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 Men1,2

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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.
6Abbreviations 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.
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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.

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 periods. For the duration of the study, 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. 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 midpoint 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 study day. Participants consumed a high-fat, high-fructose meal along with a specific combination of study supplements or study test meal; antibiotic replacement.

To ensure a random assignment of treatment order, the 24 potential 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.

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 midpoint 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.

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:lyngcitein. 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.

TABLE 1  Study test meal composition

<table>
<thead>
<tr>
<th>Component</th>
<th>Value</th>
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<tbody>
<tr>
<td>Energy, kJ</td>
<td>4887</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>130</td>
</tr>
<tr>
<td>Total fat, g</td>
<td>52</td>
</tr>
<tr>
<td>SFA, g</td>
<td>20</td>
</tr>
<tr>
<td>Trans fat, g</td>
<td>2</td>
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<tr>
<td>Cholesterol, mg</td>
<td>446</td>
</tr>
<tr>
<td>Protein, g</td>
<td>40</td>
</tr>
</tbody>
</table>

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.
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-Immuo 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 (Synchrone 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.

Sample 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/m²) 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

| Age, y | 56.2 ± 6.18 |
| Height, m | 1.78 ± 0.06 |
| Body weight, kg | 96.0 ± 6.97 |
| BMI, kg/m² | 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.
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. 1A). 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. 1A). 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. 1B).

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 the FO 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 at any 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 is comparable with other isoflavone consumption studies (55–58).

**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

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>FO</th>
<th>ISO</th>
<th>FO + ISO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kJ</td>
<td>11502 ± 1617</td>
<td>11347 ± 768</td>
<td>10577 ± 1282</td>
<td>9811 ± 513</td>
</tr>
<tr>
<td>Carbohydrate, g</td>
<td>350.5 ± 45.2</td>
<td>362.2 ± 34.6</td>
<td>338.3 ± 42.9</td>
<td>313.1 ± 21.2</td>
</tr>
<tr>
<td>Total fat, g</td>
<td>103.4 ± 17.5</td>
<td>99.8 ± 7.01</td>
<td>88.2 ± 12.0</td>
<td>85.6 ± 4.87</td>
</tr>
<tr>
<td>SFA, g</td>
<td>40.1 ± 9.88</td>
<td>32.8 ± 2.68</td>
<td>31.8 ± 6.09</td>
<td>26.4 ± 1.45</td>
</tr>
<tr>
<td>Monounsaturated fat, g</td>
<td>23.6 ± 5.77</td>
<td>23.8 ± 3.45</td>
<td>21.0 ± 4.4</td>
<td>19.4 ± 2.06</td>
</tr>
<tr>
<td>PUFA, g</td>
<td>9.96 ± 1.90</td>
<td>11.7 ± 2.43</td>
<td>8.55 ± 1.21</td>
<td>9.98 ± 0.94</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>271.9 ± 73.9</td>
<td>262.2 ± 45.1</td>
<td>263.4 ± 61.5</td>
<td>224.5 ± 26.5</td>
</tr>
<tr>
<td>Protein, g</td>
<td>95.0 ± 14.5</td>
<td>97.9 ± 6.22</td>
<td>97.4 ± 11.4</td>
<td>89.7 ± 8.84</td>
</tr>
<tr>
<td>Dietary fiber, g</td>
<td>29.1 ± 3.74</td>
<td>28.1 ± 2.03</td>
<td>30.6 ± 3.50</td>
<td>26.8 ± 3.04</td>
</tr>
</tbody>
</table>

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.

**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

<table>
<thead>
<tr>
<th></th>
<th>0 h</th>
<th>4 h</th>
<th>Δ (4 h – 0 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPA</td>
<td>82.1 ± 9.93</td>
<td>92.7 ± 10.3</td>
<td>+10.6 ± 1.66*</td>
</tr>
<tr>
<td>FO</td>
<td>83.4 ± 11.9</td>
<td>134.4 ± 28.8</td>
<td>+51.0 ± 25.8*</td>
</tr>
<tr>
<td>ISO</td>
<td>81.1 ± 10.6</td>
<td>89.7 ± 11.6</td>
<td>+8.61 ± 2.98*</td>
</tr>
<tr>
<td>ISO + FO</td>
<td>69.2 ± 4.30</td>
<td>146.4 ± 39.4</td>
<td>+78.6 ± 38.1*</td>
</tr>
<tr>
<td>DHA</td>
<td>190.6 ± 17.1a</td>
<td>220.7 ± 18.0</td>
<td>+30.2 ± 5.49*</td>
</tr>
<tr>
<td>FO</td>
<td>179.6 ± 18.3abc</td>
<td>221.0 ± 15.6</td>
<td>+41.5 ± 13.4a</td>
</tr>
<tr>
<td>ISO</td>
<td>187.2 ± 18.3abc</td>
<td>214.6 ± 18.9</td>
<td>+27.4 ± 3.96*</td>
</tr>
<tr>
<td>ISO + FO</td>
<td>170.7 ± 15.2ab</td>
<td>228.1 ± 20.4</td>
<td>+57.3 ± 14.3b</td>
</tr>
<tr>
<td>Total (n-3) fatty acids</td>
<td>348.0 ± 37.8</td>
<td>580.2 ± 35.8</td>
<td>+80.0 ± 23.2a</td>
</tr>
<tr>
<td>FO</td>
<td>470.6 ± 42.9</td>
<td>635.0 ± 66.7</td>
<td>+164.7 ± 55.8a</td>
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<tr>
<td>ISO</td>
<td>477.1 ± 44.8</td>
<td>564.1 ± 56.1</td>
<td>+88.0 ± 16.8a</td>
</tr>
<tr>
<td>FO + ISO</td>
<td>448.1 ± 26.8</td>
<td>638.2 ± 72.5</td>
<td>+189.9 ± 66.7a</td>
</tr>
</tbody>
</table>

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).
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.

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

**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

<table>
<thead>
<tr>
<th>Serum HDL-C (mmol/L)</th>
<th>0 h</th>
<th>2 h</th>
<th>4 h</th>
<th>6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>1.11 ± 0.04a</td>
<td>1.07 ± 0.04b</td>
<td>1.02 ± 0.04b</td>
<td>1.01 ± 0.03b</td>
</tr>
<tr>
<td>FO</td>
<td>1.10 ± 0.04a</td>
<td>1.08 ± 0.04a</td>
<td>1.03 ± 0.05b</td>
<td>1.02 ± 0.04b</td>
</tr>
<tr>
<td>ISO</td>
<td>1.11 ± 0.04a</td>
<td>1.08 ± 0.04a</td>
<td>1.03 ± 0.03b</td>
<td>1.02 ± 0.10b</td>
</tr>
<tr>
<td>FO + ISO</td>
<td>1.12 ± 0.04a</td>
<td>1.11 ± 0.04a</td>
<td>1.05 ± 0.04b</td>
<td>1.02 ± 0.03b</td>
</tr>
</tbody>
</table>

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.
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.

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Literature Cited


