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Postprandial response of adiponectin, interleukin-6, tumor necrosis factor-α, and C-reactive protein to a high-fat dietary load

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Abstract

Objective: Circulating levels of adiponectin are low in obesity and metabolic disorders associated with increasing fat mass including insulin resistance and dyslipidemia. Body fat stores may be positively related to intake of dietary fat, but little is known of mechanisms by which serum adiponectin may be regulated through diet. We investigated acute effects of a high-fat load and changes in fatty acid saturation on circulating adiponectin and associated mediators of inflammation including interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and C-reactive protein (CRP).

Methods: A high-fat test meal (59 $\pm$ 4 g fat; 71% of energy as fat) containing a high (~71:29) or low (~55:45) ratio of saturated:unsaturated fatty acids was given at breakfast on two occasions. Blood samples were collected at 0 (baseline), 1, 3, and 6 h for measurement of adiponectin, IL-6, TNF-α, and high-sensitivity CRP. A fat-exclusion lunch, snack, and dinner were also given and blood samples collected at 10 and 24 h.

Results: Eighteen healthy, lean men completed the trial. There was no evidence of acute change in circulating adiponectin in response to the lipid bolus or a differential effect of fatty acid saturation on adiponectin, high-sensitivity CRP, or IL-6 ($P > 0.05$). IL-6 increased over 6 h on both treatments (time, $P < 0.05$). TNF-α decreased on the high saturated:unsaturated fatty acid treatment (treatment by time, $P < 0.05$). There were no significant correlations between circulating adiponectin and insulin on either dietary treatment in these normoglycemic subjects.

Conclusion: Acute changes in the content of saturated and unsaturated fatty acids had no adverse effect on postprandial circulation of the adipose-related factors adiponectin, IL-6, TNF-α, or high-sensitivity CRP.

Keywords: Adiponectin; Saturated fatty acids; Postprandial; Inflammatory cytokines

Introduction

Adiponectin is an adipocyte-derived peptide with evidence of diabetic and cardioprotective properties [1,2], with low circulating levels in the obese [3,4] and in metabolic disorders associated with increased fat mass including hyperglycemia, insulin resistance, dyslipidemia, and vascular inflammation [5–9]. Low circulating levels of adiponectin in obesity remains something of a paradox because it is of adipocyte origin, but antagonistic suppression by inflammatory adipokines that increase in parallel with adipose mass such as tumor necrosis factor-α (TNF-α) may be involved [4]. Exogenous administration of globular adiponectin has been shown to increase fatty acid β-oxidation and decrease fat mass in rodents [10], hence, the possibility that it may

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play a role in weight gain whereby low circulating levels enhance adiposity.

Since dietary fat is associated with increased lipid storage, weight gain, and obesity [11,12], there has been interest in determining the responsiveness of serum adiponectin to acute dietary fat loads. Suppression of adiponectin expression and/or release into circulation in individuals with a large adipose mass may be confounded by a concomitant high dietary fat intake. Trials that have investigated the effects of high lipid loads on circulating adiponectin have shown a decrease [13] or no response [14] postprandially, and little is yet known of mechanisms by which adiponectin may be regulated through diet. There is however preliminary evidence that proinflammatory cytokines TNF-α and interleukin-6 (IL-6) may change after an acute fat bolus [15–17], particularly in metabolically compromised individuals [17,18], and are purported to be involved in adiponectin regulation. Moderate fat (20–25 g) mixed meals appear to have little postprandial effect on circulating adiponectin, at least in lean individuals [19,20]. Because fatty acid composition significantly affects established markers of cardiovascular disease and type 2 diabetes mellitus (T2DM) such as dyslipidemia, glucose, and insulin control, in this trial we wanted to investigate acute effects of change in lipid saturation during high-energy high-fat loading on adiponectin and the proinflammatory peptides IL-6, TNF-α, and C-reactive protein (CRP). We hypothesized that a fatty load may adversely affect postprandial response of adiponectin and the markers of inflammation and that this response may be exacerbated when the fatty acid profile of the lipid bolus is primarily saturated.

