No Evidence of an Effect of Alterations in Dietary Fatty Acids on Fasting Adiponectin Over 3 Weeks

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Objective: Little is known about the effects of alterations in fatty acid classes on adiponectin, a hormone secreted by the adipocyte known to be important in the development of diabetes and cardiovascular disease (CVD). Any factor, including diet, that may positively influence adiponectin gene expression or increase circulating levels might be useful for improving such metabolic abnormalities. We investigated the effects of alterations in dietary fatty acid saturation on fasting serum adiponectin and associated peptides.

Methods and Procedures: Double-blind, randomized, crossover, 2 × 3-week residential intervention trial where 18 mildly hyperlipidemic adult men were provided with a high saturated:unsaturated fat (SFA:USFA) and lower SFA:USFA treatment separated by an uncontrolled 4-week washout. Only fatty acid profile was altered between treatments. Fasting blood samples were collected on days 0, 1, 7, 14, 21, 22 of each intervention period for the measurement of adiponectin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), high-sensitivity C–reactive protein (hsC-RP), leptin, and ghrelin.

Results: Body weight was kept constant (±1 kg) throughout each treatment. There was no detectable difference in fasting adiponectin at baseline (mean day 0 + day 1) between the treatment groups (mean ± s.d.; $high_{SFA:USFA}$ = 7.0 ± 1.7 vs. $low_{SFA:USFA}$ = 6.7 ± 1.4 μg/ml, $P > 0.05$). There were neither significant between-treatment effects of fatty acid saturation (diet × time, $P > 0.05$) on serum adiponectin nor any significant between-treatment effects on serum TNF-α, IL-6, hsC-RP, leptin, or ghrelin ($P > 0.05$).

Discussion: Fasting serum adiponectin was not detectably affected by alterations in dietary fatty acid profile in mildly hyperlipidemic men. There was no evidence that an increase in SFA content of the diet significantly worsened fasting serum adiponectin over a 3-week intervention period.

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INTRODUCTION

Adipose tissue–derived adiponectin is an insulin-sensitizing hormone that is associated with decreased risk of cardiovascular disease (CVD) and type 2 diabetes (T2DM) (1,2). Circulating levels of adiponectin have been shown to be inversely correlated with total levels (3,4), and visceral adiposity (5) and adverse metabolic states such as dyslipidemia (6), hyperglycemia, and insulin resistance (2,7,8). A suppressed level of adiponectin gene expression in the adipose tissue of obese subjects has also been demonstrated (3,9). Importantly, adiponectin is linked to fat metabolism *per se* whereby exogenous administration of globular adiponectin can increase free fatty acid oxidation by 11–19% in muscle in mice, increase removal of free fatty acid from the circulation, and lead to the loss of adipose mass (10). In addition, it has been shown that chronic infusion of adiponectin produced from *Escherichia coli* significantly ameliorated insulin resistance and improved lipid profiles in both lipoatrophic diabetic mice and diet-induced obese mice (11). Therefore, any factor that may positively influence adiponectin gene expression or circulating levels of this hormone might be a useful agent for improving insulin resistance, risk factors for T2DM, and CVD, or potentially even decrease the adipose mass in obese individuals.

Alterations in diet have been shown to affect the markers of disease risk such as glucose, lipids, and insulin. The relationship between dietary fat, especially saturated fatty acid (SFA),

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and CVD has long been understood (12,13), and it is known that a diet rich in SFA can lead to increases in total cholesterol and low-density lipoprotein-cholesterol (12), both of which are independent risk factors for CVD. A diet rich in SFA is also known to adversely affect the risk of T2DM (14). In light of the inverse association between adiponectin (both circulating and gene expression) and CVD and T2DM, and the positive association between dietary fat intake and CVD and T2DM, it seems pertinent to explore the link between fasting serum adiponectin and fat intake, and in particular alterations in fatty acid saturation.

Long-term changes in dietary intake can influence adiponectin indirectly through changes in adipose mass (3,4), but whether diet *per se* has an independent effect on this hormone has yet to be established. Intervention trials, which make acute changes in diet, have shown mixed results with the evidence of both an increase (15) and a decrease (16) in circulating adiponectin up to 4 h following consumption of meal. Alternately, other trials, including one from our own laboratory (17), have shown that circulating adiponectin does not alter postprandially in response to a meal (18,19). The relationship between medium-term dietary manipulation and adiponectin has been investigated in two trials. Circulating levels have been shown to be unaffected by alteration in the fat content of a eucaloric diet over 7 days involving 21 subjects (20). Bonnet and colleagues have demonstrated higher levels of the hormone in the individuals supplemented with high ω-3 polyunsaturated fatty acids (PUFAs) fatty fish at least three times per week in addition to 20 g high monounsaturated fatty acids (MUFAs) rapeseed oil per day over 10 weeks compared with baseline levels (21).

