The Journal of Nutrition
Nutrition and Disease

Acute Fish Oil and Soy Isoflavone Supplementation Increase Postprandial Serum (n-3) Polyunsaturated Fatty Acids and Isoflavones but Do Not Affect Triacylglycerols or Biomarkers of Oxidative Stress in Overweight and Obese Hypertriglyceridemic Men[1,2]

Heather E. C. Hanwell,[3] Colin D. Kay,[4] Johanna W. Lampe,[5] Bruce J. Holub,[3] and Alison M. Duncan[3]*

[3]Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, Ontario, Canada, N1G 2W1; [4]School of Medicine, Diet and Health Group, University of East Anglia, Norwich, UK, NR4 7TJ; and [5]Fred Hutchinson Cancer Research Center, Seattle, WA 98109

Downloaded from jn.nutrition.org at Univ of Guelph Library GST# R134892330 on July 8, 2009

Abstract

Chronic consumption of fish and fish oil high in (n-3) PUFA reduces triacylglycerols (TG) but may increase oxidative stress, whereas consumption of soy isoflavones may reduce oxidative stress. Elevated serum TG and oxidative stress are considered cardiovascular disease (CVD) risk factors, but the effects of acute (n-3) PUFA and soy isoflavones on these CVD risk factors are unknown. The purpose of the study was to determine the effects of acutely supplementing a high-fat, high-fructose meal with fish oil and isoflavone placebo (FO) and fish oil placebo and soy isoflavones (ISO). In a randomized, double-blind, placebo-controlled, crossover study, 10 overweight or obese men consumed a high-fat, high-fructose meal with 4 dietary supplement combinations: fish oil placebo and isoflavone placebo (placebo); fish oil and isoflavone placebo (FO); fish oil placebo and isoflavones (ISO); and fish oil and isoflavones (FO + ISO). Serum collected at baseline and at 2, 4, and 6 h postprandially was analyzed for fatty acids, isoflavones, TG, and oxidative stress biomarkers (lipid hydroperoxides, oxidized-LDL, total antioxidant status). FO significantly increased serum (n-3) PUFA and ISO increased serum isoflavones. The study meal significantly increased serum total fatty acids and TG without affecting oxidative stress biomarkers. Serum TG and oxidative stress biomarkers did not differ between treatments. The FO and ISO were bioavailable but did not attenuate the postprandial rise in serum TG. Neither the study meal nor the FO or ISO induced significant changes in oxidative stress biomarkers. The current study adds to a limited literature on the acute effects of FO and ISO interventions on postprandial biomarkers of CVD risk. J. Nutr. 139: 1128–1134, 2009.

Introduction

Studies indicate that supplementation with fish oil rich in (n-3) PUFA (1) and soy rich in isoflavones (2) reduces cardiovascular disease (CVD)[6] risk. Numerous mechanisms for this protection have been explored, 2 of which include (n-3) PUFA-induced reduction in circulating triacylglycerols (TG) (1,3) and soy isoflavone-induced reduction in biomarkers of oxidative stress (2). The effects of chronic consumption of (n-3) PUFA or soy isoflavones on CVD risk biomarkers have predominantly been studied in the fasted state; however, the postprandial state is also relevant (4), particularly because commonly consumed high-fat, high-fructose meals significantly increase circulating TG (5–9) and biomarkers of oxidative stress (10–12). Taken together, these ideas have prompted scientific interest in the exploration of interventions targeted for the postprandial period to reduce CVD risk (4).

Consumption of fish or fish oil, rich in the (n-3) PUFA, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), reduces fasting and postprandial serum TG (7,13,14) and is inversely associated with CVD risk (15–17). However, (n-3) PUFA can also increase oxidative stress and susceptibility of

[1] Supported by the Hannam Utilization Soybean Fund, Heart and Stroke Foundation of Ontario, and donations from Ocean Nutrition Canada and Archer Daniels Midland Company.

[2] Author disclosures: H. E. C. Hanwell, C. D. Kay, J. W. Lampe, B. J. Holub, and A. M. Duncan, no conflicts of interest.

[6] Abbreviations used: BIA, bioelectrical impedance analysis; CVD, cardiovascular disease; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FO, fish oil and isoflavone placebo; FO + ISO, fish oil and isoflavones; HDL-C, HDL-cholesterol; iAUC, incremental area under the curve; ISO, fish oil placebo and soy isoflavones; LOOH, lipid hydroperoxide; ox-LDL, oxidized LDL; placebo, fish oil placebo and isoflavone placebo; TAS, total antioxidant status; TC, total cholesterol; TG, triacylglycerol.

* To whom correspondence should be addressed. E-mail: amduncan@uoguelph.ca.

0022-3166/08 $8.00 © 2009 American Society for Nutrition.
Manuscript received January 28, 2009. Initial review completed February 5, 2009. Revision accepted March 4, 2009.
First published online April 1, 2009; doi:10.3945/jn.109.105171.

LDL to oxidation (18–23). Therefore, the combination of antioxidants with (n-3) PUFA represents a potential dietary strategy to confer the benefits of reduced serum TG while mitigating the potential for increased oxidative stress.

Soy isoflavones are candidate antioxidants for combination with (n-3) PUFA to mitigate the potential for increased oxidative stress. The molecular structure of soy isoflavones contains multiple hydroxyl groups capable of conferring antioxidant activity (24–27). The ability of soy isoflavones to reduce biomarkers of oxidative stress has been demonstrated in cell culture (28–30), animal (31–33), and human (34–39) studies. In addition to their inherent antioxidant activity, soy isoflavones can also elevate the expression and activity of antioxidant enzymes (40–47). Thus, there is rationale to hypothesize that soy isoflavones may mitigate the adverse prooxidant effects of (n-3) PUFA consumption.

The surge of scientific and public interest in the role of dietary supplements in human health has evolved into the consideration of dietary supplement combinations that have the potential to elicit additive or synergistic effects. This concept is exemplified by combining the serum TG-lowering effect of fish oil (n-3) PUFA with the antioxidant effect of soy isoflavones to counteract the (n-3) PUFA-induced increase in oxidative stress, thereby overall maximizing the reduction of CVD risk. In particular, the effects of these 2 dietary supplements could be combined during the postprandial period following a high-fat, high-fructose meal known to elevate both serum TG and oxidative stress. Thus, the purpose of this study was to investigate the effect of acutely supplementing a proatherogenic high-fat, high-fructose meal with fish oil (n-3) PUFA, soy isoflavones, and their combination on postprandial serum TG and oxidative biomarkers in overweight and obese participants with elevated fasting serum TG.

