The effects of fish oil and isoflavones on delayed onset muscle soreness

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ABSTRACT

LENN, J., T. UHL, C. MATTACOLA, G. BOISSONNEAULT, J. YATES, W. IBRAHIM, and G. BRUCKNER. The effects of fish oil and isoflavones on delayed onset muscle soreness. *Med. Sci. Sports Exerc.*, Vol. 34, No. 10, pp. 1605–1613, 2002. **Introduction/Purpose:** Fish oils (FO) have been shown to modulate the inflammatory response through alteration of the eicosanoid pathway. Isoflavones (ISO) appear to reduce the inflammatory pathway through their role as a tyrosine kinase inhibitor. Delayed onset muscle soreness (DOMS) develops after intense exercise and has been associated with an inflammatory response. Therefore, we hypothesized that physical parameters associated with DOMS could be decreased via the modulation of the inflammatory response by supplementing subjects with either FO or ISO. **Methods:** 22 subjects were recruited and randomly assigned to one of three treatment groups: FO (1.8 g of omega-3 fatty acids·d^{-1}), ISO (120 mg soy isolate·d^{-1}), or placebo (PL) (Western fat blend and/or wheat flour). All treatment groups received 100-IU vitamin E·d^{-1} to minimize lipid peroxidation of more highly unsaturated fatty acids. Subjects were supplemented 30 d before the exercise and during the week of testing and were instructed to refrain from unusual exercise. DOMS was induced by 50 maximal isokinetic eccentric elbow flexion contractions. Strength parameters, pain, arm circumference, and relaxed arm angle (RANG) were measured at 48, 72, and 168 h post exercise. Cortisol, creatine kinase (CK), interleukin-6 (IL-6), tumor necrosis factor (TNFα), malondialdehyde (MDA), and serum iron were measured before supplementation, after supplementation, and post exercise. **Results:** Significant decreases were observed in RANG and strength 48 h postexercise among all groups, and there were significant increases in pain and arm circumference. There were no significant changes among all groups from baseline at 168 h (7 d) post exercise. There were no significant treatment effects between groups for the physical parameters or for cortisol, CK, IL-6, TNFα, MDA, or serum iron. **Conclusion:** These data indicate FO or ISO, at the doses supplemented, were not effective in ameliorating DOMS with the above-cited protocol. **Key Words:** EPA, DHA, PHYTOESTROGENS, EICOSAPENTAENOIC ACID, DOCOSAHEXAENOIC ACID, ECCENTRIC, EXERCISE, DOMS, INFLAMMATION, IL-6, TNF-α, MALONDIALDEHYDE, IRON, CYTOKINES, STRENGTH

Muscle soreness is a common ailment that can occur in any individual independent of physical fitness level and can occur any number of times throughout one's life. The performance of unaccustomed exercise of moderate or high intensity and duration will result in muscle soreness experienced during and after the exercise, which is referred to as delayed onset muscle soreness (DOMS). Immediate soreness may be due to biochemical end products of metabolism affecting free nerve endings (6,27) or by temporary hypoxia due to muscle ischemia (26). DOMS normally increases in intensity in the first 24 h after exercise, peaks from 24–72 h, and then subsides so that by 5–7 d postexercise it is gone (4,5). The severity of DOMS is variable, ranging from mild discomfort to extreme soreness that limits the use of muscles by reducing one's ability to produce force (12) and by reducing one's relaxed arm angle (RANG) (45). Elevations in creatine kinase (CK) begin around 24 h (2), peak around 48–72 h, and have been used as a marker of muscle damage (4,7).

Armstrong (3) suggests that the breakdown of the structural and contractile proteins and the membrane results in an inflammatory response. Smith (58) also suggests that the damage to the connective and/or contractile tissue initiates an inflammatory response. Monocytes migrate to the injured area, differentiate to macrophages in the inflammatory environment, and secrete large quantities of pro-inflammatory prostaglandins. The increase in prostaglandins and increased histamines, kinins, and surrounding edema are then thought to activate nociceptors (group III and IV afferents) and result in the sensation of DOMS (4,58).

Due to the general uncertainties regarding DOMS, many management modalities have been proposed. Studies of stretching (13), massage (64) cryotherapy (53), and ibuprofen (22) have been ineffective at decreasing muscle soreness.

Fish oils are rich in omega-3-polyunsaturated fatty acids and have been shown to reduce joint tenderness in patients with rheumatoid arthritis and reduce the production of pro-inflammatory substances (25). The polyunsaturated fatty acids, particularly 20:5n3 (eicosapentaenoic acid or EPA) and 22:6n3 (docosahexaenoic acid or DHA), have been

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shown to become incorporated into the cellular membranes. This process can alter the release of the more pro-inflammatory 2 series prostaglandins, thromboxanes, and prostacyclins.

