initial attempt at understanding the mechanism underlying the unexpected drug effects on muscle growth showed there were no drug-specific effects on muscle COX enzyme levels after training (59).

The impetus for our human studies in this area was the early studies of Rodemann and Goldberg (46), Palmer and colleagues (39, 40), and Vandenburg and colleagues (63) in isolated animal muscle and muscle cell culture. They showed an increase in arachidonic acid flux through the COX enzyme, either through stretch or direct arachidonic acid supplementation, promoted the muscle production of $PGF_{2\alpha}$ and PGE_2, which in turn stimulated muscle protein synthesis or degradation, respectively. Interestingly, in the aforementioned training study only muscle exposed to both exercise and either of the two drugs over the 12 wk experienced this supplemental growth (59), suggesting a muscle stretch and/or loading-related mechanism. Although we speculated that the COX inhibitors may have had a stronger effect on reducing PGE_2-related protein degradation (59), the actual mechanism behind the drug effects on muscle growth is still unknown. Understanding how these drugs work is important not only for the millions of individuals worldwide that consume these drugs regularly along with healthcare professionals that may prescribe chronic consumption of these drugs, but it also provides insight into the regulation of skeletal muscle adaptations to exercise.

The goal of the current investigation was to take a comprehensive, yet targeted, approach to understand the COX-inhibiting drug effects on muscle growth (59). Including our previous measurements of the COX enzymes (59), we examined 27 components (enzymes, receptors, cytokines, myokines, transcription factors, and growth factors) involved in the regulation of the PG/COX pathway and muscle mass. In the muscle biopsy samples taken before and after training we examined the COX pathway enzymes that are upstream and downstream of COX and responsible for enhancing general prostaglandin production (i.e., PGH_2, the product of COX and precursor to all prostaglandins) and specific enzymes and receptors that regulate the production and initiate the effects of $PGF_{2\alpha}$ and PGE_2,

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respectively (Fig. 1). Specifically, we examined phospholipase A_2 (PLA) enzymes [cyctosolic ($cPLA_2$) and secreted ($sPLA_2$)] that convert membrane phospholipids to the COX-substrate arachidonic acid (8, 20, 23, 50); the enzymes that convert PGH_2 to $PGF_{2\alpha}$ ($PGF_{2\alpha}$ synthase) (29, 30, 36, 68) and PGE_2 to $PGF_{2\alpha}$ (PGE_2 to $PGF_{2\alpha}$ reductase) (34, 51, 68); the enzymes that convert PGH_2 to PGE_2 (PGE_2 synthase-1, -2, and -3) (37, 41, 47, 70); the $PGF_{2\alpha}$ receptor (1, 6, 35, 38); and PGE_2 receptor-4 (6, 55) (Table 1). We also examined several cytokines and myokines that have been shown to regulate skeletal muscle metabolism and adaptation, specifically, tumor necrosis factor (TNF)-α, IL-1β, IL-6, IL-8, and IL-10 (42, 43). Additionally, we measured several known regulators of muscle growth [myogenin, myogenic regulatory factor-4 (MRF4), myostatin] (16, 25, 48) and atrophy [Forkhead box O3A (FOXO3A), atrogin-1, muscle RING finger protein 1 (MuRF-1), and inhibitory κB kinase β (IKKβ)] (16, 17, 31, 48). The findings from these analyses resulted in two proposed mechanisms as to how COX-inhibiting drugs promote muscle mass gains during resistance training in older individuals.

MATERIALS AND METHODS

Overall Study Design

This study was a randomized, placebo-controlled, double-blind 12-wk investigation. During the 12 wk subjects completed a progressive resistance training program of the lower extremities three times per week and consumed a placebo (n = 12, 67 ± 2 yr), acetaminophen (4.0 g/day; n = 11, 64 ± 1 yr), or ibuprofen (1.2 g/day; n = 13, 64 ± 1 yr). The study was conducted at the Human Performance Laboratory at Ball State University and Ball Memorial Hospital and approved by the Institutional Review Boards of both institutions. All study procedures, risks, and benefits were explained to the subjects before giving written consent to participate. A detailed presentation of the subject characteristics, screening, and exclusion criteria; resistance training program; COX-inhibitor consumption, compliance, and side-effect monitoring; and the muscle size (via MRI) and strength measurements and related findings have been reported previously (59).

Targeted muscle mRNA measurements of potential COX inhibitor-related regulators of muscle mass were examined in previously obtained muscle biopsies taken before and after training (59). In addition, we completed microdialysis-based measurements of muscle proteolysis (21, 22, 24, 56) to address the hypothesis that resistance exercise training would reduce the previously reported age-related elevation (58) and provide supplementary information on the potential impact of the COX-inhibiting drugs on basal resting proteolysis.