Materials and Methods

Study design. This study utilized a randomized, double-blind, placebo-controlled crossover design. Participants (n = 10) consumed a high-fat, high-fructose breakfast test meal along with a specific combination of dietary supplements on each of 4 study days separated by 1-wk washout periods. For the duration of the study, participants were instructed to maintain their habitual lifestyle and dietary habits with the exception of avoiding dietary supplements as well as any sources of fish, (n-3) PUFA, soy, and isoflavones. Participants were also instructed to avoid over-the-counter medications, strenuous activity, and alcohol for 48 h before each study day and to consume a standardized meal on the evenings before each study day. For 12 h prior to each study day, participants avoided all food and drink except for water. The study received ethical approval from the Human Research Ethics Board of the University of Guelph and Clinical Trial approval from the Natural Health Products Directorate of Health Canada.

Participant recruitment and screening. Participants were recruited through newspaper advertisements, e-mails, recruitment posters, and word of mouth. To maximize the likelihood of observing an increase in postprandial TG and oxidative stress, overweight or obese (BMI 25–34.9 kg/m^2) healthy men > 45 y old with fasting serum TG > 1.5 mmol/L were recruited. Individuals were excluded if they reported: current or previous disease, including any form of CVD; use of medications that could affect the study endpoints; use of dietary supplements (a 4-wk prestudy washout was permitted); use of tobacco or recreational drugs; a >5-kg body weight change within the past 6 mo; allergy to fish, soy, or other components of the study supplements or study test meal; antibiotic use within the past 6 mo; or elite athletic training. All participants attended a study orientation, were provided with a comprehensive study handbook, and provided written consent.

Study test meal and dietary supplements. On each of the 4 study days, participants consumed a high-fat, high-fructose meal (**Table 1**) that consisted of 2 sausage and egg McMuffins (McDonald's) and a KoolAid drink that contained 0.75 g crystalline fructose/kg body weight to increase the magnitude of postprandial TG elevation (48–50).

Along with the study test meal, participants consumed 1 of 4 dietary supplement combinations in a randomized order: fish oil placebo and isoflavone placebo (placebo); fish oil and isoflavone placebo (FO); fish oil placebo and isoflavones (ISO); and fish oil and isoflavones (FO + ISO). To ensure a random assignment of treatment order, the 24 potential treatment orders were placed in an envelope and as each participant was enrolled into the study, an order was drawn from the envelope without replacement.

The fish oil supplement was provided by Ocean Nutrition Canada in a capsule form containing 1000 mg of refined fish oil concentrate providing 400 mg EPA and 200 mg DHA. The fish oil treatment consisted of 7 fish oil capsules for a total dose of 7.0 g refined fish oil containing 2.8 g EPA and 1.4 g DHA. The fish oil placebo supplement, also provided by Ocean Nutrition Canada, was identical in appearance to the fish oil supplement but contained 1000 mg of corn oil providing ~540 mg linoleic acid, 300 mg oleic acid, 110 mg palmitic acid, and <10 mg linolenic acid.

The soy isoflavone supplement was provided by Archer Daniels Midland Company in a powder form (NovaSoy 400 Soy Isoflavone Extract) that contained 336 mg NovaSoy providing 150 mg glycoside isoflavones (equivalent to 96 mg aglycone isoflavones) in isoflavone proportions of 1.05:1.0:0.29 for genistein:daidzein:glycitein. The NovaSoy 400 powder was dissolved with the KoolAid drink. The soy isoflavone placebo supplement was also in a powder form and consisted of graham cracker crumbs selected to closely match the soy isoflavone powder in taste, color, and texture.

Data collection

Anthropometrics. Height was measured at the beginning of the study using a metric measuring tape posted against the wall while participants were barefoot. Body weight was measured on every study day on a calibrated digital scale (ES200L, OHAUS) with participants barefoot in light clothing. For participants whose body weight exceeded 100 kg (n = 3), a calibrated imperial beam scale (Health-O-Meter, Continental Scale) was used. Waist and hip circumferences were measured at the beginning of the study using a metric measuring tape; waist circumference was measured at the midway point between the iliac crest and the lowest rib and hip circumference was measured around the widest part of the buttocks. Blood pressure and heart rate were measured at the beginning of the study using a blood pressure monitor (UA-767PC Blood Pressure Monitor, A&D Medical) after 5 min of rest. Body composition was measured at the beginning of the study using bioelectrical impedance analysis (BIA) (BodyStat 1500, BodyStat). To optimize BIA results, participants were instructed to consume at least 2 glasses of water the evening before their BIA measurement and to avoid strenuous exercise on the morning of their BIA measurement.

Food records. Participants completed food records for 3 consecutive days (2 weekdays and 1 weekend day) during the week prior to each

TABLE 1 Study test meal composition[1,2]

Energy, *kJ*	4887
Carbohydrate, *g*	130
Total fat, *g*	52
SFA, *g*	20
Trans fat, *g*	2
Cholesterol, *mg*	445
Protein, *g*	40

[1] Values are based on the mean body weight of participants at study entry, because energy and carbohydrate intake varied due to the provision of fructose at 0.75 g/kg body weight.

[2] Meal composition represents a combination of values from McDonald's and the amount of fructose in the study KoolAid drink.

Downloaded from jn.nutrition.org at Univ of Guelph Library GST# R134892330 on July 8, 2009

study day. Detailed instructions were provided and completed food records were reviewed to ensure clarity and accuracy. Food records were analyzed using ESHA Food Processor (version 9.81, ESHA Research) and 3-d means were calculated prior to statistical analysis.

Blood samples. Blood samples were collected on every study day at baseline (time 0) and at 2, 4, and 6 h following consumption of the study test meal. Samples were collected into anticoagulant-free collection tubes, left to clot at room temperature for 30 min then centrifuged at 1500 × *g*; 15 min at 5°C. The collection tubes were then placed on ice while the serum was extracted, pooled, and aliquotted into cryovials, which were flash-frozen in liquid nitrogen and stored at −80°C until analysis.

