

JEPonline  
Journal of Exercise Physiologyonline

Official Journal of The  
American Society of Exercise Physiologists (ASEP)

ISSN 1097-9751  
An International Electronic Journal  
Volume 7 Number 1 February 2004

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Nutrition and Exercise

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CHRONIC SUPPLEMENTATION WITH FISH OIL INCREASES FAT OXIDATION  
DURING EXERCISE IN YOUNG MEN

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ABSTRACT

CHRONIC SUPPLEMENTATION WITH FISH OIL INCREASES FAT OXIDATION DURING EXERCISE IN YOUNG MEN. **Derek M. Huffman, Jody L. Michaelson, Tom R. Thomas.** JEPonline. 2004;7(1):48-56. Recent evidence suggests that omega-3 fatty acids from fish oil (FO) stimulate fat oxidation in liver and perhaps skeletal muscle. Our purpose was to examine the effect of an acute high-dose and a chronic low-dose of FO on fat oxidation during exercise. Seven recreationally active males (age 21-27 yr) jogged for 60 min at 60 % VO<sub>2</sub>max in three trials administered in random order: 1) no meal (NM), 2) 4 h following a high-fat meal (HFM), and 3) 4 h following an isocaloric HFM partly substituted with FO (HFM+FO). The FO supplement contained 60 % eicosapentaenoic acid, and 40 % docosahexaenoic acid. Subjects then supplemented 4 g/day of FO for 3 wk and while remaining on the supplementation regimen, repeated the same three trials in random order. Indirect calorimetry was used for the determination of oxygen consumption, respiratory exchange ratio, and energy expenditure from fat and carbohydrate. Heart rate, and rating of perceived exertion were also monitored for each test. The acute high-dose FO had no significant affect on fat use during exercise. In contrast, chronic supplementation significantly augmented total fat energy expenditure as compared to trials before supplementation in each of the three treatments versus trials prior to chronic supplementation (NM, 269.1 ± 49.8 v. 245.7 ± 36.2 Kcal, *P* = 0.009; HFM, 295.2 ± 40.2 v. 260.8 ± 36.4, *P* = 0.001; HFM+FO, 299.0 ± 38.7 v. 280.4 ± 35.9 Kcal, *P* = 0.002). These data suggest that chronic, but not acute FO supplementation enhanced the contribution of lipid during exercise in young active males.

**Key Words** Omega-3 fatty acids, Fat metabolism, Respiratory exchange ratio, Peroxisomal poliferator-activated receptor-alpha

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## INTRODUCTION

Omega-3 fatty acids (n-3fa) in the form of fish oil (FO) have garnered much attention for their triglyceride (TG) lowering affect (1,2). The mechanistic action of FO and other n-3fa is believed to involve the suppression of enzymes engaged in TG synthesis and stimulation of beta-oxidation in the liver (1,2). Recent studies have demonstrated novel pathways that polyunsaturated fatty acids (PUFA), and in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from FO, enhance fatty acid oxidation (3). PUFA such as EPA and DHA are believed to mediate the repartitioning of metabolic fuels by stimulating fatty acid oxidation and ketogenesis, inhibiting fatty acid and TG synthesis, and reducing concentrations of malonyl-CoA. In vitro studies suggest that metabolites of EPA and DHA are more potent activators of fat oxidation than other fatty acids via the binding of the transcription factor, peroxisomal poliferator-activated receptor-alpha (PPAR-a) (3).

Stimulation of PPAR-a leads to the induction of several genes involved in thermogenesis, fatty acid transport, and oxidation (4,5,6). Studies performed in animals (4) and humans (5) have reported enhanced fat oxidation and reductions in body fat mass with FO supplementation. However, no previous study has examined the effect of FO supplementation on fat oxidation during exercise. Since FO use appears to enhance fat oxidation at rest, we performed a pilot study to determine if FO supplementation resulted in increased fat use during exercise. Such an effect of FO could have potential health benefits for individuals with cardiovascular disease, diabetes, as well as an athletic performance benefit. We hypothesized that fat energy expenditure during exercise would be augmented with acute supplementation of FO, and further magnified with chronic FO supplementation.

