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COMMENT

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PGs have been shown to modulate skeletal muscle protein metabolism as well as inflammation and pain. In nonskeletal muscle tissues, the over the counter analgesic drugs ibuprofen and acetaminophen function through suppression of PG synthesis. We previously reported that ibuprofen and acetaminophen inhibit the normal increase in skeletal muscle protein synthesis after high intensity eccentric resistance exercise. The current study examined skeletal muscle PG levels in the same subjects to further investigate the mechanisms of action of these drugs in exercised skeletal muscle. Twenty-four males (23 ± 3 yr) were assigned to 3 groups that received the maximal over the counter dose of ibuprofen (1200 mg/d), acetaminophen (4000 mg/d), or a placebo after 10–14 sets of 10 eccentric repetitions at 90% of concentric 1 repetition maximum using the knee extensors. Preexercise and 24 h postexercise biopsies of the vastus lateralis revealed that the exercise-induced change in PGF$_{2\alpha}$ in the placebo group (77%) was significantly different ($P < 0.05$) from those in the ibuprofen (-1%) and acetaminophen (-1%) groups. However, the exercise-induced change in PGE$_2$ in the placebo group (64%) was only significantly different ($P < 0.05$) from that in the acetaminophen group (-16%). The exercise-induced changes in PGF$_{2\alpha}$ and PGE$_2$ were not different between the ibuprofen and acetaminophen groups. These results suggest that ibuprofen and acetaminophen have a comparable effect on suppressing the normal increase in PGF$_{2\alpha}$ in human skeletal muscle after eccentric resistance exercise, which may profoundly influence the anabolic response of muscle to this form of exercise. (J Clin Endocrinol Metab 86: 5067–5070, 2001)

CONSOMPTION OF IBUPROFEN (IBU) and acetaminophen (ACET), two common over the counter analgesics, after unaccustomed muscular exercise that causes muscle soreness is very prevalent. However, there are few data concerning the mechanisms of action of either of these drugs in humans with regard to muscle metabolism. It is believed that both of these drugs act through inhibition of PG synthesis in either the skeletal muscle or the central nervous system.

PGs are synthesized in skeletal muscle and have been shown to have profound effects on skeletal muscle protein turnover (1–3). Specifically, PGF$_{2\alpha}$ and PGE$_2$ increase skeletal muscle protein synthesis and degradation, respectively (1–3). PGs are also considered to be modulators of inflammation and pain (4, 5). PGE$_2$ has been shown to be algesic itself (5, 6) as well as to stimulate the algesic properties of several nociceptive stimuli (6).

PG synthesis is regulated at two levels: 1) by controlling the activity of several lipases (i.e., A$_2$, C, and D) (7, 8) that release the PG precursor arachidonic acid (AA) from membrane phospholipids, and 2) controlling the activity of PG endoperoxide GH synthase (EC 1.14.99.1; now known as cyclooxygenase), the enzyme that converts AA to PGs (9–12). Cyclooxygenase is regulated by several commonly consumed analgesic drugs (e.g., ibuprofen, acetaminophen, and acetylsalicylic acid (aspirin)) (11, 13) as well as by mechanical stimulation and stretch (14, 15). Therefore, it is likely that consumption of cyclooxygenase-inhibiting drugs would alter PG levels in tissues such as skeletal muscle. It is thought that cyclooxygenase-inhibiting drugs have varying efficacy that is tissue specific (10, 11, 16–19). For example, nonsteroidal antiinflammatory drugs, such as ibuprofen, have a major effect on cyclooxygenase in the peripheral tissues (10, 11, 16–19), and similar drugs, such as indomethacin and meclofenamic acid, have been shown to have significant effects in skeletal muscle in rats and rabbits (2, 20). However, it is believed that the major site of cyclooxygenase inhibition by acetaminophen is in the central nervous system, and that this drug has little peripheral effect (10, 11, 16–19).

Recently, we reported the normal increase in skeletal muscle protein synthesis 24 h after high intensity eccentric resistance exercise was significantly attenuated in individuals that consumed over the counter doses of ibuprofen and acetaminophen (21). We also found that neither drug had any effect on the rating of perceived muscle soreness in the days following the exercise (21). Considering these findings and the aforementioned mechanisms of action of ibuprofen and acetaminophen in nonskeletal muscle tissue, we measured PGF$_{2\alpha}$ and PGE$_2$ levels in these same subjects to further investigate the mechanisms of action of these drugs in exercised skeletal muscle. Before the study we hypothesized that a group that consumed no drug (placebo) or acetamin-
ophen would elicit larger increases in skeletal muscle PGF$_{2\alpha}$ and PGE$_2$ than the ibuprofen group, which would respond with a relatively blunted skeletal muscle PG response.