Materials and methods

Subjects

Eighteen men (mean body mass index [BMI] 22.9 $\pm$ 2.0 kg/m^2, range 19–26 kg/m^2) 19–33 y of age were recruited into the trial through newspaper advertisement. All subjects completed both arms of the intervention. All were healthy as indicated by normal biochemistry (lipid profile, liver function, thyroid function, plasma glucose) and blood pressure and none were taking medications for lipid, blood pressure, or metabolic disorders or had a history of treatment for significant disease. Human ethics approval for this study was obtained from the Auckland ethics committee, Auckland, New Zealand.

Protocol

This was a double-blinded, randomized trial. On two occasions subjects spent 24 h at the University of Auckland Human Nutrition Unit. On the morning of day 1, subjects were given a high-fat test meal and blood samples were collected over 24 h. The test meals contained butter fat with a high (~71:29) or low (~55:45) saturated:unsaturated fatty acid (SFA:USFA) ratio (Table 1). The major alterations in dietary lipid were a decrease in the saturated fats palmitic acid (16:0, −13% total fat) and myristic acid (C14:0, −4%) and an increase in the monounsaturated fatty acid (MUFA) oleic acid (18:1, +11%) and the polyunsaturated fatty acids (PUFAs) linoleic acid (18:2ω-6, +6%) and α-linolenic acid (C18:3ω-3, +2%) in the low$_{\text{SFA:USFA}}$ product. Details of industrial preparation of this product have been given in a previous publication [21]. Participants were randomized using stratification to ensure that half were given high$_{\text{SFA:USFA}}$ and half low$_{\text{SFA:USFA}}$ on entry into the study. All subjects then crossed over to the other treatment arm. Treatments were separated by a minimum 3-d washout period. Subjects were confined to the human nutrition facility at the University of Auckland throughout each 24-h treatment during which time they were provided with all meals and snacks. Subjects were maintained in energy balance over 24-h based on an estimate of basal metabolic rate (BMR) and energy expenditure for a sedentary day (1.4 $\times$ BMR). They arrived fasted at the nutrition unit at 0730 h, an indwelling venous cannula was inserted, and a baseline blood sample was collected. At 0800 h the high-fat breakfast was served and subjects were asked to consume the breakfast within 15 min. Blood samples were collected at 1, 3, 6, and 10 h. Lunch was served immediately after the 6-h blood sample, an afternoon snack at 8 h, and dinner immediately after the 10-h blood sample. Subjects slept at the unit that evening and a final blood sample was collected fasted the following morning at 24 h.

Table 1
Composition of dietary lipids in the test meals showing the major fatty acid constituents*

	High SFA:USFA	Low SFA:USFA
Total SFAs (%fat)	70.5	54.4
Lauric C12:0	3.8	2.7
Myristic C14:0	12.0	8.3
Palmitic C16:0	31.5	18.8
Stearic C18:0	10.1	13.4
Total MUFAs (%fat)	22.1	32.0
C18:1$_{\text{total}}$	18.6	30.0
C18:1$_{trans}$	4.3	4.7
Total PUFAs (%fat)	3.0	10.5
Linoleic C18:2	1.2	7.2
α-Linolenic C18:3	0.8	2.3
Cholesterol mg/100 g butter	222	191

%fat, percentage of total fat; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; USFA, unsaturated fatty acid

* Minor peaks not shown.