## METHODS

### Participants

Subjects were seven men, ages 21-27 yr, their characteristics are presented in Table 1. All had been participating in a regular exercise program that included aerobic and weight training for 3-5 days/wk for at least 1 yr. Although the mode of aerobic exercise differed slightly among subjects, each was primarily engaged in some form of weight-bearing activity (i.e. basketball, soccer, jogging). All subjects provided informed consent approved by the University of Missouri Health Sciences IRB, and completed a health history questionnaire, physical activity questionnaire, and dietary history/daily supplement questionnaire. Any person having more than one major CVD risk factor, or any other disease symptom (7), or if they were currently taking FO supplements were not allowed to participate.

### Maximal testing

Each volunteer completed a running maximal oxygen consumption ( $VO_{2max}$ ) test on a treadmill. The protocol that was used to determine  $VO_{2max}$  has been used previously in our lab (8). All data were collected via a computer-interfaced system comprising an Ametek S3A/I oxygen analyzer, a Parvo Medics  $CO_2$ -100 gas analyzer, and a Hewlett Packard 47303A Digital Pneumotach (Vertek Series). Computations of indirect calorimetry were performed using custom programmed software (Rayfield, Inc., Vermont). Oxygen uptake, heart rate (HR), rating of perceived exertion (RPE), and the electro-cardiogram (ECG) were all monitored and recorded during each test.

### Body composition

Body composition was assessed by underwater weighing. Measurements were carried out according to methods previously described (9). Weight in air was measured using a standard Toledo scale, and weight in water was taken while in the seated position. Residual volume was determined by the helium dilution technique using a Collins Modular Lung Analyzer that includes a helium analyzer. The highest three underwater weight measurements were averaged and used to determine percentage body fat using the Siri formula (10).

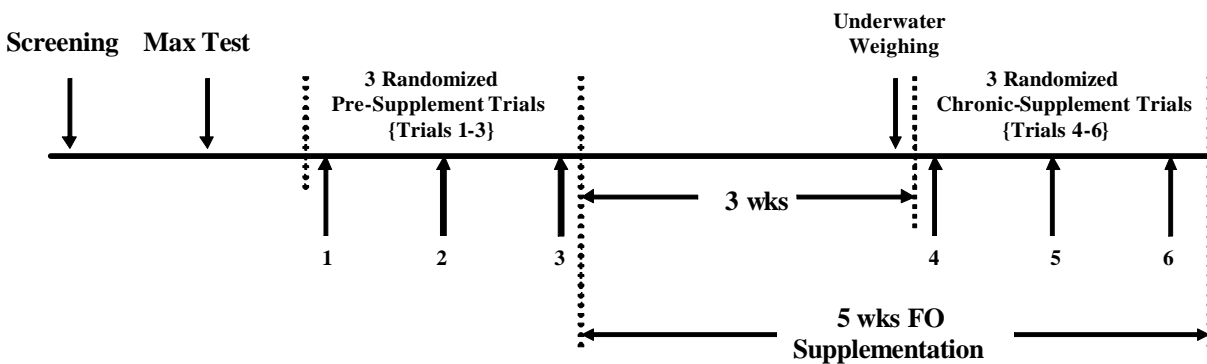
### Subject Preparation

In order to better standardize food intake, subjects were asked to write down everything eaten during the three days prior to their first trial and to consume only easily accessible foods during the third day which served as the 24 hr control. Each was then asked to follow their diet record for the three days, which included the 24 hr control preceding all remaining trials. For the 24 hr control, subjects consumed the exact foods they recorded

during the third day of their first diet record in both quantity and quality. In addition, a second diet record was collected prior to the last trial in order to assess the consistency of food intake throughout the study. Subjects were also asked to get adequate sleep, abstain from vigorous activity for 48 hr, and refrain from alcohol and caffeine use for 24 hr prior to testing. Telephone calls and e-mails were made at least three days beforehand as a reminder of the upcoming trial, and to encourage adherence to all guidelines. In addition, subjects were advised to maintain their exercise and dietary habits throughout the course of the study.

