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Effect of dietary n–3 polyunsaturated fatty acids on plasma total and high-molecular-weight adiponectin concentrations in overweight to moderately obese men and women[1–3]

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ABSTRACT

Background: Recent studies indicated that dietary n–3 polyunsaturated fatty acids (PUFAs) increase circulating adiponectin concentrations in rodents.

Objective: We aimed to investigate whether a diet rich in n–3 PUFAs increased plasma concentrations of total or high-molecular-weight (HMW) adiponectin in healthy overweight-to-moderately obese men and women.

Design: Sixteen women and 10 men with a body mass index (in kg/m^2) between 28 and 33 were randomly assigned to consume a diet rich in n–3 PUFAs (3.5% of energy intake) from both plant and marine sources or a control diet (0.5% of energy intake from n–3 PUFAs). For the first 2 wk, these diets were consumed under isocaloric conditions; then followed a 12-wk period of ad libitum consumption that was associated with a moderate loss of ≈3.5% of body weight in both groups. Total and HMW adiponectin plasma concentrations were measured before and after each diet phase.

Results: Plasma fasting adiponectin concentrations did not change during the isocaloric period, but they increased modestly (≈10%) during the ad libitum period when subjects lost weight [$P = 0.009$ for time in repeated-measures analysis of variance] and to a similar extent in subjects consuming the control ($\bar{x} \pm$ SD: 0.42 ± 0.69 μg/mL) and n–3 PUFA (0.45 ± 0.85 μg/mL) diets ($P = 0.920$ for time × treatment interaction). Plasma concentrations of HMW adiponectin did not change significantly during the study.

Conclusion: Dietary n–3 PUFAs consumed at levels of 3.5% of energy intake do not significantly increase plasma or HMW adiponectin concentrations in overweight-to-moderately obese healthy men and women over the course of 14 wk. *Am J Clin Nutr* 2008; 87:347–53.

KEY WORDS Adiponectin, fatty acids, omega-3, n–3, diet, obesity, overweight, humans

INTRODUCTION

The hormone adiponectin is specifically and abundantly secreted by adipocytes (1, 2). Adiponectin is present in plasma in the form of low-molecular-weight trimers, mid-molecular-weight hexamers, and high-molecular-weight (HMW) 12- or 18-mers (1). Although adiponectin is secreted by mature adipocytes, plasma adiponectin concentrations show a negative correlation with body fat mass (3). Adiponectin receptors are ubiquitously expressed, and they appear most abundantly in liver and muscle (4). The binding of adiponectin to its receptor has been shown to induce fatty acid β-oxidation and to increase cellular insulin sensitivity (4). Consistent with this finding, plasma adiponectin concentrations have been found to be positively associated with systemic insulin sensitivity and to be reduced in states characterized by insulin resistance, such as obesity (4). Furthermore, adiponectin exhibits protective properties with regard to cardiovascular disease, and a low plasma adiponectin concentration has been shown to be an independent risk factor for coronary heart disease and hypertension (5). Factors associated with elevated plasma concentrations of adiponectin include treatment with thiazolidinediones, a class of drugs that act as ligands of the transcription factor peroxisome proliferator–activated receptor γ (PPARγ) (6); moderate alcohol intake (7, 8); and marked weight loss such as that associated with gastric bypass surgery (9, 10).

In the past few years, it has been reported that the feeding of diets containing large amounts of fish oil increases plasma adiponectin concentrations in rats (11) and mice (12). Neschen et al (12) showed that the expression of the adiponectin gene was increased in epididymal but not subcutaneous fat in mice fed fish oil, and their results indicated that the increase in plasma adiponectin concentrations was likely mediated by the activation of PPARγ. The only identified natural ligand of PPARγ is a derivative of the n–6 fatty acid linoleic acid (13, 14), which suggests that the components in fish oil that are capable of raising adiponectin production most likely are also fatty acids; potential

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candidates include fish oil–specific long-chain n–3 polyunsaturated fatty acids (PUFAs).