Test meals

The high-fat breakfast comprised a sweet blueberry muffin, a milk and sugar-free decaffeinated hot beverage, and/or glass of cold water. The test meal was scaled to body size

Table 2
Twenty-four–hour energy and macronutrient intakes that were matched between treatments for each subject*

	Energy (kJ)	Fat (%en)	Fat (g)	CHO (%en)	Protein (%en)
Test meal	3130 ± 193	70.8 ± 4.9	59.1 ± 3.7	23.2 ± 1.7	4.9 ± 0.5
Lunch	3476 ± 209	3.4 ± 0.2	3.1 ± 0.2	78.8 ± 5.5	14.2 ± 1.4
Snack	1456 ± 87	1.8 ± 0.1	0.7 ± 0.04	90.6 ± 6.3	7.2 ± 0.7
Dinner	3201 ± 192	2.8 ± 0.2	2.4 ± 0.1	85.1 ± 5.9	9.9 ± 0.9

%en, percentage of energy; CHO, carbohydrate
* Mean ± SD.

based on daily energy requirements and each subject was given 5.3 g of butter fat per megajoule intake. The average dietary intake was 11.2 ± 0.2 MJ/d, the average energy content of the high-fat breakfast was 3.13 ± 1.9 MJ, and the average butter fat in the test meal was 59 ± 4 g, equivalent to approximately 73 g of dairy butter (Table 2). A fat-exclusion lunch (3.1 g of fat, comprising vegetarian pasta, bread roll, orange juice) was served after the 6-h blood sample; a fat-exclusion snack (0.7 g of fat, comprising fruit cake, apple juice) was served midafternoon, 8 h after breakfast; a fat-exclusion dinner (1.3 g of fat, comprising vegetarian risotto, raspberry desert, carbonated beverage) was served after the 10-h blood sample. These meals and snacks were designed to be identical on both treatment arms.

Analytical methods

Blood samples were centrifuged and serum was stored at −80°C until later batch analyses. Samples were analyzed for adiponectin, IL-6, TNF-α, and high-sensitivity CRP (hs-CRP). Adiponectin was analyzed by enzyme-linked immunosorbent assay using an inhouse system. Serum samples were diluted 1:5000 with phosphate buffered saline and then applied to 96-well microliter plates coated with monoclonal antibody against human adiponectin (R&D System, Minneapolis, MN, USA). After incubation at room temperature for 120 min, wells were washed and incubated for another 60 min with the biotinylated monoclonal antibody against adiponectin (R&D System). The wells were again washed and incubated with streptavidin-conjugated horseradish peroxidase for 60 min and then reacted with tetramethyl benzidine reagent for 15 min. One hundred microliters of 3 M HCl was then added to each well to stop the reaction and absorbance at 450 nm was measured. Intra- and interassay coefficients of variation were 6.2–7.9% and 3.8–6.3%, respectively. Lower limits of detection for the assay were 0.5–2 ng/mL of adiponectin protein. Serum IL-6 and TNF-α were analyzed by enzyme-linked immunosorbent assay using a Duoset commercial kit (R&D System). One hundred microliters of sample was applied to 96-well microtiter plates and incubated with the individual coating antibodies for 2 h at room temperature. After washing with phosphate buffered saline, the detection antibodies were applied for another 2 h at room temperature. The bound immune complexes were detected at 450 nm. The standard curve was generated for every set of samples assayed using the standards provided in the kit. The hs-CRP was analyzed using a Pointe Scientific (Canton, Michigan, USA) immunoturbidimetric commercial kit. Latex particles coated with antibody specific to human CRP aggregate to form immune complexes. Increased light scattering, proportional to the concentration of analyte, was measured on a COBAS Mira autoanalyzer (Roche Diagnostics, Basle, Switzerland). CRP concentration was calculated using a calibration curve of CRP standards and Prism software (GraphPad, San Diego, CA, USA) used to fit third-order polynomials to the curve to calculate sample concentration. The assay range was 0.05–10 mg/L with a sensitivity of 0.1 mg/L. Serum insulin concentrations were measured by radioimmunoassay using a commercial kit (Peninsula Laboratories, Belmont, CA, USA). Polyclonal antibodies from guinea pig antiserum raised against bovine insulin were incubated with the human insulin standard Actrapid (Novo Nordisk A/S, Bagsvæd, Denmark) and unknowns. Iodinated ^{125}I tracer was added, samples were centrifuged, and the bound fraction was counted on a Wallac 1480 Wizard 3 gamma counter (Wallac Finland Oy, Turku, Finland).