### Study design

The study design is illustrated in Figure 1. Participants completed six exercise bouts. The first three trials were performed in random order: 1) no meal (NM Trial), 2) 4 hr following a HFM (HFM Trial), and 3) 4 hr following an isocaloric HFM partly substituted with FO (HFM+FO Trial). Each was then started on a 3 wk interval of FO supplementation (4 g/day). After the completion of the 3 wk interval, the remaining three trials were performed while remaining on FO supplementation. These three trials are referred to as: 4). Chronic-no meal (C-NM Trial), 5). Chronic-HFM (C-HFM Trial), and 6). Chronic-HFM+FO (C-HFM+FO Trial); (“C” denotes Chronic for each Chronic Trial abbreviation). Trials in each group of three (ie. pre-supplementation trials, chronic-supplement trials) were separated by approximately 1 week.



**Figure 1: Study timeline for all trials and testing.** Subjects were initially screened and tested for maximal oxygen consumption on visits one and two to the lab respectively. Prior to chronic supplementation, three trials (1-3) were performed in randomized order (NM, HFM, HFM+FO), and each were separated by approximately 1 wk. Chronic supplementation (4 g/day) of FO only was then initiated for 3 wks and was maintained during the second round of trials (4-6) until study completion. The supplementation trials (4-6) were also performed in random order (C-NM, C-HFM, C-HFM+FO), and each were separated by approximately 1 wk. Completion time for each subject was 9 wks. Max Test = maximal oxygen consumption test; FO = fish oil; Pre-Supplementation Trials = trials performed prior to chronic FO supplementation; Chronic-Supplement Trials = trials performed during chronic FO supplementation.

### Fish oil supplementation

FO supplementation was administered in the form of gel capsules (Super EPA-500, Bronson Pharmaceutical, St. Louis, MO). Each tablet contained 300 mg of eicosapentaenoic acid (EPA) and 200 mg of docosahexaenoic acid (DHA) for a total of 500 mg of n-3fa. For the high-dose of FO (i.e., HFM+FO Trial and C-HFM+FO Trial), subjects consumed the appropriate number of capsules relative to body weight (0.2 g/kg body weight). Since the mean body weight was 89 kg (Table 1), a typical amount consumed was 32-44 capsules with the test meal, for a total of 16-22 g of FO. This value approaches those used in a study by Harris et al. (2), which amounted to 24-28 g/day of FO. For the chronic dose, each subject consumed eight capsules with their meals over the course of the day (4 g/day); for example, two with breakfast, three with lunch and two with dinner. Our group has used this low-dose supplementation regimen previously (8).

### Test meals

High-fat meals (HFM) administered were similar to those used previously (8). The test meals were given 4 hr prior to exercise and consisted of a specialty ice cream and heavy whipping cream blended together in

milkshake form (84.3 % calories from fat). In addition, an isocaloric HFM with FO capsules was given in which the FO substituted for approximately 15.4 % of the whipping cream, such that the caloric value and fat content of each test meal were identical. The HFM contained primarily long-chain TG which were composed chiefly of the most common dietary forms of fatty acids (palmitic, 16:0; steric, 18:0; oleic, 18:1). However the two high-dose FO meals which contained 0.2 g of FO per kg body weight was substituted for 15.4 % of the fat from the whipping cream, and thus contained lesser amounts of these fatty acids. Each shake consisted of 1.3 g fat, 0.3 g carbohydrate (CHO), and 0.05 g protein (PRO) per kg body weight for a total of 14.5 Kcal/kg body weight. Thus, an 89 kg male would have consumed: 1285 Kcal, 115 g of fat, 30 g of CHO, and 5 g of PRO.