Muscle Biopsy

Subjects underwent a muscle biopsy (3) of the *m. vastus lateralis* before and at the end of the resistance training program in the basal state (i.e., no testing or training was completed for 3 days before each biopsy). Biopsies were obtained in the early morning (~7 AM) after at least 30 min of supine rest and after an overnight fast of ~12 h. The evening meals before the biopsy were supplied in liquid form (Ensure Plus, 57% carbohydrate, 15% protein, and 28% fat) and provided 50% of the estimated daily caloric need (1.5 times the predicted resting metabolic rate) to standardize the composition, amount, and timing (i.e., duration of the fast) of the final meal consumed before the biopsy. At the end of training, drug consumption (59) continued until the biopsies were obtained. After the biopsy, excess blood, visible fat, and connective tissue were removed, and a portion of the muscle to be used for mRNA analysis was immediately stored in 0.5 ml RNAlater (Ambion, Austin, TX) at 4°C for 24 h and then placed at −20°C until analysis.

Muscle mRNA Measurements

Quantitative PCR (qPCR) was completed to determine the mRNA levels of the components listed in Table 1. Decisions regarding the specific components of the prostaglandin/COX pathway were based on relevant literature and our recent skeletal muscle transcriptome analysis on a relatively large cohort of young and old men and women (45). mRNA analyses were completed on 33 individuals (minus one subject from each group).

Total RNA extraction and RNA quality check. Total RNA was extracted in TRI Reagent (Molecular Research Center, Cincinnati, OH). The quality and integrity (RIN of 8.12 ± 0.03) of extracted RNA

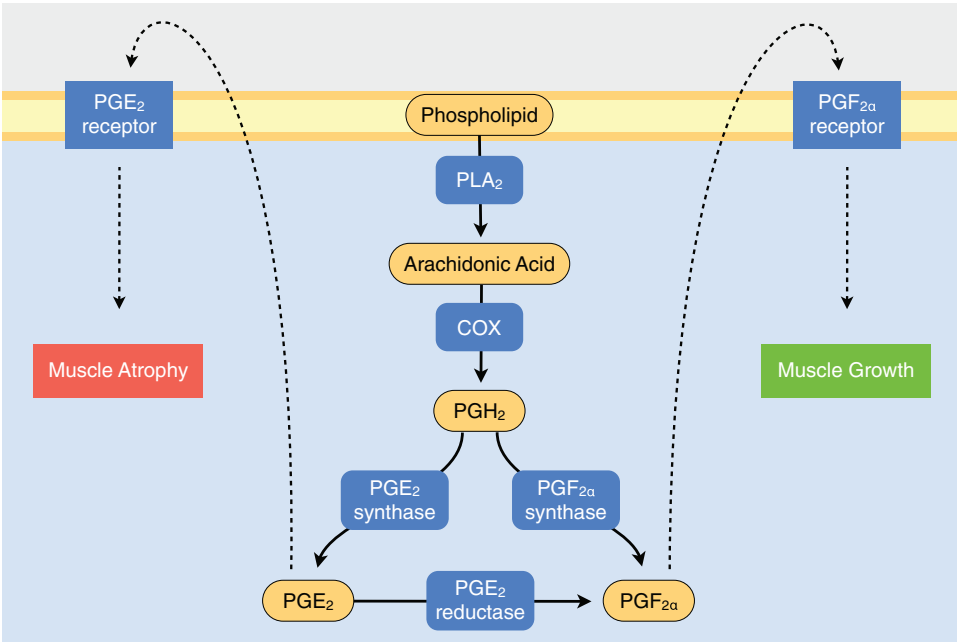

Fig. 1. Schematic of the prostaglandin (PG) producing cyclooxygenase (COX) pathway and specific receptors that influence growth and atrophy in skeletal muscle. See Table 1 for additional nomenclature, isoform, variant, and gene information. All of the enzymes and receptors in blue were measured in the current study.

