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Fasting triacylglycerol status, but not polyunsaturated/saturated fatty acid ratio, influences the postprandial response to a series of oral fat tolerance tests☆

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Abstract

Elevated postprandial lipemia is emerging as a risk factor for obesity-related chronic diseases, such as type 2 diabetes and cardiovascular disease, and is associated with alterations in several metabolic biomarkers of disease. Our goal was to examine the effects of specific polyunsaturated/saturated fatty acid (P/S) ratios on postprandial triacylglycerol (TAG) concentrations and metabolic biomarkers in men with different fasting TAG concentrations through a series of oral fat tolerance tests (OFTT) consisting solely of emulsified lipid. Otherwise healthy men with high (>1.69 mmol/L) fasting TAG (HTAG, n=8) and low fasting TAG (LTAG, n=8) underwent three OFTTs with specific P/S ratios of 0.2, 1.0 and 2.0, respectively, and a total lipid load of 1 g/kg subject body mass. All subjects received each treatment separated by at least 1 week. Postprandial plasma TAG fatty acid composition reflected fatty acids present in the OFTT. All other metabolic responses were independent of the P/S ratio ingested. An accelerated increase in postprandial TAGs was observed in HTAG compared to LTAG. Interleukin (IL)-6 and soluble intercellular adhesion molecule (sICAM)-1 were significantly elevated in HTAG at baseline (P<.05). IL-6 increased significantly following each OFTT (P<.05) in both groups. Postprandial glucose and CRP were significantly exaggerated (P<.05) in HTAG. Overall, HTAG subjects had an accelerated postprandial TAG response and increased concentrations of several inflammatory markers following an OFTT, in the absence of an insulin response. However, P/S ratio had no influence on postprandial lipid and inflammatory parameters.

Keywords: Polyunsaturated/saturated fatty acid ratio; Triacylglycerol; Metabolic biomarkers; Oral fat tolerance test; Postprandial; Lipids

1. Introduction

Elevated plasma triacylglycerol (TAG, >1.69 mmol/L) is a criterion of metabolic syndrome [1], a clustering of obesity-related risk factors associated with chronic disease such as type 2 diabetes and cardiovascular disease (CVD) [2]. Furthermore, postprandial lipemia is established as a risk factor for CVD [3–7]. Individuals with elevated fasting TAG demonstrate an exaggerated postprandial lipemia [8,9]; therefore, the postprandial period represents an ideal target for disease risk assessment.

Chronic low-grade inflammation observed in obesity has been implicated in the development of CVD [10], type 2 diabetes [11] and the metabolic syndrome [12,13]. Dietary fats have been well established as regulators of pro-inflammatory gene expression [14,15], and thus the contribution of chronic low-grade inflammation to disease

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risk during the postprandial response to dietary fat warrants further study. Recent evidence has shown the postprandial period to be a pro-inflammatory [16–18], pro-atherogenic [17,19] and pro-thrombotic [20,21] state. The postprandial response to dietary fat of several of these inflammatory markers is of particular interest. IL-6 is an inflammatory cytokine that is associated with insulin resistance [22] and type 2 diabetes [11] and that has been shown to increase in response to dietary fat ingestion [17,23–25]. The adhesion molecules sICAM-1 [26] and sVCAM-1 [27] are implicated in CVD [26] and type 2 diabetes [27], and both have been shown to increase following a high fat meal [17,19]. The acute phase protein CRP [28] and PAI-1 [29], a fibrinolytic antagonist, have been linked to CVD [28,29] and may [20,30,31] or may not [21,23,24] be regulated by dietary fat throughout the postprandial period.

We have previously developed a novel series of OFTTs to study biomarkers for disease during the postprandial response to fat [32]. OFTTs with specific polyunsaturated/saturated fatty acid (P/S) ratios of 0.2, 1.0 and 2.0 (respectively, P/S 0.2, P/S 1.0 and P/S 2.0) can be prepared to assess the postprandial response to a range of P/S ratios. The OFTTs contain only lipid, allowing us to focus specifically on postprandial TAG and inflammatory responses to a lipid load. This allows us to study three different P/S ratios within the same mode of delivery, namely, an emulsified beverage. This is important as there is conflicting evidence that saturated fatty acids (SFA) decrease [33–35], increase [36] or have no effect [37] on postprandial TAG concentrations. The influence of P/S ratio on inflammatory markers in the postprandial state is unknown. The present study was designed to test two hypotheses: (1) that men with high fasting TAG concentrations would exhibit an exaggerated postprandial response in lipid and inflammatory parameters compared to men with low fasting TAG and (2) that exposure to a high SFA load (P/S 0.2) would elicit a greater inflammatory response compared to a high polyunsaturated (P/S 2.0) OFTT in both groups of men.

2. Methods and materials

2.1. Subjects and preliminary screening

This study was approved by the University of Guelph Research Ethics Board. Eighteen males were recruited to participate in this study through a combination of newspaper, poster and website advertisements. Subjects were required to be over 45 years of age, nonsmokers and sedentary or participating in a low frequency (<3×30 min) of aerobic exercise per week. Individuals taking medication for control of blood lipids were excluded from participation in this study. Subjects were asked to read and complete an Informed Consent Form identifying potential risks associated with participation in the study. Subjects were required to attend one pretrial session to complete a Subject Screening Questionnaire and to have weight and height measured. All subjects with a BMI greater than 27 kg/m^2 were required to undergo a screening oral glucose tolerance test (OGTT) to assess glucose tolerance. Any persons meeting the criteria for type 2 diabetes were excluded from the study and instructed to consult their physician. Subjects had waist circumference measured and body composition determined using bioelectrical impedance analysis (Bodystat, Tampa, FL, USA). In total, 18 subjects were recruited for the study. Two subjects withdrew during the study owing to palatability concerns. The OFTTs were well tolerated by the remaining subjects (n=16). Subjects were divided into two groups based on fasting TAG concentrations above (HTAG, n=8) or below (LTAG, n=8) the National Cholesterol Education Program Adult Treatment Panel III cut-off of 1.69 mmol/L [1].

2.2. Materials

The palm stearine, soybean oil and bleaching clay (Engelhard F105) were generously provided by Bunge Canada (Toronto, ON). Sodium methoxide (Sigma Aldrich) and citric acid (VWR International) were used in the lipid interesterification. Myverol 18-99K and Tween80 (both from Acatris, Oakville, ON, Canada) were used to prepare the emulsified OFTT beverages which included red food coloring and cherry flavoring, purchased from a local grocery store. Aspartame was supplied by Cambrian Chemicals (Cambridge, ON, Canada).