### Exercise Trials

Each trial consisted of jogging for 60 min at 60 %  $\text{VO}_2\text{max}$ ; the appropriate exercise intensity was achieved within the first 10 min of exercise. Intensity was then controlled on ensuing trials by using the same progression in treadmill speed. Data collection included HR and RPE; expiratory gases were collected and used to determine oxygen consumption and energy expenditure. The respiratory exchange ratio (RER) was used to estimate substrate oxidation from fat and carbohydrate. Values were calculated by taking 5 min averages at 15 min intervals throughout the 60 min of exercise.

### Statistical Analysis

Within-group analyses were performed using a two-way ANOVA (trial x time) with repeated measures design. Dietary variables were compared using paired t-tests. Significant F ratios ( $P < 0.05$ ) were followed up using planned contrasts. Based upon a previous study (5), it was determined that a statistical power of 0.75 would be necessary to detect significant differences between treatments with a sample size of  $n=7$ . All values are expressed as means  $\pm$  SD.

## RESULTS

The results for 3-day diet records are presented in Table 2. There were no significant differences in food intake between diet records taken before trials 1 and 6 respectively, but trends were observed for dietary fat ( $P=0.065$ ) and CHO intake ( $P=0.079$ ). The data from measures taken during the exercise bouts including HR (beats/min),  $\text{VO}_2$  (L/min), and RPE are presented in Table 3. HR (beats/min),  $\text{VO}_2$  (L/min), and RPE were not different among trials, but all significantly increased across time,  $P < 0.05$ . For all trials, average caloric expenditure was  $689 \pm 5$  Kcal, oxygen consumption was  $2.43 \pm 0.02$  L/min (59.0 % of  $\text{VO}_2\text{max}$ ), at a mean HR of  $150 \pm 2$  beats/min and RPE of  $11.7 \pm 0.2$ .

**Table 1. Physical Characteristics of Subjects.**

Variable	Mean $\pm$ SD
Age, y	23.5 $\pm$ 1.9
Body weight, kg	88.6 $\pm$ 12.4
Body fat, %	19.5 $\pm$ 8.7
BMI, kg/m <sup>2</sup>	28.1 $\pm$ 2.1
Activity, times/wk	4.7 $\pm$ 1.1
Activity, hr/wk	5.5 $\pm$ 2.9
$\text{VO}_2\text{max}$ , ml/kg/ $\dot{V}$ min	46.1 $\pm$ 4.5
$\text{VO}_2\text{max}$ , L/min	4.2 $\pm$ 0.5

**Table 2. Composition of Diet from 3-Day Diet Records.**

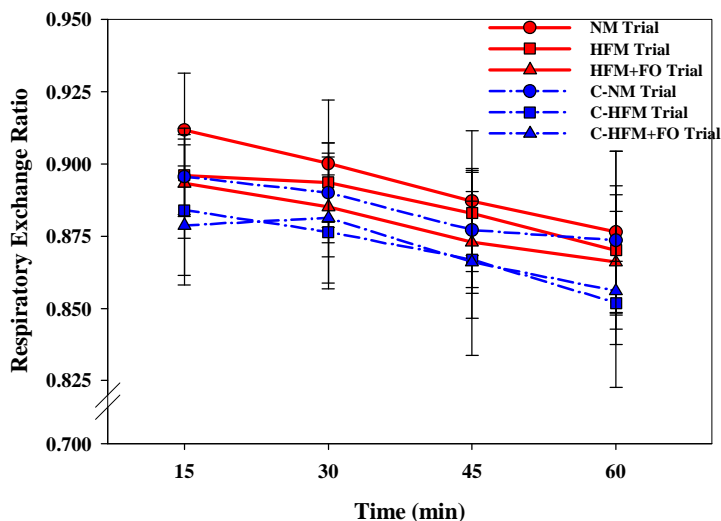
Variable	Trial 1	Trial 6	P
<b>Total Energy, (Kcal/day)</b>			
<b>Dietary fat, %</b>	27.9 $\pm$ 4.5	32.2 $\pm$ 5.6	0.065
<b>Saturated fat, %</b>	8.9 $\pm$ 1.6	10.3 $\pm$ 1.9	0.318
<b>Monounsaturated fat, %</b>			
<b>Polyunsaturated fat, %</b>	4.2 $\pm$ 1.6	5.1 $\pm$ 1.6	0.172
<b>Protein, %</b>	16.2 $\pm$ 4.0	16.0 $\pm$ 2.9	0.695
<b>Carbohydrate, %</b>	54.1 $\pm$ 6.3	50.7 $\pm$ 7.1	0.079

