

Anti-Inflammatory Medication after Muscle Injury

A TREATMENT RESULTING IN SHORT-TERM IMPROVEMENT BUT SUBSEQUENT LOSS OF MUSCLE FUNCTION*

BY DEV K. MISHIRA, M.D., JAN FRIDÉN, M.D., PH.D., MARY C. SCHMITZ, M.S.E.,
AND RICHARD L. LIEBER, PH.D., SAN DIEGO, CALIFORNIA

Investigation performed at the Departments of Orthopaedics and Bioengineering, Biomedical Sciences Graduate Group, Veterans Administration Medical Center and University of California, San Diego School of Medicine, San Diego

ABSTRACT: We studied the effect of flurbiprofen, a non-steroidal anti-inflammatory drug, on muscles that had been subjected to exercise-induced injury. The muscles of the anterior compartment in the limbs of rabbits were cyclically activated as the ankle was simultaneously moved through passive plantar flexion every two seconds for thirty minutes. This treatment imposed acute passive lengthening (eccentric contractions) of the maximally contracted muscles of the anterior compartment. After the eccentric contraction-induced muscle injury, one group of rabbits was treated with oral administration of flurbiprofen, two times a day for six days, while the other group of rabbits served as untreated controls. The contractile, histological, and ultrastructural properties of the muscles were measured before the initial exercise and at three, seven, and twenty-eight days afterward.

The group that was treated with flurbiprofen demonstrated a more complete functional recovery than the untreated controls at three and seven days but had a deficit in torque and force generation at twenty-eight days. The administration of flurbiprofen also resulted in a dramatic preservation of the intermediate filament protein desmin. After three days, the proportion of fibers of the extensor digitorum longus that lost desmin-staining was significantly greater in the untreated controls than in the treated animals (34 ± 4.1 compared with 2.9 ± 1.7 per cent) ($p < 0.001$), a finding that supports the concept of a short-term protective effect. However, the muscles in the treated animals still mounted a dramatic regenerative response, as indicated by the expression of embryonic myosin. Early in the recovery period (at three days), significantly

fewer fibers of the extensor digitorum longus (2.2 ± 1.4 per cent) expressed embryonic myosin in the treated animals than in the untreated controls (11.8 ± 1.9 per cent) ($p < 0.001$). However, at seven days, the expression of embryonic myosin by the muscles from the treated animals (19.5 ± 11.9 per cent) actually exceeded that of the muscles from the untreated controls (16.2 ± 4.1 per cent). This finding suggests either a delayed or an ineffectual regenerative response by the muscles in the treated animals.

CLINICAL RELEVANCE: Non-steroidal anti-inflammatory drugs are used to provide analgesia and sometimes to improve performance after sports-related soft-tissue injuries. This study demonstrates that the effects of flurbiprofen are time-dependent. The contractile properties and histological data suggest that non-steroidal anti-inflammatory drugs cause a short-term gain but a subsequent functional loss. It is not possible to determine if this loss persists or to what extent recovery occurs in the long term. Nonetheless, these results may prompt rethinking of the liberal prescription of non-steroidal anti-inflammatory drugs as treatment for muscle injury.

Exercise-induced muscle injury is a common problem in sports and in the workplace and accounts for substantial disability. The condition improves with time in most individuals, but chronic symptoms sometimes develop. Delayed soreness after unaccustomed exercise is also common. Numerous studies have demonstrated that muscle damage and soreness are more frequent following exercise involving eccentric contractions (lengthening of activated muscles) than following exercise involving either isometric or concentric contractions^{2,4,10,12,13,15,24}.

Previous studies revealed the nature of damage of human quadriceps muscles after exercise involving eccentric contractions¹⁵. Specific ultrastructural abnormalities included disruption of the contractile apparatus and focal disorganization of the Z band (the boundary between sarcomeres). The results of subsequent studies, involving a variety of animal models, demonstrated fiber-type-specific damage and provided insight into the damage mechanism itself^{22,24}.

Disruption of muscle fibers after eccentric exer-

*Although none of the authors have received or will receive benefits for personal or professional use from a commercial party related directly or indirectly to the subject of this article, benefits have been or will be received but are directed solely to a research fund, foundation, educational institution, or other non-profit organization with which one or more of the authors are associated. Funds were received in total or partial support of the research or clinical study presented in this article. The funding sources were Upjohn, Kalamazoo, Michigan; the Department of Veterans Affairs; and National Institutes of Health Grant AR 40050.

†Department of Orthopaedics, Veterans Administration Medical Center and University of California, San Diego School of Medicine, 3350 La Jolla Village Drive, San Diego, California 92161.

‡Hand Surgery Unit, Gothenburg University, Gothenburg, Sweden.

ercise would be expected to provide a substantial inflammatory stimulus. However, since inflammation (which includes proteolysis by infiltrating neutrophils and macrophages) can itself cause damage in excess of that originally sustained by the tissue, prevention of inflammation might improve the status of the muscle after injury. On the basis of this assumption, non-steroidal anti-inflammatory drugs are commonly prescribed to provide analgesia and to improve performance. The effects of these drugs on muscle function are, however, poorly understood and are difficult to study in humans since their analgesic effect may permit improved performance²⁵. Thus, the purpose of this study was to quantify the effect of non-steroidal anti-inflammatory drugs on skeletal muscles in rabbits after eccentric contraction-induced injury.

Materials and Methods

Experimental Design

Skeletal-muscle injury was induced non-invasively in forty-five male New Zealand White rabbits. Pre-exercise data on contractile properties, muscle mass, and blood chemistry were obtained to serve as a baseline for comparison after exercise. After the muscle injury, twenty-five rabbits were treated with approximately nine milligrams of flurbiprofen a day. The remaining twenty animals were permitted normal cage activity and served as untreated controls. The duration of treatment was three days for the animals studied at three days and seven days for those studied at seven and twenty-eight days. The contractile properties of the isolated tibialis anterior and extensor digitorum longus muscles after exercise were measured at three days (nine of the treated animals and six of the untreated controls), seven days (six of the treated animals and eight of the untreated controls), and twenty-eight days (ten of the treated animals and six of the untreated controls). Blood was drawn immediately before all contractile testing. All of the experimental procedures were performed in accordance with the guidelines set forth by the National Institutes of Health²⁶.

The time-periods for the administration of flurbiprofen were chosen on the basis of the common clinical practice of providing the drug in the early post-injury period. The protocol for the twenty-eight-day testing was designed to determine the longer-term effects of a short period (seven days) of treatment with the drug.