Isoflavones have been found to have hypocholesterolemic effects, inhibit low-density lipoprotein (LDL) oxidation, and decrease endothelial cell proliferation and angiogenesis, which all have a favorable effect on the cardiovascular system (8). Isoflavones may also have other health benefits (9,56) as well as having antioxidant properties (31), which may be beneficial in ameliorating the negative effects of free radical generation during exercise. In studies of myocardial ischemia-reperfusion injury, soybean isoflavones were found to be protective and significantly reduce TNF-α (21), a pro-inflammatory cytokine (51). In addition, the isoflavone genistein is known as a potent tyrosine kinase inhibitor (28), thus possibly inhibiting the postreceptor intracellular cascade of events.

Fish oils, vitamin E, and the phytoestrogens are naturally occurring substances, which are found in any normal diet. They can also be purchased in supplemental forms in any of the health food, grocery, or pharmacy stores. These nutritional supplements serve as a safe and, possibly, therapeutically viable options to minimize DOMS with no known adverse affects. The purpose of this study was to determine the effects of nutritional supplementation with fish oils or isoflavones (genistein and daidzein) versus a placebo before and after a maximal-effort eccentric exercise bout on the signs and symptoms of DOMS (elevation in CK, pain, and swelling, and a decrease in strength and RANG).

Therefore, we hypothesized that: 1) supplementing subjects with fish oils, high in EPA and DHA, should decrease pain perception, decrease further destruction through this, otherwise, cyclical pathway, and decrease the associated biochemical markers; and 2) supplementing with the isoflavone genistein could limit or decrease the inflammatory response through tyrosine kinase inhibition, and decrease DOMS and/or the associated biochemical markers.

METHODS

Subjects

Thirteen men (age: 22.7 $\pm$ 3.92, BMI: 24.1 $\pm$ 2.7) and nine women (age: 24.5 $\pm$ 5.47, BMI: 23.6 $\pm$ 5.3) were recruited from the University of Kentucky to participate in this study. Exclusion criteria included: 1) history of significant pain in the arms or shoulders, or any severe breaks or fractures in the arms or shoulders within the past 3 yr; 2) currently participating in, or have participated in, a strength-training program within 60 d before this study; 3) known impaired sensitivity to pain; 4) internal or external appliances in the arms or shoulders; 5) pacemaker; 6) pregnancy; 7) acute trauma or inflammation, bleeding disorders, infection, severe ischemia, or poor thermal regulation; 8) HIV positive status; and 9) currently taking oral contraceptives or hormone replacement therapy. All subjects were fully informed of the purpose of this study, the nature of the potential risks associated with the procedures, and were required to read and sign an informed consent form approved by the University of Kentucky Institutional Review Board before participating in this study.

TABLE 1. Fatty acid composition (%) of treatment fat sources.

	Fish Oil	Western Fat Blend
C14:0	8.66	2.13
C16:0	21.71	24.03
C16:1	11.85	2.3
C18:0	4.16	16.48
C18:1	11.10	41.14
C18:2	1.7	13.92
C18:3n3	1.11	0.00
C20:5n3	22.11	0.00
C22:5n3	2.64	0.00
C22:6n3	14.95	0.00
Total	100.00	100.00

Fatty acids with area $< 0.1\%$ are not included.

Subjects were randomly assigned to: 1) fish oil group (FO; $N = 7$) receiving 1.8 g of omega-3 fatty acids$\cdot d^{-1}$ and wheat flour (soy control), 2) isoflavone group (ISO; $N = 8$) receiving 120 mg soy isolate (1.3:1 genistein: daidzein)$\cdot d^{-1}$ and Western fat blend (fish oil control), and 3) placebo group (PL; $N = 7$) receiving comparable amounts of Western fat blend and/or wheat flour. Three men and three women did not complete the experimental protocol, resulting in FO ($N = 5$), ISO ($N = 6$), and PL ($N = 5$). Fatty acid composition for the fish oil and western fat blend capsules are depicted in Table 1. All three groups received 100 IU of d-α tocopherol/dL-α tocopheryl acetate to minimize long chain unsaturated fatty acid oxidation. Although some studies suggest that vitamin E may modulate muscle soreness or damage after exercise by decreasing lipid peroxidation, these effects are regarded as inconclusive (29). Subjects were supplemented 30 d before the exercise, during the week of testing, and were instructed to refrain from resistance training 30 d before and during the study.

