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# Modifying the n-6/n-3 polyunsaturated fatty acid ratio of a high–saturated fat challenge does not acutely attenuate postprandial changes in inflammatory markers in men with metabolic syndrome

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**Abstract**

Metabolic syndrome (MetS) features chronic inflammation and exaggerated postprandial triacylglyceride (TAG) responses. Fasting concentrations of interleukin-6 (IL-6) and C-reactive protein (CRP), key inflammatory mediators, decrease after sustained n-3 polyunsaturated fatty acid (PUFA) intake; however, the ability of n-3 PUFA to attenuate postprandial inflammatory responses is not well studied. Thus, we examined the acute effect of modifying the n-6/n-3 PUFA ratio of a high–saturated fatty acid (SFA) oral fat tolerance test (OFTT) on postprandial TAG and inflammatory responses in men with MetS. Men (n = 8, ≥45 years old) with MetS ingested 2 high-SFA OFTTs (1 g fat per kilogram body weight), with either a 20:1 (low n-3) or 2:1 (high n-3) n-6/n-3 PUFA ratio, and a water control in a randomized crossover design. Blood samples were collected for 8 hours after treatment to measure postprandial TAG, free fatty acids, IL-6, soluble IL-6 receptor, and CRP. Postprandial TAG increased at the same rate after ingestion of the low–n-3 and high–n-3 OFTTs; however, both OFTTs were significantly different from the water control. There were no differences in the rate at which IL-6 concentrations increased after ingestion of either of the OFTTs compared with water. Furthermore, neither time nor treatment affected circulating soluble IL-6 receptor or CRP concentrations. Thus, increasing the n-3 PUFA content of a high-SFA OFTT does not acutely change postprandial TAG or inflammatory responses in men with MetS.


## 1. Background

The National Cholesterol Education Program–Adult Treatment Panel (ATP) III defines metabolic syndrome (MetS) as the presence of at least 3 of the 5 following cardiovascular disease (CVD) risk factors: abdominal obesity, impaired fasting glucose, elevated fasting triacylglycerides (TAG), decreased high-density lipoprotein (HDL) cholesterol, and high blood pressure (≥130/85 mm Hg) [1]. As currently defined, MetS affects between 15% and 25% of North American adults [2] and confers a 5- and 2-fold risk of developing type 2 diabetes mellitus (T2D) and CVD, respectively, compared with individuals without MetS [3].

Elevated fasting TAG are an independent risk factor for CVD; and exaggerated postprandial lipemia has recently emerged as a risk factor for T2D and CVD, indicating that these diseases are, in part, a postprandial phenomenon [4,5]. Although not defined by the ATP III criteria, other features of MetS include chronic low-grade inflammation, indicated by elevated circulating levels of inflammatory proteins, and dysregulated postprandial metabolism, manifested as exaggerated elevated glucose and/or TAG after a meal [6,7]. Elevated fasting concentrations of key inflammatory proteins interleukin-6 (IL-6) and C-reactive protein (CRP) are a hallmark of MetS and have recently been recognized as predictors of T2D and CVD [8-10]. Diets high in total fat and saturated fat (SFA) have been shown to exacerbate the chronic inflammatory state of MetS [11] and are established as regulators of proinflammatory gene expression [12]. Furthermore, there is convincing evidence that the postprandial period is a proinflammatory state [13,14]. However, abnormal postprandial inflammatory responses are not

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clearly defined; and a lack of standard methods and differences in test meal composition encumber interpretation of existing literature.

The n-6 and n-3 polyunsaturated fatty acids (PUFA) are fatty acids of particular interest in terms of health and chronic disease. The n-6/n-3 PUFA ratio in the Western diet has increased in parallel with the prevalence of obesity, T2D, and CVD [15], whereas high intakes of n-3 PUFA have been associated with significant benefits in terms of chronic disease [16,17]. Today, the n-6/n-3 PUFA ratio in the typical Western diet is estimated to be between 10 and 25:1, a substantial increase compared with the estimated n-6/n-3 PUFA ratio of 1:1 to 2:1 in the diets on which humans evolved [15]. Specifically, decreasing the dietary n-6/n-3 PUFA ratio for 6 months has been shown to lower fasting and postprandial TAG concentrations [18]; and high intakes of n-3 PUFA are associated with reduced fasting concentrations of IL-6 and CRP [19,20].