Biochemical analyses

Serum fatty acids. Serum samples from all treatments at time points 0 and 4 h were analyzed in 1 batch for fatty acids including EPA, DHA, and total (n-3) fatty acids using a method adapted from Stark and Holub (51). Briefly, serum samples underwent the Folch extraction prior to analysis on a Varian 3400 gas-liquid chromatograph with a 60-m DB-23 capillary column (0.32-mm internal diameter). Intra-assay variability, reported as percent relative SD, was 2.65% for EPA, 2.94% for DHA, and 2.85% for total (n-3) fatty acids.

Serum isoflavones. Serum samples from the ISO and FO + ISO treatments at time point 4 h were analyzed for genistein and daidzein using Labmaster Time Resolved Fluoro-Immuno Assay kits with fluorescence measured on a Wallac Victor 2 model 1420 spectrofluorometer (52). Intra- and interassay variability was 7.9% and 14.8% for genistein and 7.2% and 14.0% for daidzein, respectively.

Serum lipids. Serum samples from all treatments at time points 0, 2, 4, and 6 h were analyzed in 1 batch for TG, total cholesterol (TC), and HDL-cholesterol (HDL-C) using an auto-analyzer (Synchron CX systems; Beckman Coulter) at an absorbance of 520 nm. Incremental area under the curve (iAUC) for TG was calculated using GraphPad Prism (GraphPad Software). Intra-assay variability was 2.01% for TG, 0.76% for TC, and 1.13% for HDL-C.

Serum biomarkers of oxidative stress. Serum samples from all treatments at time points 0, 2, 4, and 6 h were analyzed for lipid hydroperoxides (LOOH) and oxidized-LDL (ox-LDL) using methods adapted from Orem et al. (53). Serum LOOH were analyzed using a commercial spectrophotometric assay (Alpco Diagnostics). Serum ox-LDL was analyzed using a commercially available competitive ELISA (Alpco Diagnostics). Interassay variability was 15.4% for LOOH and 6.59% for ox-LDL.

Serum samples from all treatments at time points 0, 2, 4, and 6 h were analyzed for serum total antioxidant status (TAS) using a commercially available kit (Randox) in combination with a Hitachi 911 Auto-analyzer (Roche Diagnostics) based on methods reported by Kay and Holub (54). Intra- and interassay variability for TAS were 6.56% and 1.90%, respectively.

Statistical analysis. All statistical analyses were performed using the SAS, version 9.1 (SAS Institute). Examination of all data for normality using box plots, stem leaf diagrams, and residual plots revealed that serum TG and LOOH were not normally distributed and required log transformation to comply with the assumptions of the statistical analyses.

Body weight and BMI were compared among treatments using repeated-measures ANOVA, controlling for participant, treatment order, and treatment, followed by the Tukey's test for multiple comparisons. Energy and nutrient intakes were compared among the treatment washout periods using repeated-measures ANOVA, controlling for participant, treatment order, and treatment, followed by the Tukey's test for multiple comparisons.

The effect of study treatment on serum fatty acids was determined using repeated-measures ANOVA on the calculated change between time point 4 h postprandially and baseline, controlling for participant, treatment order, and treatment, followed by the Tukey's test for multiple comparisons. Serum fatty acids were further compared between the fish oil treatments (FO, FO + ISO) and fish oil placebo treatments (placebo, ISO) using the SAS contrast statement.

To ensure that the washout periods between treatments were sufficient, repeated-measures ANOVA was performed on d 1 values for all serum lipid and oxidative stress endpoints. The effect of time on serum lipid and oxidative stress endpoints was determined using repeated-measures ANOVA within each treatment, controlling for participant and time point, followed by the Tukey's test for multiple comparisons. The effect of treatment on serum lipid and oxidative stress endpoints was determined using repeated-measures ANOVA within each time point, controlling for participant, treatment order, and treatment, followed by the Tukey's test for multiple comparisons.

$P < 0.05$ was considered significant and data that were log transformed were exponentiated back to the natural scale following statistical analysis. Values in the text are presented as means ± SEM, with the exception of body weight that is presented as means ± SD.

Results

Participant characteristics. A total of 10 participants started and completed the study. Baseline characteristics indicate that all participants were overweight or obese (BMI > 25 kg/m^2) with elevated fasting serum TG (>1.5 mmol/L) (**Table 2**). Body weight for each treatment period was 96.4 ± 2.43 kg (placebo), 97.1 ± 2.34 kg (FO), 96.7 ± 2.35 kg (ISO), and 96.6 ± 2.32 kg (FO + ISO). Body weight and BMI (data not shown) did not differ between treatments.

Energy and nutrient intakes. Daily energy, macronutrient, SFA, monounsaturated fat, PUFA, cholesterol, and dietary fiber intakes did not differ between the 1-wk washout periods preceding each study day (**Table 3**).

Serum fatty acids. Baseline EPA and total (n-3) fatty acids did not differ, but baseline DHA was significantly lower for the FO + ISO treatment than the placebo treatment (**Table 4**). Comparison of the change between 4 h postprandially and baseline showed that serum EPA, DHA, and total (n-3) fatty acids increased at 4 h postprandially within the FO treatments (FO, FO + ISO) compared with the FO placebo treatments (placebo, ISO) (P = 0.01, 0.04, and 0.02, respectively), providing evidence for absorption of the (n-3) PUFA from the FO treatments (Table 4).

Serum isoflavones. Serum genistein and daidzein at 4 h postprandially were 1027 ± 121.6 nmol/L and 838.2 ± 95.8 nmol/L, respectively, for the ISO treatment and 1185 ± 78.6 nmol/L and 1017 ± 45.7, respectively, for the FO + ISO treatment. These concentrations are comparable to previous studies that have examined pharmacokinetics of isoflavones

TABLE 2 Characteristics at study entry of overweight and obese men[1]

Age, *y*	56.2 ± 6.18
Height, *m*	1.78 ± 0.06
Body weight, *kg*	96.0 ± 6.97
BMI, *kg/m*^{2}	30.3 ± 1.87
Body fat, *%*	28.5 ± 2.97
Waist circumference, *cm*	108 ± 6.53
Hip circumference, *cm*	113 ± 4.15
Waist:hip circumference ratio	0.95 ± 0.04
Serum TG, *mmol/L*	2.40 ± 0.78

[1] Data are means ± SD, n = 10.