Values are means  $\pm$  SD. 3-day diet records were completed prior to the first and last (sixth) trial. The diet record administered prior to trial 1 was intended to provide a template for all trials, and the diet record given prior to trial 6 was used to verify consistency in food intake over the course of the study. There were no significant differences for dietary variables between diet records;  $P > 0.05$ .

**Table 3. Comparison of Exercise Monitors Between Trials.**

Variable	Trial	15 min	30 min	45 min	60 min
<b>Oxygen Consumption</b> (%VO <sub>2</sub> max)	NM	57.4 ± 2.0	58.7 ± 2.7	59.6 ± 3.0	59.8 ± 3.3
	HFM	57.5 ± 3.3	58.4 ± 2.6	58.6 ± 2.5	59.4 ± 2.8
	HFM+FO	57.8 ± 1.6	59.3 ± 1.2	60.2 ± 1.7	60.5 ± 1.2
	C-NM	57.0 ± 2.0	58.6 ± 2.0	58.6 ± 2.7	59.8 ± 1.7
	C-HFM	56.8 ± 1.5	58.6 ± 1.3	59.5 ± 2.7	60.3 ± 1.7
	C-HFM+FO	57.9 ± 2.2	59.9 ± 1.7	60.1 ± 1.7	60.3 ± 2.7
<b>Heart rate</b> (beats/min)	NM	141 ± 11	147 ± 10	151 ± 11	155 ± 13
	HFM	141 ± 14	145 ± 12	151 ± 14	154 ± 15
	HFM+FO	145 ± 15	152 ± 15	156 ± 16	159 ± 16
	C-NM	139 ± 7	146 ± 6	150 ± 8	153 ± 10
	C-HFM	143 ± 8	150 ± 7	153 ± 9	156 ± 9
	C-HFM+FO	141 ± 8	150 ± 8	153 ± 9	156 ± 10
<b>Rating of Perceived Exertion (RPE)</b>	NM	10.6 ± 0.5	11.4 ± 0.8	12.0 ± 1.2	12.4 ± 1.0
	HFM	10.4 ± 0.7	11.4 ± 0.5	11.9 ± 0.7	12.3 ± 1.4
	HFM+FO	10.7 ± 0.6	11.6 ± 0.8	12.3 ± 1.4	12.9 ± 1.3
	C-NM	10.4 ± 0.5	10.7 ± 0.8	11.6 ± 0.8	12.3 ± 1.1
	C-HFM	10.7 ± 0.5	11.0 ± 0.6	11.6 ± 1.0	12.3 ± 1.3
	C-HFM+FO	10.4 ± 0.5	11.1 ± 0.9	12.4 ± 1.6	12.7 ± 1.5

Values are means ± SD. There were no significant differences between trials,  $P > 0.05$ . NM Trial = no meal trial, HFM Trial = high fat meal trial; HFM+FO Trial = high-fat meal with high-dose FO; C-NM Trial = no meal with chronic FO; C-HFM Trial = high-fat meal with high-dose FO trial; C-NM Trial = no meal with chronic FO trial; C-HFM Trial = high-fat meal with chronic FO trial; C-HFM+FO Trial, high-fat with high-dose FO and chronic FO trial.