Physical Measurements

All subjects underwent a familiarization session on the KinCom Dynamometer (Chattex, Chattanooga, TN) just before testing in the Nutter Football Training Facility at the University of Kentucky, Lexington, KY. After 30 d of supplementation, all subjects participated in four sessions lasting approximately 30–60 min each. All subjects were evaluated four times during the course of the investigation. Baseline criterion measures of perceived soreness, upper arm circumference, RANG, and isokinetic and isometric muscle strength were measured on the first day (0 h). Muscle soreness was then induced into the nondominant arm by the performance of 50 maximal effort eccentric contractions at a $90°\cdot s^{-1}$ using the KinCom Dynamometer. Subjects returned on days 2, 4, and 7 after day 0 for the measurement of perceived muscle soreness, upper arm circumference, RANG, and strength.

The model we selected for this study was based on a modification of a more intense preliminary protocol done in our lab, which resulted in extreme soreness and swelling.

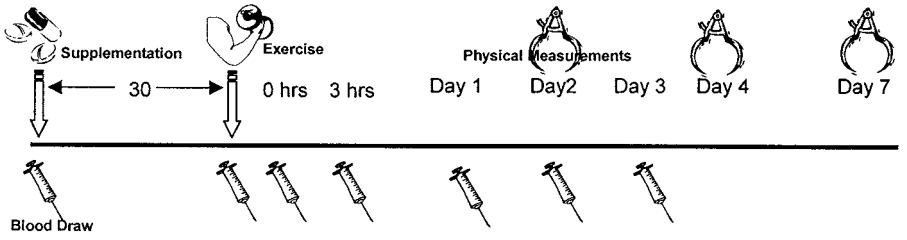

FIGURE 1—Experimental protocol.

The adjustments made were based on the necessity for a model that would induce DOMS but would not overwhelm the system with an extreme secondary inflammatory response. Of particular concern was an excessive amount of damage or severe inflammatory response, which might mask any of the beneficial effects of the two nutritional supplements. Therefore, the current protocol utilized a lesser number of repetitions and a slightly faster speed.

Muscle soreness evaluation. Each subject was asked to evaluate his or her level of perceived muscle soreness using a 100-mm visual analog scale (VAS). The rating was as follows: 0 cm, complete absence of pain; and 10 cm, extremely sore with noticeable pain and stiffness at all times, and the muscle and arm are difficult to use. Subjects placed a mark on the scale indicative of their level of perceived soreness, and a measurement (in cm) was made from the zero to the subjects' mark.

Circumference. The upper arm circumference measurements were determined at three different locations (10, 70, and 100 mm from the olecranon process) by using an anthropometric tape measure.

Range of motion. Using a standard universal goniometer, the RANG of the elbow joint was determined at a relaxed elbow angle (the angle at the elbow as the arm is hung relaxed at the side).

Isokinetic strength. Isokinetic strength measurement consisted of both eccentric (-30, $-90°\cdot s^{-1}$) and concentric (30, $90°\cdot s^{-1}$) contractions on a KinCom Dynamometer. Each subject performed two warm-up contractions at each angular velocity and then performed three measured trials at each velocity. The three curves were then averaged for each velocity and served as the maximal contraction force at that velocity. The order of the speed and the type of contraction were counterbalanced between subjects; however, the order of the speed and type of contraction performed by the subject was maintained for each subsequent visit.

Isometric strength. Isometric strength was measured at an elbow angle of 90°. The subject performed two 3-s maximal voluntary (isometric) contractions (MVCs) with at least 1 min of rest between efforts. The average of the two MVCs was recorded.

Blood Measurements

All subjects had blood drawn seven times during the course of this investigation. There was an initial blood draw before supplementation. The next blood draw was before the induction of the muscle soreness, and the next five blood draws were immediately after, 3 h, 24 h, 48 h, and 72 h after the exercise bout (Fig. 1). Seven tubes of venous blood, 10 mL each, were collected into plain red top tubes. Either trained investigators or trained phlebotomists performed collection.

Muscle damage. Cortisol and CK were measured by the University of Kentucky Chandler Medical Center Clinical Laboratory.

Inflammatory markers. Il-6 was measured using Pierce Endogen (Rockford, IL) ImmuLux human Il-6 fluorescent ELISA kit no. 84602. This kit has a sensitivity of $<$ 0.3 $pg\cdot mL^{-1}$ and is specific for the measurement of natural and recombinant human IL-6. It does not cross react with IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-7, IL-8, IL-10, IL-12, GMCSF, IFNα, IFNγ, or mouse IL-6.

TNFα was measured using Endogen (Woburn, MA) ELISA kit no. EH2-TNFA. The sensitivity is <5 $pg\cdot mL^{-1}$ and is specific for the measurement of natural and recombinant human TNFα. It does not cross react with IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-6, IL-7, IL-8, TNFβ, IFNα, IFNγ, or mouse TNFα.