Adiponectin has been shown to be closely related to cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). Although both of these hormones play an important role in carbohydrate and lipid metabolism (22,23), unlike adiponectin they are positively correlated with T2DM, CVD, and obesity. Dietary fatty acid composition has been shown to affect circulating TNF-α, IL-6, and C–reactive protein (C-RP). Intervention trials, which last from 4 to 12 weeks, have demonstrated either an increase (24) or a decrease (25–28) in TNF-α and IL-6 as a result of feeding diets high in PUFA. Similarly, C-RP has been shown to increase (29), decrease (30,31), or not change (32) in response to alterations in fatty acid profile. Alterations in diet where body fat remains unchanged have been shown not to affect fasting leptin concentrations (33), and it has been proposed that circulating leptin may be driven by total adipose mass (34) as opposed to being affected by diet. There is little evidence to indicate that ghrelin responds to medium/longer-term feeding, especially alterations in fatty acid profile.

Hence, this trial was designed to investigate whether fasting levels of serum adiponectin and the associated peptides TNF-α, IL-6, C-RP, leptin, and ghrelin are altered by changes in dietary fatty acid saturation made over a 3-week period in a group of hyperlipidemic men known to be at risk of CVD through elevation of blood cholesterol.

METHODS AND PROCEDURES

Subjects

Eighteen men with mild hyperlipidemia (recruited on the basis of low-density lipoprotein-cholesterol 3.0–5.0 mmol/l) who were otherwise healthy completed both arms of the trial. At the time of participation in the trial, they were taking no medication for hyperlipidemia or any other condition, nor had they any current or previous history of CVD, T2DM, or any other metabolic disorder. Written consent was obtained from each participant, and ethical approval obtained from the Auckland Ethics Committee, Auckland, New Zealand.

Protocol and diets

All subjects in this randomized, double-blind, crossover intervention trial were required to reside at the University of Auckland Human Nutrition and Metabolic Unit for both dietary periods, which comprised two treatment arms each of 3-week duration. Work from our own (35) and other laboratories (36) have demonstrated that 3 weeks is of sufficient duration to see a decrease in serum total cholesterol and low-density lipoprotein-cholesterol when dietary SFA are replaced by unsaturated fats. It is reasonable to hypothesize that if such traditional risk factors change within a 3-week time frame, emerging risk factors such as adiponectin may change within the same period.

Between treatments, subjects returned home for a washout period of 4 weeks, during which time they were asked to resume their habitual diet and exercise patterns. The energy and macronutrient content of the diet was initially calculated using the dietary program, Foodworks (version 2.05, New Zealand Edition, Xyris Software, Brisbane, Australia, 1999), and then verified by direct chemical analyses of duplicate diet samples. Eight duplicate 5-day diets were collected at intervals throughout the trial, homogenized, and an aliquot frozen for later chemical analysis. This enabled the composition of the diet to be verified, and also demonstrated that there were no seasonal trends in composition.

Subjects were randomized using stratification to ensure that half were given the high saturated fatty acid:unsaturated fatty acid treatment ($high_{SFA:USFA}$) and half the low SFA:USFA ($low_{SFA:USFA}$) on entry into the study. All subjects then crossed over on to the other treatment arm. Subjects were provided with breakfast, lunch, and dinner in addition to between-meal snacks in the form of a 5-day rotation diet. Subjects were maintained in energy balance throughout the trial based on an estimate of basal metabolic rate (37) and energy expenditure, and food intake was altered daily to maintain a constant body weight (±1 kg). Subjects weighed themselves daily after an overnight fast and after voiding the bladder. Breakfast and dinner were taken under supervision at the Human Nutrition and Metabolic Unit, while lunch was packed and subjects could consume this meal at their place of work or study. Data on 24-h urinary nitrogen balance collected on day 10 and 20 of each intervention period were used to assess independent dietary compliance. Urinary losses of nitrogen were directly compared with dietary protein intake using para-amino benzoic acid to verify the completeness of collection (38–40). Decaffeinated, energy-free beverages were freely available throughout the intervention, and alcohol was prohibited at all times. Subjects were required to eat only and all of the foods provided and no others. The dietary compositions of both treatments are presented in **Table 2**.