2.3. Oral fat tolerance test preparation

To study postprandial metabolic responses, we produced an emulsified, artificially flavored and sweetened beverage that contained 1 g of lipid per kilogram of subject body weight and was devoid of protein or carbohydrate as previously described in detail [32]. Briefly, prior to the experimental trials, three fat blends of palm stearine and soybean oil were prepared to achieve the desired P/S ratio. The ratios of P/S 0.2, P/S 1.0 and P/S 2.0 were selected to reflect low, intermediate and high concentrations of polyunsaturated fatty acids, respectively. The blends were chemically interesterified in order to achieve a completely random distribution of the fatty acids present (for review, see Ref. [38]).

2.4. Study design

2.4.1. Beverage preparation

On each trial day, 1 g of lipids per kilogram of subject body weight of the desired P/S fatty acid blend was measured into a glass mug. The fat was warmed to approximately 50°C and subsequently emulsified in water (73% wt/wt). To maintain the emulsified pure fat challenge, the monoglyceride Myverol (2% wt/wt) and polysorbate Tween 80 (0.15% wt/wt) were added using a handheld homogenizer. Each OFTT was sweetened with nonnutritive aspartame and flavored with commercial cherry flavoring.

2.5. Experimental trials

The study was a double-blind randomized design in which subjects and the primary investigators were blinded to the treatment. Subjects visited the laboratory on three separate occasions separated by at least 1 week. An acute bout of exercise prior to a postprandial TAG test has a well-established TAG-lowering effect [39]. On each occasion, subjects were required to abstain from alcohol and exercise for 48 h prior to the trial and to maintain dietary records for 3 days prior to each trial. Subjects were provided a standardized meal (17±1% protein, 55±1% carbohydrate, 28±1% fat) on the evening before each trial day and reported to the laboratory after an overnight (12 h) fast. Upon arrival at the laboratory, a catheter was inserted into a forearm vein to allow for the withdrawal of blood samples. Intravenous saline was administered to maintain the catheter for repeated blood sampling. On each day, following an initial fasting blood sample (0 h), the subjects ingested one of three OFTT emulsions (P/S 0.2, P/S 1.0 or P/S 2.0). The composition of the three OFTTs was measured by gas chromatography using a Shimadzu GC-8A with flame ionization detector (Shimadzu, Kyoto, Japan) after preparation of fatty acid methyl esters, according to the method of Bannon et al. [40], and is shown in Table 1. Subjects consumed the OFTT within 10 min. Blood samples were taken at 1, 2, 3, 4, 5, 6 and 8 h after the OFTT, and subjects were allowed to consume only water during this time. Not all end points were measured at every postprandial time point.

2.6. Whole blood measurement

At each time point, a blood sample was drawn in a heparinized tube for the analysis of whole blood metabolites. Blood lipids, including total TAGs, total cholesterol and HDL cholesterol, were analyzed using Cholestech LDX lipid cassettes (Cholestech, Hayward, CA, USA). LDL-cholesterol concentrations were calculated using the Friedewald equation. This calculation was performed automatically by the Cholestech LDX. Blood glucose was analyzed in duplicate by a glucose oxidase method (YSI 2300 Stat Plus Glucose Analyzer, Yellow Springs, OH, USA).

Table 1
Fatty acid composition (% wt/wt of total fatty acids) of OFTT beverages

Fatty acid[a]	P/S 0.2	P/S 1.0	P/S 2.0
12:0	0.24	0.01	0.01
14:0	1.07	0.54	0.27
16:0	49.93	29.42	18.78
18:0	5.74	5.36	5.52
18:1n-9	26.74	24.44	24.00
18:2n-6	13.32	33.81	43.16
18:3n-3	1.82	6.14	7.63
20:0	0.22	0.29	0.38
SFA	57.19	35.62	24.96
MUFA	26.74	24.44	24.00
PUFA	15.14	39.95	50.79
P/S Ratio[b]	0.26	1.12	2.03

[a] Only major (i.e., >0.15 wt.%) fatty acids are shown.
[b] P/S Ratio was calculated by dividing total PUFA by total SFA.

Table 2
Subject characteristics and fasting blood measurements

	All subjects	LTAG (n=8)	HTAG (n=8)
Age (years)	57±2	58±3	57±2
Height (cm)	171±1	169±2	173±2
Weight (kg)	92±4	89±4	95±8
BMI (kg/m^2)	31.5±1.3	31.3±1.4	31.6±2.2
Body fat (%)	29.9±1.2	29.3±1.7	30.5±1.9
Waist circumference (cm)	106±3	107±3	106±5
Triacylglycerol (mmol/L)	2.0±0.31	1.17±0.1	2.84±0.45*
FFA (μmol/L)	490±28	485±45	495±35
HDL Cholesterol (mmol/L)	1.0±0.5	0.99±0.08	1.02±0.08
LDL Cholesterol (mmol/L)[a]	3.6±0.3	3.7±0.3	3.5±0.4
Total cholesterol (mmol/L)	5.4±0.2	5.3±0.4	5.6±0.3
Glucose (mmol/L)	5.0±0.2	4.9±0.2	5.1±0.3
Insulin (pM/L)	92.0±14.8	70.2±10.4	113.8±26.3
HOMA[b]	1.8±0.3	1.3±0.2	2.2±0.5
IL-6 (pg/ml)	2.6±0.5	1.9±0.3	3.2±0.9
CRP (mg/L)	3.5±0.7	2.8±0.6	4.3±1.3
PAI-1 Activity (activity units/ml)	15.5±2.9	14.0±4.3	17.4±4.1
PAI-1 Total (pg/ml)	22.3±2.3	21.0±3.6	23.4±3.0
sICAM-1 (pg/ml)	240±13	222±12	257±22
sVCAM-1 (pg/ml)	638±39	613±68	662±40

Data are presented as mean±S.E.M.
[a] LDL was calculated using the Friedewald equation.
[b] Updated HOMA as a marker of insulin sensitivity.
* P<05, HTAG vs. LTAG.

2.7. Other blood measurements

Blood samples were drawn into three tubes: 3 ml was drawn into a nontreated tube, allowed to clot at room temperature, centrifuged at 1340×g (2400 rpm) for 10 min (Beckman Allegra X-12R, Fullerton, CA, USA) and supernatant frozen at −20°C for analysis of serum insulin, soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule (sVCAM-1), serum FFA and C-reactive protein (CRP); 5 ml was drawn into an EDTA-treated tube on ice, centrifuged at 4°C and 1000×g for 15 min and supernatant was stored at −80°C for analysis of plasma interleukin (IL)-6; 4 ml was drawn into a citrate-treated tube, centrifuged at 4°C and 1340×g (2400 rpm) for 10 min and the supernatant was stored at −80°C for analysis of plasma total plasminogen activator inhibitor (PAI)-1 total antigen and PAI-1 activity. Blood samples were analyzed for specific end points at each time point, as indicated in the results.