Research evaluating postprandial inflammatory marker responses to the ingestion of specific fatty acids remains limited [6], and whether increasing the n-3 PUFA content of a high-SFA load will acutely improve postprandial inflammatory responses has not been investigated. Thus, there is strong rationale to investigate the impact of modifying the n-6/n-3 ratio on both the magnitude and duration of postprandial TAG and inflammatory responses. We have previously developed and used a novel series of oral fat tolerance tests (OFTTs) to examine changes in different disease biomarkers in the postprandial period after fat ingestion [21]. The OFTT contains only lipid, allowing us to investigate postprandial responses to a high-fat load of specific fatty acid composition. We have also included a water control trial, allowing comparison between postprandial and natural increases in end points of interest. The objective of this study was to investigate whether increasing the n-3 PUFA content of a high-SFA OFTT would impact postprandial TAG, IL-6, CRP, and soluble IL-6 receptor (sIL-6R) concentrations in men with MetS.

## 2. Methods

### 2.1. Subjects and preliminary screening

This study was approved by the University of Guelph Research Ethics Board, and all subjects provided written informed consent. Men, at least 45 years old, were recruited from Guelph, Ontario, and surrounding communities using newspaper advertisements. Potential subjects were screened for T2D using a standard 2-hour oral glucose tolerance test (Trutol Custom Laboratories, Baltimore, MD); and fasting glucose, TAG, and HDL cholesterol were analyzed, along with subject height, weight, body mass index (BMI), waist circumference, and blood pressure. Only subjects that met at least 3 ATP III criteria for MetS were included in the study [1]. Subjects taking antihypertensive medication were considered to meet the ATP III hypertension criteria. Individuals were excluded if they smoked, used lipid-lowering or anti-inflammatory medication, used medication to control diabetes, had any disease or condition known to affect study end points, or were consuming large amounts of n-3 PUFA through the diet or supplements. Individuals were also excluded if they had a BMI less than 25 kg/m$^2$, were dieting, had significant weight gain or loss (±6 kg) within the last 6 months, or were physically active (>30 minutes, 3 d/wk of moderate physical activity). A total of 8 subjects were recruited and completed the study. A sample size calculation ($P = .05$, power = 0.8) for TAG area under the curve revealed that n = 8 or 9 was sufficient to demonstrate significant differences between the high–n-3 OFTT vs water and low–n-3 OFTT vs water, respectively.

### 2.2. Experimental trials

This study used a randomized crossover design and consisted of 3 trial days, each separated by a 1-week washout period. Subjects were instructed to maintain their usual lifestyle and eating habits and to avoid consumption of products high in n-3 PUFA for the duration of the study period. Immediately preceding each trial day, subjects completed a 3-day food record that was subsequently analyzed using ESHA Food Processor 9.5 (Salem, OR).

Subjects were required to abstain from alcohol, caffeine, over-the-counter medications, and exercise for 48 hours before each trial. Subjects were provided with a standardized meal (550 kcal, 13.1% protein, 52.4% carbohydrate, 17.2% fat) consisting of lasagna, a cereal bar, and a bottle of water to consume in their home the evening before each trial day. Subjects then reported to the laboratory at 8:00 AM after a 12-hour overnight fast. Upon arrival at the laboratory, a Teflon catheter was inserted into a forearm vein; and intravenous saline was administered to maintain catheter integrity and to allow for repeated blood sampling. After an initial fasting blood sample (0 hour), subjects ingested either an OFTT (1 g fat per kilogram body weight) or an equal volume of water. Blood samples were collected over the following 8 hours, as indicated below, for each assay. Subjects consumed water ad libitum during the 8-hour postprandial period. Body composition was determined on the third trial day for all subjects using bioelectrical impedance analysis (Bodystat, Tampa, FL).