Blood sampling and analyses

Blood and urine samples were routinely collected throughout both intervention periods. Fasting venous blood samples were collected on the morning of days 0 and 1 (pre-intervention baseline), 7, 14, 21, and 22. Once collected, blood samples were centrifuged and serum stored at −80 °C until later batch analyses. Samples were analyzed for adiponectin, TNF-α, IL-6, high-sensitivity C-RP (hsC-RP), leptin, and ghrelin. Adiponectin was analyzed by enzyme-linked immunosorbent assay using an in-house assay. Serum samples were diluted 1:5000 with phosphate-buffered saline and then added to 96-well microliter plates coated with monoclonal antibody against

human adiponectin (R & D System, Minneapolis, MN). 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, Minneapolis, MN). The wells were again washed and incubated with streptavidin-conjugated horseradish peroxidase for 60 min and then reacted with tetra-methyl benzidine reagent for 15 min. To each well, 100 µl of 3 M HCl was then added to stop the reaction and the absorbance at 450 nm was measured. Intra- and inter-assay 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, Minneapolis, MN). Then, 100 µl of sample was applied to 96-well microtitre 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 read at 450 nm. The standard curve was generated for every set of samples assayed using the standards provided in the kit. hsC-RP was analyzed using a Pointe Scientific (Lincoln Park, MI) immuno-turbidometric commercial kit. A solution of latex particles coated with the antibody specific to human C-RP aggregate was added to form immune complexes. Increased light scattering, proportional to the concentration of analyte, was measured on a COBAS Mira auto-analyzer (Roche Diagnostics, Basel, Switzerland). C-RP concentration was calculated using a calibration curve of C-RP standards and Prism software (GraphPad, San Diego, CA) used to fit third-order polynomials to the curve to calculate sample concentration. Assay range was 0.05–10 mg/l with a sensitivity of 0.1 mg/l.

Total ghrelin concentrations were measured by radioimmunoassay using a Linco Research commercial kit (Saint Charles, MI), using ^{125}I-labelled ghrelin as the tracer. Primary antibody was rabbit anti-ghrelin, secondary antibody was goat anti-rabbit IgG, and precipitation was achieved using polyethylene glycol (PEG) in a phosphate and Triton-X100 buffer. Hundred microliters of sample and standards were incubated with primary antibody at 4 °C overnight. After 24 h, 100 µl of tracer was added and incubated overnight at 4 °C. After 48 h, 1.0 ml of precipitating reagent was added, tubes incubated at room temperature for 20 min, and then centrifuged at 1,700*g* for 20 min at 4 °C. The supernatant was decanted and the pellets were counted on a Wallac 1480 Wizard gamma counter (Wallac Finland Oy, Turku, Finland). Linco Research reagents were prepared and stored according to the manufacturer's instructions. Curve fitting and sample concentration were computed using the MultiCalc software supplied with the counter. Coefficient of variation for this assay across the full standards range was 3.3%. The lowest level of detection was 0.311 ng/ml.

Serum leptin was measured by enzyme-linked immunosorbent assay using a Duoset commercial kit (R & D System, Minneapolis, MN). Hundred microliters of sample was added to 96-well microtitre 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 measured at 450 nm. The standard curve was generated for every set of samples assayed using the standards provided in the kit.

Statistical analyses

Paired *t*-test analyses (two tailed) were used to identify any differences in dietary energy or macronutrient composition between the low$_{SFA:USFA}$ and high$_{SFA:USFA}$ diets as taken by the subjects. All anthropometric and metabolic variables including body weight, adiponectin, TNF-α, IL-6, hsC-RP, leptin, and ghrelin were analyzed using linear mixed model ANOVA (SAS: PROC MIXED, SAS version 8.0, SAS Institute, Cary, NC, 2001). The dietary treatment, the arm of the trial (stratum), the intervention period, and the study day within period factors were explicitly modeled as fixed factors, as was the treatment/day interaction, which addressed whether the trajectory over time during the intervention period differed between treatments (diet × time). Subjects within strata were treated as random, as were their interactions with day and intervention period. Repeat baseline measures before intervention (d0, d1) were combined into a single mean value to reduce variability at baseline. Repeat data collected at the end of the intervention (d21, d22) were not combined. Variable intervals between blood collections were also included in the analyses such that the unequal number of days between measurements was modeled as an autoregressive order 1 process with constant day-to-day correlation. Biochemical assays were analyzed in triplicate and presented as a mean ± s.e.m. Statistical significance was based on 95% limits ($P < 0.05$).