2.8. Fatty acid analysis

The heparinized blood remaining after the whole blood measurements was centrifuged at 1340×g (2400 rpm) (Beckman Allegra X-12R) and the supernatant frozen at −20°C for TAG fatty acid analysis at a later date. Composition of plasma TAG fatty acids was assessed at 0, 4 and 8 h. Plasma fatty acids were extracted using

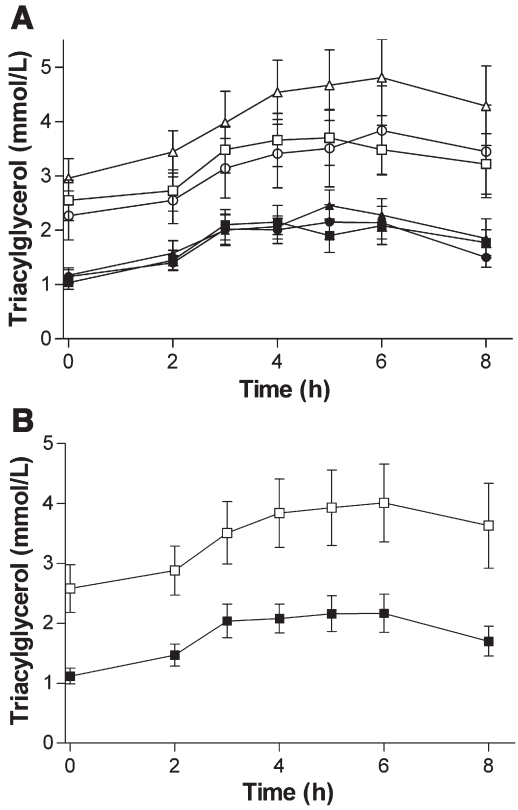

Fig. 1. (A) Postprandial TAG response to three OFTTs of different P/S ratios in HTAG (fasting TAG >1.69 mmol/L, n=8) and LTAG (fasting TAG ≤1.69 mmol/L, n=8) subjects. Open squares (□) represent HTAG following P/S 0.2; open triangles (△) represent HTAG following P/S 1.0; open circles (○) represent HTAG following P/S 2.0; closed squares (■) represent LTAG following P/S 0.2; closed triangles (▲) represent LTAG following P/S 1.0; closed circles (●) represent LTAG following P/S 2.0; Data are presented as geometric mean±transformed S.E.M. TAGs increased significantly following the ingestion of the OFTT in all treatments and in both groups (P<05). There was no effect of treatment in either group, so treatments were collapsed to facilitate group comparisons. (B) Average postprandial TAG response to three OFTTs in LTAG (n=8) and HTAG (n=8) subjects. Open squares (□) represent HTAG; closed squares (■) represent LTAG. Data are presented as geometric mean±transformed S.E.M. TAGs in HTAG were significantly greater than LTAG throughout the postprandial period (intercept term, P<05); the rate of change (slope term) was significantly higher in the HTAG vs. LTAG (P<05).

chloroform/methanol and TAG was isolated on silica gel G plates [41]. A standard fatty acid (C17:0; 5 μg/ml) was added to the scraped silica band and the sample was methylated using 14% (wt/v) BF_3/methanol reagent. Fatty acid methyl esters were separated by automated gas-liquid chromatography (Vista 6010; Varian Instruments, Georgetown, Canada) on a fused-silica BP20 capillary column (25 m×0.25 mm internal diameter; Varian Instruments). Fatty acid methyl ester peaks were identified by comparison with standards purchased from Supelco Canada and Sigma companies. Saturated, monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid content of plasma TAG were calculated on a quantitative and percent (wt/wt^{-1}) basis [42].

2.9. Assays

All samples were analyzed in duplicate. Serum insulin was determined using a RIA method (Coat-a-Count, Diagnostic Products, Los Angeles, CA, USA). Serum

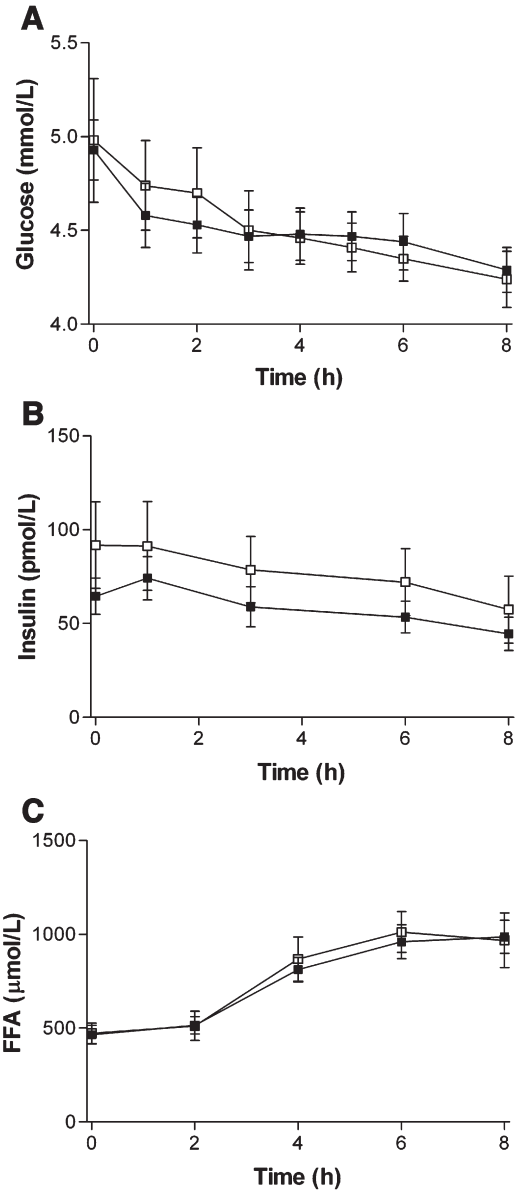

Fig. 2. (A) Mean postprandial glucose response to three OFTTs in LTAG (n=8) and HTAG (n=8) subjects. Open squares (□) represent HTAG; closed squares (■) represent LTAG. Data are presented as geometric mean±transformed S.E.M. There is a significant difference in response during the postprandial period in the HTAG subjects vs. LTAG subjects (rate of change, P<05). (B) Mean postprandial insulin response to three OFTTs in LTAG (n=8) and HTAG (n=8) subjects. Open squares (□) represent HTAG; closed squares (■) represent LTAG. Data are presented as geometric mean±transformed S.E.M. There is a significant decrease in insulin (P<05) in both groups throughout the postprandial period. (C) Mean postprandial FFA response to three OFTTs in LTAG (n=8) and HTAG (n=8) subjects. Open squares (□) represent HTAG; closed squares (■) represent LTAG. Data are presented as arithmetic mean±S.E.M. There is a significant in increase in FFA throughout the postprandial period (P<05).