**Experimental Subjects**

Twenty-four recreationally active males were recruited and randomly assigned to three groups of eight subjects: placebo (PLA), IBU, or ACET (Table 1). All subjects were accepted into the study after giving informed consent and following a screening for any metabolic abnormalities via blood and urine analyses, and medical history questionnaire. The investigation was approved by the institutional review board of the University of Arkansas for Medical Sciences.

**Materials and Methods**

**Eccentric exercise protocol**

Each subject underwent a bout of unilateral high intensity eccentric exercise with each leg 2 d after and approximately 24 h before a muscle biopsy for the measurement of PGF$_{2\alpha}$ and PGE$_2$. The maximal load that each subject could lift concentrically with their knee extensors was first determined, and the eccentric workload was set at 120% of the concentric maximum. The eccentric exercise consisted of 10–14 sets of 10 repetitions with 60-sec rest between sets of knee extensor exercise on a muscle dynamometer in the isometric mode (Cybex Norm, Lumenex, Ronkonkoma, NY). The range of 10–14 sets was achieved as a result of the variation in fatigue of the muscles of each subject. When the weight was lowered in less than 0.5 sec, the subject completed that set and was deemed fatigued, and the protocol was stopped.

**Drug dose and administration**

Drugs were administered in double blind, placebo-controlled fashion. On the day of the eccentric exercise protocol each drug was administered in three doses (0.8 mg, 1.0 mg, and 2.0 mg) corresponding to the maximal over the counter daily dose (IBU: 400 mg/dose, total of 1200 mg, ACET, 120 mg, and 2.0 mg, total of 400 mg). The first dose was given at the start of the eccentric exercise protocol. A fourth dose was given the following morning approximately 5 h before the second muscle biopsy (see below), which corresponded to the 0800 h dose the day before. The PLA group was given the same number of pills, and they were indistinguishable from the drug doses. The times of the doses were chosen to divide the maximal over the counter dose evenly over the day and as a result of the pharmacokinetic studies that had previously been completed on these drugs (22–24).

**Muscle biopsy and PG measurement**

Muscle biopsies (25) were taken from the vastus lateralis 2 d before (dominant leg) and approximately 24 h after (nondominant leg) the eccentric exercise protocol. The muscle was cleansed of excess blood, connective tissue, and fat and immediately frozen in liquid nitrogen. The tissue was stored in liquid nitrogen (−196°C) until analysis. For the measurement of PGF$_{2\alpha}$ and PGE$_2$, a piece of muscle weighing approximately 60 mg wet weight was homogenized on ice for 30 sec in 10 vol ice-cold buffer containing 25 mM HEPES, 4 mM EDTA, 25 mM benzamidine, 1 μM leupeptin and pepstatin, 0.15 mM aprotenin, 2 mM phenylmethylsulfonyl fluoride, and 10 μg/ml methionine to prevent any further production of PGs from AA. The homogenate was acidified to pH 3.5 with 2.0 mg HCl and centrifuged at 3000 × g at 4°C for 15 min. The supernatant was then applied to a column containing 200 mg mixed anion exchange resin (Whatman, St. Louis, MO) at 4°C. The column was washed with 10 ml distilled H$_2$O, followed by 10 ml ethanol and 10 ml hexane, and the eluates were discarded. The PGs were eluted from the column with 10 ml 90% ethanol and stored at −80°C. The ethanol-acetone fraction was dried under N$_2$ gas and reconstituted with 50 μl ethanol and 950 μl Tris buffered saline (assay buffer, catalogue no. 80016, Assay Designs, Ann Arbor, MI). Concentrations of PGF$_{2\alpha}$ and PGE$_2$ were determined by enzyme immunoassay (Assay Designs, Ann Arbor, MI). The efficiency of the PG extraction procedure was determined to be more than 97% after extraction, radioactive analysis, and PG concentration determination by enzyme immunoassay of [5,6,8,11,12,14,15-N$^3$-H]PGF$_{2\alpha}$ (Amersham Pharmacia Biotech, Little Chalfont, UK).

**Statistics**

Subject characteristics (height, weight, age, and percent body fat) and percent change in PG concentration from pre- to postexercise among the groups were compared using one-way ANOVA. PG concentrations before and after exercise among the groups were compared by two-way ANOVA with repeated measures over time. When a significant interaction was obtained, a Newman-Keuls post hoc analysis was used to determine the location of the differences. Significance was accepted at a level of P < 0.05. Data are presented as the mean ± SE.