It is not known whether a diet rich in fish oil or n–3 PUFAs increases plasma adiponectin concentrations in humans. In the only published study investigating this question in humans, there was a trend toward modestly (7.6%) higher plasma adiponectin concentrations ($P = 0.086$) when nonobese, weight-stable, healthy men and women consumed a portion of fatty salmon daily for 4 wk than was seen when they consumed a diet not containing fish (15). From this study, it remained unclear, however, whether the trend may have been a result of increased n–3 PUFA intake or of changes in other dietary variables associated with fish consumption, such as a higher protein intake. The objective of the present study was to determine whether plasma adiponectin concentrations are altered in overweight-to-moderately obese healthy men and women during consumption of a diet enriched in n–3 PUFAs or a control diet. Subjects were assessed after both isocaloric dietary intake resulting in stable body weight and ad libitum intake resulting in a modest loss of body weight. We measured total adiponectin and HMW adiponectin because the amount or the proportion of HMW adiponectin relative to total adiponectin in plasma has been proposed to be more closely associated with enhanced insulin sensitivity than are total adiponectin concentrations (10, 16). We also analyzed whether the concentrations of adiponectin at the previously reported diurnal peak (ie, at 1000) and diurnal nadir (ie, at 0400) (17) were altered during the control or n–3 PUFA diets.

SUBJECTS AND METHODS

Subjects

The primary aim of this study was to test the hypothesis that a diet rich in n–3 PUFAs lowers body weight in overweight and moderately obese men and women. The sample size calculation showed that, if 36 subjects completed the study, we would have 80% power to detect a 2.25-kg difference in body weight between the control and n–3 PUFA groups at $P < 0.05$. Using a sequential clinical trial design, we performed one interim analysis after 26 subjects had completed the study. The result of this interim analysis was that we accepted the null hypothesis of no difference between the groups and we stopped the study early on the grounds of futility, because the 2 groups had very similar weight loss. We here present plasma and HMW adiponectin data for these 26 subjects throughout the study.

All 33 subjects entering the study had a body mass index (BMI: in kg/m^2) between 28 and 33; they were weight stable [±2.27 kg (5 pounds) in the past 6 mo] and were within 4.54 kg (10 pounds) of their lifetime maximum weight. Exclusion criteria included the use of tobacco or recreational drugs, alcohol abuse, a history of cardiovascular disease or diabetes mellitus, the presence of any chronic illness or psychiatric illness, restrictive eating behavior, pregnancy, and the intake of selective serotonin-reuptake inhibitors, lipid-lowering drugs, β-blockers, glucocorticoids, or anabolic steroids. Three subjects withdrew because of dislike of the study diet or because the required visits conflicted with their work schedules. One subject failed to comply with the study diet and was excluded after 8 wk. One subject was excluded because fasting glucose concentrations measured at the end of the study suggested that she had overt type 2 diabetes mellitus throughout the study. Two subjects admitted to having started smoking during the study and were therefore excluded. Plasma concentrations of adiponectin were measured in the 26 remaining subjects (16 F, 10 M). We used a block randomization procedure, separately for men and women, to randomly assign the subjects. Of the 26 subjects included in this analysis, 13 received the diet enriched in n–3 PUFAs, and 13 received the control diet. Baseline characteristics are shown, separately for the 2 diet groups, in **Table 1**.

All subjects provided written informed consent. All procedures were approved by the University of Washington Human Subjects Committee.

Study design

In the 2-wk lead-in period, all subjects first consumed the control diet, which was rich in monounsaturated fatty acids (**Table 2**). The caloric content of the diet was calculated, on the basis of a 3-d diet record completed by the subjects and the use of the Mifflin formula (18), to maintain each subject's weight within 1 kg of baseline. The control diet consisted of conventional foods typically found in a mixed American diet. The principal sources of fat during this period were high-oleic safflower and sunflower oils and margarines based on these oils. The plant oils and the margarines were used for the preparation of all meals and snacks. In addition, subjects were given capsules containing high-oleic safflower oil. Subjects were instructed to consume all food provided, not to eat any additional food, to take a specific number of the oil capsules each day, and to complete a daily diary of food intake. Subjects were asked to come in twice a week to be weighed and to pick up food. Adjustments in the caloric content of the diet were made as required to meet the target for weight stability. On the morning of day 14 of the control diet and after an overnight fast, subjects were admitted to the University of Washington General Clinical Research Center (CRC) for visit #1 (CRC1).