Table 3
Fasting fatty acid composition (% wt/wt) of plasma TAG[a] in HTAG and LTAG subjects

Fatty acid	HTAG 0h	LTAG 0h
	Average	Average
12:0	0.2±0.1*	0.5±0.1
14:0	1.9±0.2	1.6±0.3
16:0	26.5±0.8*	22.9±1.3
18:0	4.3±0.3	4.0±0.4
18:1(n-9)	38.1±1.7	37.7±1.4
18:2(n-6)	15.9±1.2	18.1±1.2
18:3(n-3)	1.0±0.1	1.0±0.2
20:0	0.3±0.2	0.1±0.1
20:4(n-6)	0.8±0.2	0.5±0.2
SFA	33.8±1.1	30.7±1.3
MUFA	46.1±1.7	45.7±1.3
PUFA	19.9±1.5	22.7±1.4
n-6[b]	18.2±1.3	20.4±1.2
n-3[c]	1.7±0.3*	2.3±0.4
n-6/n-3[d]	12.8±1.8	11.6±2.1

Values are mean±S.E.M. of three OFTTs in HTAG (fasting TAG >1.69 mmol/L, n=8) and LTAG (fasting TAG ≤1.69 mmol/L, n=8) subjects.
[a] Only major fatty acids that are consistent with OFTT composition are reported.
[b] Sum of n-6 fatty acids.
[c] Sum of n-3 fatty acids.
[d] n-6/n-3 fatty acid ratio was calculated by dividing total n-6 fatty acids by total n-3 fatty acids.
* Within a row, significantly different (P<05) from LTAG.

sICAM-1 and sVCAM-1 and plasma IL-6 were determined using separate high-sensitivity quantitative sandwich enzyme immunoassays (Quantikine HS, R&D, Minneapolis MN, USA). Serum FFA was calculated by colorimetric assay (NEFA kit, Wako Bioproducts, Richmond, VA, USA). Serum CRP was determined by quantitative sandwich enzyme immunoassay (ADI, San Antonio, TX, USA). Plasma total PAI-1 antigen was determined using a quantitative enzyme immunoassay (Tintelize, Biopool, Sweden). Plasma PAI-1 activity was determined using a bio immunoassay (Chromolize, Biopool).

2.10. Calculations and statistical analysis

All subject characteristics (height, weight, age, BMI, waist circumference, percent body fat) were analyzed for group differences using a two-tailed t test. Food record analysis was performed with ESHA Food Processor SQL v. 9.04. Fasting blood measurements were averaged across the three trials and then analyzed for group differences with a two-tailed t test. The updated homeostasis model assessment (HOMA) of insulin sensitivity was calculated using the HOMA calculator for Windows XP v. 2.2 from the Diabetes Trials Unit, University of Oxford. A mixed-effects linear regression model (Proc Mixed, SAS systems version 9.1) was used to analyze time curves of biological interest. This approach utilized curve fitting at the linear, quadratic and

Table 4
Fatty acid composition (% wt/wt) of plasma TAG[a] following three OFTTs of different P/S fatty acid ratios

Fatty acid	0h			4h			8h		
	0.2	1.0	2.0	0.2	1.0	2.0	0.2	1.0	2.0
12:0*	0.4±0.1	0.4±0.1	0.3±0.1	0.3±0.1	0.3±0.1	0.2±0.1	0.1±0.0	0.3±0.1	0.1±0.0
14:0*,†,‡,§	1.7±0.2	2.1±0.2	1.5±0.2	1.4±0.1	1.8±0.1	1.2±0.1	1.2±0.1	1.5±0.2	1.0±0.2
16:0	24.0±0.7	25.9±0.9	24.1±1.0	30.7±0.5†,‖	28.3±1.0†,‖	23.6±0.8	32.9±1.0*,‖,¶	28.2±1.1‖	23.3±1.0
18:0	3.8±0.3	4.2±0.2	4.4±0.3	4.3±0.3	4.1±0.2	4.0±0.2	4.9±1.5	5.2±0.8	3.9±0.3
18:1(n-9)*,†,‡	38.7±1.3	37.0±0.7	38.0±1.3	35.8±0.9	34.4±1.1	35.0±1.2	37.5±1.1	31.1±1.4	34.2±1.0
18:2(n-6)	17.2±0.7	17.0±1.1	16.8±0.8	17.3±0.9‖	19.2±0.9‖	24.0±0.8†	14.9±0.5‖,¶	20.3±1.8‖	27.7±1.8*,#
18:3(n-3)	1.1±0.1	0.9±0.2	1.0±0.1	0.8±0.1‖	1.3±0.2	1.7±0.1†	0.8±0.1‖	1.1±0.2‖	2.0±0.3*
20:0	0.2±0.2	0.1±0.0	0.3±0.1	0.2±0.1	0.4±0.2	0.1±0.1	0.1±0.0	0.4±0.2	0.1±0.0
20:4(n-6)	0.7±0.2	0.6±0.1	0.8±0.1	0.6±0.1	0.5±0.1	0.7±0.2	0.6±0.1	0.6±0.1	0.6±0.1
SFA	31.3±1.0	33.8±0.8	31.6±1.1	37.7±0.7†,‖	36.1±0.9‖	30.0±0.9	39.8±1.6*,‖,¶	36.1±1.0‖	28.9±1.3
MUFA*,†,‡	46.3±1.2	45.2±0.9	46.2±1.1	41.1±0.8	39.9±1.2	40.7±1.2	42.0±1.4	36.3±1.4	38.4±1.1
PUFA	21.3±1.0	21.0±1.2	21.5±1.0	20.8±1.0‖	22.9±1.2†,‖	28.6±1.0†	17.8±0.7*,‖,¶,#	26.1±1.7*,‖	32.5±1.7*,#
n-6[b]	19.6±0.9	19.0±1.0	19.3±0.9	19.0±1.0‖	21.0±1.0†,‖	25.9±1.0†	16.5±0.6*,‖,¶,#	23.9±1.5*,‖	29.7±1.6*,#
n-3[c,‡,§]	1.8±0.1	2.0±0.3	2.2±0.3	1.8±0.2	1.8±0.2	2.7±0.2	1.2±0.2	2.1±0.4	2.9±0.4
n-6/n-3[d]	12.3±1.3	13.2±1.6	11.0±1.4	13.3±2.1	13.2±1.4	10.6±1.0	15.8±1.6	18.1±4.1	18.3±6.0