Table 1. *Nomenclature, gene information, and mRNA primer characteristics*

	Gene Name	Accession No.	Sequence (5′ → 3′)	Amplicon Size, bp	mRNA Region, bp	Annealing Temp, °C
			Prostaglandin/COX pathway and receptors			
Cytosolic phospholipase A_2 ($cPLA_2$)	PLA2G4A	NM_024420.2	CCTTACCAGCACATTATAGTGGAGCACC GGAGTATCAAGCATGTCACCAAAGGC	110	221–330	60
Secreted phospholipase A_2 ($sPLA_2$)	PLA2G10	NM_003561.1	CTGTGCGGACCGGCAGAGAACAAAT GCGAGTCCGGCTCACATAGGAACT	136	789–924	59
$PGF_{2\alpha}$ synthase[1]	AKR1C3	NM_003739.5[b]	ACTCAAGTACAAGCCTGTCTGCAACC TTCGGGTCCACCCATCGTTTGTC	150	612–761	60
PGE_2 to $PGF_{2\alpha}$ reductase[2]	CBR1	NM_001757.2	ATAAAACCCCAAGGGAGAGTGGTGAACG CAGCTCCTCCTCAGTGATGGTCTCA	120	514–633	60
PGE_2 synthase-1[3]	PTGES	NM_4878.4	CGGAAGAAGGCCTTTGCCAACC GGGTAGATGGTCTCCATGTCGTTCC	125	171–295	59
PGE_2 synthase-2[4]	PTGES2	NM_025072.6[c]	AGAGCAGGCACCGCCTCCAGG ACGCAGCACGCCATACACCGC	146	1359–1504	61
PGE_2 synthase-3[5]	PTGES3	NM_006601.5	AGGCCCGCCCACCAGTTCGC AGTCCCTTCGATCGTACCACTTTGCAG	82	254–335	61
$PGF_{2\alpha}$ receptor[6]	PTGFR	NM_000959.3[d]	CTGTATTTGTTGGAGCCCATTTCTGGTTAC CATGTTGCCATTCGGAGAGCAAAA	111	1011–1121	60
PGE_2 receptor-4[7]	PTGER4[a]	NM_000958.2	GCTCGTGGTGCGAGTATTCGTCAACC TCCAGGGGTCTAGGATGGGGTTCA	122	1453–1574	60
			Cytokines and myokines			
TNF-α	TNF	NM_000594.2	CCCAGGCAGTCAGATCATCTTCTCGAA CTGGTTATCTCTCAGCTCCACGCCATT	149	390–538	58
IL-1β	IL1B	NM_000576.2	GGATATGGAGCAACAAGTGGTG CGCAGGACAGGTACAGATTCT	113	661–773	61
IL-6	IL6	NM_000600.3	CTATGAACTCCTTCTCCACAAGCGCCTT GGGGCGGCTACATCTTTGGAATCTT	127	61–187	59
IL-8	IL8	NM_000584.3	GCTCTGTGTGAAGGTGCAGTTTTGCCAA GGCGCAGTGTGGTCCACTCTCAAT	135	153–287	60
IL-10	IL10	NM_000572.2	GGCGCTGTCATCGATTTCTTCC GGCTTTGTAGATGCCTTTCTCTTG	101	430–530	60
			Muscle growth and atrophy regulators			
Myogenin	MYOG	NM_002479.4	CAGTGCACTGGAGTTCAGCGCCAA TTCATCTGGGAAGGCCACAGACACAT	139	599–737	60
MRF4	MYF6	NM_002469.2	CCCCTTCAGCTACAGACCCAAACAAGAA CCCCCTGGAATGATCGGAAACAC	100	542–641	60
Myostatin	MSTN	NM_005259.2	GACCAGGAGAAGATGGGCTGAATCCGTT GCTCATCACAGTCAAGACCAAAATCCCTT	96	861–956	60
FOXO3A	FOXO3	NM_201559.2[e]	GAACGTGGGGAACTTCACTGGTGCTA GGTCTGCTTTGCCCACTTCCCCTT	98	2278–2375	59
Atrogin-1	FBXO32	NM_058229.3[f]	TATTGCACCCTGGGGGAAGCTTTCAA TCCAACAGCCGGACCACGTAGTTAAA	92	481–572	59
MuRF-1	TRIM63	NM_032588.3	CTCAGTGTCCATGTCTGGAGGCCGTT GGCCGACTGGAGCACTCCTGTTTGTA	147	328–474	58
IKKβ	IKBKB	NM_001556.2[g]	ATGTCATCCGATGGCACAATCAGG TGGGTCAGCCTTCTCATGATCTGG	127	260–386	60