On completion of CRC1, subjects were randomly assigned to 1 of 2 groups: for 2 more wk, one of these groups continued to consume the control diet, and the other group received the diet enriched in n–3 PUFA (Table 2). All food was provided to the subjects. The n–3 PUFA diet was identical to the control diet in

TABLE 1
Baseline characteristics of participants[1]

	Control group ($n = 13$)	n–3 PUFA group ($n = 13$)
F/M	8/5	8/5
Age (y)	37.8 ± 13.6[2]	37.5 ± 14.0
Body weight (kg)	85.4 ± 9.3	87.6 ± 12.9
BMI (kg/m^2)	30.1 ± 1.1	30.5 ± 1.6
Fasting plasma glucose (mg/dL)	97 ± 7	97 ± 9
Fasting plasma insulin (μU/mL)	13.7 ± 6.0	28.9 ± 44.6
Fasting plasma adiponectin (μg/mL)	4.33 ± 1.90	4.21 ± 2.02
Fasting plasma HMW adiponectin (μg/mL)	1.89 ± 1.33	1.85 ± 1.24
HMW/total adiponectin ratio in fasting plasma	0.40 ± 0.13	0.41 ± 0.18

[1] PUFA, polyunsaturated fatty acids; HMW, high-molecular-weight. The 2 groups did not differ significantly with regard to any of these variables (in independent-sample t tests or Mann-Whitney U tests).

[2] $\bar{x}$ ± SD (all such values).

TABLE 2
Composition of the diets[1]

	Lead-in period (2 wk)	Isocaloric period (2 wk)		Ad libitum period (12 wk)	
	Control diet (n = 26)	Control diet (n = 13)	n–3 PUFA diet (n = 13)	Control diet (n = 13)	n–3 PUFA diet (n = 13)
Protein (% of energy)	15.9 ± 0.1	15.9 ± 0.0	15.8 ± 0.1	15.8 ± 0.5	16.0 ± 0.4
Carbohydrates (% of energy)	49.8 ± 0.4	49.8 ± 0.2	49.5 ± 0.2	49.5 ± 1.0	49.4 ± 1.4
Alcohol (% of energy)	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.7 ± 0.7	0.7 ± 1.1
Fat (% of energy)	34.3 ± 0.4	34.3 ± 0.2	34.7 ± 0.1	34.0 ± 0.8	33.9 ± 0.8
SFA (% of energy)	8.6 ± 0.1	8.5 ± 0.1	8.4 ± 0.0	8.5 ± 0.3	8.4 ± 0.3
MUFA (% of energy)	18.6 ± 0.5	18.7 ± 0.1	15.5 ± 0.0	18.3 ± 0.4	15.0 ± 0.5
n–6 PUFA (% of energy)	4.8 ± 0.0	4.8 ± 0.0	4.9 ± 0.0	4.8 ± 0.2	4.8 ± 0.1
n–3 PUFA (% of energy)	0.5 ± 0.0	0.5 ± 0.0	3.6 ± 0.1	0.5 ± 0.0	3.4 ± 0.1
α-Linolenic acid (% of energy)	0.5 ± 0.0	0.5 ± 0.0	2.2 ± 0.0	0.5 ± 0.0	2.0 ± 0.1
Long-chain n–3 PUFA (% of energy)	0.0 ± 0.0	0.0 ± 0.0	1.4 ± 0.1	0.0 ± 0.0	1.4 ± 0.1
Vitamin E (mg α-TE/1000 kcal)	25.8 ± 1.1	26.3 ± 0.5	26.5 ± 0.7	26.1 ± 0.8	26.4 ± 1.7
Cholesterol (mg/1000 kcal)	67.5 ± 2.6	67.1 ± 2.6	60.1 ± 0.2	69.6 ± 3.4	64.5 ± 3.0
Fiber (g/1000 kcal)	11.1 ± 0.1	11.0 ± 0.1	11.1 ± 0.0	10.7 ± 0.8	10.6 ± 0.4

[1] All values are $\bar{x}$ ± SD. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TE, tocopherol equivalents.

all respects except the principal sources of dietary fat, in that canola and flaxseed oils were substituted for high-oleic safflower and sunflower oils. Both canola and flaxseed oils are rich in the n–3 PUFA α-linolenic acid. In addition, margarine rich in α-linolenic acid was provided. Subjects in the n–3 PUFA group were asked to take a certain number of capsules containing fish oil, instead of capsules containing high-oleic safflower oil. The number of fish-oil capsules that subjects were asked to take was calculated to provide 1.4% of energy in the form of long-chain n–3 PUFA. At a total daily food intake of 1800 kcal, subjects were asked to take 12 capsules, each containing 725 mg fish oil or 240 mg long-chain n–3 PUFA. Subjects continued to pick up food, to be weighed, and to turn in food records twice a week. The total caloric content of dispensed food items was identical (isocaloric) to the amount that led to weight stability in the lead-in diet period. The subjects were again instructed to consume all of the food and not to eat any other food. On day 14 of this period, subjects were readmitted to the CRC for visit #2 (CRC2).