Non-Invasive Muscle-Injury Model

A non-invasive means of producing a physiological eccentric contraction injury has been developed (Fig. 1). The rabbits were anesthetized with a subcutaneous injection of ketamine (fifty milligrams per kilogram of body mass), xylazine (five milligrams per kilogram of body mass), and acepromazine (one milligram per kilogram of body mass). Immediately after sedation, blood was drawn for a complete blood-cell count and serum

chemistry studies. The exercise session was performed with the rabbit under halothane anesthesia. Heart and respiratory rates were continuously monitored to ensure adequate anesthesia. After induction of the anesthesia, the right foot was placed in a specially designed sandal and secured to a dual-mode servomotor (model 6400; Cambridge Technologies, Cambridge, Massachusetts) with Velcro straps. The motor axis of rotation was aligned with the center of rotation of the ankle. The fibular head was located, and sterile percutaneous electrodes were placed in this region to activate the common peroneal nerve. Increasing voltage was used to determine the threshold voltage, at which twitch tension was first observed. The voltage was then increased until no additional increase in tension was measured (the maximum voltage). Subsequent tests were performed at twice this maximum voltage, eliciting supramaximum stimulation of the nerve and activation of all muscle fibers.

To mimic intense exercise, the common peroneal nerve was activated at a rate of forty hertz for 400 milliseconds followed by a rest period of 1600 milliseconds. During the 400-millisecond activation period, the ankle was passively moved through 100 degrees of plantar flexion, which resulted in eccentric contraction of

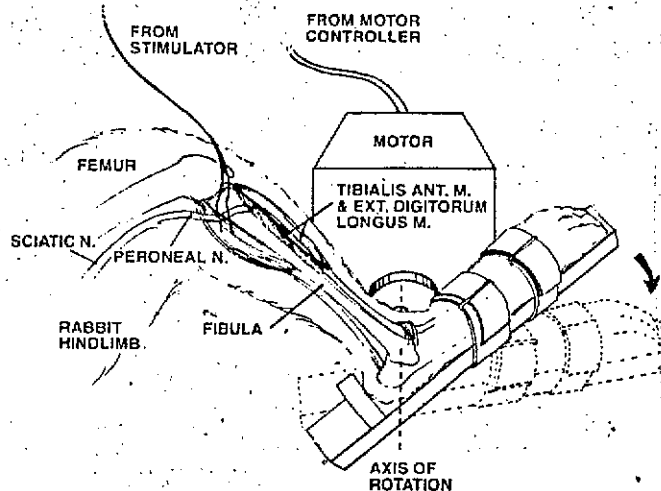


FIG. 1

Diagram of the non-invasive testing and exercising apparatus. The rabbit hindlimb is strapped onto a sandal. The muscle is activated through percutaneous electrodes that are placed in the region of the peroneal nerve, causing dorsiflexion. The motor output plantar flexes the foot, causing eccentric contractions.

the anterior compartment or, in other words, acute passive lengthening of the contracted muscle. During the 1600-millisecond rest period, the angle of the ankle was reset to the starting position in the absence of activation of the peroneal nerve. This cycle was repeated every two seconds for thirty minutes. After the exercise, the rabbit was monitored during recovery on a heating pad and was returned to its cage. A similar experimental model has been used for human subjects, who per-

formed voluntary exercise, resulting in eccentric exercise of the quadriceps muscles, on a bicycle equipped with an ergometer. This animal model is considered analogous to previous studies of intense exercise by humans.

Measurement of Isometric Dorsiflexion Torque

Animals were killed at three, seven, and twenty-eight days after the initial eccentric exercise, and muscle contractile properties were measured in a terminal experiment. In all of the treated animals and untreated controls, maximum isometric joint torque was first measured non-invasively while the anterior compartment was activated through the peroneal nerve, with the joint held at the neutral ankle-angle (about 10 degrees of plantar flexion). Since the joint was held at a fixed angle, this torque reflects the maximum isometric torque generated by the dorsiflexor muscles of rabbits.

Measurement of Muscle Contractile Force

After the torque was measured, the tendons of the tibialis anterior and extensor digitorum longus muscles were operatively isolated and attached to force transducers, as described previously²². Briefly, the distal ends of the tendons were secured to dual-mode servomotors (models 6400 and 640; Cambridge Technologies) and were aligned with the measuring and translation axis of the motors. The common peroneal nerve was then isolated, a stimulating electrode was placed around it, and the maximum stimulation voltage was determined. The temperature of the muscles was then maintained at 37 degrees Celsius with use of radiant heat, mineral oil, and a servotemperature controller (model 73A; Yellow Springs Instrument, Yellow Springs, Ohio). Under computer control²³, the lengths of the muscles were adjusted to the lengths at which twitch tension was maximum.

Contractile properties were measured by stimulation of the muscles over the frequency range of five to 200 hertz. Force-versus-frequency curves were generated after stimulation frequencies of five, ten, fifteen, twenty, forty, sixty, eighty, 100, and 200 hertz. From these data, the maximum isometric torque, the maximum tetanic tension, and the half-fusion frequency were determined. The maximum isometric torque was defined as the maximum torque developed during the force-frequency testing in the non-invasive model. The maximum tetanic tension was defined as the maximum tension developed during isolated muscle force-frequency testing (usually occurring at 200 hertz). Half-fusion frequency was defined as the frequency at which the tension was 50 per cent of the maximum tetanic tension, as determined by linear interpolation of the force-frequency curve with use of only the data points immediately above and below 50 per cent of maximum tetanic tension, as previously described²². The peak joint torque or muscle force achieved during

stimulation of the peroneal nerve in all animals was recorded.

Hematological and Blood-Chemistry Studies

Blood was collected for analysis at three points during the course of treatment: immediately after the subcutaneous sedation for the initial non-invasive exercise session and before administration of the halothane, immediately (usually within five minutes) after completion of the exercise session, and just before terminal testing — again, immediately after the subcutaneous sedation.

Whole blood for a complete blood-cell count and determination of the hemoglobin level was collected in EDTA Microtainer Tubes (Becton Dickinson, Rutherford, New Jersey) and was analyzed on a Serono-Baker System 9000 device (Serono-Baker, Allentown, Pennsylvania). The white blood-cell differential was manually counted by a technician who was experienced with this method and who was blinded to the treatment protocol.

Blood for serum-chemistry analysis was collected in an inert gel Serum Separator Microtainer Tube (Becton Dickinson), immediately centrifuged, and analyzed with a Kodak Ektachem DT60 system (Eastman Kodak, Rochester, New York). Serum creatinine, creatine kinase, and lactate dehydrogenase concentrations were measured.