Downloaded from jn.nutrition.org at Univ of Guelph Library GST# R134892330 on July 8, 2009

TABLE 3 Daily energy, macronutrient, cholesterol, and dietary fiber intakes of overweight and obese men during the study day washout periods between acute consumption of fish oil and soy isoflavones[1,2]

	Placebo	FO	ISO	FO + ISO
Energy, *kJ*	11502 ± 1617	11347 ± 768	10577 ± 1282	9811 ± 513
Carbohydrate, *g*	350.5 ± 45.2	362.2 ± 34.6	338.3 ± 42.9	313.1 ± 21.2
Total fat, *g*	103.4 ± 17.5	99.6 ± 7.01	88.2 ± 12.0	85.6 ± 4.87
SFA, *g*	40.1 ± 9.68	32.8 ± 2.66	31.8 ± 6.09	26.4 ± 1.45
Monounsaturated fat, *g*	23.6 ± 5.77	23.6 ± 3.45	21.0 ± 4.4	19.4 ± 2.06
PUFA, *g*	9.96 ± 1.90	11.7 ± 2.43	8.55 ± 1.21	9.98 ± 0.94
Cholesterol, *mg*	271.9 ± 73.9	262.2 ± 45.1	263.4 ± 61.5	224.5 ± 26.5
Protein, *g*	95.0 ± 14.5	97.9 ± 6.22	97.4 ± 11.4	89.7 ± 8.84
Dietary fiber, *g*	29.1 ± 3.74	28.1 ± 2.03	30.6 ± 3.50	26.8 ± 3.04

[1] Data are means ± SEM, n = 10, and based on the mean of 3-d food records completed during the week before each study day.
[2] Energy, carbohydrate, total fat, SFA, monounsaturated fat, PUFA, cholesterol, protein, and dietary fiber intakes did not differ between treatments.

following their consumption (*55–58*), providing evidence for absorption of the isoflavones from the ISO supplements.

Serum lipids. Serum TG significantly increased at 2, 4, and 6 h postprandially relative to baseline within every study treatment (**Fig. 1***A*). Furthermore, within every study treatment, serum TG significantly increased at 4 and 6 h compared with 2 h postprandially but did not significantly differ between 4 and 6 h postprandially (Fig. 1*A*). Serum TG did not significantly differ between any of the treatments at any time point, nor did TG iAUC significantly differ between any of the treatments (Fig. 1*B*).

Serum TC did not significantly differ between time points within any treatment or between treatments at any time point (data not shown). Serum HDL-C significantly decreased at time points 4 and 6 h postprandially compared with baseline within every treatment but did not significantly differ between treatments at any time point (**Table 5**).

Serum biomarkers of oxidative stress. Serum LOOH and ox-LDL did not significantly differ between time points within any treatments or between treatments at any time point (data not shown). Serum TAS significantly increased at 2 h (1.56 ± 0.02 mmol/L) and 4 h (1.56 ± 0.03 mmol/L) but not at 6 h (1.55 ± 0.02 mmol/L) compared with baseline (1.48 ± 0.03 mmol/L) within the FO treatment. Serum TAS was significantly lower at baseline within the FO treatment compared with all other treatments (1.54 ± 0.02, 1.53 ± 0.03, and 1.51 ± 0.03 mmol/L for the placebo, ISO, and FO + ISO treatments, respectively), but did not significantly differ between treatments at any other time point (data not shown).

Discussion

This study investigated the effects of acutely supplementing a high-fat, high-fructose study meal with fish oil (n-3) PUFA, soy isoflavones, and their combination on postprandial CVD biomarkers in overweight or obese hypertriglyceridemic men. This study was designed to test 4 hypotheses: *1*) the high-fat, high-fructose study meal would increase postprandial TG and oxidative stress; *2*) supplementation of the high-fat, high-fructose study meal with fish oil (n-3) PUFA would reduce the postprandial increase in TG and further increase oxidative stress; *3*) supplementation of the high-fat, high-fructose study meal with soy isoflavones would mitigate the postprandial increase in oxidative stress; and *4*) the combined supplementation of the high-fat, high-fructose study meal with fish oil (n-3) PUFA and soy isoflavones would mitigate the postprandial increase in TG and oxidative stress. This is one of the few studies to evaluate the effects of acute fish oil (n-3) PUFA supplementation and the first, to our knowledge, to evaluate the effects of acute soy isoflavone supplementation on postprandial CVD biomarkers. Although fish oil (n-3) PUFA and soy isoflavones can individually reduce CVD risk (1,2), the acute effect of their combination on postprandial CVD biomarkers has not been examined.

Serum (n-3) PUFA significantly increased within the FO treatments, providing evidence of their absorption, which is in agreement with previous studies of acute (n-3) PUFA supplementation (7,59,60). Similarly, within the ISO treatments, postprandial serum isoflavones circulated at concentrations comparable with other isoflavone consumption studies (*55–58*). The presence of the (n-3) PUFA and isoflavones in the serum confirmed a rationale for examining their effects on biomarkers of CVD risk.

TABLE 4 Serum fatty acids at baseline (0 h) and 4 h postprandially following consumption of fish oil and soy isoflavones in overweight and obese men[1,2]

	0 h	4 h	Δ (4 h − 0 h)
EPA		*μmol/L*	
Placebo	82.1 ± 9.93	92.7 ± 10.3	+10.6 ± 1.66[a]
FO	83.4 ± 11.9	134.4 ± 28.8	+51.0 ± 25.8[b]
ISO	81.1 ± 10.6	89.7 ± 11.6	+8.61 ± 2.98[a]
ISO+ FO	69.2 ± 4.30	146.4 ± 39.4	+76.8 ± 39.1[b]
DHA			
Placebo	190.6 ± 17.1[a]	220.7 ± 18.0	+30.2 ± 5.49[a]
FO	179.6 ± 18.3[ab]	221.0 ± 15.6	+41.5 ± 13.4[b]
ISO	187.2 ± 18.3[ab]	214.6 ± 18.9	+27.4 ± 3.96[a]
FO+ ISO	170.7 ± 15.2[b]	228.1 ± 20.4	+57.3 ± 14.3[b]
Total (n-3) fatty acids			
Placebo	490.0 ± 38.7	580.2 ± 35.8	+89.0 ± 23.2[a]
FO	470.6 ± 42.9	635.0 ± 66.7	+164.7 ± 55.8[b]
ISO	477.1 ± 44.8	564.1 ± 56.1	+88.0 ± 16.8[a]
FO + ISO	448.1 ± 26.8	638.2 ± 72.5	+189.9 ± 66.7[b]

[1] Data are means ± SEM, n = 10. Means within a column within a fatty acid without a common letter differ, $P < 0.05$.
[2] The contrast method in SAS was used to compare fish oil treatments (FO, ISO + FO) with fish oil placebo treatments (placebo, ISO).