### 2.3. Oral fat tolerance tests

The OFTTs were devoid of carbohydrate and protein and differed in n-6/n-3 PUFA ratio while maintaining a PUFA/SFA ratio of approximately 0.2. Before the trial days, palm stearin (57% 16:0, palmitic acid) (generously provided by Bunge Canada, Toronto, Ontario) and safflower oil (65% 18:2 n-6, linoleic acid) (President's Choice, purchased at Zehrs Markets, Guelph, Ontario) were blended in amounts that would achieve n-6/n-3 PUFA ratios of approximately 20:1 (low n-3) and 2:1 (high n-3) after the addition of refined fish oil concentrate (48% 20:5 n-3, eicosapentaenoic acid;

Table 1
Fatty acid composition of high–n-3 and low–n-3 PUFA OFTTs

| Fatty acid | High n-3[a] | Low n-3[a] |
|---|---|---|
| | % wt/wt | |
| 12:0 | 0.27 | 0.26 |
| 14:0 | 1.08 | 1.10 |
| 16:0 | 49.25 | 50.02 |
| 18:0 | 5.40 | 5.35 |
| 18:1 (9-*cis*) | 25.04 | 25.63 |
| 18:2 (9-*cis*, 12-*trans*) | 0.12 | 0.13 |
| 18:2 (n-6) | 10.77 | 13.25 |
| 18:3 (n-3) | 0.19 | 0.15 |
| 20:0 | 0.37 | 0.35 |
| 20:4 (n-6) | 0.12 | 0.02 |
| 20:4 (n-3) | 0.11 | 0.02 |
| 20:5 (n-3) | 2.25 | 0.37 |
| 22:5 (n-3) | 0.11 | 0.01 |
| 22:6 (n-3) | 0.98 | 0.16 |
| SFA | 56.56 | 57.27 |
| PUFA | 15.13 | 14.36 |
| n-6 PUFA | 10.96 | 13.27 |
| n-3 PUFA | 3.84 | 0.74 |
| PUFA/SFA ratio | 0.27 | 0.25 |
| n-6/n-3 ratio | 2.85 | 17.93 |

Final fatty acid compositions of lipid blends prepared for the OFTTs were analyzed using gas-liquid chromatography. Values are % wt/wt of total fatty acids, except for ratios. Minor fatty acids are not reported; therefore, totals do not add up to 100%.

[a] *High n-3* refers to OFTT with n-6/n-3 PUFA ratio of approximately 2:1; *Low n-3* refers to OFTT with n-6/n-3 PUFA ratio of approximately 20:1.

25% 22:6 n-3, docosahexaenoic acid) (generously provided by Ocean Nutrition Canada, Dartmouth, Nova Scotia).

On each trial day, the prepared lipid blend was warmed to approximately 40°C; and 1 g fat per kilogram body weight was aliquoted for each subject. The appropriate amount of fish oil was then added to the fat blend. The final fatty acid composition of the lipid blend prepared for each OFTT was analyzed by gas-liquid chromatography and is presented in Table 1.

To maintain the pure fat challenge, the lipid blend was subsequently emulsified in water (73 wt%) using the monoglyceride Myverol (2 wt%) and polysorbate Tween80 (0.15 wt%) (Acatris, Oakville, Ontario) and mixed with a standard handheld homogenizer. Nonnutritive aspartame (Cambrian Chemicals, Cambridge, Ontario), artificial lemonade flavor (1.2 wt%) (David Michael, Aurora, Ontario), and 2 drops of commercial yellow food coloring (Club House Food Preparation Colour; McCormick Canada, London, Ontario) were added to the OFTT. The OFTT was maintained at 40°C and was consumed by subjects within 15 minutes of its preparation.

### 2.4. Blood metabolites and inflammatory markers

For analysis of whole blood lipids and glucose, blood was collected in 4-mL sodium heparin BD Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Whole blood lipids, including TAG, total cholesterol, and HDL cholesterol, were analyzed at 0, 2, 3, 4, 6, and 8 hours using Cholestech LDX lipid cassettes (Cholestech, Hayward, CA). Whole blood glucose was analyzed in duplicate at 0, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hours by a glucose oxidase method (YSI 2300 Stat Plus Glucose Analyzer; Yellow Springs International, Yellow Springs, OH).

At time 0, 2, 4, 6, and 8 hours, blood was drawn into 6-mL no-additive vacutainer tubes and left to clot at room temperature for 30 minutes. The clotted sample was then centrifuged (1200*g*) at 4°C for 10 minutes. The serum supernatant was aliquoted and frozen at −20°C for subsequent analysis of free fatty acids (FFA), CRP, and fasting insulin (0 hour). The FFA concentrations were measured using an in vitro enzymatic colorimetric method (NEFA-C Test Kit; Wako Chemical, Richmond, VA). The CRP concentration was measured using a Human CRP ELISA Kit (Alpha Diagnostic International, San Antonio, TX). The minimum detectable CRP concentration using this method is 0.35 ng/mL. The intra- and interassay variations (reported by manufacturer) are 3.2% and 4.9%, respectively. Fasting serum insulin was measured using a solid phase $^{125}$I-radioimmunoassay (Coat-A-Count; Diagnostic Products, Los Angeles, CA).