Electron Microscopic Study

Portions of the tibialis anterior and extensor digitorum longus muscles were fixed overnight in 2.5 per cent glutaraldehyde in isotonic phosphate buffer. One-millimeter-thick tissue blocks were postfixed for two hours in 1 per cent osmium tetroxide, dehydrated in graded alcohols, and infiltrated with Spurr embedding resin (Polysciences, Warrington, Pennsylvania). The blocks were oriented so that the muscle fibers could be sectioned either longitudinally or transversely. Survey sections of one micrometer were stained with toluidine blue, and a region was selected, trimmed, and sectioned for electron microscopic study. Section thickness was kept as close to sixty nanometers as possible. Myofibrillar diameter was measured from tissue samples obtained from the untreated controls and the treated animals at twenty-eight days. Two micrographs were made of each muscle, and ten myofibrils that were clearly sectioned at the A band and the I band (Fig. 2) were digitized by one observer who was blinded with regard to the identity of the sample.

Histochemistry and Immunohistochemistry

The mid-part of each muscle belly was transversely cut into a block approximately three millimeters thick, frozen in isopentane cooled by liquid nitrogen (-159 degrees Celsius), and stored at -80 degrees Celsius for histochemical processing. Eight-micrometer-thick cross

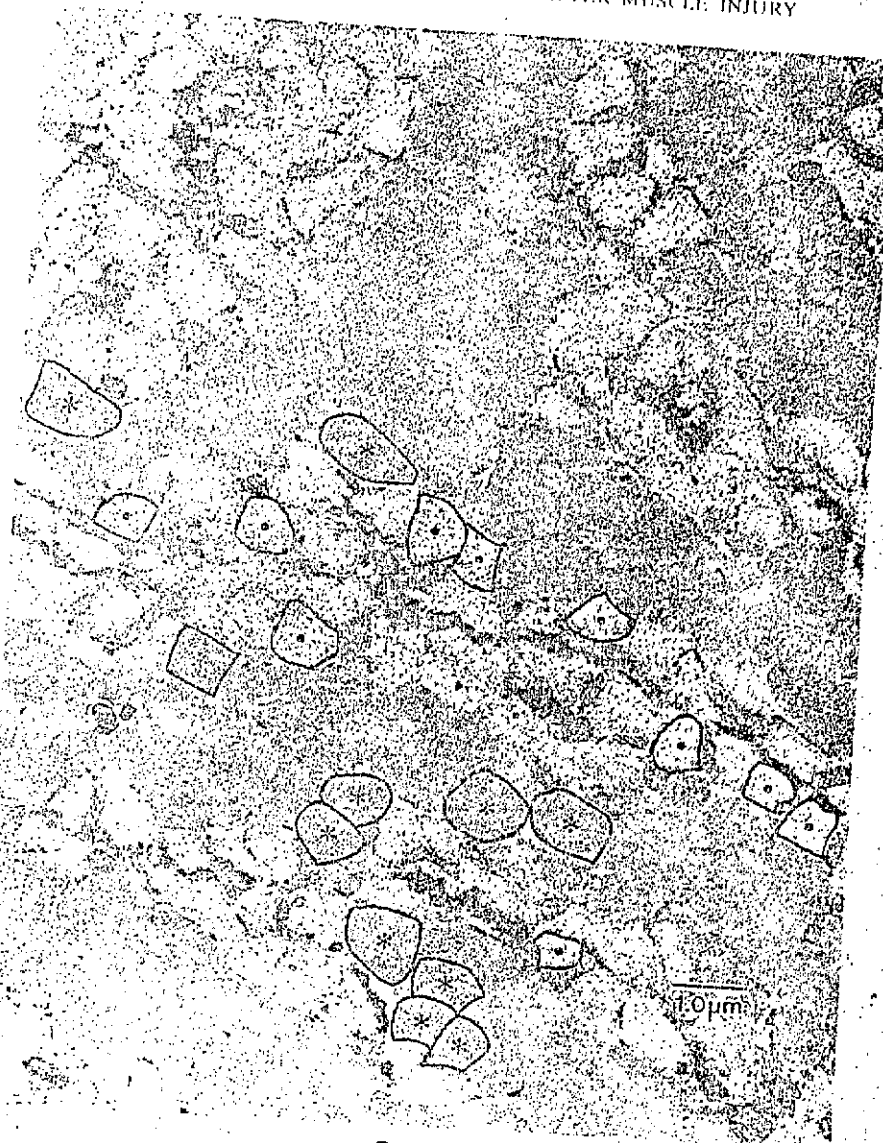


FIG. 2

Electron micrograph of a section of the extensor digitorum longus from a treated rabbit (twenty-eight days after exercise). Ten myofibrils in the A band (*) note the actin and myosin filaments) and ten in the I band (* note the actin filaments only) were digitized for calculation of the myofibrillar diameter.

sections of the muscle were stained with hematoxylin and eosin. To assess the qualitative tissue infiltration with macrophages, other sections were stained for acid phosphatase.

Cryosections were stained with antibodies against laminin to visualize the basal lamina of the fiber; against fibronectin to detect intracellular deposits, indicating lesions in the membrane; against vimentin to demonstrate inflammatory cells²²; and against desmin for evaluation of the structural integrity of the cytoskeletal network²³.

To quantify the size and distribution of the fibers, morphometry was performed on laminin-stained sections after fiber-typing with use of an interactive image-analysis system connected to a Zeiss Axiophot light microscope (Carl Zeiss, Oberkochen, Germany), which was equipped with an MTT tube camera (Dage-MTI, Michigan City, Indiana). Measurements were

performed with a calibrated digitizing pad in conjunction with a computer-controlled morphometry program (IBAS; Kontron, Eching, Germany). Again, one observer digitized all muscle sections and was blinded with regard to the identity of the sample.

A monoclonal antibody against embryonic myosin was used as the marker for muscle regeneration. In all animals, antibody-binding was visualized with use of indirect peroxidase-antiperoxidase technique (Dakopatts, Copenhagen, Denmark).

Stereology

For each animal, one section was taken from the central muscle portion. Within this section, nine fascicles were sampled at each depth: superficial, middle, and deep. Approximately sixty fibers per fascicle were measured (with use of stereological point-counting). This corresponded to approximately 10 per cent of the entire

muscle and far exceeded that recommended in standard stereological texts²⁸.

Administration of Flurbiprofen

Flurbiprofen (2-[2-fluoro-4-biphenyl]propionic acid) is a member of the phenylpropionic acid class of compounds and is a commonly prescribed anti-inflammatory drug for human use (Ansaïd; Upjohn, Kalamazoo, Michigan). It was supplied by the manufacturer as a white powder crystalline solid. Because of its relative insolubility in water (less than one milligram per milliliter), the drug was suspended in common clover honey, which proved to be a palatable preparation for oral administration to the rabbits. Dosage was established elsewhere²⁹ as 3.0 milligrams per kilogram of body mass a day, given in two doses. For a 3.0-kilogram rabbit, the total dose was 9.0 milligrams a day. Therefore, half of this dose (4.5 milligrams) was suspended in approximately 2.0 milliliters of honey and was administered orally with a syringe twice a day for the treatment period.