Downloaded from jn.nutrition.org at Univ of Guelph Library GST# R134892330 on July 8, 2009

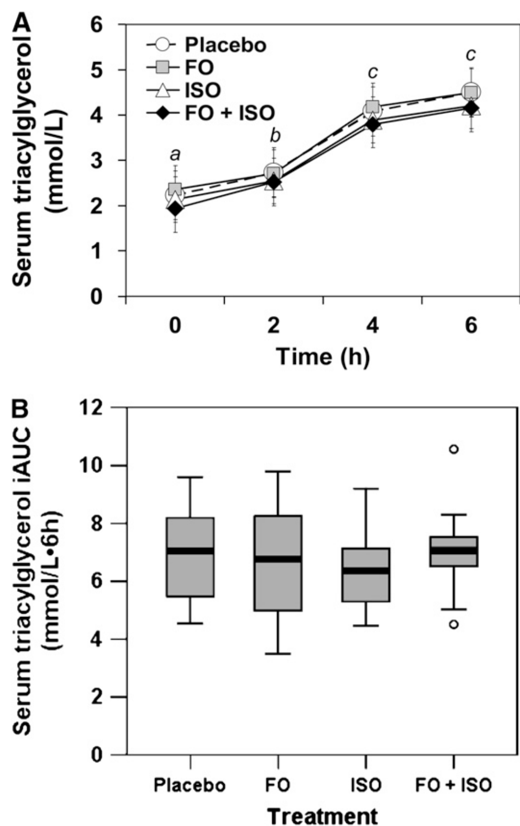

FIGURE 1 Postprandial serum TG following consumption of fish oil and soy isoflavones in overweight and obese men, n = 10. Data are presented as geometric means ± SEM at each time point in the line graph (*A*) and as iAUC as box plots (*B*). Serum TG data were log-transformed prior to statistical analysis. Within the line graph, values with without a common letter different between time points for all 4 treatments; treatments did not differ at any time point (*A*). Within the box plots, the median is indicated by the crossbar, 50% of all values lie within the box, whiskers indicate nonextreme values, and white circles indicate extreme values. iAUC did not differ between treatments (*B*).

Serum TG significantly increased following consumption of the high-fat, high-fructose study meal within the placebo treatment, confirming part of the first hypothesis that the study meal would increase postprandial TG, which was consistent with previous studies that found significantly increased TG following consumption of fructose or a high-fat meal (7,9,13,59–61). However, the current study's second hypothesis was not supported in that supplementing the high-fat, high-fructose study meal with fish oil (n-3) PUFA did not significantly reduce the postprandial increase in TG. This result is in agreement with 3 previous acute postprandial studies that also did not find a significant decrease in postprandial TG following consumption of meals that provided an acute dose of fish oil (n-3) PUFA compared with meals with varying lipid compositions (7,59,60). On the other hand, it contrasts an acute study that found postprandial TG was significantly reduced following consumption of a meal rich in fish oil (n-3) PUFA compared with a meal rich in mixed oil (62), although the fish oil meal included a blend of 40 g test oil with a rice base. It is noteworthy that chronic fish oil (n-3) PUFA supplementation consistently decreases postprandial and fasting TG (8,63–67). The differential effect of acute and chronic fish oil supplementation on TG suggests that (n-3) PUFA exert long-term changes in enzymatic regulation rather than affecting absorption or clearance. Nevertheless, the effect of acute fish oil (n-3) PUFA supplementation on postprandial TG warrants further study.

TABLE 5 Serum HDL-cholesterol at baseline (0 h) and time points 2, 4, and 6 h postprandially following consumption of fish oil and soy isoflavones in overweight and obese men[1]

	0 h	2 h	4 h	6 h
Serum HDL-C		*mmol/L*		
Placebo	1.11 ± 0.04^{a}	1.07 ± 0.04^{ab}	1.02 ± 0.04^{bc}	1.01 ± 0.03^{c}
FO	1.10 ± 0.04^{a}	1.08 ± 0.04^{a}	1.03 ± 0.05^{b}	1.02 ± 0.04^{b}
ISO	1.11 ± 0.04^{a}	1.08 ± 0.04^{a}	1.03 ± 0.03^{b}	1.02 ± 0.10^{b}
FO + ISO	1.12 ± 0.04^{a}	1.11 ± 0.04^{a}	1.05 ± 0.04^{b}	1.02 ± 0.03^{b}

[1] Data are means ± SEM, n = 10. Means within a row without a common letter differ, $P < 0.05$. Serum HDL-C did not differ between treatments at any time point.

There were no significant effects of either time or treatment on serum TC in response to the high-fat, high-fructose study meal, which agrees with previous postprandial studies that fed high-fat meals (6) in combination with fish oil (n-3) PUFA (59) or soy isoflavones (68). Serum HDL-C was significantly reduced at time points 4 and 6 h postprandially within all treatments, possibly due to parallel elevations in chylomicrons and VLDL.

Serum biomarkers of oxidative stress were not significantly affected by consumption of the high-fat, high-fructose study meal in the current study. This refutes part of the first hypothesis that the study meal would increase postprandial oxidative stress and contrasts studies that have found increased oxidative stress following consumption of high-fat meals (10,54). Limited assay sensitivity or elevated basal oxidative stress may have challenged detection of an increase in postprandial oxidative stress. Part of the second hypothesis of the current study was also not supported in that there were no further increases in postprandial serum LOOH or ox-LDL when the high-fat, high-fructose study meal was supplemented with fish oil (n-3) PUFA. Although this is consistent with a study of healthy men that demonstrated no significant differences in postprandial oxidative stress between an EPA-supplemented, high-fat meal and an oleic acid-rich meal (60), chronic studies of fish oil (n-3) PUFA supplementation have found significant increases in oxidative stress (18–20,23). Overall, results of these and the current study rationalize further investigation into effects of fish oil (n-3) PUFA on oxidative stress.