Lipid peroxidation. Malondialdehyde (MDA) and thiobarbituric acid reacting substances (TBARS) were determined fluorometrically according to the modified method of Li and Chow (37) by using a Gilford Fluoro IV spectrofluorometer (Gilford Instrument, Oberlin, OH). For the majority of the samples, 0.5-mL serum was added to 1.0 mL of 0.2% thiobarbituric acid in 0.2 N HCl and 0.3 mL of 0.2% butylated hydroxytoluene in ethanol, vortex-mixed, and incubated at 60°C for 60 min. The mixture was then extracted with 2 mL of isobutanol, and the fluorescence intensity was measured with excitation 515 nm and emission at 550 nm. 1,1,3,3-Tetramethoxypropane was used as a standard to construct the standard curve.

Serum iron. Total serum iron, unsaturated iron binding capacity, total iron binding capacity, and % saturation iron were determined using a Sigma Diagnostics (St. Louis, MO) iron and iron binding capacity kit no. 565-A.

Statistical Analysis

Data were statistically analyzed (ANOVA) using the Statistical Package for the Social Sciences (SPSS, Chicago, IL) version 10.0. All statistical analysis were conducted in the null form, and the alpha level of $P < 0.05$ was determined as statistically significant. Power analysis based on previous studies reported on the effects of weight training on DOMS and decreased pain (pain perception) was used to arrive at

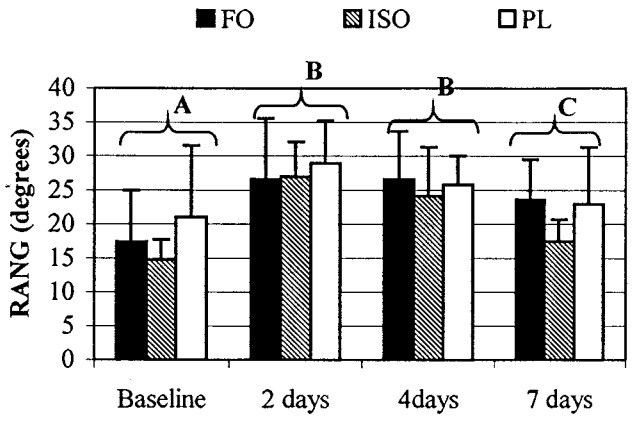

FIGURE 2—Relaxed arm angle was measured using a goniometer at a relaxed angle 0, 48, 96, and 168 h post exercise. There were no significant differences between treatment groups. When treatment groups were combined, there were significant differences ($P < 0.008$) as indicated by values having different letters.

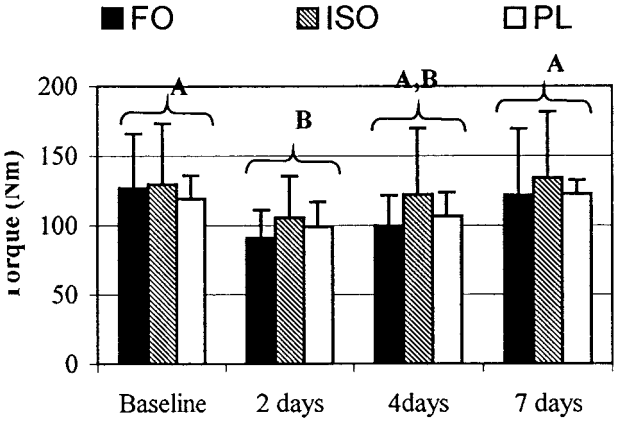

FIGURE 4—Isometric strength was measured at an elbow angle of 90° on a KinCom Dynamometer. Values are the average of two 3-s trials. There were no significant differences between treatment groups. When treatment groups were combined, there were significant differences ($P < 0.008$) as indicated by values having different letters.

the number of subjects needed. This analysis was completed with the help of our Biostatistics Unit using nQuery Advisor. When the sample size in each of the three groups is five, a one-way ANOVA will have 90% power to detect at the 0.05 level, a difference in means characterized by the variance of means, assuming that the common standard deviation is 1.064. *Post hoc* tests were conducted using a multiple *t*-test with Bonferroni correction, which adjusted a $P < 0.05$ significance level to $P < 0.008$ for significant differences.

RESULTS

Physical Measurements

There were no significant differences among all treatment groups, and there were significant differences when treatment groups were combined as follows:

RANG. Significant decreases in RANG at 2, 4, and 7 d were noted compared with baseline. Significant decreases were also noted between 2 and 7 d and between 4 and 7 d (Fig. 2).