Values are mean±S.E.M. of three OFTTs in HTAG (fasting TAG >1.69 mmol/L, n=8) and LTAG (fasting TAG ≤1.69 mmol/L, n=8) subjects.
[a] Only major fatty acids that are consistent with OFTT composition are reported.
[b] Sum of n-6 fatty acids.
[c] Sum of n-3 fatty acids.
[d] n-6/n-3 fatty acid ratio was calculated by dividing total n-6 fatty acids by total n-3 fatty acids.
* Significantly different (P<05), 0 vs. 8 h.
† Significantly different (P<05), 0 vs. 4 h.
‡ Significantly different (P<05) between 0.2 and 1.0 OFFTs without interaction with time.
§ Significantly different (P<05) between 1.0 and 2.0 OFFTs without interaction with time.
‖ Significantly different (P<05) from 2.0 with interaction with time.
¶ Significantly different (P<05) from 1.0 with interaction with time.
# Significantly different (P<05), 4 vs. 8 h.

cubic terms, and fit was accepted at the highest order term where $P<.05$. This statistical approach is useful in making complex comparisons on a dataset based on rate of change through the comparison of fitted time curve shapes between groups and treatments. Plasma fatty acid composition was analyzed at three time points (0, 4 and 8 h) for effects of group, treatment and group-by-treatment interactions using a two-way repeated measures ANOVA. When statistical significance was indicated, a Tukey *post hoc* test was used for multiple comparisons. When plasma fatty acid composition was not different between groups, groups were pooled and the effects of treatment, time and treatment-by-time interactions were assessed using a two-way repeated measures ANOVA for the overall group ($n=16$). Pearson product moment correlations were made between blood lipid parameters (fasting TAG, and TAG fatty acid composition) and fasting concentrations of inflammatory markers (IL-6, CRP, sICAM, sVCAM, PAI-1 total and activity). Statistical significance was set at $P<.05$. All data that were normally distributed are presented as arithmetic mean±S.E.M. All time course data that were not normally distributed were log transformed and are presented as the geometric mean±average transformed S.E.M., as indicated.

3. Results

3.1. Subject characteristics and fasting blood measurements

Subject physical and clinical characteristics are summarized in Table 2. There were no significant differences in age, height, weight, BMI, waist circumference or percent body fat between HTAG and LTAG subjects. There was no difference in terms of daily food consumption in the three study days leading up to the trial, with mean energy intake (percent total±S.E.M.) reported as 17±1% protein, 52±2% carbohydrates, 30±1% fat and 1±1% alcohol.

Fasting blood measurements are summarized in Table 2. By study design, subjects in the HTAG group had significantly higher fasting TAGs than those in the LTAG group ($P<.001$). There were no other statistically significant differences in fasting blood measurements. Although not statistically significant, fasting insulin, IL-6, sICAM-1 and HOMA were higher by 62% ($P=.14$), 68% ($P=.17$), 16% ($P=.18$) and 69% ($P=.15$), respectively, in HTAG vs. LTAG subjects.

3.2. Postprandial TAG, glucose, insulin and FFA

For all OFTT treatments, blood TAGs increased significantly during the postprandial period in both HTAG and LTAG subjects (Fig. 1A, $P<.05$). There was no significant treatment effect on postprandial TAG response. A significant difference ($P<.05$) in postprandial TAG response between HTAG and LTAG was observed for the intercept and slope terms, meaning that during the postprandial period HTAG maintained a significantly elevated TAG concentration with an accelerated rate of change compared to LTAG (Fig. 1B).

Mean glucose and insulin decreased significantly ($P<.05$) during the postprandial period after the ingestion of each OFTT (Fig. 2A and B). Glucose was significantly higher during the OFTT at the quadratic and cubic terms ($P<.05$) in the HTAG vs. the LTAG, meaning that the HTAG and LTAG have similar intercept and slope terms but demonstrate significantly different curve shapes suggesting a difference in the rate of change of blood glucose in the postprandial period. This can be further interpreted that the decrease in blood glucose occurred later in the HTAG compared with the LTAG group. As expected, serum FFA concentrations increased significantly ($P<.05$) from fasted levels in both subject groups (Fig. 2C). There were no differences in terms of postprandial FFA concentration between HTAG and

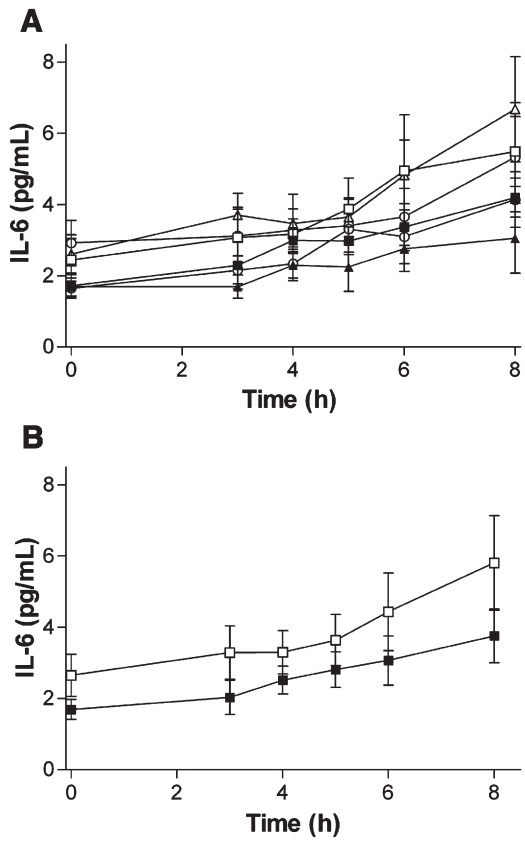

Fig. 3. (A) Postprandial IL-6 response to three OFTTs of different P/S ratios in HTAG (fasting TAG >1.69 mmol/L, $n=8$) and LTAG (fasting TAG ≤1.69 mmol/L, $n=8$) subjects. Open squares (□) represent HTAG following P/S 0.2; open triangles (△) represent HTAG following P/S 1.0; open circles (○) represent HTAG following P/S 2.0; closed squares (■) represent LTAG following P/S 0.2; closed triangles (▲) represent LTAG following P/S 1.0; closed circles (●) represent LTAG following P/S 2.0. Data are presented as geometric mean±transformed S.E.M. IL-6 increased significantly following the ingestion of the OFTT in all treatments and in both groups ($P<.05$). There was no effect of treatment in either group, so treatments were collapsed to facilitate group comparisons. (B) Mean postprandial IL-6 response to three OFTTs in LTAG ($n=8$) and HTAG ($n=8$) subjects. Open squares (□) represent HTAG; closed squares (■) represent LTAG. Data are presented as geometric mean±transformed S.E.M. IL-6 in HTAG was significantly greater than in LTAG throughout the postprandial period (intercept term, $P<.05$).