The fact that neither the high-fat, high-fructose study meal itself nor the study meal supplemented with fish oil (n-3) PUFA caused an increase in oxidative stress compromised the ability to test the third hypothesis that soy isoflavones would mitigate a postprandial increase in oxidative stress. Because other studies support the antioxidant potential of soy isoflavones (34–39), the current study does not preclude further investigations into their antioxidant potential.

A unique aspect of the current study was its investigation of the effects of a combination of fish oil (n-3) PUFA and soy isoflavone supplementation to a high-fat, high-fructose study meal. However, results did not support the fourth hypothesis that the combination of these dietary supplements to the high-fat, high-fructose study meal would mitigate the postprandial increase in TG and oxidative stress. Despite this, it is informative that both supplements were circulating at 4 h postprandially and did not cause adverse effects. Furthermore, the combination of these dietary supplements could impart benefit on parameters not measured or could benefit lipid and oxidative parameters when administered chronically. Thus, there is rationale to

Downloaded from jn.nutrition.org at Univ of Guelph Library GST# R134892330 on July 8, 2009

further study the concept of combinatorial dietary supplementation approaches for CVD risk reduction.

In summary, although this randomized, double-blind, placebo-controlled, cross-over human intervention study did not substantiate its hypotheses, it does provide important data regarding the postprandial effects of consuming a high-fat, high-fructose study meal, the acute bioavailability of fish oil (n-3) PUFA and soy isoflavones, and the acute effects of these dietary supplements on CVD risk. Importantly, the results indicate that supplementing a high-fat meal with 7 g of highly oxidizable PUFA does not increase postprandial oxidative stress. Overall, this well-controlled investigation of the effects of 2 common dietary supplements, fish oil (n-3) PUFA and soy isoflavones, both alone and in combination, adds needed research to an emerging field of interest in the realm of nutrition and CVD prevention.

Acknowledgments
We thank Megan Olson, Colleen Fuller, Lucas Burke, and Mehrnoosh Kashani for their assistance with data collection.