Statistical analyses

Metabolic outcomes were analyzed using a linear mixed model analysis of variance (PROC MIXED, SAS 8.0, SAS Institute, Cary, NC, USA). Repeated measure analysis of variance tested within-diet and between-diet interactions over periods of 1, 6, and 24 h. Treatment group, identity, time of sample, run order, and block effects were included in the analysis. In all measurements, when there was no differential effect of fat quality, the two treatments were combined and the quantitative effects of the dietary lipid analyzed over time. Statistical significance was based on 95% limits ($P < 0.05$).

Results

All subjects randomized into this crossover trial completed both treatment arms. No subjects withdrew or were excluded for non-compliance. Baseline characteristics of the 18 participants are listed in Table 3. Subjects were young, healthy by clinical review, and predominantly lean.

Table 3
Subject characteristics of the 18 lean men at baseline*

No. of subjects	18
Age (y)	23 ± 4.2
Body weight (kg)	72.8 ± 6.7
Body mass index (kg/m^2)	22.9 ± 2.0
Waist circumference (cm)	79.5 ± 6.1
Systolic blood pressure (mmHg)	123 ± 10.5
Diastolic blood pressure (mmHg)	78 ± 9.0
Fasting plasma glucose (mmol/L)	4.7 ± 0.3
Fasting insulin (pmol/L)	126 ± 73.1
HOMA-ir†	4.4 ± 2.6
Total cholesterol (mmol/L)	4.3 ± 0.8
LDL cholesterol (mmol/L)	2.5 ± 0.7
HDL cholesterol (mmol/L)	1.4 ± 0.4
TAG (mmol/L)	0.8 ± 0.3
Adiponectin (ug/ml)	7.02 ± 2.1
IL-6 (pg/mL)	27.9 ± 69
TNF-α (pg/mL)	719 ± 668
hs-CRP (mg/L)	0.61 ± 0.8

HDL, high-density lipoprotein; HOMA-ir, homeostasis model assessment of insulin resistance; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; LDL, low-density lipoprotein; TAG, triacylglycerol; TNF-α, tumor necrosis factor-α

* Mean ± SD.

† HOMA-ir = (fasting insulin [pmol/L] × fasting glucose [mmol/L])/135.

However, there was indication of mild inflammation that manifested in raised cytokine levels in several subjects and some evidence of raised fasting insulin concentrations. Fasting serum adiponectin was negatively correlated with BMI (Pearson's correlation, $P < 0.05$; Fig. 1), an indirect estimate of adiposity, in this group of predominantly lean men. Mean ± SD fasting adiponectin was 7.0 ± 2.5 μg/mL before the oral fat load (range 2–15 μg/mL) and the change from baseline over 24 h after the high$_{SFA:USFA}$ and low$_{SFA:USFA}$ treatments is shown in Figure 2 ($P > 0.05$). When analyzed independent of treatment, there was no significant change in circulating adiponectin at 1, 3, or 6 h postprandially (time, $P > 0.05$) and no significant between-treatment effects of fatty acid saturation at 1, 3, or 6 h (treatment by time, $P > 0.05$; Table 4). There was a significant change in

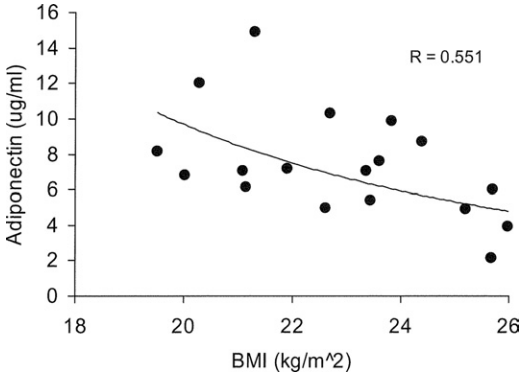

Fig. 1. Fasting serum adiponectin was negatively correlated with adiposity as estimated by BMI ($P < 0.05$). BMI, body mass index.