Other common aliases: [1]PGH_2 9,11-endoperoxide reductase; [2]PGE_2 9-ketoreductase; [3]microsomal prostaglandin E synthase-1 (mPGES-1); [4]microsomal prostaglandin E synthase-2 (mPGES-2); [5]cytosolic prostaglandin E synthase (cPGES); [6]FP; [7]EP4. Top sequence reflects the Forward primer and the bottom sequence reflects the Reverse primer. [a]PTGER4 was the only PGE_2 receptor examined, as our recent transcriptome analysis of a relatively large cohort of young and old adults showed infrequent and low level expression of PTGER1, PTGER2, and PTGER3; while PTGER4 was abundantly expressed in all of the individuals studied (45). [b]Primers detect both variant 1, isoform 1 (NM_003739.5) and variant 2, isoform 2 (NM_001253908.1). [c]Primers detect variant 1, isoform 1 (NM_025072.6). [d]Primers detect both variant 1, isoform a (NM_000959.3) and variant 2, isoform b (NM_001039585.1). [e]Primers detect both variant 1 (NM_001455.3) and variant 2 (NM_201559.2). [f]Primers detect all variants: variant 1, isoform 1 (NM_058229.3); variant 2, isoform 2 (NM_148177.2) and variant 3, isoform 3 (NM_001242463.1). [g]Primers detect all variants: variant 1, isoform 1 (NM_001556.2); variant 2, isoform 2 (NM_001190720.2) and variant 7, isoform 5 NM_001242778.1). See text for definitions of abbreviations.

(113.28 ± 4.51 ng/μl) were evaluated using a RNA 6000 Nano LabChip kit on an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA) as we have previously described (7, 59, 69).

qPCR. Oligo (dT) primed first-strand cDNA was synthesized (150 ng of total RNA) using SuperScript II RT (Invitrogen, Carlsbad, CA). Quantification of mRNA levels (in duplicate) was performed in a 72-well Rotor-Gene 3000 Centrifugal Real-Time Cycler (Corbett Research, Mortlake, NSW, Australia). Housekeeping gene GAPDH was used as a reference gene, as we have previously described (28, 44, 69). All primers used in this study were mRNA specific (on different exons and/or crossing over an intron) and designed for qPCR (Vector NTI Advance 9 software, Invitrogen) using SYBR Green chemistry. Details about primer characteristics are presented in Table 1. A melting curve analysis was generated for all qPCR runs to validate that only one product was present. A serial dilution curve (cDNA made from 500 ng of total RNA of human skeletal muscle; Ambion) was generated for each qPCR run to evaluate reaction efficiencies. The amplification calculated by the Rotor-Gene software was specific and highly efficient (efficiency = 1.07 ± 0.02; R^2 = 0.99 ± 0.00; slope = 3.19 ± 0.04). Gene expression before and after the 12 wk of resistance exercise training was compared using the $2^{-\Delta\Delta C_T}$ (fold change) relative quantification method (7, 32, 33, 44).

Muscle Microdialysis Measurements

Resting skeletal muscle proteolysis was examined with microdialysis sampling of interstitial 3-methyhistidine (3MH) concentration (21, 22, 24, 56, 58) before and after training the same morning as the muscle biopsies but from the opposite leg. These secondary measurements focused on the placebo group, but due to the double-blind nature of the protocol, all subjects were examined. As we have previously described in detail (24, 58), a preperfused CMA 60 microdialysis catheter was placed in the *m. vastus lateralis*, followed by an unperfused 2.5-h rest period, and then perfused with a calibrated microinfusion pump for 1.5 h at 2.0 μl/min with sterile Ringer supplemented with Dextran-70. The initial 30-min dialysate sample was discarded, followed by four 15-min samples, with the first two used for 3MH determination. Dialysate weights were similar to the expected weight across drug groups and pre- to posttraining (overall average: 30.0 ± 0.1 mg), suggesting no net fluid transport across the microdialysis membrane (19). Dialysate 3MH concentration was determined in duplicate by HPLC as previously described (24, 56, 58) and corrected for probe recovery based on our previously obtained nonexercising recovery data for 3MH in the *m. vastus lateralis* of older subjects (58), and the two 15-min determinations were averaged to represent the pre- or posttraining value for that subject. Subjects with subcutaneous fat thickness over the thigh region that prevented microdialysis sampling of only the muscle (determined with MRI) were excluded; therefore, 26 total individuals were studied [placebo: 9 (8 males, 1 female), acetaminophen: 10 (9 male, 1 female), ibuprofen: 7 (7 male)].

Statistical Analysis

Data were analyzed with two-way (group and time) analysis of variance ANOVA with repeated measures, and post hoc comparisons were made with Tukey's test. Significance was accepted at $P < 0.05$. Data are presented as means ± SE.

RESULTS

Prostaglandin/COX Pathway and Receptors

The prostaglandin-producing COX pathway components and the receptors for $PGF_{2\alpha}$ and PGE_2 are presented in Table 2 and Fig. 2. Several components of the COX pathway were increased ($P < 0.05$) due to training ($cPLA_2$: 120%, $PGF_{2\alpha}$ synthase: 222%, PGE_2 to $PGF_{2\alpha}$ reductase: 99%). $sPLA_2$ and PGE_2 synthase-1, -2, and -3 remained unchanged ($P > 0.05$) from pre- to posttraining in all three groups. The $PGF_{2\alpha}$ receptor was upregulated ($P < 0.05$) only in the drug groups (~70%) over the 12 wk. PGE_2 receptor-4 was upregulated ($P < 0.05$) by 60% due to training.