After CRC2, subjects continued for another 12 wk to consume the diets to which they had been randomly assigned. All food was provided. Subjects continued to pick up food and to be weighed twice a week. The amount of food provided during this period was 115% of the amount provided in the previous 2 diet periods. Subjects were specifically instructed to eat only when they were hungry, to eat only as much as they needed to feel comfortably satiated (ie, ad libitum), and to return any foods not eaten. These returned foods were weighed to assess the amount consumed by each subject on each day of the study. During this period, as an approach to increase compliance with the study diet, subjects were allowed to exchange one study meal/wk for a meal of personal choice and to have ≤3 alcoholic beverages/wk. Subjects were asked to provide accurate information on the type and amount of alcoholic beverages consumed and of the food eaten during the meal of personal choice. On day 84 of this diet period, subjects were readmitted to the CRC for visit #3 (CRC3).

During each of the 3 admissions to the CRC, subjects were weighed while wearing a hospital gown. Breakfast, lunch, dinner, and a snack were served at 0800, 1200, 1730, and 2000, respectively, and subjects were asked to complete each meal within 30 min. An intravenous line placed in the forearm was used to sample 5 mL blood every half-hour between 0800 and 2100 and every hour between 2100 and 0800 the next morning. We created a "pool" sample by combining 50 μL plasma from each sample drawn at 30-min intervals and 100 μL plasma from each sample drawn at 1-h intervals. Additional fasting blood samples were drawn at 0800 on day 2 of each CRC visit. Blood samples were placed on ice and centrifuged (15 min, 1600 × g, 4 °C) immediately after the fasting blood draws had been completed. Plasma was kept frozen at −70 °C until analyses were conducted. During CRC1 and CRC3, we also performed a whole-body dual-energy X-ray absorptiometry scan with the use of a GE Lunar Prodigy scanner (General Electric Healthcare, Waukesha, WI) to assess body fat mass.

Laboratory methods

We measured total adiponectin concentrations in fasting plasma samples and in samples that were pooled from the 38 samples drawn throughout each 24-h visit to the University of Washington CRC (the pool sample). HMW adiponectin concentrations were measured in fasting samples only. We measured total and HMW adiponectin concentrations in duplicate by using a multimeric enzyme-linked immunosorbent assay (Alpco Diagnostics Inc, Salem, NH). This method has been validated against Western blotting for measurement of adiponectin multimers (19). Total adiponectin in fasting and pool samples also was measured in duplicate with the use of a radioimmunoassay (Linco Research Inc, St Charles, MO). In our hands, the Linco radioimmunoassay kit yielded values for total adiponectin that were much higher (≈2.5-fold) than the values obtained with the Alpco enzyme-linked immunosorbent assay. However, the correlation between the 2 methods was very high (r = 0.94; 95% CI: 0.90, 0.96; $P < 0.0001$), and in all cases, we took care to perform repeated measurements on samples from the same subject within the same assay run.

Fasting glucose was measured by using the hexokinase method on a Hitachi 917 autoanalyzer (Roche Diagnostics, Mannheim, Germany), and fasting insulin was measured with an immunoassay on a different autoanalyzer (AIA 600 II; Tosoh

Bioscience Inc, San Francisco, CA). Insulin resistance was measured by using homeostatic model assessment (HOMA) and calculated as the product of the fasting plasma insulin concentrations (in μU/mL) and the fasting plasma glucose concentration (in mmol/L), divided by 22.5.

Statistical analysis

All statistical analyses were performed with SPSS software (version 11.5; SPSS Inc, Chicago, IL). Distribution of variables was analyzed by checking histograms and normal plots of the data, and normality was tested by means of Kolmogorov-Smirnov and Shapiro-Wilk tests. Pearson correlation coefficients were calculated to investigate the association between the change in body weight and plasma adiponectin, the association between adiponectin concentrations in fasting and pool samples, and the association between adiponectin plasma concentrations and the HOMA insulin resistance (IR) index. Baseline characteristics of the groups were compared by means of independent-sample *t* tests or Mann-Whitney *U* tests. Changes in body weight, body fat mass, total and HMW adiponectin concentrations, and the ratio of HMW to total adiponectin were compared by using repeated-measures analysis of variance (RM-ANOVA) with the 3 time points of the 3 visits (CRC1, CRC2, and CRC3) as the 3 levels of the within-subjects factor (time) and with treatment (control versus n–3 PUFA) as the between-subjects factor. Post hoc, we performed another RM-ANOVA with a reduced number of levels of the within-subjects factor by including either just CRC 1 and CRC2 or just CRC2 and CRC3. If the assumption of sphericity did not hold, we used the Greenhouse-Geisser correction factor to adjust the df. The distribution of residuals of these analyses was again tested by checking histograms and normal plots of the data, and normality was tested by means of Kolmogorov-Smirnov and Shapiro-Wilk tests. Friedman tests were performed separately for each group if the residuals were not normally distributed, which was the case for fasting plasma insulin, HOMA, and total adiponectin data as measured by the Linco radioimmunoassay kit. Friedman tests were followed up with Wilcoxon's signed-ranks tests after adjustment for multiple testing. The level of significance was $P < 0.05$.