Literature Cited

1. Holub BJ. Clinical nutrition. 4. Omega-3 fatty acids in cardiovascular care. CMAJ. 2002;166:608–15.
2. Lichtenstein AH. Soy protein, isoflavones and cardiovascular disease risk. J Nutr. 1998;128:1589–92.
3. Jacobson TA. Role of n-3 fatty acids in the treatment of hypertriglyceridemia and cardiovascular disease. Am J Clin Nutr. 2008;87:S1981–90.
4. Karpe F. Postprandial lipoprotein metabolism and atherosclerosis. J Intern Med. 1999;246:341–55.
5. Couillard C, Bergeron N, Prud'homme D, Bergeron J, Tremblay A, Bouchard C, Mauriege P, Despres JP. Postprandial triglyceride response in visceral obesity in men. Diabetes. 1998;47:953–60.
6. Cohen JC, Noakes TD, Benade AJ. Serum triglyceride responses to fatty meals: effects of meal fat content. Am J Clin Nutr. 1988;47:825–7.
7. Harris WS, Connor WE, Alam N, Illingworth DR. Reduction of postprandial triglyceridemia in humans by dietary n-3 fatty acids. J Lipid Res. 1988;29:1451–60.
8. Westphal S, Orth M, Ambrosch A, Osmundsen K, Luley C. Postprandial chylomicrons and VLDLs in severe hypertriacylglycerolemia are lowered more effectively than are chylomicron remnants after treatment with n-3 fatty acids. Am J Clin Nutr. 2000;71:914–20.
9. Callow J, Summers LK, Bradshaw H, Frayn KN. Changes in LDL particle composition after the consumption of meals containing different amounts and types of fat. Am J Clin Nutr. 2002;76:345–50.
10. Ursini F, Zamburlini A, Cazzolato G, Maiorino M, Bon GB, Sevanian A. Postprandial plasma lipid hydroperoxides: a possible link between diet and atherosclerosis. Free Radic Biol Med. 1998;25:250–2.
11. Ursini F, Sevanian A. Postprandial oxidative stress. Biol Chem. 2002;383:599–605.
12. Kay CD, Holub BJ. The postprandial effects of dietary antioxidants in humans. Curr Atheroscler Rep. 2003;5:452–8.
13. Kelley DS, Siegel D, Vemuri M, Mackey BE. Docosahexaenoic acid supplementation improves fasting and postprandial lipid profiles in hypertriglyceridemic men. Am J Clin Nutr. 2007;86:324–33.
14. Qi K, Fan C, Jiang J, Zhu H, Jiao H, Meng Q, Deckelbaum RJ. Omega-3 fatty acid containing diets decrease plasma triglyceride concentrations in mice by reducing endogenous triglyceride synthesis and enhancing the blood clearance of triglyceride-rich particles. Clin Nutr. 2008;27:424–30.
15. McLaughlin J, Middaugh J, Boudreau D, Malcom G, Parry S, Tracy R, Newman W. Adipose tissue triglyceride fatty acids and atherosclerosis in Alaska Natives and non-Natives. Atherosclerosis. 2005;181:353–62.
16. Iso H, Kobayashi M, Ishihara J, Sasaki S, Okada K, Kita Y, Kokubo Y, Tsugane S. Intake of fish and n3 fatty acids and risk of coronary heart disease among Japanese: the Japan Public Health Center-Based (JPHC) Study Cohort 1. Circulation. 2006;113:195–202.
17. Mozaffarian D. Fish and n-3 fatty acids for the prevention of fatal coronary heart disease and sudden cardiac death. Am J Clin Nutr. 2008;87:S1991–6.
18. Brown JE, Wahle KW. Effect of fish-oil and vitamin E supplementation on lipid peroxidation and whole-blood aggregation in man. Clin Chim Acta. 1990;193:147–56.
19. Finnegan YE, Minihane AM, Leigh-Firbank EC, Kew S, Meijer GW, Muggli R, Calder PC, Williams CM. Plant- and marine-derived n-3 polyunsaturated fatty acids have differential effects on fasting and postprandial blood lipid concentrations and on the susceptibility of LDL to oxidative modification in moderately hyperlipidemic subjects. Am J Clin Nutr. 2003;77:783–95.
20. Grundt H, Nilsen DW, Mansoor MA, Nordoy A. Increased lipid peroxidation during long-term intervention with high doses of n-3 fatty acids (PUFAs) following an acute myocardial infarction. Eur J Clin Nutr. 2003;57:793–800.
21. Spiteller G. Linoleic acid peroxidation: the dominant lipid peroxidation process in low density lipoprotein–and its relationship to chronic diseases. Chem Phys Lipids. 1998;95:105–62.
22. Dimitrova-Sumkovska J, Dosic-Markovska B, Zafirova-Roganovic D, Anastasovska V. Effects of different dietary fatty acid supplements upon lipoprotein metabolism and lipid peroxides production in hyperlipidemic rats. Prilozi. 2006;27:67–86.
23. Allard JP, Kurian R, Aghdassi E, Muggli R, Royall D. Lipid peroxidation during n-3 fatty acid and vitamin E supplementation in humans. Lipids. 1997;32:535–41.
24. Vedavanam K, Srijayanta S, O'Reilly J, Raman A, Wiseman H. Antioxidant action and potential antidiabetic properties of an isoflavonoid-containing soyabean phytochemical extract (SPE). Phytother Res. 1999;13:601–8.
25. Hwang J, Sevanian A, Hodis HN, Ursini F. Synergistic inhibition of LDL oxidation by phytoestrogens and ascorbic acid. Free Radic Biol Med. 2000;29:79–89.
26. Rohrdanz E, Ohler S, Tran-Thi QH, Kahl R. The phytoestrogen daidzein affects the antioxidant enzyme system of rat hepatoma H4IIE cells. J Nutr. 2002;132:370–5.
27. Anbazhagan V, Kalaiselvan A, Jaccob M, Venuvanalingam P, Renganathan R. Investigations on the fluorescence quenching of 2,3-diazabicyclo [2.2.2]oct-2-ene by certain flavonoids. J Photochem Photobiol B. 2008;91:143–50.
28. Hodgson JM, Croft KD, Puddey IB, Mori TA, Beilin LJ. Soybean isoflavonoids and their metabolic products inhibit in vitro lipoprotein oxidation in serum. J Nutr Biochem. 1996;7:664–9.
29. Rimbach G, De Pascual-Teresa S, Ewins BA, Matsugo S, Uchida Y, Minihane AM, Turner R, VafeiAdou K, Weinberg PD. Antioxidant and free radical scavenging activity of isoflavone metabolites. Xenobiotica. 2003;33:913–25.
30. Liu J, Chang SK, Wiesenborn D. Antioxidant properties of soybean isoflavone extract and tofu in vitro and in vivo. J Agric Food Chem. 2005;53:2333–40.
31. Oh HY, Lim S, Lee JM, Kim DY, Ann ES, Yoon S. A combination of soy isoflavone supplementation and exercise improves lipid profiles and protects antioxidant defense-systems against exercise-induced oxidative stress in ovariectomized rats. Biofactors. 2007;29:175–85.
32. Aoki H, Otaka Y, Igarashi K, Takenaka A. Soy protein reduces paraquat-induced oxidative stress in rats. J Nutr. 2002;132:2258–62.
33. Park E, Shin JI, Park OJ, Kang MH. Soy isoflavone supplementation alleviates oxidative stress and improves systolic blood pressure in male spontaneously hypertensive rats. J Nutr Sci Vitaminol (Tokyo). 2005;51:254–9.
34. Djuric Z, Chen G, Doerge DR, Heilbrun LK, Kucuk O. Effect of soy isoflavone supplementation on markers of oxidative stress in men and women. Cancer Lett. 2001;172:1–6.
35. Jenkins DJ, Kendall CW, Jackson CJ, Connelly PW, Parker T, Faulkner D, Vidgen E, Cunnane SC, Leiter LA, et al. Effects of high- and low-isoflavone soyfoods on blood lipids, oxidized LDL, homocysteine, and blood pressure in hyperlipidemic men and women. Am J Clin Nutr. 2002;76:365–72.
36. Jenkins DJ, Kendall CW, Vidgen E, Vuksan V, Jackson CJ, Augustin LS, Lee B, Garsetti M, Agarwal S, et al. Effect of soy-based breakfast cereal on blood lipids and oxidized low-density lipoprotein. Metabolism. 2000;49:1496–500.

Downloaded from jn.nutrition.org at Univ of Guelph Library GST# R134892330 on July 8, 2009