Table 2. *Fold changes from pre- to posttraining in the placebo and two drug groups*

	Placebo	Acetaminophen	Ibuprofen
Prostaglandin/COX pathway and receptors			
$cPLA_2$			
Pre	1.23 ± 0.25	1.12 ± 0.18	1.20 ± 0.24
Post*	2.63 ± 0.83	2.78 ± 0.75	1.31 ± 0.18
$sPLA_2$			
Pre	1.19 ± 0.23	1.05 ± 0.11	1.11 ± 0.16
Post	1.39 ± 0.26	1.63 ± 0.41	0.91 ± 0.11
$PGF_{2\alpha}$ synthase			
Pre	1.12 ± 0.17	1.15 ± 0.18	1.27 ± 0.26
Post*	1.67 ± 0.22	5.81 ± 3.20	1.67 ± 0.62
PGE_2 to $PGF_{2\alpha}$ reductase			
Pre	1.13 ± 0.17	1.02 ± 0.06	1.05 ± 0.11
Post*	1.41 ± 0.18	3.61 ± 1.64	1.45 ± 0.14
PGE_2 synthase-1			
Pre	1.12 ± 0.17	1.24 ± 0.26	1.21 ± 0.23
Post	2.09 ± 0.87	1.10 ± 0.17	1.21 ± 0.14
PGE_2 synthase-2			
Pre	1.04 ± 0.08	1.07 ± 0.12	1.06 ± 0.10
Post	0.93 ± 0.08	1.32 ± 0.22	0.89 ± 0.09
PGE_2 synthase-3			
Pre	1.07 ± 0.13	1.03 ± 0.09	1.18 ± 0.27
Post	0.83 ± 0.09	1.16 ± 0.23	0.85 ± 0.07
PGE_2 receptor-4			
Pre	1.24 ± 0.25	1.17 ± 0.21	1.18 ± 0.18
Post*	1.68 ± 0.39	2.24 ± 0.80	1.46 ± 0.22
Cytokines and myokines			
TNF-α			
Pre	1.39 ± 0.32	1.13 ± 0.19	1.47 ± 0.39
Post*	1.99 ± 0.40	2.13 ± 0.48	1.35 ± 0.18
IL-1β			
Pre	1.38 ± 0.29	1.24 ± 0.29	1.38 ± 0.32
Post*	1.95 ± 0.44	2.41 ± 0.47	1.42 ± 0.32
IL-8			
Pre	1.06 ± 0.10	1.17 ± 0.20	1.18 ± 0.20
Post*	2.33 ± 0.33	2.63 ± 0.65	1.74 ± 0.34
Muscle growth and atrophy regulators			
Myogenin			
Pre	1.09 ± 0.14	1.07 ± 0.15	1.08 ± 0.12
Post	1.04 ± 0.12	0.90 ± 0.13	0.97 ± 0.08
MRF4			
Pre	1.11 ± 0.15	1.13 ± 0.19	1.07 ± 0.12
Post	1.51 ± 0.24	1.57 ± 0.56	0.99 ± 0.10
Myostatin			
Pre	1.37 ± 0.30	1.13 ± 0.18	1.23 ± 0.20
Post	1.40 ± 0.27	1.02 ± 0.25	1.12 ± 0.13
FOXO3A			
Pre	1.10 ± 0.15	1.10 ± 0.14	1.08 ± 0.12
Post	1.12 ± 0.12	1.28 ± 0.26	0.86 ± 0.09
Atrogin-1			
Pre	1.05 ± 0.10	1.06 ± 0.12	1.04 ± 0.09
Post	1.11 ± 0.10	0.94 ± 0.09	0.91 ± 0.07
IKKβ			
Pre	1.06 ± 0.11	1.03 ± 0.09	1.06 ± 0.11
Post*	1.54 ± 0.21	1.28 ± 0.16	1.05 ± 0.10

See Figures 2 and 3 for other prostaglandin/COX pathway and receptors ($PGF_{2\alpha}$ receptor), cytokines and myokines (IL-6 and IL-10), and muscle growth and atrophy regulators (MuRF-1). *$P < 0.05$, main effect for training. See text for definitions of abbreviations.