At 0, 2, 4, 6, and 8 hours, blood was drawn into two 6-mL vacutainer tubes containing 10.8 mg K2 EDTA and centrifuged (1000*g*) at 4°C for 15 minutes within 30 minutes of the blood draw. Plasma supernatant was then aliquoted and frozen at −80°C for subsequent IL-6 (Quantikine HS Human IL-6 High Sensitivity ELISA; R&D systems, Minneapolis, MN) and sIL-6R (Quantikine Human IL-6sR ELISA, R&D systems) analysis. The minimum detectable IL-6 and sIL-6R concentrations were 0.039 and 6.5 pg/mL, respectively. The intra- and interassay variations for IL-6 and sIL-6R (reported by manufacturer) were 7.4% and 7.8%, and 7.0% and 7.8%, respectively.

For analysis of CRP, IL-6, and sIL-6R, all samples from one subject were analyzed in duplicate using the same ELISA kit. A pooled serum sample was used as an internal control for CRP, whereas a pooled EDTA plasma sample was used as an internal control for sIL-6R. For logistical reasons, it was not possible to use an internal control for IL-6; however, previous work in our laboratory has demonstrated the reliability of this assay, with the intra-and interassay variability found to be consistently within the range reported by the manufacturer.

### 2.5. Statistical analysis

All statistical analyses were performed using Statistical Analysis System version 9.1 (SAS Institute, Cary, NC). Data were examined for normality using residual error plots. The residuals were normally distributed for glucose, TAG, FFA, IL-6, sIL-6R, and CRP. Statistical difference was set at $P \leq .05$. Data are reported as mean ± SEM.

Dietary intake variables during the 1-week washout period between each trial day were compared using a 1-way repeated-measures analysis of variance, taking into account

Table 2
Subject baseline physical characteristics and fasting blood measurements

| Baseline characteristics | |
|---|---|
| Age (y)[a] | 56 ± 3 |
| Height (cm)[a] | 176 ± 2 |
| Weight (kg)[a] | 103.4 ± 6.8 |
| BMI (kg/m$^2$)[a] | 33.9 ± 2.4 |
| Body fat (%)[a] | 30.7 ± 2.0 |
| Waist circumference (cm)[a] | 114 ± 5 |
| Systolic blood pressure (mm Hg)[b] | 152 ± 9 |
| Diastolic blood pressure (mm Hg)[b] | 85 ± 4 |
| Glucose (mmol/L)[b] | 5.11 ± 0.13 |
| TAG (mmol/L)[b] | 2.84 ± 0.30 |
| Total cholesterol (mmol/L)[b] | 5.70 ± 0.27 |
| LDL cholesterol (mmol/L)[b] | 3.85 ± 0.21 |
| HDL cholesterol (mmol/L)[b] | 0.94 ± 0.16 |
| Total/HDL[b] | 7.48 ± 1.22 |
| Insulin (pmol/L)[b] | 90.4 ± 17.0 |
| FFA ($\mu$mol/L)[b] | 656 ± 29 |
| CRP (mg/L)[b,c] | 6.4 ± 1.4 |
| IL-6 (pg/mL)[b,c] | 2.7 ± 0.2 |
| sIL-6R (ng/mL)[b,c] | 48.3 ± 2.5 |

LDL indicates low-density lipoprotein.
[a] Data are means ± SEM; n = 8.
[b] Data are presented as the mean ± SEM of fasting values (0 hour) averaged over 3 trial days; n = 8.
[c] Data for 1 subject's water trial were excluded because of elevated fasting values; therefore, n = 7.

subject and treatment effect, followed by a Tukey post hoc test. A mixed-effects linear regression model (Proc Mixed, SAS systems version 9.1; SAS Institute) was used to examine the relationship between each end point and each treatment. This statistical approach is useful in making comparisons in a data set based on the rate of change of an end point and compares fitted longitudinal curves between treatments. Differences in the fitted longitudinal curves were examined by statistical tests performed on polynomial terms (cubic, quadratic, and linear) beginning at the highest-order term. Fit was accepted at the highest-order term where $P < .05$. This model is useful when comparing repeated measurements made over time, as it includes the fixed effects, time and treatment, as well as random effects.

