Ibuprofen and Acetaminophen: Effect on Muscle Inflammation after Eccentric Exercise

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ABSTRACT

PETERSON, J. M., T. A. TRAPPE, E. MYLONA, F. WHITE, C. P. LAMBERT, W. J. EVANS, and F. X. PIZZA. Ibuprofen and Acetaminophen: Effect on Muscle Inflammation after Eccentric Exercise. *Med. Sci. Sports Exerc.*, Vol. 35, No. 6, pp. 892–896, 2003. **Purpose:** We examined the influence of ibuprofen and acetaminophen on muscle neutrophil and macrophage concentrations after novel eccentric contractions. **Methods:** Twenty-four males (25 ± 3 yr) were divided into three groups that received the maximal over-the-counter dose of either ibuprofen (1200 mg·d^{-1}), acetaminophen (4000 mg·d^{-1}), or a placebo after eccentric contractions of the knee extensors. Biopsies from the vastus lateralis were taken before and 24 h after exercise. Inflammatory cells were quantified in muscle cross-sections using immunohistochemistry. **Results:** Macrophage concentrations were elevated by 1.5- to 2.5-fold ($P < 0.05$) at 24 h postexercise relative to preexercise concentrations, whereas neutrophil concentrations were not significantly elevated. Muscle inflammatory cell concentrations were unaffected by treatment with ibuprofen or acetaminophen when compared with placebo. **Conclusions:** Maximal over-the-counter doses of ibuprofen or acetaminophen, when administered therapeutically, do not affect muscle concentrations of neutrophils or macrophages 24 h after a novel bout of eccentric contractions. **Key Words:** NEUTROPHILS, MACROPHAGES, NONSTEROIDAL ANTIINFLAMMATORY DRUGS, ANALGESICS, MUSCLE INJURY, RESISTANCE EXERCISE

Contraction-induced skeletal muscle injury and the resulting muscle soreness, inflammation, and dysfunction is often treated with nonsteroidal antiinflammatory drugs (NSAIDs; e.g., ibuprofen) and/or analgesics (e.g., acetaminophen). The mechanism for the therapeutic effect of NSAIDs and analgesics in treating inflammation and pain has largely been ascribed to their ability to inhibit the synthesis of prostaglandins (14). In addition to inhibiting prostaglandin synthesis, NSAIDs also interfere with aspects of inflammatory cell function. Specifically, NSAIDs have been demonstrated to reduce both neutrophil chemotaxis and activation *in vitro* (1,7,19). Prostaglandin inhibition via NSAIDs is thought to act both centrally and peripherally to reduce the perception of pain and attenuate the inflammatory response, respectively (14). Analgesics, alternatively, possess minimal antiinflammatory actions and provide relief from pain mainly by blocking prostaglandin synthesis centrally (14). Despite their known mechanisms of action, few investigators have examined whether NSAIDs or analgesics influence inflammatory cell concentrations and prostaglandins in muscle after injurious exercise in humans.

The majority of previous investigators who have examined the efficacy of NSAIDs or analgesics in treating contraction-induced muscle injury have investigated their influence on muscle soreness, muscle dysfunction, and blood creatine kinase activity in the hours to days after injurious exercise. Although a few investigators have reported a reduction in either muscle soreness (8,12), muscle dysfunction (8,12), or blood creatine kinase activity (20) after contraction-induced muscle injury, the majority of studies failed to demonstrated a beneficial effect of NSAIDs or analgesics (3,10,20,24). This finding may be attributable to their inability to blunt muscle inflammatory cell concentrations after injurious exercise. The purpose of the present study was to quantify the influence of acetaminophen and ibuprofen on muscle neutrophil and macrophage concentrations after contraction-induced muscle injury in humans.

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METHODS

Subjects. The 24 males (age 25 ± 3 yr, height 180 ± 6 cm, weight 81 ± 6 kg and body fat 17 ± 8%) used in the present study were the same subjects and part of the same protocol used by Trappe et al. (27,28). Subjects were prescreened to rule out cardiovascular, metabolic, and neuromuscular disease, and had not participated in a resistance exercise training program for at least 6 months before this study. Qualifying volunteers then signed a written informed consent form. Institutional approval was obtained at The University of Arkansas for Medical Sciences and The University of Toledo.