RESULTS

Subjects

Subject characteristics are shown in **Table 1**. One subject withdrew from the trial on day 13 of the high$_{SFA:USFA}$ treatment and was replaced in the randomization. All other subjects completed the trial. Body weight was kept constant and was strictly maintained within the limits of ±1 kg during the two intervention arms. There was neither any significant difference in body weight at day 0 between the two treatments ($P > 0.05$) nor any change during the intervention, which would have affected the fasting parameters ($P > 0.05$), as shown in **Figure 1**.

Diet

The calculated macronutrient composition of the diet was 38%, 49%, and 13% of total energy for fat, CHO, and protein, respectively. Dietary cholesterol was lower on the low$_{SFA:USFA}$ treatment, but there was no difference between total fat, CHO, and protein between treatments ($P > 0.05$). An independent measure of dietary compliance using the 24-h nitrogen balance method was carried out on four occasions for each

Table 1 Baseline characteristics of the 18 men who completed both arms of the intervention. All measurements made at screen visit

Baseline	Mean	s.d.	Range
n	18		
Age (years)	39.7	13.9	23.0–67.0
Body weight (kg)	81.9	13.1	62.4–114.6
BMI (kg/m^2)	25.9	4.2	20.1–36.6
Waist (cm)	93.8	9.9	78.0–114.0
Hip (cm)	101.4	7.8	90.0–118.0
Waist:hip ratio	0.9	0.0	0.8–1.0
Total cholesterol (mmol/l)	5.8	0.7	4.8–6.6
LDL-cholesterol (mmol/l)	3.9	0.5	3.1–4.6
HDL-cholesterol (mmol/l)	1.2	0.3	0.9–1.8
TAG (mmol/l)	1.5	0.7	0.5–3.1
TC:HDL-C ratio	4.9	1.1	3.3–6.9
SBP (mm Hg)	127	21	104–182
DBP (mm Hg)	81	7	68–92
Glucose (mmol/l)	4.8	0.4	4.0–5.3

BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; SBP, systolic blood pressure; TAG, triacylglycerides; TC:HDL-C ratio, Total cholesterol:high-density lipoprotein–cholesterol ratio.

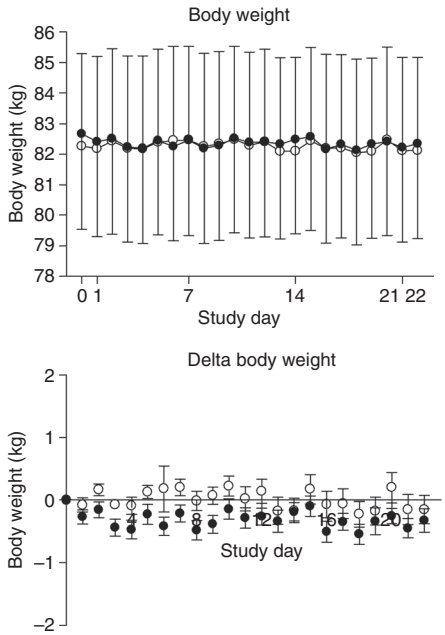

Figure 1 No significant change in body weight during 3 weeks on high$_{SFA:USFA}$ (open circles) and low$_{SFA:USFA}$ (closed circles) treatments. Mean ± s.e.m.

subject. There was reasonable long-term dietary compliance. The mean ± s.e.m. of urinary *N* as a percentage of dietary *N* was 74 ± 4%. The composition of the diet on both treatments as measured by direct chemical analysis is shown in **Table 2**. SFAs, MUFAs, and PUFAs accounted for 53, 32, and 30 g/d of the total diet in the high$_{SFA:USFA}$ treatment, respectively, and 38, 41, and 31 g/d of the total diet in the low$_{SFA:USFA}$. When calculated as a percentage of total daily energy intake (%), total saturates were 5% lower and total unsaturates 3% higher on the low$_{SFA:USFA}$ treatment. The most substantial differences were in palmitic (C16:0, −10.7 g/d), myristic (C14:0, −3.4 g/d), oleic (C18:1, +10.8 g/d) acids.