## 3. Results

### 3.1. Subjects

Subject physical characteristics and fasting blood data are summarized in Table 2. As a group, subjects met the MetS inclusion criteria based on ATP III guidelines [1]. Of the 8 subjects, 6 had elevated fasting TAG (≥1.7 mmol/L), 1 had elevated fasting whole blood glucose (≥5.6 mmol/L), 5 were taking daily antihypertensive medication, and 3 were taking daily medication for treatment of depression. None of the subjects reported changes in n-3 PUFA intake; and there were no significant differences in energy, carbohydrate, protein, total fat, saturated fat, dietary fiber, alcohol, and caffeine intake, either between trials (ie, week to week) or between treatment groups (data not shown), as determined from analysis of 3-day food records.

### 3.2. TAG, glucose, insulin, and FFA

There were no significant differences in fasting TAG concentration between treatments (Fig. 1). The relationship between postprandial TAG concentration and time, for 8 hours after ingestion of the OFTT, was significant at the quadratic term for all 3 treatments ($P < .01$). There was a significant interaction between time and treatment ($P < .05$),

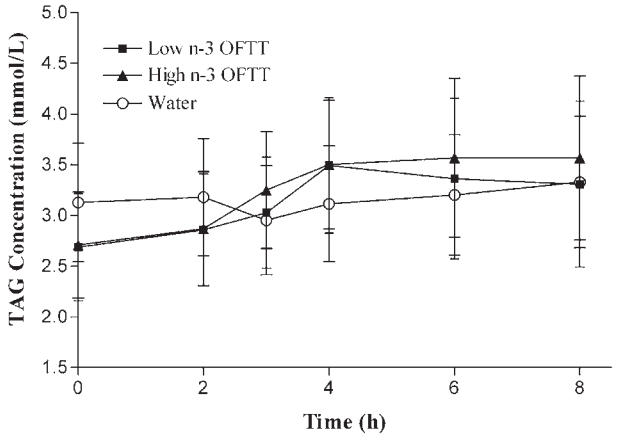


Fig. 1. Whole blood TAG concentrations over 8 hours after ingestion of either a high–n-3 or low–n-3 PUFA OFTT or water. Data are presented as means ± SEM (n = 8). There were no significant differences in fasting TAG concentration between treatments. There was a significant interaction ($P < .05$) between time and treatment. Blood TAG increased ($P < .05$) after consumption of the high–n-3 (▲) and low–n-3 (■) OFTTs, but not after water (○) ingestion. There was no difference in postprandial TAG response between the high–n-3 and low–n-3 PUFA OFTT.

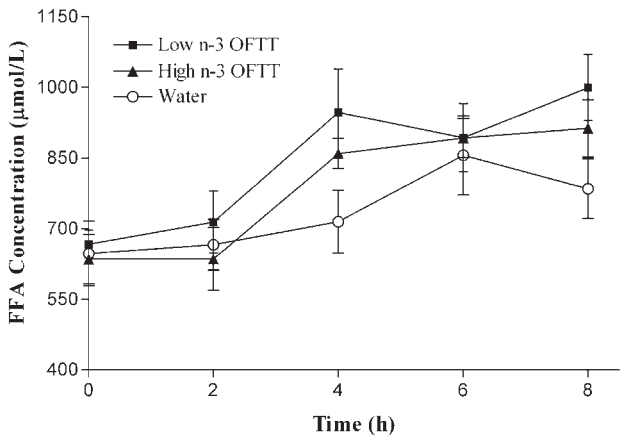


Fig. 2. Serum FFA concentrations over 8 hours after ingestion of either a high–n-3 or low–n-3 PUFA OFTT or water. Data are presented as means ± SEM (n = 8). There were no significant differences in fasting FFA concentrations between treatments. Serum FFA increased significantly after ingestion of the high–n-3 (▲) and low–n-3 (■) OFTTs and water (○). Increases in FFA were not affected by treatment. There was no significant time by treatment interaction. The rate of change in FFA fit the same longitudinal curve for all treatments.