RESULTS

Body weight changed significantly throughout the study (**Table 3**), with no effect of treatment group on this change. During the 2 wk of the isocaloric diet period when subjects were asked to consume as many calories as they had required to be weight stable in the lead-in period, body weight decreased by 0.4 kg. Although this decrease was significant (post hoc RM-ANOVA CRC1 compared with CRC2: $P = 0.001$ for time), it was minimal (<0.5% of body wt) and did not differ significantly between the 2 groups ($P = 0.538$ for time × treatment). During the 12 wk when subjects consumed these diets ad libitum, body weight decreased by a mean of 3.1 ± 3.8 kg in the control group and by 2.2 ± 3.6 kg in the n–3 PUFA group. This change was significant overall (post hoc RM-ANOVA CRC2 compared with CRC3: $P = 0.001$ for time) but did not differ significantly between the 2 groups (P = 0.541 for time × treatment). Body fat mass decreased by 2.6 ± 3.5 kg in the control group and by 1.8 × 2.9 kg in the n–3 PUFA group, changes that were significant overall but that did not differ significantly between treatment groups ($P = 0.002$ for time; $P = 0.583$ for time × treatment).

Total plasma adiponectin concentrations changed significantly ($P = 0.011$) over time, but there was no effect of treatment group on this change ($P = 0.268$). Post hoc RM-ANOVA showed that plasma total adiponectin increased significantly during the ad libitum period when subjects lost weight (CRC2 compared with CRC3: $P = 0.009$ for time) and that increases in the control and n–3 PUFA groups did not differ significantly ($P = 0.920$ for time × treatment).

The plasma concentrations of HMW adiponectin neither changed significantly throughout the study ($P = 0.129$ for time) nor differed in any way between the 2 diet groups ($P = 0.497$ for

TABLE 3
Body weight, body fat mass, fasting total and high-molecular-weight (HMW) adiponectin, and the ratio of HMW to total adiponectin during the study[1]

	After the lead-in period (CRC1)	After the isocaloric period (CRC2)	After the ad libitum period (CRC3)	*P*[2]	
				Time	Time × treatment
Body weight (kg)				<0.001	0.583
Control (n = 13)	84.9 ± 9.3[3]	84.5 ± 9.1	81.4 ± 9.1		
n–3 PUFA (n = 13)	87.3 ± 12.9	86.7 ± 12.7	84.5 ± 13.7		
Body fat mass (kg)				0.002	0.583
Control (n = 13)	31.5 ± 4.8	—	28.9 ± 6.5		
n–3 PUFA (n = 13)	33.9 ± 5.6	—	32.0 ± 4.9		
Plasma total adiponectin (μg/mL)				0.011	0.268
Control (n = 13)	4.33 ± 1.90	4.06 ± 1.53	4.48 ± 1.55		
n–3 PUFA (n = 13)	4.21 ± 2.02	4.35 ± 1.98	4.80 ± 1.88		
HMW adiponectin (μg/mL)				0.129	0.497
Control (n = 13)	1.89 ± 1.33	1.92 ± 1.36	2.01 ± 1.38		
n–3 PUFA (n = 13)	1.85 ± 1.24	2.08 ± 1.24	2.16 ± 1.27		
HMW/total adiponectin				0.170	0.875
Control (n = 13)	0.40 ± 0.13	0.43 ± 0.17	0.40 ± 0.17		
n–3 PUFA (n = 13)	0.41 ± 0.18	0.46 ± 0.16	0.42 ± 0.13		

[1] PUFA, polyunsaturated fatty acids; CRC1, CRC2, and CRC3, visits 1, 2, and 3 to the Clinical Research Center.
[2] Repeated-measures ANOVA.
[3] $\bar{x}$ ± SD (all such values).

time × treatment). Similarly, the ratio of HMW to total adiponectin did not change significantly ($P = 0.170$ for time), and there was no significant difference between the 2 treatment groups ($P = 0.875$ for time × treatment).