**Figure 2. Comparison of respiratory exchange ratio among trials.** Values are means ± SD. There were no significant differences observed among trials,  $P > 0.05$ . NM Trial = no meal trial, HFM Trial = high fat meal trial; HFM+FO Trial = high-fat meal with high-dose FO; C-NM Trial = no meal with chronic FO; C-HFM Trial = high-fat meal with high-dose FO trial; C-NM Trial = no meal with chronic FO trial; C-HFM Trial = high-fat meal with chronic FO trial; C-HFM+FO Trial, high-fat with high-dose FO and chronic FO trial.

The results for RER are illustrated in Figure 2. A weak trend for trial differences in RER was observed ( $P=0.171$ ), but this was not statistically significant. Total caloric expenditure was also not significantly different among trials (Figure 3); but a significant main effect for Trial was observed for fat energy expenditure ( $P<0.001$ , Figure 4) as well as a significant Trial $\times$ Time interaction for fat energy expenditure (data not shown),  $P<0.001$ .

In regards to fat energy expenditure, *post hoc* comparisons revealed a significant increase in fat use for all exercise trials with chronic FO intake as compared to similar treatments before chronic supplementation (Figure 4). Specifically, total fat energy expenditure (Kcal) was significantly higher with chronic FO supplementation versus before chronic supplementation for the NM ( $269.1 \pm 49.8$  v.  $245.7 \pm 36.2$  Kcal,  $P = 0.009$ ), HFM ( $295.2 \pm 40.2$  v.  $260.8 \pm 36.4$ ,  $P = 0.001$ ), and

HFM+FO treatment ( $299.0 \pm 38.7$  v.  $280.4 \pm 35.9$  Kcal,  $P = 0.002$ ) respectively.

## DISCUSSION

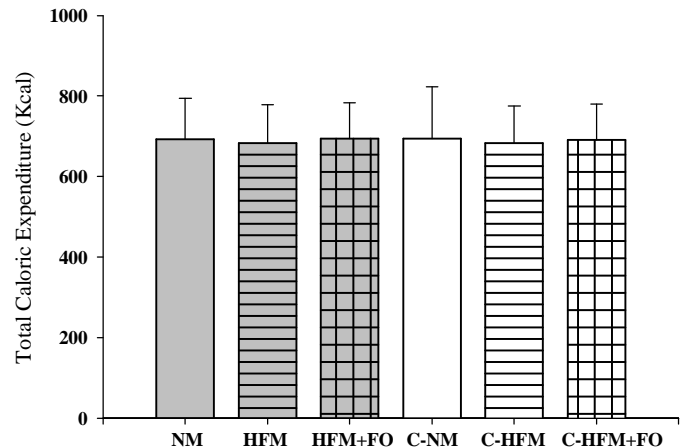
Our purpose was to explore the impact of FO supplementation on the metabolic response to a single bout of aerobic exercise. Chronic FO intake significantly augmented fat energy expenditure during exercise between each of the three types of trials (NM, HFM, HFM+FO). However, compared to the HFM, no significant effect was observed on fat use when FO was given as a bolus 4 h prior to exercise.