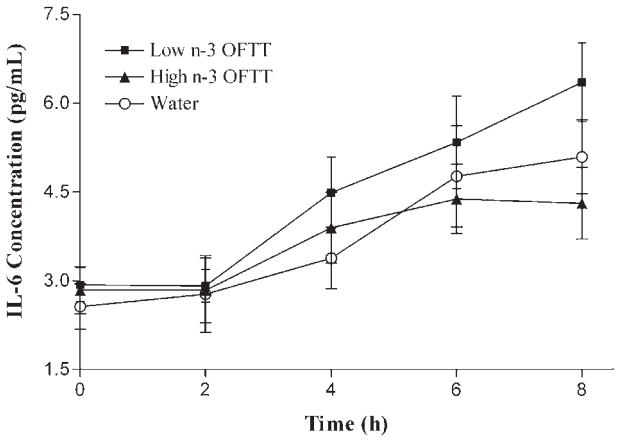


Fig. 3. Plasma IL-6 over 8 hours after ingestion of either a high–n-3 or low–n-3 PUFA OFTT or water. Data are presented as means ± SEM (n = 8). Data for 1 subject's water trial were excluded because of elevated fasting values (n = 7). There were no significant differences in fasting IL-6 concentrations between treatments. Plasma IL-6 increased significantly after ingestion of the high–n-3 (▲) and low–n-3 (■) OFTTs and water (○). Increases in circulating IL-6 were not affected by treatment. There was no significant time by treatment interaction. The rate of change in IL-6 fit the same longitudinal curve for all treatments.

with a significant increase in TAG after consumption of the high–n-3 and low–n-3 OFTTs, but not after water ingestion.

There were no significant differences in fasting glucose concentration between treatments; and the relationship between postprandial glucose concentration and time, for 8 hours after ingestion of the OFTT, was significant at the cubic term ($P < .01$). Blood glucose concentration decreased after ingestion of all treatments. There was no significant interaction between time and treatment, indicating that the rate of change in blood glucose concentration fit the same longitudinal curve for all treatments (data not shown). There were no differences in fasting insulin concentrations between treatments (data not shown).

There were no significant differences in fasting FFA concentrations between treatments. Serum FFA increased after ingestion of the high–n-3 and low–n-3 OFTTs and water. There was no significant interaction between time and treatment, indicating that the rate of change in FFA fit the same longitudinal curve for all treatments; and the relationship between postprandial FFA concentration and time, for 8 hours after ingestion of the OFTT, was significant at the linear term ($P < .0001$) (Fig. 2).

### 3.3. Inflammatory markers

There were no significant differences in fasting CRP, IL-6, or sIL-6R concentrations between treatments. Interleukin-6 concentrations increased significantly for 8 hours after ingestion of the high–n-3 and low–n-3 OFTTs and water. The relationship between time and treatment was significant at the linear term ($P < .0001$) (Fig. 3). There was no significant interaction between time and treatment in terms of IL-6 concentrations, indicating that the rate of change in IL-6 after both the high– and low–n-3 OFTTs and water fit the same longitudinal curve and that increases in circulating IL-6 concentrations were not affected by treatment. Data for sIL-6R and CRP were not significant at the linear, quadratic, or cubic terms; and there was no interaction between time and treatment for either end point (Table 3).

## 4. Discussion

To our knowledge, the current study is unique because it is the first to evaluate the acute impact of modifying the n-6/n-3 PUFA ratio in a high-SFA load on postprandial TAG and inflammatory marker responses associated with T2D and CVD risk in men with MetS. We included a water control trial to discern whether changes in inflammatory marker concentrations observed in the 8-hour postprandial period after OFTT ingestion were due to fat ingestion per se. Contrary to our hypothesis, increasing the n-3 PUFA content of an OFTT did not elicit different postprandial inflammatory responses; and, interestingly, the postprandial changes in inflammatory marker concentrations were not different between the OFTTs and water. Our data are in contrast with interpretations of previously reported data showing increases in postprandial IL-6 after a high-fat meal [22,23] and suggest