Blood markers

There was no significant difference at baseline (mean day 0 + day 1) between the high$_{SFA:USFA}$ and the low$_{SFA:USFA}$ treatments in serum adiponectin (mean ± s.d.; 7.0 ± 1.7 vs. 6.7 ± 1.4 µg/ml, $P = 0.19$), TNF-α (563.1 ± 372.2 vs. 606.4 ± 425.3 pg/ml, $P = 0.09$), IL-6 (20.8 ± 28.9 vs. 18.9 ± 24.9 pg/ml, $P = 0.19$), hsC-RP (1.4 ± 1.3 vs. 1.4 ± 0.9 mg/l, $P = 0.72$), or ghrelin (783.2 ± 131.2 vs. 785.3 ± 211.0 pg/ml, $P = 0.95$). Leptin was significantly higher at baseline (mean day 0 + day 1) on the high$_{SFA:USFA}$ treatment (2186.4 ± 536.6 vs. 2002.7 ± 338.5, $P = 0.04$). **Table 3** shows the effects of both treatments on fasting levels of these hormones between day 0 and the end of the intervention period (day 22). **Figure 2** shows the change relative to d0 in adiponectin, TNF-α, IL-6, and hsC-RP. **Figure 3** shows the change relative to d0 for leptin and ghrelin. Fasting adiponectin levels were not affected by treatment, demonstrating that alterations in fatty acid saturation did not affect the circulating adiponectin (diet × time, $P = 0.10$). TNF-α (diet × time, $P = 0.74$), IL-6 (diet × time, $P = 0.22$), and hsC-RP (diet × time, $P = 0.39$) were also not affected by fatty acid saturation of the diet, and there was no significant effect of fatty acid saturation on leptin (diet × time, $P = 0.56$) or ghrelin (diet × time, $P = 0.65$).

Table 2 Composition of the high$_{SFA:USFA}$ and the low$_{SFA:USFA}$ treatments as measured by direct chemical analysis; mean ± s.d.[a]

	High$_{SFA:USFA}$	Low$_{SFA:USFA}$	δ
Energy intake, EI (range, MJ/d)	10.0–16.0	10.0–16.0	
EI (mean, MJ/d)[b]	13.3 ± 1.5	13.3 ± 1.6	0
CHO (% of energy)			
Calculated[d]	49	49	0
Measured[b]	53 ± 2	53 ± 3	0
Protein (% of energy)			
Calculated[d]	13	13	0
Measured[b]	13 ± 1	13 ± 1	0
Fat (% of energy)			
Calculated[d]	38	38	0
Measured[b]	34 ± 1	34 ± 3	0
Cholesterol (mg/d)			
Measured[b,c]	232 ± 93	236 ± 55	+4
Total SFA (calculated, en%)[d]	18 ± 0.4	13 ± 0.4	−5
SFA profile (g/d)			
Total	52.9 ± 5.5	37.9 ± 2.9	−15
C12:0	3.1 ± 1.1	1.6 ± 0.2	−1.5
C14:0	8.3 ± 1.3	4.9 ± 0.6	−3.4
C16:0	28.0 ± 2.7	17.3 ± 1.3	−10.7
C18:0	11.8 ± 1.5	13.3 ± 1.3	+1.5
Total MUFA (calculated, en%)[d]	10 ± 0.7	12 ± 0.7	+2
MUFA profile (g/d)			
Total	32.3 ± 5.6	41.2 ± 4.4	+8.9
C16:1	1.8 ± 0.3	0.8 ± 0.9	−1.0
C18:1$_{total}$	29.7 ± 4.9	40.5 ± 3.8	+10.8
Total PUFA (calculated, en%)[d]	7 ± 1	8 ± 1	+1
PUFA profile (g/d)			
Total	30.2 ± 4.3	31.4 ± 6.2	+1.2
C18:2	27.1 ± 3.5	27.7 ± 5.0	+0.6
C18:3	3.1 ± 1.1	3.7 ± 1.3	+0.6

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid; USFA, unsaturated fatty acid.
[a]Results are the mean values for eight duplicate portions analyzed from both high$_{SFA:USFA}$ and low$_{SFA:USFA}$ treatments; [b]no significant difference between treatments ($P > 0.05$). Minor fractions of fatty acids not shown; [c]cholesterol intake shown for a 13.3 MJ diet; [d]calculated from Foodworks dietary program.

