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COMMENT

Skeletal Muscle $PGF_{2\alpha}$ and PGE_2 in Response to Eccentric Resistance Exercise: Influence of Ibuprofen and Acetaminophen

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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 (25 ± 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 120% 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 (−14%) 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)

CONSUMPTION 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 aracadonic 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 acetacylic 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-

Abbreviations: AA, Aracadonic acid; ACET, acetaminophen; IBU, ibuprofen; PLA, placebo.

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 isotonic 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 (0800, 1400, and 2000 h) corresponding to the maximal over the counter daily dose (IBU, 400 mg/dose, total of 1200 mg; ACET, 1500, 1500, and 1000 mg, total of 4000 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 (−190 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 aprotinin, 2 mM phenylmethylsulfonylfluoride, and 10 μg/ml meclofenamic acid to prevent any further production of PGs from AA. The homogenate was acidified to pH 3.5 with 2.0 M HCl and centrifuged at 3000 × *g* at 4 C for 15 min. The supernatant was then applied to a column containing 200 mg octadecylsulfate (Sigma, St. Louis, MO) at 4 C. The column was washed with 10 ml distilled H_2O, followed by 10 ml ethanol and 10 ml hexane, and the eluates were discarded. The PGs were eluted from the column with 10 ml ethyl acetate and stored at −80 C. The ethyl acetate fraction was dried under N_2 gas and reconstituted with 50 μl ethanol and 950 μl Tris-buffered saline (assay buffer, catalogue no. 80010, 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]PGE_2 (Amersham Pharmacia Biotech, Little Chalfont, UK).

TABLE 1. Subject characteristics

Group	Age (yr)	Ht (cm)	Wt (kg)	% Body fat	Eccentric load (N/m)[a]
ACET	26 ± 4	179 ± 5	78 ± 5	16 ± 9	208 ± 50
IBU	24 ± 3	181 ± 5	78 ± 10	14 ± 6	216 ± 58
PLA	25 ± 3	181 ± 8	87 ± 23	20 ± 9	203 ± 52

Values are the mean ± SD.
[a] Nondominant leg.

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

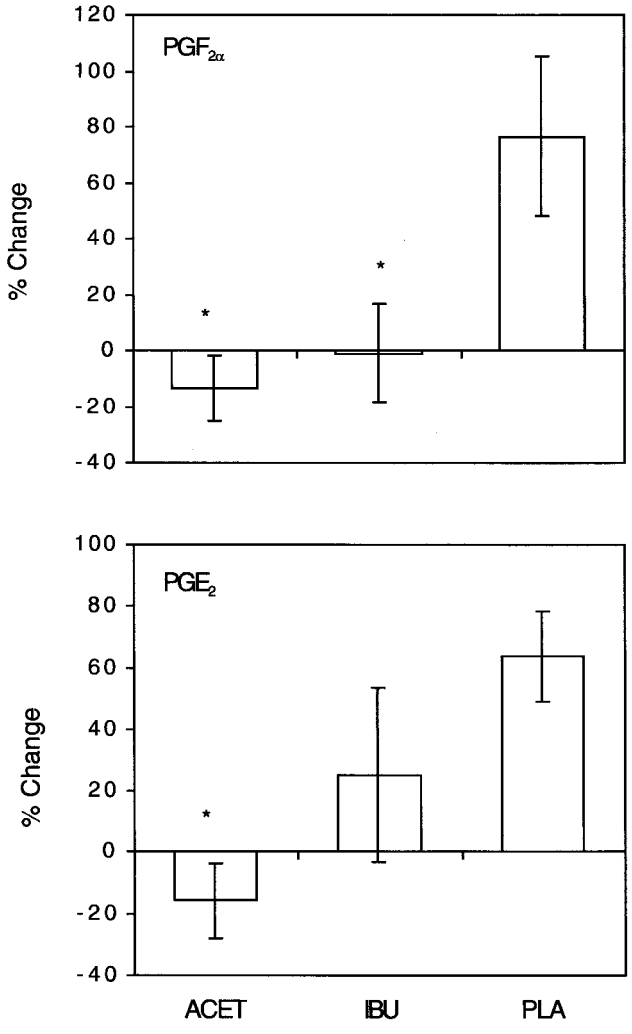

FIG. 1. Relative changes in $PGF_{2\alpha}$ and PGE_2 from pre- to postexercise. *, Significantly different ($P < 0.05$) from PLA. The change from pre- to postexercise was not different between ACET and IBU for $PGF_{2\alpha}$ or PGE_2.

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 $PGF_{2\alpha}$ is increased in human skeletal muscle after eccentric resistance 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.

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