Table 3
Plasma sIL-6R and CRP concentrations over 8 hours after ingestion of either a low–n-3 or high–n-3 PUFA OFTT or water (control)

| Time (h) | 0 | 2 | 4 | 6 | 8 |
|---|---|---|---|---|---|
| sIL-6R (ng/mL) | | | | | |
| Low n-3 | 56.5 ± 16.0 | 54.0 ± 17.6 | 62.8 ± 28.5 | 53.6 ± 16.9 | 53.5 ± 15.2 |
| High n-3 | 55.2 ± 15.7 | 55.1 ± 17.9 | 55.4 ± 19.2 | 55.3 ± 15.7 | 58.5 ± 14.8 |
| Water[a] | 49.3 ± 14.0 | 52.2 ± 15.9 | 49.7 ± 12.6 | 52.6 ± 13.3 | 49.6 ± 16.7 |
| CRP (mg/L) | | | | | |
| Low n-3 | 3.0 ± 1.4 | 3.6 ± 1.3 | 3.4 ± 1.4 | 4.1 ± 1.4 | 4.7 ± 1.5 |
| High n-3 | 2.5 ± 1.5 | 2.9 ± 1.9 | 5.1 ± 2.4 | 5.1 ± 2.4 | 4.8 ± 2.3 |
| Water[a] | 2.9 ± 1.6 | 2.2 ± 1.4 | 3.4 ± 2.1 | 2.7 ± 1.3 | 2.8 ± 1.6 |

All data are presented as means ± SEM. There were no significant changes over time in either sIL-6R or CRP concentrations. There were no significant differences between treatments within an end point. *Low n-3* refers to the OFTT with n-6/n-3 PUFA ratio of 20:1; *high n-3* refers to the OFTT with n-6/n-3 PUFA ratio of 2:1.

[a] Data for 1 subject's water trial were excluded because of elevated fasting concentrations.

that previously observed postprandial increases in IL-6 may reflect diurnal variations in this inflammatory marker as opposed to a true postprandial response.

Our previous work has shown that subjects who meet the MetS criteria for elevated fasting TAG (≥1.7 mmol/L) [1] have exaggerated postprandial TAG responses to a high-SFA OFTT compared with subjects with fasting TAG levels below the clinical cutoff [21]. In our sample of men with MetS, circulating TAG increased after ingestion of a high-SFA load; and the rate of increase was not affected by increasing the n-3 PUFA content of the OFTT. Although we did not include a control group of healthy men, failure of circulating TAG to return to fasting concentrations by 8 hours certainly suggests dysregulated postprandial TAG metabolism in this population, regardless of the fatty acid composition of the OFTT. Our data also confirm previous work from our group demonstrating that neither the circulating TAG nor FFA fraction is altered by the types of fatty acids ingested in the OFTT [21]. Although the absence of postprandial glucose and insulin responses raises questions regarding the physiologic relevance of the OFTT, the protocol we used is similar to the oral glucose tolerance test (ie, pure carbohydrate load) commonly used to assess carbohydrate metabolism and fits with our goal of isolating the impact of specific fatty acids on postprandial metabolism.

Previous studies suggest that both the amount and type of fatty acids in a meal may affect the magnitude and duration of the postprandial TAG response [6]. Although sustained n-3 PUFA supplementation has consistently been shown to reduce fasting plasma TAG and improve postprandial TAG responses [24-26], the ability of n-3 PUFA to attenuate postprandial TAG responses without prior n-3 PUFA supplementation has not been examined. Sustained n-3 PUFA intake may improve circulating TAG concentrations by decreasing the rate of very low-density lipoprotein synthesis [25] and/or by inhibiting fatty acid oxidation via peroxisome proliferator–activated receptor–α activation [27]; however, in the current study, the postprandial TAG response was not different between the high– and low–n-3 OFTTs, suggesting that 8 hours does not provide sufficient time for n-3 PUFA to exert these beneficial effects. Nonetheless, increases in TAG after consumption of the OFTTs, but not water, provide a physiologic basis for further investigating the impact of fat ingestion on circulating inflammatory mediators in the postprandial state because a relationship between postprandial TAG and inflammatory cytokines has been previously demonstrated [6,28].