Pain scale. There were significant increases between baseline and 2 d. Pain scales measurements returned to baseline at 7 d, with significant decreases between 7 d and 2 and 4 d (Fig. 3).

Circumference. Significant increases in arm circumference (40 mm and 70 mm) were noted at 4 d (27.9 ± 3.1 and 29.3 ± 3.2, respectively) relative to baseline (26.6 ± 3.0 and 28.4 ± 3.2, respectively) In addition, significant increases were noted in arm circumference (70 and 100 mm) at 2 d (29.2 ± 3.3 and 30.2 ± 3.5, respectively) compared with baseline (28.4 ± 3.2 and 29.7 ± 3.4, respectively).

Strength. There were significant differences for the 90° isometric strength at 2 d from baseline and 7 d compared with 2 d (Fig. 4). There was a significant difference for the eccentric and concentric contraction between day 2 (26 ± 14.2 and 30.5 ± 32, respectively) and day 7 (38.2 ± 22 and 34.8 ± 33.5, respectively) at $30°\cdot s^{-1}$. There was a significant difference for the eccentric contraction at $90°\cdot s^{-1}$ when day 2 (31 ± 19.2) and 7 (39.3 ± 18) were compared.

Blood Measurements

Fatty acid analysis. Supplementation resulted in significant increases in serum content of 20:5n3 and 22:6n3 for

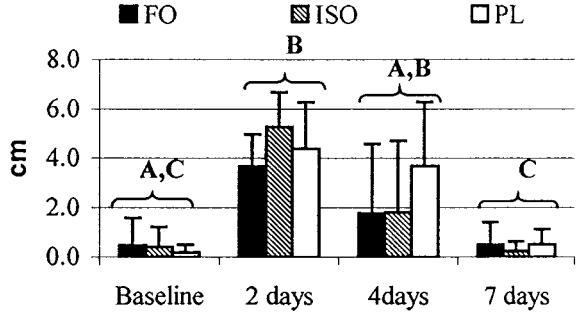

FIGURE 3—Muscle soreness was assessed using a 10-cm visual analog, 0 being complete absence of pain and 10 being extremely sore. There were no significant differences between treatment groups. When treatment groups were combined, there were significant differences ($P < 0.008$) as indicated by values having different letters.

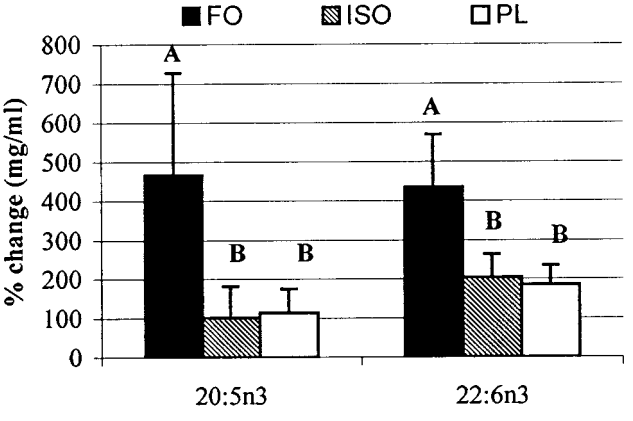

FIGURE 5—Serum fatty acid composition was calculated as % change from baseline. Supplementation resulted in significant increases in 20:5n3 and 22:6n3 fatty acids for the fish oil group at $P < 0.05$.

TABLE 2. Values for blood chemistry.