Pharmacokinetic studies have shown that, in most species, orally administered flurbiprofen is absorbed rapidly. Peak plasma or serum drug concentrations are generally observed thirty minutes to five hours after a single dose³⁰. The disappearance half-life for rabbits is estimated to be six to seven hours³⁰.

To ensure that the drug had been absorbed by each rabbit, serum drug assays were performed from samples drawn at the time of the terminal testing. Analysis was performed with high-pressure liquid chromatography (Phoenix International Life Sciences, Montréal, Québec, Canada).

Statistical Analysis

Data were stored in a format that was processed with the SuperANOVA and StatView 4.0 statistical packages (Abacus Concepts, Berkeley, California). To determine relative differences between the rabbits that had been treated with flurbiprofen and the untreated controls, a one-way analysis of variance was performed. To determine possible time and treatment-dependent interactions, a two-way analysis of variance was performed. Data are presented as means and standard errors. The level of significance was $p = 0.05$. Power analysis revealed that statistical power for these experiments exceeded 60 per cent for most parameters.

Results

Time-Course of Change in Torque

The natural history of exercise-induced muscle injury was studied in the untreated animals. Torque was measured before the exercise and at one, two, three, seven, fourteen, and twenty-eight days afterward. Torque decreased precipitously in the untreated controls one and two days after the exercise (Fig. 3). The magnitude of the decline was approximately 25 per cent, with near recovery at two weeks. In contrast, the treated

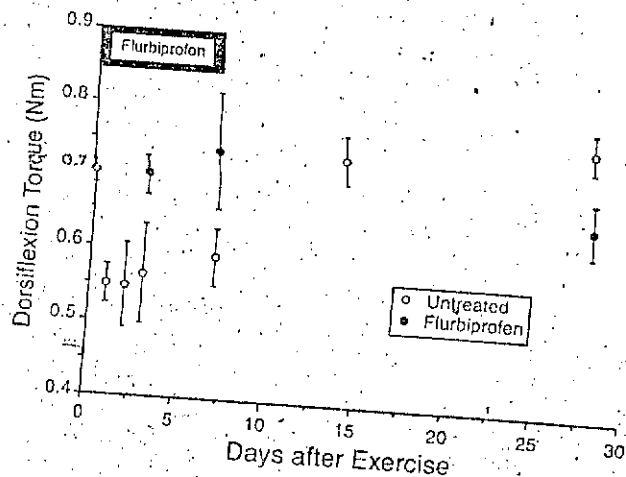


FIG. 3

Time-course of the change in torque for muscles from the treated animals and the untreated controls. Isometric torque was measured non-invasively with the servomotor holding the ankle fixed in approximately 10 degrees of plantar flexion and activating the peroneal nerve. The muscles in the untreated controls had a transient decrease in torque that returned to normal at approximately fifteen days. In contrast, the muscles in the treated animals generated a significantly higher torque early in the treatment period (at three and seven days) ($p < 0.001$) and a significantly lower torque later in the experimental period ($p < 0.001$). The values are given as the mean and the standard error. The box in the upper left corner represents the time-period of administration of the flurbiprofen.

animals demonstrated a remarkable recovery at three and seven days but showed a decline in torque generation at twenty-eight days. In support of this treatment-dependent effect, two-way analysis of variance revealed a highly significant relationship between treatment and time ($p < 0.01$). Thus, the muscles in the treated animals demonstrated increased torque at the early time-periods but decreased torque at twenty-eight days.

Muscle Contractile Properties

To understand the underlying basis for the changes in torque, the contractile properties of the isolated tibialis anterior and extensor digitorum longus were determined, as described earlier. The maximum tetanic tension in the tibialis anterior muscle was greater in the treated animals than in the untreated controls at three and seven days (Fig. 4), while no difference was seen for the extensor digitorum longus at three days. However, the maximum tetanic tension in the tibialis anterior and extensor digitorum longus in the treated animals was diminished, compared with that in the untreated animals, at twenty-eight days (Fig. 4). The mean maximum tetanic tension was 2017 ± 257 , 2469 ± 463 , and 1649 ± 128 grams at three, seven, and twenty-eight days, respectively, in the tibialis anterior muscles from the treated animals and 1441 ± 58 , 1929 ± 112 , and 2115 ± 90 grams in those from the untreated controls (Table I). The mean maximum tetanic tension was 2705 ± 244 , 2548 ± 389 , and 3626 ± 305 grams at three, seven, and twenty-eight days, respectively, in the extensor digitorum longus muscles from the treated animals and 2736 ± 478 , 3234 ± 481 ,

TABLE I
CONTRACTILE PROPERTIES*

Parameter	3 Days after Exercise		7 Days after Exercise		28 Days after Exercise	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
Tibialis ant.						
Maximum tetanic tension (g)	2017 ± 257	1441 ± 58	2469 ± 463	1929 ± 112	1649 ± 128	2115 ± 90
Muscle mass (g)	3.2 ± 0.15	2.9 ± 0.12	3.1 ± 0.30	3.4 ± 0.12	3.4 ± 0.13	3.2 ± 0.11
Half-fusion frequency (Hz)	43.3 ± 8.69	26.2 ± 1.65	29.5 ± 2.26	26.5 ± 0.85	26.9 ± 1.78	30.2 ± 2.88
Time to peak twitch tension (ms)	24.4 ± 1.64	31.2 ± 3.35	29.0 ± 1.17	27.8 ± 2.40	27.4 ± 1.34	28.8 ± 1.31
Ext. dig. longus						
Maximum tetanic tension (g)	2705 ± 244	2736 ± 478	2548 ± 389	3234 ± 481	3626 ± 305	4218 ± 167
Muscle mass (g)	3.2 ± 0.14	3.1 ± 0.16	2.5 ± 0.56	2.9 ± 0.19	3.5 ± 0.17	3.6 ± 0.07
Half-fusion frequency (Hz)	33.0 ± 3.0	30.3 ± 2.6	21.3 ± 0.26	31.1 ± 3.5	28.7 ± 2.9	37.2 ± 4.9
Time to peak twitch tension (ms)	26.7 ± 1.5	34.9 ± 3.8	29.4 ± 2.6	38.6 ± 1.0	35.3 ± 2.1	38.9 ± 0.9

*The values are given as the mean and the standard error.

and 4218 ± 167 in those from the untreated controls (Table I). Data were normalized to the physiological cross-sectional area of the muscles in order to compensate for absolute size differences among the animals²⁷. While the specific tension in the tibialis anterior from the untreated controls was approximately 2.7 kilograms per square centimeter three days after the exercise, the specific tension in the muscles from the treated animals was greater (3.6 kilograms per square centimeter). Three days after the exercise, the specific tension in the extensor digitorum longus muscle was approximately 1.5 kilograms per square centimeter in both the untreated controls and the treated animals. By twenty-eight days after the exercise, however, the tibialis anterior and extensor digitorum longus muscles from

the untreated controls showed significantly greater specific tension than those from the treated animals. Again, this result was significant in that two-way analysis of variance revealed a significant interaction between time and treatment ($p < 0.05$) for the tibialis anterior and the extensor digitorum longus. Measurements of muscle hydration revealed that cross-sectional changes due to edema could not account for the differences in physiological cross-sectional area among animals.