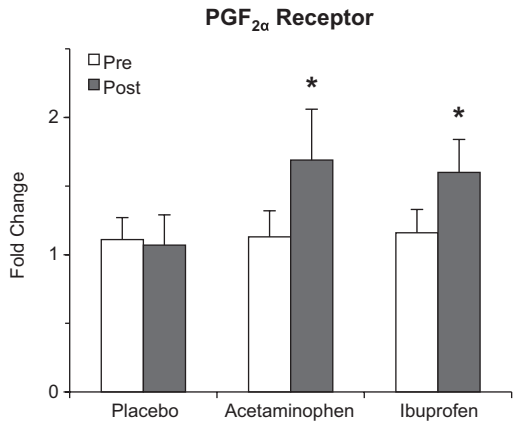

Fig. 2. Muscle mRNA levels of the $PGF_{2\alpha}$ receptor, the only component upregulated in both drug groups and not the placebo group from the beginning (pre) to the end (post) of the 12-wk resistance exercise training and drug intervention. *Significant increase in both drug groups from pretraining, $P < 0.05$.

Cytokines and Myokines

TNF-α (98%), IL-1β (132%), and IL-8 (114%) were increased ($P < 0.05$) due to training (Table 2). IL-6 (139%) and IL-10 (211%) were both upregulated ($P < 0.05$) only in the placebo group (Fig. 3).

Muscle Growth and Atrophy Regulators

The growth regulators myogenin, MRF4, and myostatin, as well as the atrophy regulators FOXO3A and atrogin-1, remained unchanged ($P > 0.05$) from pre- to posttraining in all three groups (Table 2). MuRF-1 was differentially upregulated in the placebo group compared with the acetaminophen and ibuprofen groups (Fig. 3). IKKβ was increased ($P < 0.05$) due to training (Table 2), which appeared to be driven primarily by the placebo group.

Muscle 3MH

Basal muscle myofibrillar proteolysis, as estimated from muscle interstitial levels of 3MH, was not influenced ($P > 0.05$) by resistance training or drug consumption (placebo: pre, 4.64 ± 0.34; post, 4.73 ± 0.48 nmol/ml; acetaminophen: pre; 4.03 ± 0.30; post, 4.29 ± 0.36 nmol/ml; ibuprofen: pre, 4.97 ± 0.76; post, 4.74 ± 0.91 nmol/ml; all subjects: pre, 4.60 ± 0.32; post, 4.61 ± 0.39 nmol/ml).

DISCUSSION

The main goal of this investigation was to provide mechanistic insight as to how the over-the-counter COX-inhibiting drugs acetaminophen and ibuprofen altered skeletal muscle metabolism and promoted supplemental muscle growth by 25–50% during resistance training in older individuals (59). The findings from the current analyses resulted in two proposed mechanisms: *1*) The drug-induced $PGF_{2\alpha}$ receptor upregulation helped offset the drug suppression of $PGF_{2\alpha}$-stimulated protein synthesis after each exercise bout and enhanced skeletal muscle sensitivity to this stimulation. *2*) The drug-induced suppression of intramuscular PGE_2 production increased net muscle protein balance after each exercise bout through a reduction in PGE_2-induced IL-6 and MuRF-1, both promoters of muscle loss. In addition, most of the prostaglandin/COX pathway enzymes and receptors had not previously been examined in human skeletal muscle or in response to exercise. Many of these components were elevated over the 12 wk and further suggest the prostaglandin/COX pathway is part of the adaptive response of skeletal muscle to resistance exercise training.

Our original hypothesis was that daily consumption of the COX-inhibiting drugs would chronically blunt the COX-mediated production of $PGF_{2\alpha}$ and subsequent stimulation of