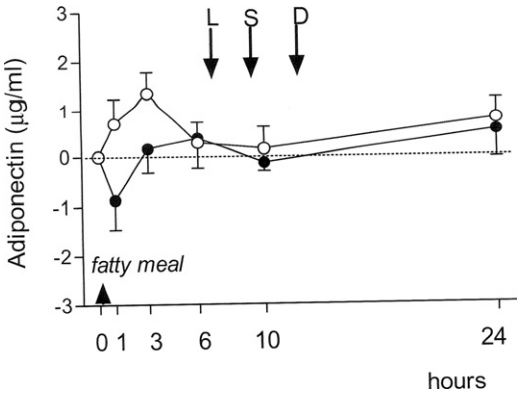

Fig. 2. Postprandial changes in circulating adiponectin after a high-fat breakfast containing a high (71:29, black circles) or low (55:45, white circles) saturated:unsaturated fatty acid profile. L, lunch; S, snack; D, dinner.

circulating insulin over 1 and 6 h in response to the test meal (time, $P < 0.001$), and also over 24 h (time, $P < 0.001$) when the hyperinsulinemic effects of the high-carbohydrate (CHO) meals and snacks served at 6, 8, and 10 h were observed. There were no effects of treatment over 6 or 24 h (treatment by time, $P > 0.05$). The glucose response to the test meal was highly variable between subjects on both treatments (change from baseline, range −1.9 to 2.2 mmol/L) but there were no significant between-treatment effects at any time point. There were also no significant correlations between circulating adiponectin and insulin or glucose on either dietary treatment at 1, 3, 6, 10, or 24 h in these normoglycemic subjects (Pearson's correlation, $P > 0.05$).

Circulating levels of the proinflammatory cytokine IL-6 varied considerably among subjects. In this group of lean men, baseline levels were typically 5–50 pg/mL, although 2 of the 18 subjects had significantly raised levels on both treatment arms of 100–300 pg/mL, indicative of a nonspecific inflammatory response, although there was no indication of infection as assessed by white blood cell count in these subjects at screening. Despite the between-subject variability, circulating levels of IL-6 significantly increased over 6 h (time, $P < 0.01$), but there were no differential effects of saturation (treatment by time, $P > 0.05$). Over 24 h circulating levels had returned to baseline (time, $P < 0.05$; Fig. 3). TNF-α, a second marker of inflammatory response, was also high in the same two subjects across both treatments. There was no increase in response on either treatment; rather circulating levels tended to decrease over 6 h on the high$_{SFA:USFA}$ treatment (treatment by time, $P = 0.04$; Fig. 3). There was no correlation between change in IL-6 and TNF-α over the initial 6-h postprandial period (Pearson's correlation, $P > 0.05$). The acute fat feeding did not alter circulating hs-CRP over 6 h on either treatment ($P > 0.05$). Over 24 h during the periods of high-CHO feeding, hs-CRP was significantly lower on high$_{SFA:USFA}$ relative to low$_{SFA:USFA}$ treatment (treatment by time, $P = 0.05$; Fig. 3).

Table 4
Postprandial effects of the two high-fat breakfasts on serum measurements*