The half-fusion frequency, which represents the speed with which tension develops in a muscle, was similar for the two groups (Table I). This result indicates that the effects of the injury itself and subsequent healing were probably independent of the type of muscle fiber.

Hematological and Serum-Chemistry Studies

Although the treated animals had decreased numbers of white blood cells compared with the values in the control animals at corresponding time-points, these differences were not significant. A significant reduction in circulating neutrophils was seen in the treated animals at twenty-eight days ($p < 0.01$) but not at the other time-points. A significant reduction in circulating lymphocytes was seen in the treated animals at seven days ($p < 0.05$) but not at the other time-points. No significant differences were seen in circulating monocytes.

The levels of serum creatine kinase and lactate dehydrogenase in the treated animals were reduced compared with the levels in the untreated controls at three and seven days. There were no substantial differences at twenty-eight days (Table II).

Morphological Appearance

The morphology of the isometrically exercised tibialis anterior muscles was normal and showed tightly packed polygonal fibers with normal amounts of ep-

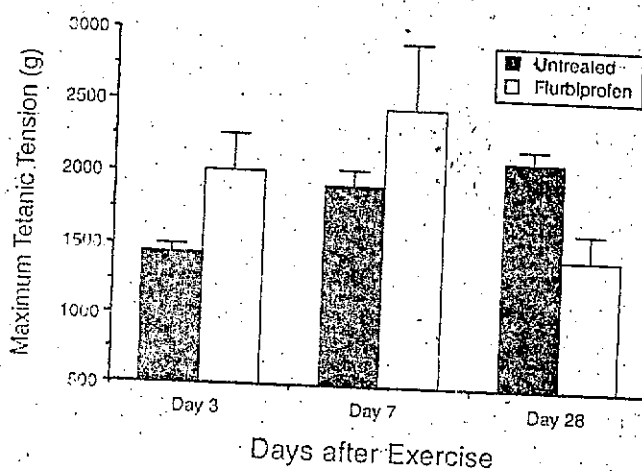


FIG. 4

Graph of the mean maximum tetanic tension of the tibialis anterior muscles from the treated animals compared with that of the untreated controls. Tetanic tension mirrors the results obtained with measurement of dorsiflexion torque (Table I) in that the treated animals generated greater muscle forces early in the treatment and smaller muscle forces later in the treatment. The error bars represent the standard error.

TABLE II
PERCENTAGE CHANGE IN SERUM ENZYMES RELATIVE TO INITIAL LEVELS*

Enzyme	3 Days after Exercise		7 Days after Exercise		28-Days after Exercise	
	Treated	Untreated	Treated	Untreated	Treated	Untreated
Creatine kinase	81 ± 82.0	365 ± 218	-48 ± 13.3	24 ± 37.9	119 ± 79	92 ± 41.1
Lactate dehydrogenase	60 ± 39.9	115 ± 59.6	-49 ± 30.8	63 ± 65.7	54 ± 51.3	94 ± 70.9

*The values are given as the mean and the standard error.

domysial and perimysial connective tissue. Muscle nuclei were peripherally placed, and inflammatory cells were not present. In contrast, the tibialis anterior muscles from both of the eccentrically exercised groups were composed of fibers that were highly rounded, lightly staining, and surrounded by an increased extracellular space. Inflammatory cells were seen in many regions, especially near capillaries. The general appearance of the muscle resembled edematous tissue, an observation supported by the fact that exercised whole muscle mass was significantly greater than whole muscle mass from non-exercised animals ($p < 0.01$).

Ultrastructurally, regions with disorganization of the myofibrillar apparatus were observed, especially in the A band and Z band, as described previously²². However, a new finding was inflammatory cells within the fibers of the untreated controls. While numerous cells were observed in the capillary endothelium, several had extravasated into the muscle fibers themselves. The large muscle fibers that had been observed previously in cross section²¹ showed morphological changes in which a portion of the muscle fiber was being digested by infiltrating macrophages, resulting in supercontraction of the remaining sarcomeres. It is known that muscle contraction is isovolumic. Thus, as the sarcomeres shorten (to less than 2.0 micrometers), the myofilaments move farther apart, increasing the diameter of the fiber. In this way, sarcomere supercontraction resulted in extreme increases in the diameters of the muscle fibers in focal regions along the length of the muscle fibers.

Immunohistochemical Findings

Muscle injury resulted in a dramatic loss of cytoskeletal desmin from certain fibers across the entire muscle, although the result was more dramatic for the extensor digitorum longus than for the tibialis anterior (Fig. 5, A). This cytoskeletal loss occurred even though myofibrillar contractile proteins were clearly present in serial sections stained immunohistochemically. The muscles from the untreated controls had a significantly greater area fraction of cells without desmin-staining (desmin-negative cells) (13 per cent), compared with those from the treated animals (2 per cent) at each time-point and during the entire experimental period ($p < 0.001$). This finding indicated a protective effect of treatment with flurbiprofen on structural proteins of muscle. For example, at three days, significantly more extensor digitorum longus fibers lost desmin-staining in the untreated controls (34 ± 4.1 per cent) than in

the treated animals (2.9 ± 1.7 per cent) ($p < 0.001$). This difference persisted at seven days (Fig. 5, A), but all muscle fibers had normal desmin-staining by the twenty-eighth day. Two-way analysis of variance revealed a significant relationship between time and treatment ($p < 0.001$), highlighting the different response time-courses for the two groups.