### Testing Standardization

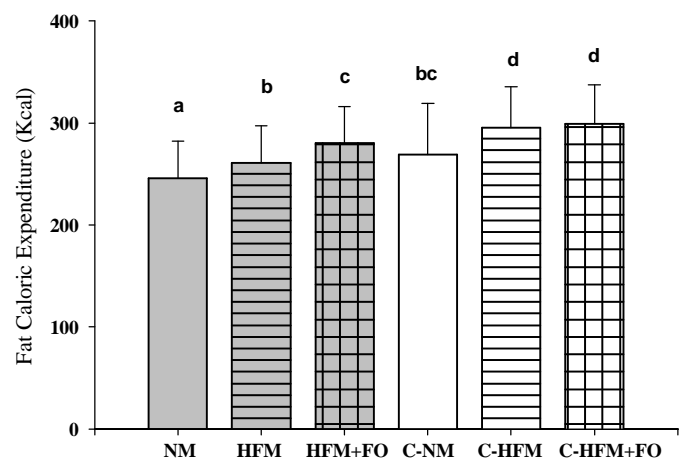
We attempted to control for several factors that could have made a significant impact on substrate use including nutrient intake, dietary and activity habits, and physical activity levels prior to exercise; however all these variables were self-reported. The FO supplementation was controlled by pill counting, and the ingestion of all test meals was monitored. We also attempted to recruit subjects of similar age, percent body fat, and training status to minimize the impact of these variables on exercise metabolism. Because of the volume and length of exercise bouts, we chose to recruit recreationally active individuals in order to minimize a training effect. A training effect might have resulted in an increase in fat use since endurance training is known to enhance lipid oxidative capacity during exercise and conserve muscle glycogen (reviewed in 11,12). The treadmill runs were somewhat longer than what most of the participants typically performed, but the exercise protocol used was of an intensity (60%  $\text{VO}_2\text{max}$ ) and duration (1 hr) that was most appropriate to enhance fat oxidation in recreationally active individuals. In addition, since the exercise intensity was relatively low and each test was separated by at least 1 wk with an additional 3 wks in between the third and fourth trial, a training-effect over the course of the study was unlikely.

### Indirect calorimetry

It might be argued that indirect calorimetry was not the optimal method for measuring substrate utilization since the type and rate of substrate oxidation are only estimates of *in vivo* metabolism using gas exchange measurements (13). Indeed RER is an indirect estimate of substrate use and cannot be generalized to any particular tissue (14). However, RER is very good when evaluating total-body substrate oxidation during exercise as opposed to single limb activity, particularly during steady-state conditions (15). Furthermore, the calculation of substrate utilization from RER measurements makes the assumption that fat and carbohydrate



**Figure 3: Comparison of total caloric expenditure among trials. Values are means  $\pm$  SD in Kcal. There were no significant differences observed among trials,  $P > 0.05$ . NM Trial = no meal trial, HFM Trial = high fat meal trial; HFM+FO Trial = high-fat meal with high-dose FO trial; C-NM Trial = no meal with chronic FO trial; C-HFM Trial = high-fat meal with chronic FO trial; C-HFM+FO Trial, high-fat with high-dose FO and chronic FO trial.**



**Figure 4. Comparison of total fat caloric expenditure among trials. Values are means  $\pm$  SD in Kcal. Different letters denote a significant difference among trials,  $P < 0.05$ . NM Trial = no meal trial, HFM Trial = high fat meal trial; HFM+FO Trial = high-fat meal with high-dose FO trial; C-NM Trial = no meal with chronic FO trial; C-HFM Trial = high-fat meal with chronic FO trial; C-HFM+FO Trial, high-fat with high-dose FO and chronic FO trial.**

comprise 100% of the substrate pool with 0% contribution from amino acids and proteins. Although it is likely that amino acids and protein were catabolized during exercise, their involvement was likely minimal due to the amount and intensity of the exercise bouts.

### **Meal Timing**

The results of this investigation are in agreement with previous studies that have shown a significant impact of a pre-exercise meal on substrate utilization during exercise (11,12). We hypothesized that by administering the HFM (84.3% fat) 4 hr prior to exercise, the amount of energy that was derived from lipid sources would be magnified and aid in the discrimination of potential differences between treatments. Binnert et al. (18) has previously demonstrated that TG and FFA concentrations peak at 3-4 hr following a meal. Thomas et al. (8) also observed a 60-70% increase in plasma TG 4 hr after ingestion of a HFM.