**Results**

There were no differences in any of the subject characteristics among the three groups (Table 1). PGF$_{2\alpha}$ (picograms per mg wet wt) was increased (P < 0.05) in the PLA group (1.12 ± 0.15 to 1.76 ± 0.17), but was unchanged (P > 0.05) in the IBU (1.20 ± 0.12 to 1.07 ± 0.12) and ACET (1.76 ± 0.29 to 1.33 ± 0.17) groups. PGE$_2$ (picograms per mg wet wt) was unchanged (P > 0.05) in the PLA (4.29 ± 0.22 to 6.87 ± 0.50), IBU (4.81 ± 0.46 to 5.43 ± 0.97), and ACET (6.70 ± 0.86 to 5.08 ± 0.57) groups. Figure 1 shows the change in PGF$_{2\alpha}$ and PGE$_2$ from pre- to postexercise. The change in PGF$_{2\alpha}$ in the PLA group was significantly (P < 0.05) different from that in the IBU and ACET groups; however, the change from pre- to postexercise was not different (P > 0.05) between the ACET and IBU groups. The change in PGE$_2$ from pre- to postexercise was significantly different (P < 0.05) between the PLA and ACET groups; however, the change from pre- to postexercise was not different between PLA and IBU or between IBU and ACET groups.

**Discussion**

The main findings of this study were 1) PGF$_{2\alpha}$ increases after eccentric resistance exercise; and 2) both IBU and ACET attenuate this increase. It was somewhat surprising that in addition to IBU, ACET had a profound attenuating effect on PGF$_{2\alpha}$, levels after the exercise bout compared with those in the placebo group. In fact, compared with placebo, ACET also blunted the PGE$_2$ response to exercise, whereas IBU did not have a significant effect. These findings were contrary to our hypothesis that ACET would have no effect in skeletal muscle, given that ACET is believed to have little effect in peripheral tissues (10, 11, 16–19). However, these findings are consistent with our previous findings that both IBU and ACET block the increase in muscle protein synthesis after the same high intensity eccentric exercise bout (21). Therefore, these data seem to suggest that ACET is effective for blocking
PG production in muscle, probably through a similar mechanism to that of IBU (9, 10, 12).

There are two likely explanations for our findings regarding the similarity of effect of these drugs on PG regulation in skeletal muscle. First, the dose of the drugs must be considered. The dose of the IBU and ACET used in the current study was based on FDA limitations on the maximal over the counter doses. These dose limitations are derived considering safety and efficacy and are probably unrelated to any previously examined effects on skeletal muscle. ACET is considered to be a pure analgesic and is not considered to elicit antiinflammatory activity (11, 19, 26). IBU is also considered to be a pure analgesic when taken at the dose used in the current study (i.e. 1200 mg/d) and reportedly only has antiinflammatory activity at doses above this level (11, 19, 26). It appears that maximal over the counter doses of these drugs inhibit cyclooxygenase activity in skeletal muscle in an equivalent manner.

The second possible explanation for the similar effects of the drugs used in the current study is the isoform(s) of cyclooxygenase that exists in skeletal muscle. The basis for the tissue specificity of ACET and IBU (10, 11, 16–19) is related to the isoform of cyclooxygenase that is present in a given tissue. Currently, two isoforms of cyclooxygenase are known to exist in humans, cyclooxygenase 1 and 2, which are tissue specific (12, 27). To our knowledge, the isoform(s) of cyclooxygenase that is present in skeletal muscle has not been characterized. Thus, there may be an unidentified isoform of cyclooxygenase, as previously suggested (19, 28), in skeletal muscle. The reason for our initial hypothesis and our subsequent contrary findings may simply be due to the fact that no previous studies exist related to cyclooxygenase regulation and isoform distribution in human skeletal muscle. In the current study we did not directly measure cyclooxygenase activity or identify the specific isoforms of cyclooxygenase present in skeletal muscle, both of which would be helpful in interpreting our data.

In summary, these findings suggest that PGF$_{2x}$ is increased in human skeletal muscle after eccentric exercise. The common analgesics IBU and ACET, when consumed at maximal over the counter doses, blunt this response. This attenuated PG response may profoundly influence the anabolic response of muscle to this form of exercise. More information is needed about the isoform(s) of cyclooxygenase in human skeletal muscle and the metabolic consequences of PG blockade in skeletal muscle.

Acknowledgments

We thank the subjects for their participation and effort.


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This work was supported by a grant from the McNeil Consumer Products Co. (to W.J.,L.) and NIH Grant AG-00831 (to T.T.).

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