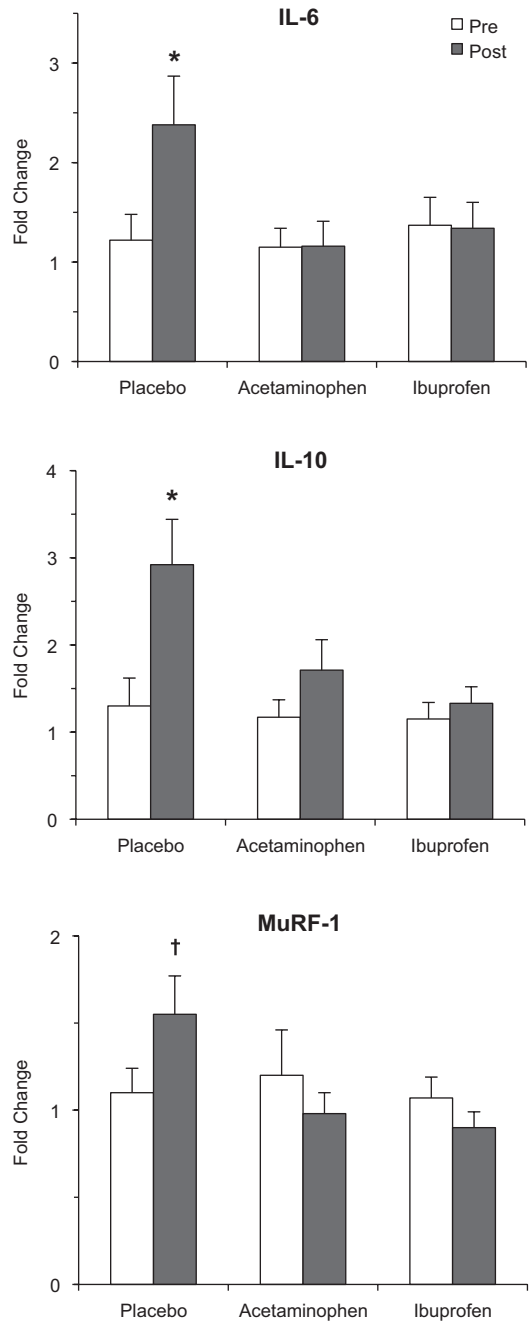

Fig. 3. Muscle mRNA levels of the components upregulated only in the placebo group and not the drug groups from the beginning (pre) to the end (post) of the 12-wk resistance exercise training and drug intervention. MuRF-1, muscle RING finger protein 1. *$P < 0.05$ from pretraining in the placebo group. †$P < 0.05$ from posttraining in the acetaminophen and ibuprofen groups.

muscle protein synthesis after resistance exercise (60, 61), ultimately blunting the amount of exercise training-induced hypertrophy. Recent studies of the $PGF_{2\alpha}$ receptor (1, 6, 38) in skeletal muscle (35), using agonists and competitive inhibitors, have confirmed that $PGF_{2\alpha}$ binding to its receptor stimulates muscle cell growth and have delineated the intracellular signaling pathways triggered by this receptor. Additional $PGF_{2\alpha}$ receptors in the muscle of the drug groups, coupled with a general training increase in COX (59) and the $PGF_{2\alpha}$-producing enzymes ($PGF_{2\alpha}$ synthase and PGE_2 to $PGF_{2\alpha}$ reductase), would make the muscle less susceptible to the same daily COX-inhibiting drug doses and more sensitive to any $PGF_{2\alpha}$ that was produced following exercise. The overall contribution of these responses to the supplementary muscle growth in the drug groups is unclear but would be better understood if the time course for the upregulation of the receptor and enzymes was known. It would also be interesting to know the related consequences of longer-term exercise training and drug consumption.

The supplemental muscle growth in the drug groups also appears to be driven by lower levels of IL-6 and MuRF-1 compared with the placebo group (Fig. 3). IL-6 has been shown in numerous population-based studies of older individuals to be associated with a reduction in muscle mass and functional independence (2, 11, 13, 49). In addition, acute IL-6 infusion in humans to raise plasma concentration to postexercise levels reduces muscle protein turnover by 50%, resulting in an increased net muscle protein degradation (62). In animals, chronic infusion of IL-6 to simulate postexercise levels and low-level inflammation retards growth (5) and promotes muscle atrophy (18). Interestingly, PGE_2 stimulates IL-6 transcription in nonskeletal muscle cells (12, 14, 15, 52, 65–67) through a nuclear factor (NF)-κB-mediated mechanism; thus a reduction in intramuscular PGE_2 production due to the COX-inhibiting drugs (60) is a plausible explanation for the lower IL-6 transcription in the drug groups. In support of this concept, Vella et al. (64) have shown an increase in NF-κB DNA binding to the IL-6 promoter region in the hours following an acute bout of resistance exercise. The IL-10 results (Fig. 3) also support the IL-6 findings, as IL-6 infusion increases IL-10 in humans (53) and IL-10 knockout animals have an exaggerated skeletal muscle IL-6 response to an inflammatory insult (26), suggesting IL-10 is a regulator of IL-6.

MuRF-1, an E3 ubiquitin ligase that mediates skeletal muscle proteolysis, has also been shown to be regulated via NF-κB (9, 48). Thus it is intriguing to speculate that the drug effects on IL-6 and MuRF-1 are linked through a common PGE_2 receptor-NF-κB-mediated mechanism. This notion is supported by the IKKβ findings (Table 2) showing the main effect for an increase with training of this NF-κB regulator (17, 31, 64) was primarily driven by the increase in the placebo group. It should be noted that the IL-6 and MuRF-1 data are generated from biopsies taken in the basal state (3 days after the final exercise bout). IL-6, and to a lessor degree MuRF-1, are substantially more elevated during the first 24 h following an acute exercise bout (33, 44, 54) than those reported here for the placebo group. Thus the proposed effects we describe here were likely influenced to a greater degree during the repeated acute responses to each exercise bout. It is also possible older individuals have an exaggerated response following exercise (57) that the drugs help dampen.