Despite the clear protective effect of flurbiprofen at three and seven days, the muscles from the treated animals still showed a significant regenerative response at these time-periods, as evidenced by expression of em-

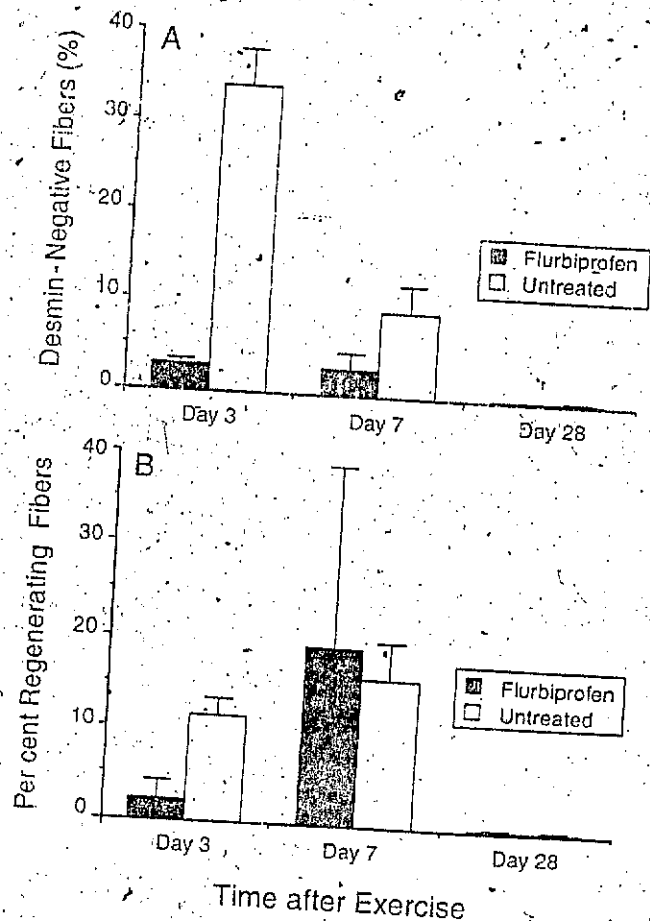


FIG. 5
(A) Graph of the mean area fraction of fibers, demonstrating the absence of the cytoskeletal protein desmin (desmin-negative fibers) on immunohistochemical sections. The untreated controls had a significantly greater area fraction of desmin-negative fibers at three and seven days ($p < 0.001$), whereas both groups had become normal by twenty-eight days after the exercise. The error bars represent the standard error. (B) Graph of the mean area fraction of fibers, demonstrating the expression of embryonic myosin on immunohistochemical sections. The error bars represent the standard error.

bryonic myosin (Fig. 5, B): While the muscles from the untreated controls expressed significantly greater amounts (11.8 ± 1.9 per cent) of embryonic myosin three days after the exercise than those from the treated animals (2.2 ± 1.4 per cent) ($p < 0.001$), at seven days this relative magnitude was reversed: the muscles from the treated animals expressed more embryonic myosin (19.5 ± 11.9 per cent) than those from the untreated controls (16.2 ± 4.1 per cent), although this difference was not significant ($p > 0.05$).

Morphometric Results

A significant decrease in myofibrillar diameter was observed in the A band ($p < 0.001$) but not in the I band ($p > 0.1$) (Fig. 6, A). The magnitude of the decrease (approximately 0.2 micrometer or approximately 10 per cent of normal) was not enough to explain the decrease in muscle force-generating capacity. Thus, the force decrement in the muscles from the treated animals at twenty-eight days did not result simply from the decreased myofibrillar diameter.

In contrast to the decreased myofibrillar diameter, the diameters of the muscle fibers measured on frozen

sections from treated animals twenty-eight days after eccentric contraction were significantly larger ($p < 0.01$) than those from the untreated controls (Fig. 6, B). This effect was most pronounced for the type-2B fibers, which maintained an area (5238 ± 137 square micrometers) that was approximately 22 per cent greater than the type-2B fibers from the untreated controls (4283 ± 132 square micrometers). Two-way analysis of variance on fiber area as a function of type of fiber revealed a significant difference depending on type ($p < 0.0001$) and depending on treatment ($p < 0.05$) but, with the numbers available for study, we could detect no significant interaction ($p > 0.2$). This suggests a fiber-type-independent increase in the diameter of the fiber.

Experimental Complications

Three of the treated animals had a myotendinous separation of the extensor digitorum longus, during contractile testing (one at three days and two at seven days). Data from these animals were excluded from calculations of the contractile properties of the extensor digitorum longus.

Discussion

Whether prescribed for amelioration of painful degenerative osteoarthritis, soft-tissue injury, or acute pain, non-steroidal anti-inflammatory agents are common in the practice of orthopaedic surgery. Although they are generally regarded as benign, there is a growing body of evidence to suggest that chronic usage of non-steroidal anti-inflammatory drugs may have adverse effects on the musculoskeletal system. Fracture-healing and bone ingrowth at the interfaces of implants inserted without cement may be impeded by anti-inflammatory drugs^{16,19}.

This study demonstrates that systemic administration of flurbiprofen after muscle injury affects, in a time-dependent manner, contractile function, the morphology of cells, and circulating levels of inflammatory cells and muscle enzymes. Treatment provided a short-term improvement (at three and seven days) but a subsequent deficit (at twenty-eight days). These data suggest that the inflammatory process itself, which may occur secondary to the initial muscle injury, also results in an additional delayed injury to the muscle and that the eventual functional recovery of the muscle is dependent on the inflammatory process. By suppressing the initial inflammatory reaction, the non-steroidal anti-inflammatory drug permits improved performance in early time-periods but appears to suppress the stimulus that may be needed for cellular remodeling in longer time-periods.

The idea that inflammation follows acute muscle injury has precedent²⁰. In a recent review, Smith proposed a scheme by which injured muscle initiates a cascade of cellular and chemical events²¹. The first event is thought to be mechanical injury to the myofibrillar apparatus and