Experimental design. Subjects were randomly assigned in a double-blind fashion to one of three experimental groups: acetaminophen, ibuprofen, or placebo. Each group (eight subjects per group) received the same total number and color of capsules. Maximal over-the-counter doses were given to the acetaminophen (three doses 1500/1500/1000 mg to equal 4000 $mg \cdot d^{-1}$) and ibuprofen (three doses of 400 mg to equal 1200 $mg \cdot d^{-1}$) groups while the placebo group received pills indistinguishable from the drug capsules. Initial drug doses were administered at the onset of the injury protocol (0800 h), with additional doses given at 6-h intervals (1400 and 2000 h, respectively). A fourth dose was administered the following morning approximately 5 h before the second biopsy (0800 h). At the doses administered in this study, ibuprofen and acetaminophen have similar pharmacokinetic properties. Ibuprofen and acetaminophen are known to appear in the plasma within 10 min, achieve peak plasma levels within 0.5–2 h, and have a half-life of approximately 2 h (2,5,15).

Experimental procedures. After a 15-min warm-up consisting of light cycling and stretching of the lower limbs, the maximal load that each subject could lift concentrically with their knee extensors was determined (Cybex Norm, Lumenex, Ronkonkoma, NY). The experimental protocol involved unilateral eccentric contractions of the knee extensors with each leg on a muscle dynamometer in the isotonic mode (Cybex Norm). To perform eccentric contractions, the leg was passively raised to 0° of flexion then lowered to 90° of flexion while subjects resisted. Each subject performed 10–14 sets of 10 repetitions at an intensity of 120% of their concentric 1-repetition maximum. Variable sets (10–14) were performed to achieve muscle fatigue on each subject. When the weight was lowered in under 0.5 s, the subject was considered fatigued, and the protocol was stopped after the completion of that set.

Muscle biopsies were performed on the vastus lateralis of the dominant leg 48 h before and on the nondominant leg 24 h after the injury protocol under local anesthetic (1% Xylocaine HCl) (4). Muscle tissue samples were placed on a cork in mounting medium (Tragacanth Gum, Sigma Chemical Inc., St. Louis, MO), frozen in isopentane cooled to the temperature of liquid nitrogen (−190°C), and then frozen and stored at −70°C.

Immunohistochemistry. Frozen cross-sections (10 μm) were cut, adhered to gelatin-coated slides (0.04% chromium (III)-potassium sulfate-0.4% gelatin), and stored at −20°C. Muscle sections were placed on a tray surrounded by wet towels, air dried for 30 min, fixed in cold acetone for 10 min, and then air dried for 10 min. After a 5-min wash with 15 mM phosphate-buffered saline (PBS), sections were incubated for 5 min with a 0.03% hydrogen peroxide solution (J. T. Baker, Phillipsburg, NJ). After a quick wash followed by a 5-min wash with PBS, sections were treated with a blocking buffer (final concentration: 29.7 $mg \cdot mL^{-1}$ bovine serum albumin, 0.05% tween 20, 0.2% gelatin in universal buffer; Sigma Chemical) for 30 min, washed again with PBS for 5 min, then the primary antibody was applied. Neutrophils and macrophages were identified using a monoclonal mouse antihuman CD15 antibody (1:50 in PBS) (Dako Laboratories, Carpinteria, CA) and a monoclonal mouse antihuman CD68 antibody (1:100 in PBS) (Dako Laboratories), respectively. Sections were then placed in an airtight container with moist towels and incubated overnight at 4°C. The following day, sections were washed twice in PBS for a total of 30 min, and the secondary antibody was applied for 30 min. Biotinylated goat antimouse IgM (1:200 in PBS) (Vector Laboratories, Burlingame, CA) and biotinylated horse antimouse IgG (1:200 in PBS) (Vector Laboratories) were used to bind the neutrophil and macrophage primary antibodies, respectively. Sections were then washed in PBS for 30 min and horseradish peroxidase-avidin D (1:1000) (Vector Laboratories) was applied for 45 min. After a 10-min wash in PBS, sections were developed using an AEC peroxidase substrate kit (3-amino-9-ethylcarbazole) (Vector Laboratories), which elicited a red reaction product that was then viewed under a light microscope. The reaction was stopped by rinsing slides with distilled water. Slides were then mounted with an aqueous mounting medium (Biomeda Corp., Foster City, CA), air dried overnight at room temperature, and stored at 4°C. Control sections were stained using an identical procedure except the primary antibodies were omitted to control for nonspecific binding of the secondary antibodies.

Inflammatory cell concentrations were quantified by counting CD15 and CD68 positive cells using a light microscope (Olympus America Inc., Melville, NY) equipped with an eyepiece counting grid and Normarski optics (400×). Area of the muscle sections were also determined (200×) by counting all y-intercepts that intersected with the muscle section on the counting grid. Inflammatory cell concentrations were expressed relative to muscle cross-sectional volume ($cells \cdot mm^{-3}$).