Many of the prostaglandin/COX pathway components measured in this study have not been previously examined in skeletal muscle or with exercise training and the training-induced effects are noteworthy. The upregulation of $cPLA_2$ (Table 2) along with COX-1 upregulation (59) suggest an overall enhanced ability to produce more arachidonic acid and PGH_2, the precursor to all prostaglandins. The increase in $PGF_{2\alpha}$ synthase and PGE_2 to $PGF_{2\alpha}$ reductase suggests an increased capacity of skeletal muscle to produce $PGF_{2\alpha}$ directly and through the conversion of PGE_2 (Fig. 1). Although the enzymes that convert PGH_2 to PGE_2 were unchanged, the PGE_2 receptor-4 was upregulated with exercise training (Table 2). Thus the potential of $PGF_{2\alpha}$ and PGE_2 effects are enhanced, albeit through different adaptive approaches (enzyme vs. receptor level increases). Overall, it appears the prostaglandin/COX pathway in skeletal muscle is part of the adaptive response to resistance exercise training in older adults.

Several of the training-induced effects (i.e., main effect for training) appeared to have a nonstatistically significant influence by one of the drugs. $cPLA_2$ and two of the classic inflammatory cytokines (TNF-α and IL-1β) were suppressed by ibuprofen and uninfluenced by acetaminophen (Table 2). While these observations do not lend insight into the drug effects on the muscle adaptations examined in the current study, they do support the classic view that ibuprofen, but not acetaminophen, has anti-inflammatory effects in peripheral tissues. These findings may have implications for interactions of acetaminophen and ibuprofen with other metabolic and inflammatory processes in skeletal muscle. In addition, the two $PGF_{2\alpha}$-producing enzymes ($PGF_{2\alpha}$ synthase and PGE_2 to $PGF_{2\alpha}$ reductase) showed a more variable response in the acetaminophen group due to a very large response in the same two individuals. This is intriguing since these two enzymes have different structures and catalytic designs (29, 30, 34, 36, 51, 68). Although speculative, this response may be related to the influence of glutathione on these enzymes (34, 68), considering glutathione is also involved in other pathways directing the removal of acetaminophen (4, 27).

The myofibrillar proteolysis measurements were added to the current study with the primary goal of determining whether resistance exercise training could reduce the previously reported age-related elevation (58). While 12 wk of resistance training was unable to influence basal myofibrillar proteolysis, some age-specific findings were apparent. Our previous investigation (58) examined young (27 ± 2 yr) compared with older individuals over the age of 70 yr (mean 75 ± 4, range: 71–83 yr), and when the age of the current study cohort is considered, those above 70 yr had a 33% higher basal proteolysis than those between 60 and 70 yr (irrespective of training: 4.39 vs. 5.83 nmol/ml). Considering the previously reported magnitude of age-related elevation (44%, 4.28 ± 0.27 vs. 6.16 ± 0.56 nmol/ml), it may be that accelerated muscle proteolysis becomes more apparent in the eighth decade of life. The proteolysis data from the drug groups suggest there was no impact of the COX-inhibiting drugs on basal resting proteolysis.

Perspectives and Significance

We propose two mechanisms through which chronic consumption of acetaminophen and ibuprofen influence skeletal muscle metabolism and promote supplemental muscle growth

during resistance training in older individuals. In addition, we define for the first time the adaptation of the prostaglandin/COX pathway enzymes and receptors, in relation to $PGF_{2\alpha}$ and PGE_2, the two prostaglandins that stimulate muscle protein turnover. These findings highlight the involvement of prostaglandins and myokines in the adaptive response to resistance exercise in older individuals and underscore the need for more acute and chronic studies in this area. Considering the potency of the drug effects on muscle mass and function, these mechanistic findings also have important implications for the understanding of sarcopenia, the age-related loss of skeletal muscle mass and function, and the further development of strategies to combat sarcopenia.

GRANTS

This investigation was funded by NIH Grant R01 AG-020532.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: T.A.T., R.A.S., and B.J. conception and design of research; T.A.T., R.A.S., B.J., and C.C.C. performed experiments; T.A.T., R.A.S., and B.J. analyzed data; T.A.T., R.A.S., B.J., C.C.C., and S.W.T. interpreted results of experiments; T.A.T. prepared figures; T.A.T. drafted manuscript; T.A.T., R.A.S., B.J., C.C.C., and S.W.T. edited and revised manuscript; T.A.T., R.A.S., B.J., C.C.C., and S.W.T. approved final version of manuscript.

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