LTAG groups, nor was there an effect of P/S ratio on postprandial glucose, insulin or FFA responses.

3.3. Plasma TAG fatty acid analysis

In addition to the magnitude of the plasma TAG response, the fatty acid composition of plasma TAGs at fasting and following the ingestion of each OFTT was quantified. For consistency, the fatty acids reported in Table 3 represent the fasting proportions of the major fatty acids in the blood that are in common with those fatty acids comprising the OFTTs. The plasma fatty acid composition was evaluated in two stages. First, the data was analyzed based on subject group independently at 0, 4 and 8 h for TAG status differences. At time 0 h, the plasma percentages of lauric (12:0) and palmitic (16:0) acids were significantly ($P<.05$) greater in HTAG than in LTAG (Table 3). Furthermore, the proportion of n-3 fatty acids (sum of all n-3 fatty acids) was significantly lower ($P<.05$) in HTAG compared to LTAG. There were no group differences observed at 4 h (data not shown). Independent of the OFTT treatment, total n-6 fatty acids and PUFA (sum of PUFAs measured) were significantly higher ($P<.05$) in the LTAG vs. HTAG at 8 h (data not shown). As there were no interactions between treatment and subject group, the data suggests that postprandial fatty acids are processed similarly by the HTAG and LTAG subjects. Thus, for the second stage of plasma fatty acid composition analysis, we combined HTAG and LTAG subjects. There were no differences between treatments in any of the measured fasting plasma fatty acid concentrations ($P>.05$). In general, postprandial fatty acid composition directly reflected OFTT composition (Table 4). More specifically, following the ingestion of P/S 0.2, the plasma percentage of palmitic acid and total SFA increased at 4 and 8 h. Likewise, P/S 2.0 elicited significant increases in the proportions of linoleic (18:2n-6) and linolenic (18:3n-3) acids and total n-6 and PUFA concentrations at 4 and 8 h, respectively. Ingestion of P/S 1.0, representing a 1:1 ratio of PUFA to SFA, resulted in a significantly higher plasma percentage of palmitic acid at 4 h, linoleic acid at 8 h and total n-6 and PUFA at 4 and 8 h, respectively, when compared with fasting concentrations.

3.4. Postprandial cytokines and inflammatory markers

The IL-6 data are summarized in Fig. 3. The OFTT P/S ratio did not significantly affect the IL-6 response in the HTAG or LTAG groups (Fig. 3A). The IL-6 time curve demonstrated a significant ($P<.01$), positive linear pattern with a significant difference between HTAG and LTAG in terms of the intercept term ($P<.05$; Fig. 3B). The slope for the two groups was not significantly different, and therefore the increase in IL-6 in response to the OFTTs appeared to occur in a parallel manner for both HTAG and LTAG.

HTAG had a significantly higher sICAM-1 concentration throughout the OFTT, as indicated by a significant difference at the intercept term ($P<.05$; Table 5). There was no

Table 5
Postprandial sICAM, sVCAM and PAI-1 total antigen and activity following three OFTTs in HTAG and LTAG subjects

Metabolite	Group[a]	OFTT P/S	0	4	6	8
sICAM (ng/ml)*	HTAG	0.2	249.2±20.7	256.1±19.0	251.0±20.5	238.4±30.8
	LTAG	0.2	215.9±11.9	196.7±13.3	210.5±13.8	217.1±17.7
	HTAG	1.0	250.4±20.6	254.1±25.5	250.8±22.2	239.9±20.3
	LTAG	1.0	220.8±13.8	216.6±12.9	213.9±12.9	221.4±12.6
	HTAG	2.0	252.0±20.7	242.6±19.0	254.6±20.5	264.8±30.8
	LTAG	2.0	220.7±14.3	207.5±18.8	211.6±11.3	199.6±20.9
sVCAM (ng/ml)	HTAG	0.2	671.8±60.2	657.8±22.2	689.4±88.0	661.9±51.7
	LTAG	0.2	583.0±65.0	577.8±59.4	559.2±54.1	578.4±39.9
	HTAG	1.0	655.5±32.1	657.6±59.1	636.0±57.0	640.3±58.4
	LTAG	1.0	573.1±76.0	590.8±69.1	589.9±62.3	592.7±62.1
	HTAG	2.0	624.5±42.9	625.1±54.1	645.1±58.8	570.7±82.1
	LTAG	2.0	588.7±76.9	570.5±33.8	564.1±63.3	636.3±70.6
PAI-1 Total (pg/ml)†	HTAG	0.2	19.4±3.6	-	14.5±2.9	11.2±3.2
	LTAG	0.2	20.7±4.3	-	12.2±1.6	13.0±3.7
	HTAG	1.0	21.8±2.7	-	14.9±2.3	17.4±3.7
	LTAG	1.0	19.9±3.2	-	10.4±1.2	11.9±2.1
	HTAG	2.0	22.5±4.3	-	14.5±2.7	14.1±1.6
	LTAG	2.0	16.2±3.2	-	12.1±2.1	13.1±3.0
PAI-1 Activity (AU/ml)†	HTAG	0.2	12.8±3.8	2.8±1.0	2.5±0.8	2.4±1.2
	LTAG	0.2	9.5±3.8	3.6±1.0	2.2±0.7	1.8±0.4
	HTAG	1.0	11.8±3.9	2.6±0.9	1.8±0.7	2.6±0.8
	LTAG	1.0	8.6±3.5	2.4±0.6	2.4±0.7	1.5±0.7
	HTAG	2.0	15.0±4.7	4.4±1.6	3.0±1.0	2.7±0.9
	LTAG	2.0	6.6±3.2	2.6±0.8	1.8±0.8	2.2±1.3

Data are presented as geometric mean±transformed S.E.M.

[a] HTAG (fasting TAG >1.69, n=8), LTAG (fasting TAG ≤1.69 mmol/L, n=8).