	Before Supplementation	After Supplementation	Postexercise				
			0 h	3 h	24 h	48 h	72 h
Serum total iron ($\mu g \cdot dL^{-1}$)							
FO	69 ± 36	85 ± 12	73 ± 32	65 ± 51	49 ± 36	96 ± 69	71 ± 41
ISO	100 ± 68	86 ± 29	92 ± 23	89 ± 14	125 ± 36	93 ± 10	127 ± 40
PL	86 ± 12	70 ± 14	78 ± 6	81 ± 24	84 ± 20	80 ± 32	100 ± 32
Total	85 ± 44	80 ± 20	83 ± 25	79 ± 31	79 ± 41	91 ± 42	97 ± 42
% Saturation							
FO	24 ± 12	29 ± 5	26 ± 10	27 ± 21	18 ± 14	28 ± 22	26 ± 16
ISO	37 ± 24	26 ± 12	30 ± 10	29 ± 5	40 ± 10	31 ± 6	42 ± 8
PL	25 ± 6	21 ± 5	24 ± 4	24 ± 8	26 ± 8	24 ± 10	32 ± 13
Total	29 ± 16	25 ± 8	28 ± 9	27 ± 11	26 ± 13	28 ± 13	33 ± 14
MDA ($nmol \cdot mL^{-1}$)							
FO	0.4 ± 0.2	0.5 ± 0.1	0.5 ± 0.3	0.5 ± 0.3	0.4 ± 0.1	0.5 ± 0.2	0.5 ± 0.1
ISO	0.3 ± 0.1	0.7 ± 0.4	0.3 ± .0	0.4 ± 0.1	0.5 ± 0.4	0.3 ± 0.1	0.3 ± 0.0
PL	0.6 ± 0.2	0.7 ± 0.2	0.91	0.4 ± 0.1	0.5 ± 0.2	0.6 ± 0.2	0.5 ± 0.3
Total	0.5 ± 0.2	0.7 ± 0.3	0.4 ± 0.2	0.4 ± 0.2	0.5 ± 0.2	0.4 ± 0.2	0.4 ± 0.2
CK ($U \cdot L^{-1}$)							
FO	172 ± 104	163 ± 111	140 ± 89	155 ± 99	212 ± 95	213 ± 7595	546 ± 787
ISO	129 ± 88	160 ± 129	163 ± 126	188 ± 118	407 ± 349	1993 ± 3079	7607 ± 11888
PL	159 ± 91	169 ± 72	108 ± 67	182 ± 96	259 ± 92	310 ± 154	358 ± 165
Total	152 ± 88	163 ± 99	146 ± 99	177 ± 97	285 ± 209	911 ± 1981	2837 ± 7261
Cortisol ($\mu g \cdot dL^{-1}$)							
FO	11 ± 2	12 ± 4	13 ± 3	10 ± 4	11 ± 2	9 ± 4	11 ± 4
ISO	10 ± 1	14 ± 6	15 ± 6	11 ± 4	9 ± 2	8 ± 1	9 ± 4
PL	13 ± 6	13 ± 7	11 ± 3	10 ± 3	11 ± 6	11 ± 6	10 ± 3
Total	11 ± 4	13 ± 6	14 ± 5	10 ± 3	11 ± 5	9 ± 4	10 ± 3
Il-6 ($pg \cdot mL^{-1}$)							
FO	235 ± 436	240 ± 397	189 ± 316	305 ± 539	294 ± 524		
ISO	1 ± 3	12 ± 13	23 ± 44	2 ± 3	3 ± 4		
PL	11 ± 11	6 ± 10	0 ± 0	25 ± 36	9 ± 9		
Total	78 ± 251	81 ± 233	73 ± 189	91 ± 284	109 ± 324		
TNF-α ($pg \cdot mL^{-1}$)							
FO	11 ± 25	11 ± 25	15 ± 30	16 ± 30	13 ± 29		
ISO	0 ± 0	0 ± 0	0 ± 0	49 ± 100	0 ± 0		
PL	2 ± 5	5 ± 10	0 ± 0	0 ± 0	0 ± 0		
Total	4 ± 14	5 ± 15	5 ± 17	21 ± 64	5 ± 17		

FO, fish oil group ($N = 5$); ISO, isoflavone group ($N = 6$); PL, placebo group ($N = 5$).

the FO group compared with the ISO and PL groups (Fig. 5).

Biochemical indices. There were no significant treatment or time effects for serum levels of cortisol, TNF-α, IL-6, CK, MDA, or iron (Table 2).

DISCUSSION

DOMS appears to be caused by two major mechanisms. The first is mechanical damage that occurs, particularly during eccentric or intense exercise, and seems to be principally in the area of the Z-disk of the sarcomere (11) in the musculotendon region. The second, subsequent to and dependent on the first, involves biochemical changes. This secondary mechanism is not as well understood, but free radical initiated damage or release of pro-inflammatory substances have been implicated. The tissue injury appears to initiate an inflammatory response resulting in a release of cytokines, localized edema due to the migration of monocytes, macrophages, PGE_2, histamines, etc., and increased blood flow and tissue permeability. The increase in edema and release of the prostaglandins and histamine may contribute to the pain sensation. The next series of events is the formation of proteases, phospholipases, and free radicals, which may lead to additional muscle tissue breakdown and pain. The last stage of events may result in a protective remodeling of the muscle to prevent muscle soreness with repeated or subsequent exercise (3,16,40). Therefore, the inflammatory response may be responsible for the initiation, amplification, and/or resolution of the skeletal muscle injury.

The model used in this study for the induction of DOMS resulted in similar measured parameters as presented by other investigators. The subjects' pain perception peaked around 24–72 h and returned to baseline by 7 d, which is consistent with other findings (4,48). The decreased ability to produce force noted in the isokinetic strength measured at $30° \cdot s^{-1}$ eccentrically and concentrically, the eccentric portion of $90° \cdot s^{-1}$, and the 90° isometric strength is similar with other findings (12). The loss of range of motion at 48, 96 h, and 168 h (7 d) compared with 0 h corroborates the findings of others (7).