Exposure to raised concentrations of IL-6 and CRP, potent inflammatory mediators, is implicated in the development of T2D and CVD [29]. Postprandial IL-6 responses are of particular interest in individuals who demonstrate elevated fasting IL-6 concentrations, as the postprandial state is repeatedly invoked throughout the day [28]. We have previously shown that IL-6 concentrations remain significantly elevated for 8 hours after ingestion of an OFTT in men with elevated fasting TAG and IL-6 concentrations compared with their healthy counterparts [30]. The sample of men with MetS in the current study demonstrated elevated fasting IL-6 concentrations (2.79 ± 0.21 pg/mL) [10], and IL-6 increased significantly from baseline at the same rate after both OFTTs and the water control. There were no differences in plasma IL-6 concentrations between the high– and low–n-3 OFTTs or between the OFTTs and the water control. Either attenuation or exaggeration of the increase in IL-6 over 8 hours, after ingestion of the OFTTs, would have suggested an effect of fat ingestion itself. Thus, our results suggest a natural increase in IL-6 over 8 hours in our sample of men with MetS, as opposed to a true postprandial response. Earlier studies have also demonstrated increases in IL-6 from morning to night irrespective of nutrient intake [14,31,32]. Our data are in accordance with data from Van Oostrom et al [14] demonstrating IL-6 increases similarly after a standardized meal and a water control. Our findings contradict those of previous studies showing that consumption of a high-fat meal leads to significant postprandial increases in plasma IL-6 in healthy and overweight subjects [22,23]. However, direct comparisons of our findings with these data are not possible given substantial differences in test meal composition and subject metabolic status, as well as the lack of a water control trial. Furthermore, Lundman et al [33] have shown that the degree of postprandial TAG response is not correlated to plasma IL-6 concentrations. Consideration of all of these findings suggests that increases in plasma IL-6 may not be dictated by postprandial factors per se.

The physiologic effects of IL-6 depend strongly on the soluble form of its receptor [34]. Despite an increase in plasma IL-6 over 8 hours, circulating sIL-6R concentrations did not change over time nor were there differences between treatments, suggesting that there is a range within which IL-6 concentrations fluctuate without affecting sIL-6R concentrations. To our knowledge, this study is the first to examine plasma sIL-6R concentrations in men with MetS after ingestion of high-SFA challenges with different n-6/n-3 PUFA ratios and a water control, precluding comparison to other studies. Nelson and Hickey [35] reported an 11% decrease in sIL-6R after a 4-day intervention replacing dietary linoleic acid (18:2n-6) with α-linolenic acid (18:3n-3) in healthy and overweight men. Comparison of our data to those of Nelson and Hickey [35] supports the theory that a longer n-3 PUFA supplementation period may be needed to observe anti-inflammatory benefits.

In innate immunity, IL-6 is regarded as a potent inducer of hepatic CRP production [36]. However, we found no significant changes in CRP despite significant increases in IL-6 over 8 hours. Other studies have also reported that IL-6 and CRP do not necessarily change in exact accordance with one another [37]; and it has been previously established that CRP does not follow a diurnal pattern in healthy individuals [38], although, to our knowledge, this has not been previously assessed in men with MetS. Because CRP concentrations are elevated in individuals with CVD and

MetS [39,40] and remain stable regardless of time of day or nutrient intake, this inflammatory mediator has gained appeal as an important biomarker for obesity-related diseases [8,9]. Our study confirms a lack of natural increases in circulating CRP over 8 hours in men with MetS and that there is no postprandial CRP response to a high-fat meal [41]. Studies examining the effect of sustained n-3 PUFA intake on serum CRP concentrations are inconsistent. Whereas some studies demonstrate a reduction in fasting CRP after n-3 PUFA supplementation [42,43], others did not observe an effect of n-3 PUFA on this inflammatory marker [44-47]. Our findings indicate that there is no acute effect of n-3 PUFA intake, in the absence of a prior n-3 PUFA intervention, on postprandial CRP concentrations in men with MetS.

Certain limitations to our study should be addressed in future study designs. Although all subjects met 3 of the 5 ATP III MetS criteria, they did not meet the same 3 criteria, contributing to heterogeneity within our sample population. Combined with our small sample size, this may have precluded our ability to observe some significant differences. Despite this, our study does contribute to the existing postprandial literature in men with MetS, as previous postprandial research focuses primarily on TAG responses in healthy subjects [6,28,29]. Yet, considering the emerging relationship between chronically elevated inflammatory markers, abnormal postprandial metabolism, and the development of T2D and CVD, further research designed to characterize postprandial responses and to discern between natural increases and true postprandial effects in individuals with MetS is warranted.

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