* Significantly higher response ($P<.05$) throughout the postprandial period in the HTAG subjects vs. LTAG subjects.

† Significant decrease throughout the postprandial period ($P<.05$) in both HTAG and LTAG groups.

difference between subject groups for sVCAM-1. Neither time nor P/S ratio significantly affected sICAM-1 or sVCAM-1.

Overall, there was no effect of P/S ratio on postprandial CRP (Fig. 4A). HTAG had a significantly different rate of change in CRP compared to LTAG during the postprandial period, as indicated by a significantly different slope term ($P<.05$; Fig. 4B).

PAI-1 activity and PAI-1 total antigen both decreased significantly from baseline throughout the 8-h postprandial period in the HTAG and LTAG groups as indicated by a significantly negative slope (Table 5).

There was no difference between HTAG and LTAG groups nor was there an effect of OFTT P/S ratio on PAI-1 activity or PAI-1 total antigen.

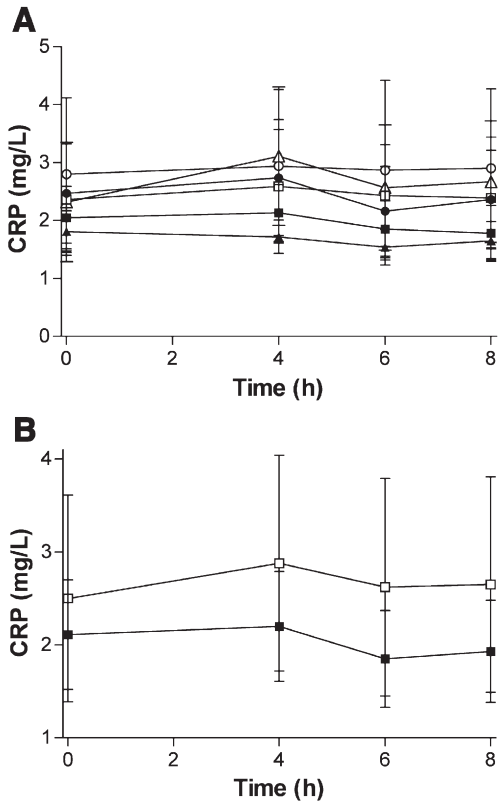

Fig. 4. (A) Postprandial C-reactive protein (CRP) response to three OFTTs of different P/S ratios in HTAG (fasting TAG >1.69 mmol/L, n=8) and LTAG (fasting TAG ≤1.69 mmol/L, n=8) subjects. Open squares (□) represent HTAG following P/S 0.2; open triangles (△) represent HTAG following P/S 1.0; open circles (○) represent HTAG following P/S 2.0; closed squares (■) represent LTAG following P/S 0.2; closed triangles (▲) represent LTAG following P/S 1.0; closed circles (●) represent LTAG following P/S 2.0. There was no effect of treatment in either group, so treatments were collapsed to facilitate group comparisons. Data are presented as geometric mean±transformed S.E.M. (B) Average postprandial CRP response to three OFTTs in LTAG (n=8) and HTAG (n=8) subjects. Open squares (□) represent HTAG; closed squares (■) represent LTAG. The rate of change (slope term) during the postprandial period was significantly greater in the HTAG vs. LTAG ($P<.05$). Data are presented as geometric mean±transformed S.E.M.

Fasting TAG was significantly ($P<.05$) correlated with fasting CRP (r=0.58). In addition, fasting total saturated fat in TAG correlated significantly ($P<.05$) with CRP (r=0.55), total n-3 fatty acids correlated significantly ($P<.05$) with PAI-1 total (r=67) and PAI-1 activity (r=0.54), and the fasting n-6/n-3 PUFA ratio in TAG correlated significantly ($P<.05$) with fasting CRP (r=−0.53) and PAI-1 total (r=−0.51). There were no additional correlations between the inflammatory markers, amount of TAG or TAG fatty acid composition.

4. Discussion

Unlike other studies that have fed dietary fatty acids as part of a mixed meal, we have used blends of fatty acids in the absence of carbohydrate or protein to isolate the postprandial impact of dietary fatty acid composition. Our primary finding is that the P/S ratio of an OFTT had no influence on postprandial lipids and inflammatory biomarkers of CVD and type 2 diabetes. We have demonstrated that men with high fasting TAG (TAG >1.69 mmol/L [1]) have an augmented postprandial TAG response compared to subjects with low fasting TAG. In addition, IL-6 and sICAM-1 remained significantly elevated throughout the postprandial period in HTAG subjects compared to their LTAG counterparts. Furthermore, slight but significant increases in glucose and CRP were observed along with elevated fasting TAG status in HTAG subjects. Collectively, our results contribute to understanding the postprandial response and the evaluation of obesity-related disease biomarkers.

To our knowledge, we are the first to compare postprandial TAG in two groups of men with different fasting TAG following the administration of three dietary P/S ratios. We found no effect of OFTT composition suggesting that the magnitude of postprandial TAG is not influenced by the fatty acid composition of a pure fat test meal. Several studies examining the TAG response to different fatty acids have demonstrated a lower postprandial TAG response to long-chain SFAs palmitic and stearic acids [33–35] or short- and medium-chain SFA found in butter [43] compared to long-chain PUFAs. One possible explanation for the differences observed in previous studies may be a lower rate of absorption of SFA [44]. However, others have reported elevated TAG following a highly SFA meal compared to a meal high in PUFA [36]. Our results support findings that fatty acid composition of a fat load has no impact on the magnitude of postprandial TAG [37]. Furthermore, our plasma fatty acid composition data suggests that the metabolism of specific fatty acids is not altered in HTAG men.

Additionally, we found that HTAG subjects exhibit higher fasting proportions of two SFA, lauric (12:0) and palmitic (16:0) acids, alongside lower fasting concentrations of total n-3 fatty acids within plasma TAG. These differences likely reflect variation in dietary patterns between LTAG and HTAG subjects, although we were unable to determine any

statistical differences in dietary fat intake between these two groups. Higher SFAs in plasma TAG have been reported to predict development of type 2 diabetes [45] and metabolic syndrome [46] in men. Since habitual dietary intake is known to alter the postprandial response, we provided a standardized meal the evening prior to the OFTT. However, in between study days subjects consumed their habitual diet under free-living conditions and therefore our data suggests that P/S ratio has no influence on postprandial parameters under typical 'daily living' conditions.