### **Fish oil and PPAR-a**

The unique finding in this study was a significant increase in fat oxidation during exercise with chronic supplementation of FO. Similar findings in animals and humans at rest have been reported with FO (4, 5) and PPAR-a agonist (fibrates) administration (19). EPA and DHA from FO have been unequivocally demonstrated to be a more potent activator of PPAR-a than other types of fatty acids of the n-6 (linoleic acid, 18:2) and n-3 (linolenic, 18:3) variety (3). Couet et al. (5) reported a significant increase in resting energy expenditure, fat oxidation, and a significant decrease in fat mass and resting RER with 6 g of FO supplementation for 3 wk. Our results suggest that EPA and DHA from FO may also play a role in modulating the partitioning of fuel sources during exercise. When performing physical activity, contracting skeletal muscle becomes the primary consumer of energy, skeletal muscle blood flow is profoundly increased, and liver blood flow is dramatically reduced (20). As a result, it is likely that such an effect on fat use during exercise would implicate the involvement of skeletal muscle PPAR-a and not hepatic PPAR-a. In addition, Muoio et al. (21) demonstrated *in vitro* that PPAR-a is abundantly expressed in skeletal muscle and that its activation caused a robust increase in lipid oxidation and reduced accumulation of TG in primary human myotubes.

Although the indirect calorimetry data is compelling, the collection of skeletal muscle biopsies for the direct measurement of malonyl-CoA, and the gene expression of fat oxidation enzymes such as pyruvate dehydrogenase kinase, and carnitine palmityltransferase-I would have provided better evidence for the involvement of PPAR-a. It is also important to note that the necessary dosage of EPA and DHA needed to activate PPAR-a in skeletal muscle has not been established. According to Clarke et al. (3), the body of evidence from human, animal, and *in vitro* work suggests that doses as low as 2-5 g/day are sufficient to alter hepatic gene expression and affect hepatic repartitioning of fuels. Our data would suggest that a 4 g dose for 3 wks is enough to influence the activities of PPAR-a in skeletal muscle.

Despite the lack of direct evidence, the likelihood of PPAR-a mediating the increase in fat oxidation during exercise is strengthened for two reasons. First, while the accepted respiratory quotient (RQ) for fat has been reported to be around 0.707 - 0.710, Livesey and Elia (22) demonstrated that the RQ for EPA and DHA were considerably higher at 0.759 and 0.762 respectively. Secondly, several investigators have reported profound reductions in blood lipids including plasma TG (2) and free fatty acids (23) with FO supplementation. These findings would suggest that FO supplementation would result in a higher RER and attenuated fat oxidation due to greater abundance of EPA and DHA and less lipid substrate available during exercise. But the evidence in the literature supports the notion of increased fat oxidation at rest (4,5) and the present investigation indicates that FO supplementation results in increased fat oxidation during exercise; each of these scenarios is likely the result of the facilitating effect of EPA and DHA on hepatic and skeletal muscle PPAR-a respectively.

### **SUMMARY**

Chronic FO intake did not significantly attenuate RER but did increase fat energy expenditure during exercise among all trials (NM, HFM, HFM+FO) in recreationally active males. However, an acute bolus of FO did not result in an independent or additive effect on fat oxidation. These data suggest that by increasing fat use during

exercise, chronic FO supplementation may have additional ergogenic benefits for health as well as athletic performance. Future studies directly measuring the effect of FO on PPAR- $\alpha$  activity in human skeletal muscle should be done to confirm the present findings. In conclusion, chronic FO supplementation increases fat energy expenditure during moderate-intensity exercise in young recreationally active males.

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## ACKNOWLEDGEMENTS

The authors thank Dr. Bryan Smith and Owen Donahue for their help with data collection and each of the subjects for their willing participation. This study was supported by the University of Missouri Research Council and the Elizabeth Hegarty Foundation.

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