DISCUSSION

Little is known about the effects of diet on serum adiponectin in humans and the extent to which dietary intake may modulate this adipokine. In this trial we have shown that a diet rich in USFA such as MUFA, compared with a diet high in SFA, has no effect on the fasting levels of total adiponectin or associated

Table 3 Effects of high$_{SFA:USFA}$ and low$_{SFA:USFA}$ dietary treatments on serum markers measured at baseline (mean day 0 + day 1) and on days 7, 14, 21, and 22

	Baseline; mean day 0 + day 1	Day 7	Day 14	Day 21	Day 22
High$_{SFA:USFA}$					
Adiponectin (μg/ml)	7.0 (0.3)	7.2 (0.5)	6.9 (0.4)	7.3 (0.4)	6.5 (0.4)
TNF-α (pg/ml)	563.1 (62.0)	566.3 (99.2)	546.6 (87.4)	560.7 (99.8)	548.4 (99.2)
IL-6 (pg/ml)	20.8 (4.8)	26.2 (8.0)	20.4 (7.0)	19.3 (6.5)	20.5 (6.9)
hsC-RP (mg/l)	1.4 (0.2)	1.7 (0.6)	1.6 (0.4)	1.9 (0.4)	1.5 (0.3)
Leptin (pg/ml)	2186.4 (89.4)*	2101.6 (121.9)	2031.7 (118.7)	2041.2 (96.9)	2182.9 (122.8)
Ghrelin (pg/ml)	783.2 (21.9)	841.8 (50.8)	888.9 (53.7)	841.3 (37.4)	847.0 (54.7)
Low$_{SFA:USFA}$					
Adiponectin (μg/ml)	6.7 (0.2)	6.9 (0.4)	6.6 (0.4)	7.0 (0.7)	7.8 (0.6)
TNF-α (pg/ml)	606.4 (70.9)	593.3 (101.7)	563.4 (93.1)	548.9 (88.5)	526.4 (84.6)
IL-6 (pg/ml)	18.9 (4.2)	19.4 (6.2)	18.5 (6.1)	21.3 (7.1)	20.8 (6.8)
hsC-RP (mg/l)	1.4 (0.2)	1.2 (0.2)	1.9 (0.6)	1.2 (0.2)	1.0 (0.2)
Leptin (pg/ml)	2002.3 (55.6)*	2049.9 (86.0)	1971.5 (87.8)	1972.0 (64.7)	2026.3 (80.1)
Ghrelin (pg/ml)	785.3 (35.2)	806.8 (45.5)	820.5 (51.7)	854.1 (65.9)	855.7 (78.9)

Significant difference at baseline, paired *t*-test. Mean (s.e.m.).
hsC-RP, high-sensitivity C–reactive protein; IL-6, interleukin-6; SFA, saturated fatty acid; TNF-α, tumor necrosis factor-α; USFA, unsaturated fatty acid.
*$P < 0.05$; no significant effects of treatment over time, ANOVA, $P > 0.05$.

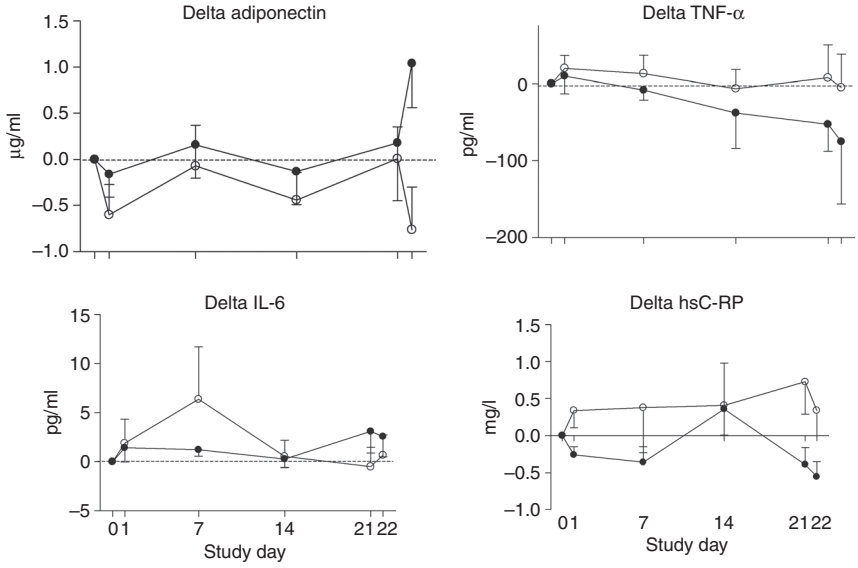

Figure 2 Change from baseline in fasting adiponectin, tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and high-sensitivity C–reactive protein (hsC-RP) during the 3-week high$_{SFA:USFA}$ (open circles) and low$_{SFA:USFA}$ (closed circles) treatments. Mean ± s.e.m.