As stated earlier, fish oils have been used to diminish other types of inflammatory conditions (rheumatoid arthritis (19,65) and atherosclerosis (33,68). Supplementing with fish oils high in long-chain polyunsaturated fatty acids, mainly EPA and DHA, has been shown to result in the incorporation of these fatty acids into the phospholipid bilayer of the cellular membrane. After the initiation of DOMS, we hypothesized the increase in EPA and DHA to result in the formation of the 3-series eicosanoids, which have less inflammatory properties than the 2-series eicosanoids formed from arachidonic acid. Concomitantly, the decrease in the amount of 2-series eicosanoids might result

in a decreased inflammatory response and less pain. However, the results from this study did not support our hypothesis with regard to the inflammatory response or perceived pain.

The levels of IL-6 seem to depend on the type of exercise involved. Brief bouts of maximal exercise (23,46) or more moderate exercise (57) do not appear to alter the levels of IL-6, whereas endurance types or prolonged exercise (24,30,51,67) and strenuous resistance eccentric exercise of larger muscle mass have generally shown significant increases in IL-6 (18,41). To our knowledge, there are no reported studies looking at the effects of eccentric exercise involving a smaller muscle mass, like the biceps brachii and IL-6 measurements. Many of the studies showing significant increases in IL-6 after eccentric exercise involve the quadriceps muscles (41) or knee flexor and extensor muscle groups (18). The endurance-type or prolonged exercises involve several of the larger muscle groups, especially with running, which can result in DOMS in the legs and back. Ostrowski et al. (52) found a positive correlation between peak IL-6 concentration and running intensity, which suggests that more intense exercises can lead to increased levels of IL-6. In a study looking at the impact of three different types of exercise on components of the inflammatory response, Brenner et al. (10) found significant increases in IL-6 only with the prolonged exercise but not with the circuit-training routine or the 5 min of cycle ergometer at 90% $\dot{V}O_{2max}$. These findings are consistent with the above-cited results. Brenner et al. (10) concluded that the lack of traditional markers of muscle injury and immune markers of the inflammatory response for the different types and intensities of exercise is due to modifications by the humoral and cardiovascular correlates of exercise. Jonsdottir et al. (35) found increased levels of IL-6 mRNA in the muscle after both eccentric and concentric contractions, suggesting that IL-6 production may not be exclusively due to muscle damage often associated with the eccentric portion of exercise. This further supports the lack of increase plasma IL-6 found in this study.

IL-6 may influence the metabolic changes in exercising muscles during longer duration types of exercise, due to its ability to induce hepatic glucose output and lipolysis (54,63). IL-6 has been shown to inhibit glycogen synthase activity and, in contrast, accelerate glycogen phosphorylase activity (36), which would lead to glycogen degradation and inhibition of glycogen synthesis. Several studies have shown that consuming carbohydrate during exercise can decrease the levels of IL-6 associated with exercise (47,49). Starkie et al. (61) reported carbohydrate ingestion attenuated plasma IL-6 production but not IL-6 mRNA in the vastus lateralis, suggesting the decreases seen in plasma IL-6 were from other tissues. Investigators looking at cycling and running (62) found that exercise-induced IL-6 production was not the result of circulating monocytes (60). More intense exercise, particularly involving the larger muscles, seems to have the highest levels of IL-6, which supports IL-6 as a beneficial chemical inductor for substrate availability, whose release may be from other tissues than muscle. The data presented show the induction of DOMS, without the increases in IL-6. Consequently, it seems that IL-6 may not be the best indicator of DOMS and may play a role in glycogen metabolism. Exercises of shorter duration and less intensity, similar to the protocol of this study, suggest that glucose availability would not be impaired. Thus, IL-6 may not be as necessary to aid in glycogen metabolism.

Prolonged exercise has been shown to result in elevations (14,30), no increases (24), or decrease in TNF-α (23). In the study by Brenner et al. (10), TNF-α was only significantly increased for the long exercise group. The results of this study did not show any significant increases in TNF-α. N-3 fatty acids have been shown to inhibit the synthesis and release of pro-inflammatory cytokines such as TNF-α, which are released during the early course of ischemic heart disease. This has been suggested as the principal cardio and neuroprotective mechanism of action(s) of n-3 fatty (20). However, the mechanism involved in DOMS may be different than the one involved in cardiac ischemia, due to the lack of treatment effect and lack of elevated TNF-α seen in this study. Prolonged types of exercise may lead to such a trauma, which may stimulate the acute phase response or the acute phase response may serve as a beneficial mechanism allowing for additional metabolic substrates necessary during these types of exercise. From the above-cited studies and the data presented, it does not appear that the TNF-α is an important consistent contributor to DOMS and may not be necessary to the inflammatory response as it relates to DOMS.