	0 h	1 h	3 h	6 h	10 h	24 h
High SFA:USFA ratio						
Adiponectin (μg/mL)	7.37 ± 0.7	6.51 ± 0.5¶	7.53 ± 0.8	7.76 ± 0.8¶	7.24 ± 0.7	7.93 ± 0.8¶
IL-6 (pg/mL)	29.3 ± 16.8	27.2 ± 16.5¶	31.1 ± 17.0	33.4 ± 16.4†	39.0 ± 17.3	29.0 ± 16.8‡
TNF-α (pg/mL)	736.1 ± 156.8	698.4 ± 164.5¶	712.8 ± 174.9	704.3 ± 163.9‖	683.7 ± 167.4	760.5 ± 173.8¶
hs-CRP (mg/L)	0.51 ± 0.1	0.42 ± 0.1¶	0.45 ± 0.1	0.44 ± 0.1¶	0.49 ± 0.13	0.25 ± 0.10¶
Insulin (pmol/L)	127 ± 15	619 ± 62§	217 ± 68	110 ± 13§	982 ± 98	183 ± 27§
Glucose (mmol/L)	4.72 ± 0.08	5.10 ± 0.24¶	4.59 ± 0.17	4.54 ± 0.07¶	5.41 ± 0.17	4.93 ± 0.10§
Low SFA:USFA ratio						
Adiponectin (μg/mL)	6.66 ± 0.4	7.36 ± 0.6¶	8.00 ± 0.8	6.95 ± 0.5¶	6.80 ± 0.6	7.48 ± 0.7¶
IL-6 (pg/mL)	26.5 ± 16.2	26.0 ± 15.7¶	26.9 ± 15.4	30.0 ± 16.3†	29.3 ± 15.4	28.5 ± 16.4‡
TNF-α (pg/mL)	701.9 ± 161.1	709.7 ± 162.2¶	704.2 ± 159.6	668.8 ± 160.7‖	713.3 ± 164.8	758.1 ± 159.7¶
hs-CRP (mg/L)	0.70 ± 0.4	0.62 ± 0.4¶	0.66 ± 0.4	0.81 ± 0.5¶	0.83 ± 0.4	0.69 ± 0.5¶
Insulin (pmol/L)	124 ± 19	539 ± 75§	251 ± 80	141 ± 26§	1034 ± 72	151 ± 12§
Glucose (mmol/L)	4.73 ± 0.08	4.55 ± 0.23¶	4.51 ± 0.16	4.76 ± 0.17¶	5.29 ± 0.19	4.99 ± 0.08§

hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; SFA, saturated fatty acid; TNF-α, tumor necrosis factor-α; USFA, unsaturated fatty acid
* Mean ± SEM.
† $P < 0.05$ for time, repeat measures analysis of variance, analyzed as 0–1 h, 0–6 h, and 0–24 h.
‡ $P < 0.01$ for time, repeat measures analysis of variance, analyzed as 0–1 h, 0–6 h, and 0–24 h.
§ $P < 0.01$ for time, repeat measures analysis of variance, analyzed as 0–1 h, 0–6 h, and 0–24 h
‖ $P < 0.05$ for treatment by time, repeat measures analysis of variance, analyzed as 0–1 h, 0–6 h, and 0–24 h.
¶ No significant effects.

Discussion

We have shown that a single high-fat meal does not elicit acute changes in serum adiponectin compared with fasting baseline levels nor does fatty acid saturation differentially affect circulating levels in healthy male subjects. Increase in saturation had no adverse affect on proinflammatory TNF-α or CRP, and although circulating levels of IL-6 transiently increased above fasting after both fatty meals, there was no differential worsening with increased saturation.