peptides, TNF-α, IL-6, C-RP, leptin, or ghrelin, when given to a group of mildly hyperlipidemic men over a 3-week period.

The adipocyte secretes adiponectin in the form of distinct complexes (41–44). Trimers are the building blocks of low-molecular weight adiponectin; two subunits of the adiponectin trimer link to form a hexamer or middle-molecular weight adiponectin (41,43), while hexamers are the raw material for the formation of high-molecular weight adiponectin. Post-translational modifications are required for folding and assembly into the higher-order structures (41). Different multimers of adiponectin trigger different signal transduction pathways and exercise distinct functions on its target tissues (45–47). Any agent or intervention, including dietary intake that causes a change in the circulating level of full-length adiponectin, would necessarily have to interfere with at least one of the steps involved in the process of producing these multimers.

Epidemiological evidence that has investigated the relationship between dietary intake and circulating full-length adiponectin is inconclusive. In a cross-sectional analysis using semiquantitative food frequency questionnaires, Pischon and colleagues (48) demonstrated in a study of 532 men that moderate alcohol intake was related to higher serum adiponectin concentrations, and conversely a diet rich in carbohydrate, which promotes glycemia, was associated with lower

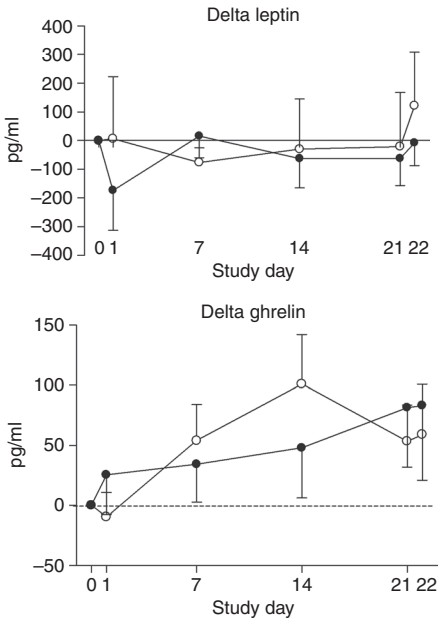

Figure 3 Change from baseline in fasting leptin and ghrelin during the 3-week high$_{SFA:USFA}$ (open circles) and low$_{SFA:USFA}$ (closed circles) treatments. Mean ± s.e.m.

concentration. Yannakoulia and co-workers (49) used 3-day food records and found no significant correlation between total energy or any macronutrient and serum adiponectin in a group of 114 individuals. However, the difficulties in obtaining reliable data on food intake by dietary interviews, records, or diaries are well known (50–52).

Intervention studies have been restricted to acute postprandial trials rather than investigating the longer-term effects of feeding on fasting levels, as in our current experiment. These postprandial trials have shown mixed results, yet the differences in the effect of feeding on postprandial adiponectin cannot readily be explained by the type of meal provided. Adiponectin both increased (15) and decreased (16) up to 4 h after test meal, while there was no effect of feeding on serum levels up to 6 h after test-meal in other trials (17–19,53). Some studies probed the effects of a mixed meal (15,19,53) while others investigated the effect of high-fat feeding *per se* (16–18). An experiment carried out in our laboratory tested the effects of a high-SFA meal vs. a low-SFA meal, and no effect of fat quality on circulating adiponectin was seen (17).

We are aware of two trials that have examined the longer-term effects of dietary fat on adiponectin in humans. In a crossover trial, Berk and colleagues (20) fed a group of lean and obese subjects a eucaloric diet comprising either 30% or 50% energy from fat each for a period of 7 days. Although circulating adiponectin did not change in response to alterations in dietary fat content over that period, there was a significant correlation between the changes in adiponectin levels for low fat to high fat with baseline insulin sensitivity. Bonnet and colleagues (21) investigated the impact of an increased intake of dietary ω-3 fatty acid (PUFA) on glucose metabolism and adipokine concentration in a group of 20 healthy subjects over a period of 10 weeks. Diets were supplemented with fatty fish at least three times per week in addition to 20 g high-MUFA rapeseed oil per day, and the results showed an increase in dietary ω-3 fatty acid consumption to be associated with a 17% increase in circulating adiponectin when compared to baseline values.