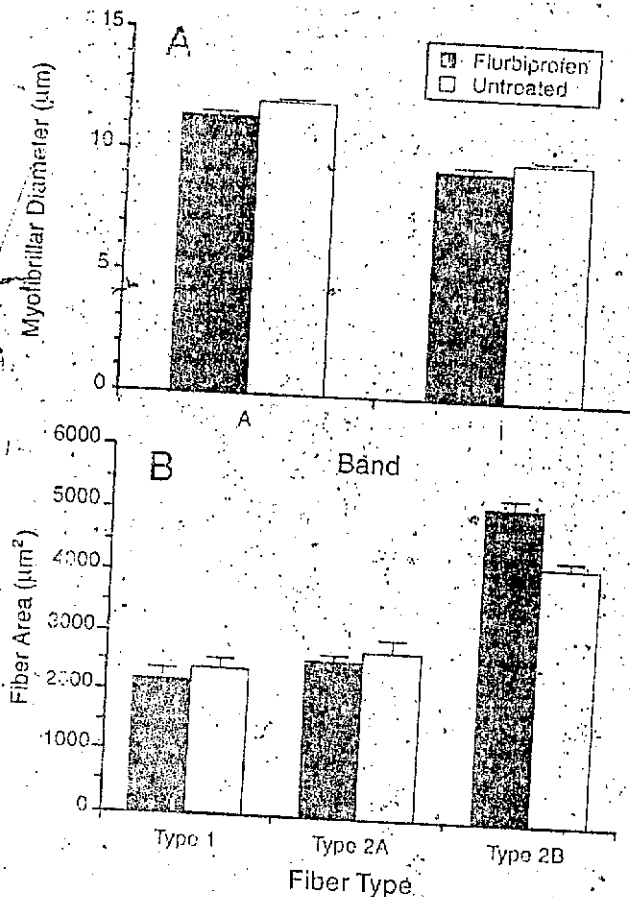


FIG. 6

Graphs of the mean morphometric properties measured from biopsy specimens of the extensor digitorum longus muscles from the treated animals and the untreated controls twenty-eight days after the eccentric exercise. The error bars represent the standard error. (A) Mean myofibrillar diameter. (B) Mean muscle fiber area.

an elevation in circulating neutrophils, which subsequently migrate to the site of the injury. The next cellular event involves circulating monocytes, which infiltrate the injured tissue and differentiate into macrophages. These cellular infiltrates release lysosomes to break down the damaged tissue. Also associated with macrophage accumulation are increases in circulating interleukin-1, acute-phase proteins, and prostaglandin E₂.

Within this context of cellular events, the prostaglandin E₂ is perhaps a central factor. Prostaglandin E₂ has been shown to increase lysosome activity in muscle *in vitro*²⁹; it also appears to increase its tissue levels in response to interleukin-1³⁰ and intracellular calcium³¹. There are also complex relationships between prostaglandin E₂ and the production of cytokines³². Prostaglandin E₂ is probably responsible for sensitization of pain afferents³³. It is therefore plausible that drugs that block the production of prostaglandin E₂ will ameliorate the pain and possibly alter the process of muscle-cell repair. Zerba et al. showed that treatment with polyethylene glycol superoxide dismutase significantly reduces the magnitude of the secondary injury, lending support to our hypothesis³⁴. Because of its toxicity, however, this drug has no applicability in clinical practice.

Non-steroidal anti-inflammatory drugs are widely used to provide analgesia and perhaps to improve performance after sports-related soft-tissue injury. Vane proposed, in 1971, that prostaglandins are necessary for the inflammatory process and that the production of prostaglandins can be blocked by inhibiting the cyclooxygenase enzyme³⁵. While this appears to be the central function of non-steroidal anti-inflammatory drugs, there is growing evidence that these drugs also function through diverse processes unrelated to prostaglandins. Some non-steroidal anti-inflammatory drugs act directly on the cell membrane to alter fluidity³⁶. Release of lysosomal enzymes and the chemotactic response of neutrophils are also inhibited by some non-steroidal anti-inflammatory drugs *in vitro*.

The appearance of myocellular enzymes in the circulation is generally considered an indication of disruption of the membranes of the muscle cells. Mild exercise, such as walking, has no effect on the serum enzyme levels³⁷. Strenuous exercise, however, can result in dramatic increases in serum levels of creatine kinase, lactate dehydrogenase, and aspartate aminotransferase^{11,23,38}.

In particular, elevated levels of serum creatine kinase are associated with muscle injury following eccentric exercise¹⁴. Typically, these values reach their peak

several days after the bout of exercise and normalize within a week or two^{11,12}. Importantly, exercise-training before eccentric exercise dramatically reduces levels of creatine kinase. Recent studies involving the release of creatine kinase in younger and older subjects have suggested that creatine kinase is not so much a direct indicator of muscle damage as it is an indirect measure of muscle turnover, which occurs following damage³⁹. Using this type of interpretation, we suggest that the muscles from the treated animals experienced the identical exercise insult but subsequently did not undergo as extensive a remodeling process in the first three to seven days because the subsequent inflammation was suppressed during the treatment period.

The fact that treatment with non-steroidal anti-inflammatory drugs protects the intermediate filament cytoskeleton from disruption may be viewed in the same manner. It is possible that cytoskeletal disruption is a prerequisite for myofibrillar reorganization. It is interesting to note that the disruption itself can occur relatively rapidly after the onset of eccentric contraction and may simply be the first cellular structural indicator of reorganization. In this regard, the delayed but exaggerated expression of embryonic myosin may represent the cell's attempt to regenerate injured myofibrils without the benefit of previous cytoskeletal preparation. Perhaps this explains the significantly ($p < 0.001$) decreased myofibrillar diameter observed twenty-eight days after eccentric contraction.

Application of these results to human exercise must be done with caution because exercise-induced muscle injury may represent one of two extremes: muscle strain, which causes a sudden, acute painful sensation¹⁷, or a perhaps more common type of injury that occurs after intense exercise and results in symptoms of muscle soreness or weakness⁴⁰. Symptoms are pronounced, especially with exercise that involves eccentric contraction. Athletes recall a period of unaccustomed heavy exercise rather than a single traumatic event. The current data probably relate more to the latter type of injury than to an acute strain.

This study demonstrates that flurbiprofen has time-dependent effects after exercise-induced muscle injury. A short period of administration provided a short-term benefit but a subsequent decrement in muscle function. However, on the basis of the current data, it is not possible to determine if this loss of muscle force persists or to what extent the muscle recovers in the long term.

Note: The authors gratefully acknowledge the assistance of Linda Kimbuchi, Abbyanne Sisk, Dr. Diane Shelton, Bridget Matabon, and Christian Giangreco.

References

1. Abramson, S. B., and Weissmann, G.: The mechanisms of action of nonsteroidal antiinflammatory drugs. *Arthritis and Rheumatism*, 32: 1-9, 1989.
2. Armstrong, R. B.: Mechanisms of exercise-induced delayed onset muscular soreness; a brief review. *Med. and Sci. Sports and Exerc.*, 16: 529-538, 1984.
3. Armstrong, R. B.; Ogilvie, R. W.; and Schwane, J. A.: Eccentric exercise-induced injury to rat skeletal muscle. *J. Appl. Physiol.*, 54: 80-93, 1983.
4. Ahnsson, E.: Observations on experimental muscular soreness. *Acta Rheumat. Scandinavica*, 2: 109-116, 1956.