Although diabetic and cardioprotective effects of adiponectin administration have been shown in animal trials, less is understood about pathways of endogenous regulation. Gene expression and serum concentrations decrease as adipose mass increases [3,4,22], which was confirmed in our trial not only during development of overweight/obesity but also across the normal body weight range, albeit assessed by proxy body mass index rather than adiposity per se. The relation of adiponectin to storage lipid mass has led to the hypothesis that an oral lipid challenge may also decrease serum adiponectin concentrations, although whether, in the face of a long half-life and as yet undefined secretion pathways that may not allow for acute responses, it is possible to modulate postprandial levels with diet is yet to be established. Four studies have investigated the effect of mixed [19,20] and high-fat [13,14] meals on postprandial serum adiponectin, and a recent study by Paniagua et al. [23] investigated the effect of high-fat and high-CHO meals on adiponectin expression. Mixed meals (20–25 g of fat) had no effect on serum levels in lean subjects [19,20] but increased those levels in obese individuals [19]. High-fat feeding failed to elicit a response in healthy subjects and first-degree relatives of diabetics [14] but decreased adiponectin in patients with T2DM [13]. Neither dose, which was lower in the T2DM study (52 g, 38% SFA) [13] than in the first-degree relatives study (80 g, 50% SFA) [14], nor fatty acid saturation (high SFA [~10–20 g of SFA/meal], high-lipid bolus [~40 g of fat/meal] was given in both trials) explained this divergence, which may be related to metabolic status. Without a low-energy or low-fat control group in our trial, the postprandial effects of high-fat feeding per se cannot be definitive; however, there was no evidence of a change in adiponectin above fasting levels in the 6 h after a fatty meal and there was no differential effect of lipid saturation. A recent trial from our laboratory has also shown that longer-term (3-wk) manipulation of fatty acid saturation had no differential effect on fasting levels of adiponectin [24]. In the study by Paniagua et al. [23] of offspring of obese patients with T2DM who were preconditioned to a high-CHO, high-MUFA or high-SFA diet, serum adiponectin did not significantly alter postprandially and there were no differential treatment effects, yet of interest was the reported increase in adipose tissue gene expression at 180 min after the meal on the two lipid treatments.

The cytokines IL-6 and TNF-α are key mediators of inflammation [25,26] that are increased in obesity, insulin resistance, and T2DM [25,27]. Although there is evidence of dietary modulation in response to changes in total fat [28,29] and fatty acid composition [30] during long-term supplementation, less is known about postprandial response. In long-term trials ω-6 PUFAs have been shown to be largely [29,31], although not entirely [30], proinflammatory, whereas marine ω-3 PUFAs may limit production of TNF-α, IL-6, and IL-1 [32,33], as may plant-derived ω-3 PUFAs such as α-linolenic acid (18:3ω-3), albeit at high