Of particular importance is the fact that in the current trial body weight was kept constant and was strictly maintained within the limits of ±1 kg during the two intervention arms. This is critical because the size of adipose tissue depots is known to be one of the key determinants of the concentration of circulating adiponectin. It is known that hormone levels are decreased in obese humans (54), restored to normal levels after weight loss (55), and negatively correlated to body fat and to waist-to-hip ratio (54). Any change in adipose tissue mass, whether an increase or decrease, can alter total adiponectin concentration. Circulating adiponectin is also affected by disease state. Although participants in the current trial were hyperlipidemic, they were taking no medication for their hyperlipidemia or any other condition, nor had they any current or previous history of CVD, T2DM, or any other metabolic disorder.

Adipose-derived pro-inflammatory cytokines, TNF-α and IL-6, have been shown to be closely related to adiponectin (56,57). In the current trial, no effect of alterations in dietary fatty acid saturation was seen on these markers. A number of large dietary supplement trials have investigated medium-term alterations in fatty acid profile, and have revealed changes in these inflammatory markers. Over an intervention period lasting 4–6 weeks, the marine-derived ω-3 PUFA eicosapentenoic acid (20:5*n*−3) was shown to inhibit the production of TNF-α and IL-6 (25,26). Although over periods of up to 12 weeks (26,27), α-linolenic acid (18:3*n*−3) an ω-3 PUFA, derived from plant sources such as flaxseed oil, may decrease TNF-α and IL-6 at high doses (28), it appears to be less effective in doing so than its marine-derived counter-parts. ώ-6 PUFAs have been shown to be predominantly pro-inflammatory (24), while healthy men (29) and individuals with T2DM (58) derive anti-inflammatory effects from fats rich in MUFA fed over a medium term.

Changes in fatty acid saturation had no effect on circulating C-RP in the current trial. In a cross-sectional study, a high-fat diet that was predominantly ω-3 PUFA was shown to be negatively associated with C-RP (59). Although C-RP has previously been shown to both increase (29) and decrease (30) in response to medium-term (4–5 weeks) feeding, an intervention study involving healthy men showed that conversion to the Mediterranean diet for 90 days did not lead to an improvement in C-RP (32) whereas in subjects with the metabolic syndrome such a dietary change lead to a decrease in C-RP after 2 years (31). Although weight loss (60) and physical activity (61–63) are known to modulate C-RP, the effects of diet on this acute-phase protein are inconclusive.

Diurnal changes in the release of leptin with a decline early morning followed by a nighttime peak, where concentrations are up to two times higher than nadir levels (64–67), complicate the interpretation of circulating leptin data. In the current

trial, leptin did not change in response to fatty acid profile. However, it might have been more appropriate to measure leptin levels more frequently than simply an early morning fasting sample at seven time-points across the two 3-week intervention periods. Long-term variations in diet, when body fat remains unchanged, have previously been shown not to alter fasting leptin concentrations (33), and it has been suggested that circulating leptin may be driven not by diet but rather by total adipose mass (34).

Ghrelin is an anorectic hormone most abundant in the stomach, the most important role of which seems to be the stimulation of appetite and regulation of energy homeostatsis (68). A number of trials have indicated that ghrelin responds postprandially to CHO feeding (69–72), although data suggest that it may not respond in the same way to fat feeding (17,71,73). There is little evidence, however, to indicate that this hormone responds to longer-term feeding, especially alterations in fatty acid profile. The lack of response of ghrelin to alterations in fatty acid profile in our trial supports this idea.

In conclusion, little is known as yet about the effects of dietary fat intake and specifically fatty acid saturation on adipokines such as adiponectin. Evidence from postprandial trials is emerging such that this adipokine is insensitive to acute intake of fat, but less is known about the effect of medium-term feeding. There was no evidence from the current trial that an increase in the SFA content of the diet significantly worsened fasting serum adiponectin, TNF-α, IL-6, C-RP, leptin, or ghrelin in this group over the 3-week intervention period.

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DISCLOSURE

The authors declared no conflict of interest.

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