Total adiponectin concentrations in the pool samples from each 24-h stay in the CRC correlated highly with total fasting adiponectin concentrations at CRC1 ($r^2 = 0.9216$, $P < 0.001$; **Figure 1**), CRC2 ($r^2 = 0.897$, $P < 0.001$), and CRC3 ($r^2 = 0.857$, $P < 0.01$). Changes throughout the study in total adiponectin concentrations in these pool samples did not differ significantly from those observed in fasting adiponectin, and, therefore, they are not presented separately.

We also measured total adiponectin concentrations at 1000 and 0400 (on the next morning), the time points during the day on which the adiponectin plasma concentration have been reported to be highest and lowest, respectively (17). Because there were again no differences between the control and n–3 PUFA groups in any of these analyses, we pooled the data for this analysis. Whereas plasma adiponectin concentrations at 1000 were significantly greater during CRC3 than during CRC1 ($P = 0.051$, post hoc Wilcoxon's test) or during CRC2 ($P = 0.030$, post hoc Wilcoxon's test), we did not observe any difference in plasma adiponectin at 0400 ($P = 0.572$, Friedman test; **Figure 2**). It is interesting that the significant difference in plasma adiponectin concentrations between 1000 and 0400 that others previously reported (17) was present in the current study only after subjects had lost weight. Thus, the diurnal plasma adiponectin amplitude, calculated as the difference between these time points, was significantly greater at CRC3 (1.58 ± 1.49 μg/mL) than at CRC1 (0.46 ± 1.22 μg/mL; $P = 0.030$, post hoc Wilcoxon's test) or at CRC2 (0.77 ± 1.10 μg/mL; $P = 0.021$, post hoc Wilcoxon's test).

Despite the increase in total plasma adiponectin and the moderate weight loss, we did not observe any change in fasting glucose or insulin concentrations or in the HOMA-IR index (**Table 4**). Moreover, we found no significant correlations between HOMA and total or HMW adiponectin, between HOMA and the ratio of HMW to total adiponectin, or between changes in HOMA and changes in total adiponectin, HMW adiponectin, the ratio of HMW to total adiponectin, or the diurnal adiponectin amplitude throughout the study. Two subjects in the n–3 PUFA group had very high fasting insulin concentrations at baseline and throughout the study. We therefore repeated all analyses involving insulin and the HOMA-IR index without these 2 subjects; the results were identical to those obtained with these 2 subjects.

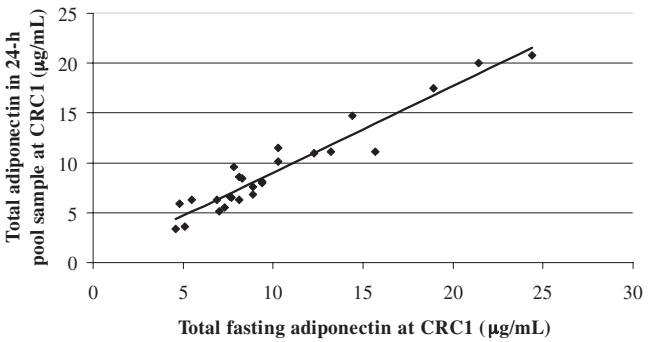

FIGURE 1. Relation between adiponectin concentrations in fasting plasma and plasma pooled from the 38 samples that were collected in each subject throughout the first 24-h Clinical Research Center visit #1 (CRC1), measured with the use of a radioimmunoassay kit (Linco Research Inc, St Charles, MO; $n = 26$). The regression line was calculated with the use of the following equation: $y = 0.8707x + 0.3282$ ($r^2 = 0.9216$, $P < 0.001$).

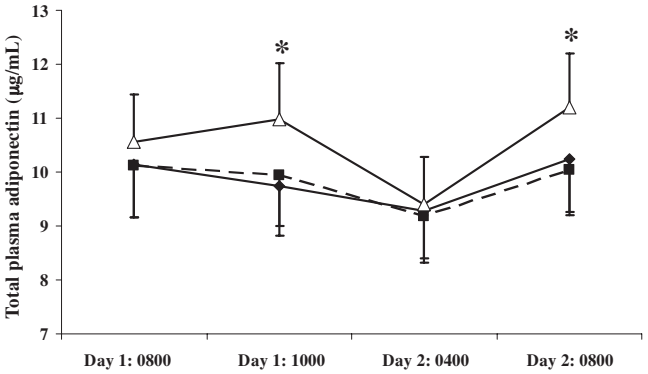

FIGURE 2. Mean plasma total adiponectin concentrations throughout the 24-h span of each of the 3 Clinical Research Center visits (CRC1, ♦; CRC2, ■; CRC3, △), measured with the use of a radioimmunoassay kit (Linco Research Inc, St Charles, MO; $n = 26$). Error bars represent SEs. For each time point, the data were analyzed with the nonparametric Friedman test: $^*P < 0.05$ for overall difference between the 3 CRC visits.