37. Tikkanen MJ, Wahala K, Ojala S, Vihma V, Adlercreutz H. Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. Proc Natl Acad Sci USA. 1998;95:3106–10.
38. Nhan S, Anderson KE, Nagamani M, Grady JJ, Lu LJ. Effect of a soymilk supplement containing isoflavones on urinary F2 isoprostane levels in premenopausal women. Nutr Cancer. 2005;53:73–81.
39. Wiseman H, O'Reilly JD, Adlercreutz H, Mallet AI, Bowey EA, Rowland IR, Sanders TA. Isoflavone phytoestrogens consumed in soy decrease F(2)-isoprostane concentrations and increase resistance of low-density lipoprotein to oxidation in humans. Am J Clin Nutr. 2000;72:395–400.
40. Kampkotter A, Wiegand C, Timpel C, Rohrdanz E, Chovolou Y, Kahl R, Watjen W. Increased expression of catalase in human hepatoma cells by the soy isoflavone, daidzein. Basic Clin Pharmacol Toxicol. 2008;102:437–42.
41. Banz W, Hauck S, Gename B, Winters T, Bartke A. Soy isoflavones modify liver free radical scavenger systems and liver parameters in Sprague-Dawley rats. J Med Food. 2004;7:477–81.
42. Mahn K, Borras C, Knock GA, Taylor P, Khan IY, Sugden D, Poston L, Ward JP, Sharpe RM, et al. Dietary soy isoflavone induced increases in antioxidant and eNOS gene expression lead to improved endothelial function and reduced blood pressure in vivo. FASEB J. 2005;19:1755–7.
43. Borras C, Gambini J, Gomez-Cabrera MC, Sastre J, Pallardo FV, Mann GE, Vina J. Genistein, a soy isoflavone, up-regulates expression of antioxidant genes: involvement of estrogen receptors, ERK1/2, and NFkappaB. FASEB J. 2006;20:2136–8.
44. Lee JS. Effects of soy protein and genistein on blood glucose, antioxidant enzyme activities, and lipid profile in streptozotocin-induced diabetic rats. Life Sci. 2006;79:1578–84.
45. Raschke M, Rowland IR, Magee PJ, Pool-Zobel BL. Genistein protects prostate cells against hydrogen peroxide-induced DNA damage and induces expression of genes involved in the defence against oxidative stress. Carcinogenesis. 2006;27:2322–30.
46. Oh HY, Kim SS, Chung HY, Yoon S. Isoflavone supplements exert hormonal and antioxidant effects in postmenopausal Korean women with diabetic retinopathy. J Med Food. 2005;8:1–7.
47. DiSilvestro RA, Goodman J, Dy E, Lavalle G. Soy isoflavone supplementation elevates erythrocyte superoxide dismutase, but not plasma ceruloplasmin in postmenopausal breast cancer survivors. Breast Cancer Res Treat. 2005;89:251–5.
48. Cohen JC, Schall R. Reassessing the effects of simple carbohydrates on the serum triglyceride responses to fat meals. Am J Clin Nutr. 1988;48:1031–4.
49. Abraha A, Humphreys SM, Clark ML, Matthews DR, Frayn KN. Acute effect of fructose on postprandial lipaemia in diabetic and non-diabetic subjects. Br J Nutr. 1998;80:169–75.
50. Singleton MJ, Heiser C, Jamesen K, Mattes RD. Sweetener augmentation of serum triacylglycerol during a fat challenge test in humans. J Am Coll Nutr. 1999;18:179–85.
51. Stark KD, Holub BJ. Differential eicosapentaenoic acid elevations and altered cardiovascular disease risk factor responses after supplementation with docosahexaenoic acid in postmenopausal women receiving and not receiving hormone replacement therapy. Am J Clin Nutr. 2004;79:765–73.
52. Frankenfeld CL, Lampe JW, Shannon J, Gao DL, Ray RM, Prunty J, Kalhorn TF, Wahala K, Patterson RE, et al. Frequency of soy food consumption and serum isoflavone concentrations among Chinese women in Shanghai. Public Health Nutr. 2004;7:765–72.
53. Orem C, Orem A, Uydu HA, Celik S, Erdol C, Kural BV. The effects of lipid-lowering therapy on low-density lipoprotein auto-antibodies: relationship with low-density lipoprotein oxidation and plasma total antioxidant status. Coron Artery Dis. 2002;13:65–71.
54. Kay CD, Holub BJ. The effect of wild blueberry (Vaccinium angustifolium) consumption on postprandial serum antioxidant status in human subjects. Br J Nutr. 2002;88:389–98.
55. Rufer CE, Bub A, Moseneder J, Winterhalter P, Sturtz M, Kulling SE. Pharmacokinetics of the soybean isoflavone daidzein in its aglycone and glucoside form: a randomized, double-blind, crossover study. Am J Clin Nutr. 2008;87:1314–23.
56. Busby MG, Jeffcoat AR, Bloedon LT, Koch MA, Black T, Dix KJ, Heizer WD, Thomas BF, Hill JM, et al. Clinical characteristics and pharmacokinetics of purified soy isoflavones: single-dose administration to healthy men. Am J Clin Nutr. 2002;75:126–36.
57. Setchell KD, Brown NM, Desai P, Zimmer-Nechemias L, Wolfe BE, Brashear WT, Kirschner AS, Cassidy A, Heubi JE. Bioavailability of pure isoflavones in healthy humans and analysis of commercial soy isoflavone supplements. J Nutr. 2001;131:S1362–75.
58. Zubik L, Meydani M. Bioavailability of soybean isoflavones from aglycone and glucoside forms in American women. Am J Clin Nutr. 2003;77:1459–65.
59. Burdge GC, Powell J, Calder PC. Lack of effect of meal fatty acid composition on postprandial lipid, glucose and insulin responses in men and women aged 50–65 years consuming their habitual diets. Br J Nutr. 2006;96:489–500.
60. Hall WL, Sanders KA, Sanders TA, Chowienczyk PJ. A high-fat meal enriched with eicosapentaenoic acid reduces postprandial arterial stiffness measured by digital volume pulse analysis in healthy men. J Nutr. 2008;138:287–91.
61. Chong MF, Fielding BA, Frayn KN. Mechanisms for the acute effect of fructose on postprandial lipemia. Am J Clin Nutr. 2007;85:1511–20.
62. Zampelas A, Peel AS, Gould BJ, Wright J, Williams CM. Polyunsaturated fatty acids of the n-6 and n-3 series: effects on postprandial lipid and apolipoprotein levels in healthy men. Eur J Clin Nutr. 1994;48:842–8.
63. Harris WS, Hustvedt BE, Hagen E, Green MH, Lu G, Drevon CA. n-3 fatty acids and chylomicron metabolism in the rat. J Lipid Res. 1997;38:503–15.
64. GISSI-Prevenzione-Investigators. Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial. Lancet. 1999;354:447–55.
65. Tinker LF, Parks EJ, Behr SR, Schneeman BO, Davis PA. (n-3) Fatty acid supplementation in moderately hypertriglyceridemic adults changes postprandial lipid and apolipoprotein B responses to a standardized test meal. J Nutr. 1999;129:1126–34.
66. Kris-Etherton PM, Harris WS, Appel LJ. Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. Circulation. 2002;106:2747–57.
67. Rose EL, Holub BJ. Effects of a liquid egg product containing fish oil on selected cardiovascular disease risk factors: a randomized crossover trial. Food Res Int. 2006;39:910–6.
68. Campbell CG, Brown BD, Dufner D, Thorland WG. Effects of soy or milk protein during a high-fat feeding challenge on oxidative stress, inflammation, and lipids in healthy men. Lipids. 2006;41:257–65.

JN THE JOURNAL OF NUTRITION

Downloaded from jn.nutrition.org at Univ of Guelph Library GST# R134892330 on July 8, 2009