Postprandial TAG dysfunction associated with hypertriglyceridemia has been observed in studies of healthy men with low or high fasting TAG status [9,47], in subjects with metabolic syndrome [8], postmenopausal women [48] and in subjects with type 2 diabetes [49]. Thus, Kolovou et al. [8] have concluded that fasting TAG status is a significant indicator of an exaggerated rise in postprandial TAG. Along with significantly higher fasting TAG in our HTAG group, we observed an augmented postprandial TAG response in these same subjects despite no differences in waist circumference, BMI or percentage body fat, a finding that is consistent within the mixed-meal literature [8,9,47,48]. This is of particular interest as visceral adipose tissue has been suggested to be a significant contributor to the degradation of postprandial TAG [50]. Individuals with the combination of elevated waist circumference and elevated fasting TAG status, termed the 'hypertriglyceridemic waist' phenotype, demonstrate exaggerated postprandial TAG and are at increased risk for CVD and type 2 diabetes [9]. Our data suggests that increased waist circumference is not requisite to observe an exaggerated postprandial TAG response in individuals with elevated fasting TAG. Further postprandial testing is needed to fully characterize an individual with regard to this emerging parameter as our results suggest that abdominal fat mass *per se* may not predict postprandial TAG dysfunction.

Perturbation in glucose metabolism is a hallmark of the metabolic syndrome [1], and FFA exposure is known to decrease glucose disposal [51]. Individuals with metabolic syndrome have demonstrated exaggerated postprandial TAG and FFA following a lipid load [52]. Our finding of significantly higher blood glucose concentrations and 62% and 69% higher (nonsignificant) fasting insulin and HOMA in HTAG suggests that glucose homeostasis is disrupted in this subject group. However, we observed no TAG status differences in postprandial FFA. Based on this evidence, the influence of glucose homeostasis and the metabolic syndrome on TAG status and postprandial lipid metabolism cannot be ruled out.

IL-6 has been shown to increase postprandially in response to a high fat meal [23–25] and in response to a high fat meal as compared to a high carbohydrate meal [17]. Contrary to these findings, van Oostrom et al. [18] concluded that the postprandial increase in IL-6 following a mixed meal was due to diurnal variation and not food intake. Overall, HTAG subjects were exposed to a significantly higher concentration of IL-6 during the postprandial period, even though fasting IL-6 concentrations were not significantly different between HTAG and LTAG, and both groups exhibited similar patterns of postprandial IL-6. IL-6 administration has been reported to stimulate hepatic secretion of TAG in rats [53]. However, it has previously been suggested that an increase in postprandial IL-6 is not determined by the degree of postprandial TAG [25]. Although our study demonstrates that TAG status and total IL-6 exposure are associated, the differential postprandial pattern of TAG, but not IL-6, between the LTAG and HTAG groups suggests that postprandial regulation of these two parameters occurs by separate and as of yet unknown mechanisms.

There is little current knowledge about the postprandial IL-6 response following specific fatty acid intake in humans. Based on our results, P/S ratio has no influence on postprandial plasma IL-6 in human subjects. Mixed meals containing high MUFA or high SFA have shown no difference in postprandial IL-6 [24,54]. On the other hand, *in vitro* work has shown an increased production of IL-6 following palmitic acid incubation in 3T3-L1 adipocytes [55], a key SFA in our P/S 0.2 OFTT. Similarly, palmitate (16:0) but not unsaturated oleate (18:1n-9) or linoleate (18:2n-6) activates IL-6 expression in human myotubes [56]. Although not statistically significant, the concentration of IL-6 in HTAG increased from fasting by 102%, 85% and 25% at 6 h and by 124%, 156% and 82% at 8 h following P/S 0.2, 1.0 and 2.0, respectively. Interestingly, LTAG did not show a similar IL-6 response pattern. In addition, fasting palmitic acid and total SFA were significantly higher in HTAG, extending the association reported between elevated palmitic acid and IL-6 seen in adolescence [57] to a population of older men. The potential relationship between fasting TAG status, SFA and postprandial IL-6 requires further study.

In the current study, sICAM-1 concentrations were significantly higher in HTAG subjects, while P/S ratio and fat loading did not have an effect on circulating sICAM-1 and sVCAM-1 concentrations. A greater postprandial increase in both sICAM-1 and sVCAM-1 has been observed with a high fat mixed meal compared to a high carbohydrate mixed meal [17] and following a high fat challenge plus OGTT compared to either fat alone or OGTT alone [19]. We were unable to detect a change in postprandial adhesion molecule concentrations using our pure fat load. We did not observe either any differences in PAI-1 antigen or activity with varying P/S ratios in older overweight males. In contrast, young healthy males administered six different fatty acids in mixed meals have shown a greater postprandial decrease in PAI-1 antigen with SFA vs. a combined MUFA/PUFA [20]. The mixed meals included substantial carbohydrate and, although unreported, likely stimulated postprandial glucose and insulin responses [20], whereas our study did not. Our adhesion molecule and PAI-1 data are accompanied by a lack of glucose and insulin responses and appear to support the idea that both fat and carbohydrate

may need to be ingested to induce changes in the postprandial state. Finally, although not the focus of our study, significant correlations were found between total n-3 and n-6/n-3 PUFA and PAI-1 concentrations, and this requires further study.

Previous oral fat loading studies have shown CRP to not change [23,58] or decrease [30] in the postprandial period. Blum et al. [31] observed a small but significant decrease in postprandial CRP with a Mediterranean-like meal (high in MUFA) compared to a Western-like meal (high in SFA) in young healthy males. In the present study, postprandial rate of change of CRP concentrations increased in HTAG and decreased in LTAG, respectively. This represents a slight, yet significantly different response between two groups of individuals that differ primarily in terms of fasting TAG concentrations. Furthermore, fasting TAG was positively associated with CRP. Whether the elevated IL-6 concentrations observed in HTAG subjects play a role in this differential CRP response is unclear, but worthy of future investigation.

Based on our results, the P/S ratio of an oral fat load has no impact on the magnitude of postprandial lipids and several inflammatory markers in two groups of older men. Our results also suggest that subjects with elevated fasting TAG have an altered TAG response to oral fat ingestion compared to similar subjects with low fasting TAG. The exaggerated TAG response is accompanied by an increased IL-6 exposure, significantly elevated sICAM-1 and glucose, and a slightly increased CRP during the postprandial response to dietary fat. Overall, the postprandial profile following OFTT administration demonstrates several unfavorable characteristics associated with multiple aspects of the metabolic syndrome, CVD and type 2 diabetes in men with elevated fasting TAG. As postprandial TAG has been reported as an independent risk factor for CVD [3–7], the postprandial lipid response to lipid ingestion should be of particular health interest. Further characterization of the physiology of the postprandial period will enhance chronic disease prevention and treatment.

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