5. Baracos, V.; Greenberg, R. E.; and Goldberg, A. L.: Influence of calcium and other divalent cations on protein turnover in rat skeletal muscle. *Am. J. Physiol.*, 250: E702-E710, 1986.
6. Baracos, V.; Rodemann, H. P.; Dinarello, C. A.; and Goldberg, A. L.: Stimulation of muscle protein degradation and prostaglandin E₂ release by leukocyte pyrogen (interleukin-1). A mechanism for the increased degradation of muscle proteins during fever. *New England J. Med.*, 308: 553-558, 1983.
7. Brooks, P. M., and Day, R. O.: Nonsteroidal antiinflammatory drugs — differences and similarities. *New England J. Med.*, 324: 1716-1725, 1991.
8. Cannon, J. G.; Orencole, S. F.; Fielding, R. A.; Meydani, M.; Meydani, S. N.; Fiatarone, M. A.; Blumberg, J. B.; and Evans, W. J.: Acute phase response in exercise: interaction of age and vitamin E on neutrophils and muscle enzyme release. *Am. J. Physiol.*, 259: 121-1219, 1990.
9. Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council: *Guide for the Care and Use of Animals*. Publication 85-23, revised 1985. Washington, D.C., United States Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, 1985.
10. Davies, C. T., and White, M. J.: Muscle weakness following eccentric work in man. *Pflugers Arch.*, 392: 168-171, 1981.
11. Evans, W. J.: Exercise-induced skeletal muscle damage. *Phys. and Sportsmed.*, 15: 89-100, 1987.
12. Evans, W. J.; Meredith, C. N.; Cannon, J. G.; Dinarello, C. A.; Frontera, W. R.; Hughes, V. A.; Jones, B. H.; and Knuttgen, H. G.: Metabolic changes following eccentric exercise in trained and untrained men. *J. Appl. Physiol.*, 61: 1864-1868, 1986.
13. Faulkner, J. A.; Jones, D. A.; and Round, J. M.: Injury to skeletal muscles of mice by forced lengthening during contractions. *Quart. J. Exper. Physiol.*, 74: 661-670, 1989.
14. Fridén, J.; Sfakianos, P. N.; and Hargens, A. R.: Short communication. Blood indices of muscle injury associated with eccentric muscle contractions. *J. Orthop. Res.*, 7: 142-145, 1989.
15. Fridén, J.; Sjöstrom, M.; and Ekblom, B.: Myofibrillar damage following intense eccentric exercise in man. *Internat. J. Sports Med.*, 4: 170-176, 1983.
16. Friedman, R. J.; Acurio, M. E.; Davis, R.; Claassen, B.; Powers, D.; Keller, J.; and Grotendorst, G.: Effects of growth factors and indomethacin on fracture healing. *Trans. Orthop. Res. Soc.*, 17: 421, 1992.
17. Garrett, W. E., Jr.: Muscle strain injuries: clinical and basic aspects. *Med. and Sci. Sports and Exerc.*, 22: 436-443, 1990.
18. Hough, T.: Ergographic studies in muscular soreness. *Am. J. Physiol.*, 7: 76-92, 1902.
19. Keller, J.; Klamer, A.; Bak, B.; Flyvbjerg, A.; He, S.-Z.; Suder, P.; and Bünger, C.: Prostaglandin E₂ stimulates callus formation and insulin-like growth factor I after fracture. *Trans. Orthop. Res. Soc.*, 17: 416, 1992.
20. Laridés, E.: Intermediate filaments as mechanical integrators of cellular space. *Nature*, 283: 249-256, 1980.
21. Lieber, R. L., and Fridén, J.: Selective damage of fast glycolytic muscle fibers with eccentric contraction of the rabbit tibialis anterior. *Acta Physiol. Scandinavica*, 133: 587-588, 1988.
22. Lieber, R. L.; Woodburn, T. M.; and Fridén, J.: Muscle damage induced by eccentric contractions of 25% strain. *J. Appl. Physiol.*, 70: 2498-2507, 1991.
23. Lieber, R. L.; Smith, D. E.; Campbell, R. C.; and Hargens, A. R.: Real-time acquisition and data analysis of skeletal muscle contraction in a multi-user environment. *Comput. Meth. and Prog. Biomed.*, 22: 259-265, 1986.
24. McCully, K. K., and Faulkner, J. A.: Injury to skeletal muscle fibers of mice following lengthening contractions. *J. Appl. Physiol.*, 59: 119-126, 1985.
25. Muckle, D. S.: Flurbiprofen for the treatment of soft tissue trauma. *Am. J. Med.*, 80: 76-80, 1986.
26. Newham, D. J.; Mills, K. R.; Quigley, B. M.; and Edwards, R. H.: Pain and fatigue after concentric and eccentric muscle contractions. *Clin. Sci.*, 64: 55-62, 1983.
27. Powell, P. L.; Roy, R. R.; Kanin, P.; Bella, M. A.; and Edgerton, V. R.: Predictability of skeletal muscle tension from architectural determinations in guinea pig hindlimb. *J. Appl. Physiol.*, 57: 1715-1721, 1984.
28. Riedall, P. C.; Adams, S. S.; Crampton, E. L.; and Marchant, B.: The disposition and metabolism of flurbiprofen in several species including man. *Xenobiotica*, 8: 691-703, 1978.
29. Rodemann, H. P., and Goldberg, A. L.: Arachidonic acid, prostaglandin E₂ and F_{2α} influence rates of protein turnover in skeletal and cardiac muscle. *J. Biol. Chem.*, 257: 1632-1638, 1982.
30. Salminen, A., and Kihlstrom, M.: Protective effect of indomethacin against exercise-induced injuries in mouse skeletal muscle fibers. *Internat. J. Sports Med.*, 8: 46-49, 1987.
31. Smith, L. L.: Acute inflammation: the underlying mechanism in delayed onset muscle soreness? *Med. and Sci. Sports and Exerc.*, 23: 542-551, 1991.
32. Thoraell, L.-E.; Eriksson, A.; and Edstrom, L.: Intermediate filaments in human myopathies. In *Cell and Muscle Motility*, pp. 85-136. Edited by R. M. Dowben and J. W. Shay. New York, Plenum Press, 1981.
33. Tidus, P. M., and Iannuzzo, C. D.: Effects of intensity and duration of muscular exercise on delayed soreness and serum enzyme activities. *Med. and Sci. Sports and Exerc.*, 15: 461-465, 1983.
34. Vane, J. R.: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature New Biol.*, 231: 232-235, 1971.
35. Weibel, E. J.: *Stereological Methods*. Vol. 1. *Practical Methods for Biological Morphometry*. London, Academic Press, 1980.
36. Wolf, P. L.: Abnormalities in serum enzymes in skeletal muscle diseases [editorial]. *Am. J. Clin. Pathol.*, 95: 293-296, 1991.
37. Zerba, E.; Komorowski, T. E.; and Faulkner, J. A.: Free radical injury to skeletal muscles of young, adult, and old mice. *Am. J. Physiol.*, 258: C429-C435, 1990.