Statistical analysis. Two-way analysis of variance with repeated measures (ANOVA; SigmaStat 2.03; Sigma Chemical Corporation) was performed to identify treatment and time effects for concentrations of neutrophils and macrophages. A one-way ANOVA was performed to identify treatment effects for percent change of neutrophils and macrophages from pre- to postexercise. Student-Newman-Keuls *post hoc* testing was utilized to locate differences when the observed F-ratio was significant ($P < 0.05$).

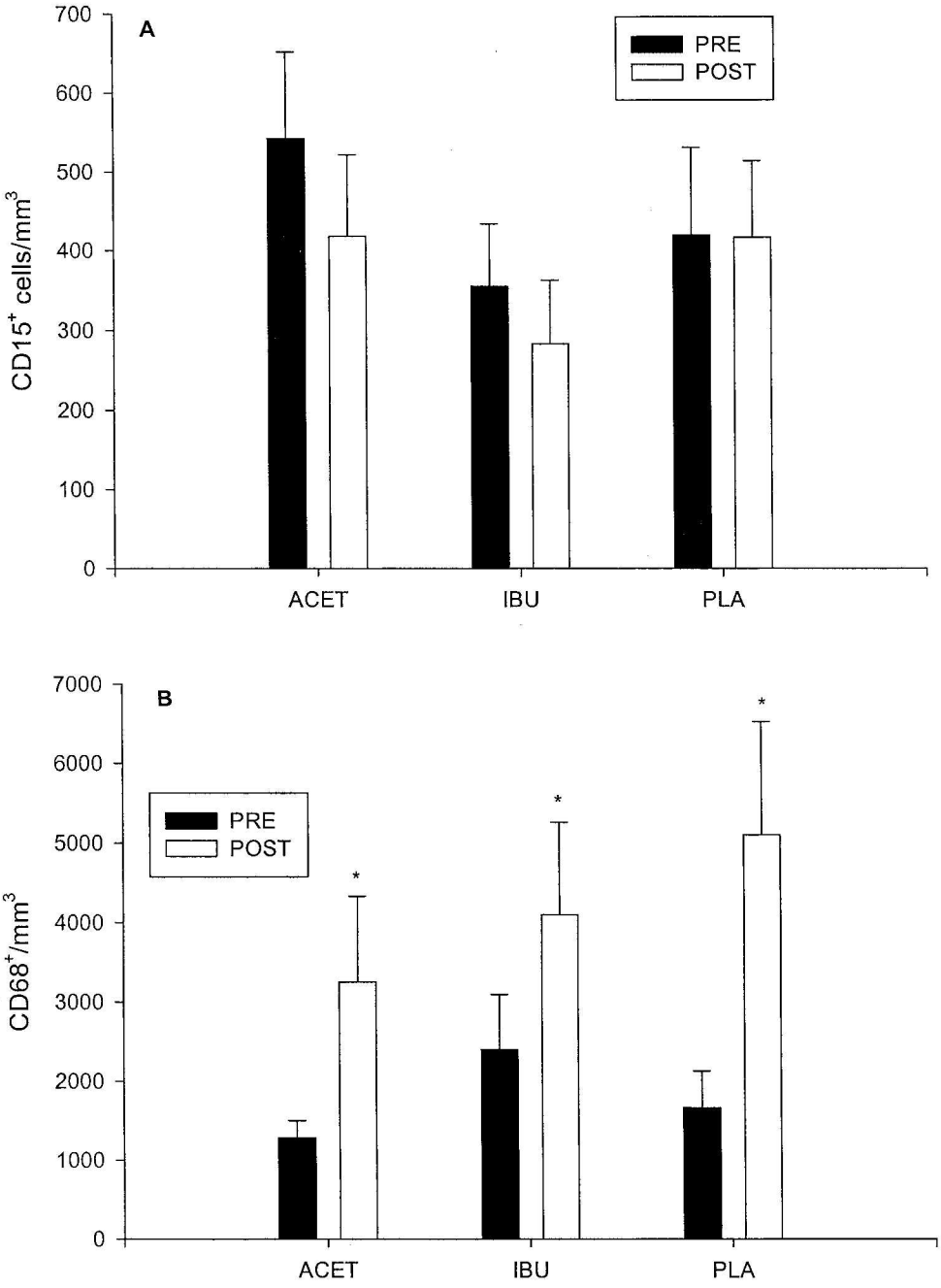

FIGURE 1—Inflammatory cell concentrations. A. Neutrophil concentrations (CD15) before and 24 h after eccentric exercise and acetaminophen (ACET), ibuprofen (IBU), or placebo (PLA) ingestion. B. Macrophage concentrations (CD68) before and 24 h after eccentric exercise and ACET, IBU, or PLA ingestion. Values are mean ± SEM. * Denotes significant time effect.