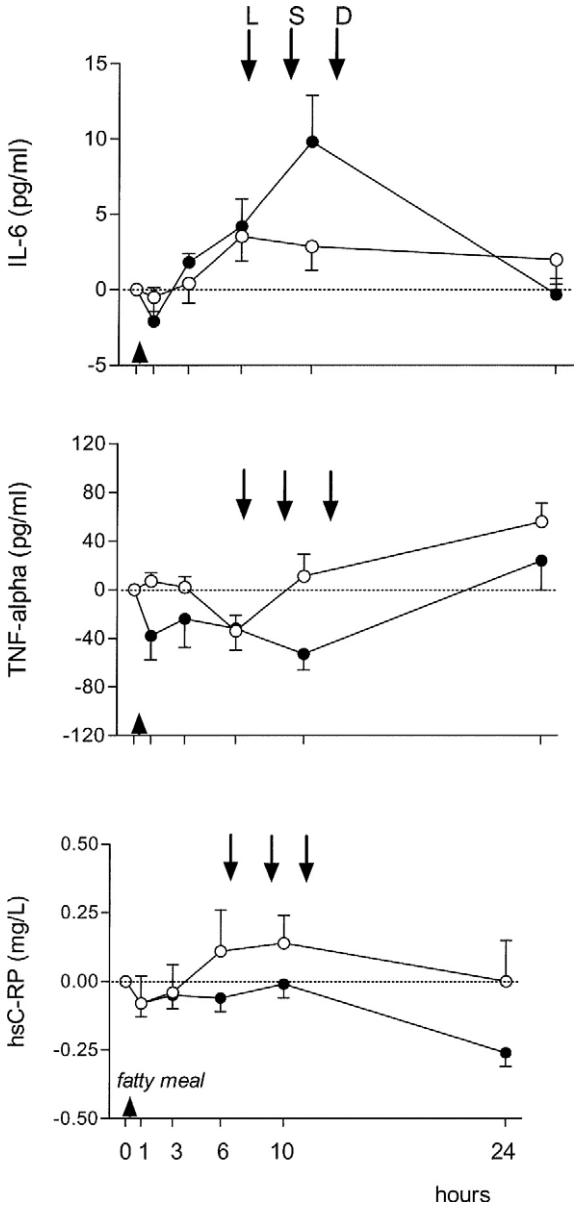

Fig. 3. Postprandial changes in circulating IL-6, TNF-α, and hs-CRP after a high-fat breakfast containing a high (71:29, black circles) or low (55:45, white circles) saturated:unsaturated fatty acid profile. D, dinner; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; L, lunch; S, snack; TNF, tumor necrosis factor.

doses (7–15 g/d) [32,34], whereas fats rich in MUFA may also have anti-inflammatory properties [35–37]. We are aware of seven studies that have investigated postprandial response of proinflammatory cytokines to a high-fat meal. IL-6 and TNF-α both increased in an early study of healthy subjects and patients with T2DM [15], as did TNF-α in a study of healthy subjects and patients with metabolic syndrome [18] and IL-6 in healthy controls [16], patients with premature coronary heart disease [16], and in the obese [17,38] in whom there was either no associated postprandial rise in TNF-α [38] or a decrease in TNF-α in a subgroup of insulin-sensitive obese men [17]. In our trial of healthy males in which we found significant lipemia after this regime of high-fat feeding [39], we also found that IL-6 increased on both high fat treatments, whereas TNF-α concentrations were unchanged or decreased. A previous trial in patients with T2DM given a mixed meal supplemented with MUFA oil or a high SFA cream also showed a transient decrease in TNF-α and an unexpected decrease in IL-6 [40], although no effect of a high-fat meal on IL-6 had previously been shown in patients with T2DM [41]. The absence of a high-CHO arm in our study precludes exclusion of changes being an energy-loading effect rather than specific to dietary fat, but this may also be the case in other recent trials [16,18]. In light of the variable response in lean, healthy, and obese subjects and patients with T2DM, no consensus can as yet be drawn about the effect of fat loading on TNF-α. The mechanism leading to the decrease in TNF-α on the high$_{SFA:USFA}$ treatment in our trial is unknown to us. It should also be noted that a recent study by Kempf et al. [42] highlighted the issue of local versus systemic changes in markers such as IL-6 during venous cannulation, where permanent insertion of a catheter beyond 3 h may lead to local tissue production of IL-6 and possible subsequent masking of minor systemic changes [42,43].

C-reactive protein, an acute-phase reactant that increases during inflammatory response [44] and is an emerging risk factor for atherosclerotic disease [45], can be modulated by weight loss and physical activity but less is known of the effect of diet [46–48]. Whether there is a causal relation between the cytokines and CRP remains unclear and alternative hypotheses that cytokines mediate acute-phase protein release from the liver or that IL-6 secretion may be induced by CRP have been proposed [46]. Certainly both are commonly raised in situations of inflammation such as atherosclerotic disease. In patients with T2DM a fatty meal has been shown to increase CRP in the absence of increased IL-6 [41], whereas in the obese there is both evidence of an increase [38] and no effect [17] of a fat bolus on CRP. Several trials in healthy subjects also have shown no postprandial effects on CRP [49–51]. No previous trials have investigated possible effects of fatty acid saturation, and there was no evidence from our trial that an acute increase in SFA content of the diet may adversely affect circulating CRP.

Conclusions

There is no evidence from this study of lean, healthy male subjects that the adipose hormone adiponectin is sensitive to acute intake of dietary lipid or to an increase in fatty acid saturation. Of the markers of inflammation, only IL-6 increased postprandially in response to high-fat feeding. There was no indication of increased levels of TNF-α or the acute-phase protein CRP. We conclude that an acute increase in the content of SFAs within a high-lipid bolus

had no adverse effect on postprandial circulation of the adipose-related factors adiponectin, IL-6, TNF-α, or hs-CRP.

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