CK has commonly been used as an indicator of muscle injury but has shown considerable variability (59). Some studies show increases in CK (10), and others show no significant differences (66). The elevations in CK seen by Brenner et al. (10) did not parallel with the cytokines, which further suggests different mechanisms involved in the release of cytokines and muscle injury. The data presented in Table 2 did not show significant increases in CK, which may be due to the high variation seen between subjects. Some subjects reached extremely high levels of CK (27,450 $U \cdot L^{-1}$ and 12,645 $U \cdot L^{-1}$; others remained relatively unchanged (62–81 $U \cdot L^{-1}$, which is consistent with other findings (50). All attempts were made to eliminate activities that might cause increases in CK, and individuals were screened for prior training, which has been shown to have a protective effect (44). However, it is difficult to completely rule out these variables.

Henning et al. (32), looking at inflammatory mediators after an intermittent static plantar flexion of the ankle, found significant increases in TXB_2. Croisier et al. (17) found mean PGE_2 remained unchanged over time and were practically equal at each time point after either concentric or eccentric exercises of the knee extensor and flexor muscle groups. Measuring eicosanoids could have enhanced this study. Unfortunately, lack of serum sample prevented the possibility of these assays.

Many of the studies showing the increases in the cytokines IL-6 and TNF-α are employing the longer duration endurance types of exercise. The studies showing the in-

creased production of these cytokines with shorter strenuous exercises require a larger muscle mass, which may be necessary to achieve measurable increases in these inflammatory markers.

It has been well documented that exercise generates free radicals. Free radicals are primarily generated in the mitochondrial membrane, due to the leakage from the electron transport chain as a result of the increased electron flow needed to meet the higher energetic needs caused by physical exercise (43). The generation of free radicals, with their destructive properties, may cause tissue damage. Several studies have shown a decrease in the amount of free radicals using several antioxidants, like α-tocopherol, ascorbic acid, glutathione, or ubiquinone (29). Polyunsaturated fatty acids, EPA, and DHA are particularly susceptible to peroxidation resulting in the production of reactive oxygen species (ROS) (42). Therefore, α-tocopherol in this study has been supplemented with the fish oils and Western fat blend to minimize the oxidation of these fatty acids. In attempts to measure the amount of muscular damage by free-radical-mediated processes MDA, a by-product of lipid peroxidation can be used as a marker of fatty acid peroxidation (5,55). Several studies have shown increases in MDA after strenuous exercise (1,15,38,39). It has also been shown that iron can lead to the increased production of MDA (34). Due to the lack of studies examining the effects of exercise on free iron, it was of interest to look at the relationship of free iron and MDA production as it possibly relates to measurements involved in DOMS.

During intense exercise, the oxygen requirement for working muscle can increase up to 200-fold (15). This dramatic increase in aerobic respiration can lead to the formation of ROS, which have damaging effects when endogenous antioxidant supplies become overwhelmed. The generation of these ROS can generate lipid peroxidation and MDA, leading to the long-term damaging effects. In a study by Child et al. (15), plasma levels of MDA were increased after a simulated half-marathon run. Liu et al. (38) found significant increases in MDA in both fast and slow muscles in rats after chronic exercise (treadmill for 2 h 5 d·wk^{-1} for 8 wk). Similar results were found in a study by Alessio et al. (1), which found significant increases in both the fast- and slow-twitch muscles of rats after both moderate intensity and high intensity, but considerably higher levels with the higher intensity. In a study with humans, significant increases in MDA were found in exhaustive maximal exercise, whereas submaximal exercise ($<$70% $\dot{V}O_{2max}$) resulted in decreases in plasma MDA (39). The MDA levels for this study remained relatively unchanged. Consequently, it appears that the mechanism involved in DOMS may not alter lipid peroxidation. Perhaps the intensity of the above protocol is not enough to overwhelm the body's endogenous antioxidant system. The lack of change with regard to iron status is not surprising, due to the results of the MDA. Thus, it does not appear that iron plays a significant role in the signs and symptoms associated with DOMS when using this particular model. Iron status would be of interest to examine when using submaximal exhaustive-types of exercise.

CONCLUSIONS

The model used in this particular study was effective at inducing DOMS and was similar to parameters as measured by others. However, there were no significant differences between treatments, suggesting that FO and ISO at the doses supplemented were not effective in ameliorating DOMS with the above-cited protocol.

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