DISCUSSION

The major finding of this randomized, controlled, dietary intervention study in overweight and moderately obese subjects was that neither total nor HMW adiponectin concentrations differed significantly in any way between subjects consuming an n–3 PUFA–enriched diet and control subjects consuming a diet with low n–3 PUFA content. The finding that weight loss resulted in the expected increase in adiponectin concentrations in both subject groups (20, 21) validates our study design and the precision of the assays used. It is interesting that this effect of weight loss was evident when we compared group means, whereas regression analysis did not find a relation between the magnitude of the increase of adiponectin and the degree of weight change in individual subjects. A possible explanation for the latter observation is that the transition to a negative energy balance plays a more important role than does the amount of fat lost in eliciting adiponectin secretion from the adipocyte. This is certainly the case for leptin, in that fasting causes a marked decline in plasma leptin concentrations before significant loss of body fat mass (22, 23). Our group (24) previously observed that circulating adiponectin concentrations do respond modestly to relatively acute changes in energy balance but also that there are sex differences in this response: the response is greater in women and less in men during a 7-d period of marked restriction of energy intake. It also may be that, with this small amount of weight loss, the changes of weight do not closely represent the decreases of body fat (particularly visceral fat mass and adipocyte size) that are likely to be more important determinants of adiponectin production (2).

Leptin serves as an example of an adipokine that displays a prominent diurnal pattern (25). Diurnal variation plays a key role in the action of certain hormones, such as cortisol and the gonadotropins. This variation also has practical implications with respect to the time at which plasma should be sampled to obtain hormone measurements that reflect integrated 24-h plasma concentrations. Adiponectin has been reported to display a diurnal rhythm in which the timing is inverse to that of leptin and in

TABLE 4
Plasma fasting glucose and insulin concentrations and homeostasis model assessment (HOMA) of the insulin resistance index throughout the study[1]

	After the lead-in period (CRC1)	After the isocaloric period (CRC2)	After the ad libitum period (CRC3)	*P*		
				Time[2]	Time × treatment[2]	Time[3]
Fasting glucose (mg/dL)				0.249	0.239	
Control (n = 13)	97 ± 7[4]	96 ± 8	96 ± 7			
n–3 PUFA (n = 13)	97 ± 9	100 ± 6	96 ± 14			
Fasting insulin (μU/mL)						
Control (n = 13)	13.7 ± 6.0	17.2 ± 10.7	19.0 ± 15.3			0.926
	13.5 (6.4–25.2)[5]	14.4 (5.3–44.7)	17.2 (4.8–56.3)			
n–3 PUFA (n = 13)	28.9 ± 44.6	28.0 ± 40.0	22.3 ± 25.0			0.635
	13.8 (7.8–162.7)	16.6 (4.7–156.5)	18.9 (7.5–101.3)			
HOMA insulin resistance index						
Control (n = 13)	3.0 ± 1.3	3.7 ± 2.4	4.0 ± 3.1			0.926
	2.8 (1.3–5.3)	3.1 (1.1–10.0)	3.4 (1.1–11.9)			
n–3 PUFA (n = 13)	6.4 ± 10.0	6.4 ± 9.5	5.0 ± 6.1			0.257
	2.9 (1.7–35.8)	4.1 (1.0–36.9)	4.0 (1.0–24.3)			

[1] CRC1, CRC2, and CRC3, visits 1, 2, and 3 to the Clinical Research Center; PUFA, polyunsaturated fatty acids.
[2] Repeated-measures ANOVA.
[3] Friedman test.
[4] $\bar{x}$ ± SD (all such values).
[5] Median; range in parentheses (all such values).