RESULTS

Subject characteristics were similar for all three groups (28). Muscle neutrophil concentrations were not significantly altered 24 h after the eccentric contractions (Fig. 1A). Muscle macrophage concentrations, however, were elevated by 1.5- to 2.5-fold in all three treatment groups 24 h after the eccentric protocol ($P < 0.01$) (Fig. 1B). Muscle inflammatory cell concentrations were unaffected by treatment with ibuprofen or acetaminophen (Fig. 1) when compared with placebo. Percent change from pre- to postexercise was also similar for neutrophils and macrophages between different drug treatments (Table 1). Although differences in preexercise inflammatory cell concentrations between groups appear large, there were no significant differences detected.

DISCUSSION

The major finding of the present study was that over-the-counter doses of ibuprofen or acetaminophen did not influence muscle inflammatory cell concentrations 24 h after eccentric contractions in humans. Muscle soreness, blood creatine kinase activity, and muscle prostaglandin E_2 (PGE_2) concentrations were similarly unaffected by drug treatment (27,28). Interestingly, both acetaminophen and ibuprofen reduced protein synthesis and prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) concentrations after eccentric contractions (27,28). Taken together, these data may indicate that therapeutic dosing of ibuprofen or acetaminophen does not influence muscle soreness and inflammation but impairs protein synthesis in humans after eccentric exercise.

TABLE 1. Percent change from pre-exercise values.

	Placebo	Acetaminophen	Ibuprofen
Neutrophils	14.6 ± 21.7	−22.2 ± 7.6	−8.7 ± 30.0
Macrophages	837.5 ± 503.7	248.6 ± 133.7	147.8 ± 126.6

Values are mean ± SEM.

Based on previous investigators who demonstrated an increase in muscle leukocyte concentrations after eccentric contractions in humans (13,16,17,25,26), we hypothesized that neutrophil concentrations would be significantly elevated postexercise. Contrary to our hypothesis, neutrophils were not significantly elevated 24 h postexercise in any of the treatment groups (Fig. 1A). MacIntyre et al. (16) reported a significant increase in radiolabeled neutrophils in muscle 2 and 4 h after eccentric contractions. The early rise in muscle neutrophils reported by MacIntyre et al. (16) is consistent with numerous investigators who reported that blood neutrophils are elevated within 12 h of recovery from eccentric contractions (6,18,20–23). These investigators also reported that blood neutrophil concentrations return to baseline by 24 h postexercise. Assuming that a temporal relationship between changes in blood and muscle neutrophil concentrations exist, it is possible that muscle neutrophils were elevated in the present study before our 24-h sampling time point.

The significant increase in muscle macrophage concentrations (Fig. 1B) in this study is consistent with Stupka et al. (26), who reported an increase in CD68 positive cells 24 h after eccentric exercise. Neither drug, however, significantly blunted macrophage accumulation indicating that muscle macrophages were not attenuated by therapeutic doses of ibuprofen or acetaminophen 24 h after novel eccentric contractions. Trappe et al. (28) reported in the same group of subjects that both ibuprofen and acetaminophen administration reduced muscle $PGF_{2\alpha}$ but not PGE_2 concentrations 24 h after contraction-induced muscle injury. The role of stable prostaglandins in muscle inflammation can be difficult to decipher *in vivo* because they are known to exert pro- (e.g., PGE_2) and anti-inflammatory (e.g., $PGF_{2\alpha}$) properties (30), and because of the myriad of factors that may influence the trafficking of inflammatory cells into injured muscle independent of prostaglandins (11). Recent data, however, indicate that $PGF_{2\alpha}$ may facilitate the resolution of inflammation (30). Therefore, the lack of drug effect on muscle macrophage concentrations in this study may reflect the inability of therapeutic doses of these drugs to blunt, in skeletal muscle, proposed mediators of inflammation (PGE_2) (9,29) while attenuating proposed modulators of inflammation ($PGF_{2\alpha}$) (9,30).

In summary, therapeutic doses of ibuprofen or acetaminophen do not blunt muscle inflammatory cell concentrations 24 h after a novel bout of eccentric contractions in humans. Interpretation of these results in combination with those of Trappe et al. (27,28) may indicate that in addition to being an ineffective treatment for muscle soreness and inflammation, therapeutic doses of ibuprofen or acetaminophen may negatively regulate muscle growth after eccentric exercise by inhibiting protein synthesis. Further investigations at earlier and later time points are needed to conclude whether over-the-counter doses of these drugs are an appropriate treatment for inflammation caused by a novel bout of eccentric contractions.

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