which adiponectin has a lower amplitude (17). Our comparison of plasma adiponectin concentrations at 1000 and 0400 showed the emergence of such a rhythm with weight loss. Although we did not find an association between the change in the diurnal adiponectin amplitude and the HOMA-IR index in the present study, it could be speculated that an increase in amplitude may play an important role in the improvement of insulin sensitivity that is seen when more insulin-resistant subjects lose body weight. It is important to note that, although fasting insulin and the HOMA-IR index appeared to improve in the n–3 PUFA group, these changes were not significant and are explained largely by the presence of the 2 subjects in that group who had very high fasting insulin concentrations at baseline and throughout the study. There was neither a consistent change in HOMA-IR over time nor a correlation between the small increase in adiponectin seen with weight loss and changes in fasting insulin or HOMA-IR. The observed increase in plasma adiponectin concentrations in the present study probably was too small to induce improvements in insulin sensitivity in subjects with mostly normal insulin sensitivity and normal glucose tolerance. The close correlation between total adiponectin measured in the fasting and the pooled 24-h plasma samples suggests that a single fasting measurement provides a reliable estimate of diurnal adiponectin concentrations over the course of a 24-h period.

In the present study, subjects in the n–3 PUFA group consumed 1.4% of total daily energy intake in the form of long-chain n–3 PUFA of marine origin, which required them to take 12–24 capsules containing 725 mg fish oil each day. This dose is roughly equivalent to the amount of n–3 PUFA in a daily portion of 125–250 g fatty fish, such as Chinook salmon, and is on the high end of what humans can be expected to consume. Neschen et al (12) fed mice diets in which 4.4%, 8.1%, and 15% of the total energy intake was in the form of long-chain n–3 PUFA, and they observed marked increases in plasma adiponectin concentrations with all 3 diets. Rossi et al (11) fed rats diets in which 16.2% of total energy intake was in the form of cod liver oil—an amount equivalent to ≈5–6% of energy intake in the form of long-chain n–3 PUFA. Although the possibility cannot be excluded, our results do not suggest that higher doses of long-chain n−3 PUFA would have led to an increase in plasma adiponectin concentrations, because there was no trend toward higher adiponectin concentrations in subjects consuming the n–3 PUFA–rich diet than in subjects consuming the control diet. It would be impractical for humans to achieve n–3 PUFA intakes much higher than those in this study, even when ingesting substantial amounts of supplements such as fish-oil capsules.

Lara et al (15) observed a trend toward moderately (7.6%) higher plasma total adiponectin concentrations when subjects consumed a daily portion of fatty salmon providing 2.4 g long-chain n–3 PUFAs—equivalent to ≈1.1% of total energy intake—for 4 wk than when subjects did not consume any fish (control diet period). The study of Lara et al was not randomized, however; all subjects consumed the salmon diet first and then the control diet. Moreover, the study was not well controlled, in that only salmon was provided, and subjects chose all other food items freely. Subjects were asked to replace the salmon with turkey, chicken, cheese, or meat during the no-fish period, and these choices probably resulted in differences in the amount of n–3 PUFA, as well as in other factors such as protein or saturated fat content. Unfortunately, the composition of the control diet was not reported by Lara et al. Their report therefore left unclear to what the modest differences in plasma adiponectin could be attributed.

Neschen et al (12) presented evidence that fish-oil feeding increased adipocyte adiponectin expression and plasma adiponectin concentrations in mice through a mechanism involving the transcription factor PPARγ. This finding is consistent with the observation that treatment with a synthetic PPARγ ligand, such as the thiazolidinedione class of drugs, increases plasma adiponectin concentrations (6). The only natural ligand of PPARγ that has been

identified is 15-deoxy-Δ12,14-prostaglandin J_2 (13, 14), a derivative of the n–6 PUFA arachidonic acid. It would therefore be plausible to assume that, because prostaglandins can be generated from the long-chain n–3 PUFA eicosapentaenoic acid as well, the dietary content of long-chain n–3 PUFA could affect the expression of PPARγ-regulated genes such as adiponectin (26). Moreover, eicosapentaenoic acid has been found to increase the expression of PPARγ itself (27). The fact that we did not find an effect of a diet rich in n–3 PUFA on plasma adiponectin concentrations suggests either that n–3 PUFA does not play a major role in PPARγ activation in humans or that the pathways regulating the intracellular trafficking or processing of n–3 PUFA that may be necessary for efficient PPARγ binding differ in humans and rodents.

A potential limitation of the present study is the fact that it was not designed and powered to detect a clinically significant difference in plasma adiponectin concentrations between the n–3 PUFA and control groups. However, whereas it is theoretically possible that we missed an effect of n–3 PUFA on plasma adiponectin concentrations because of a lack of power, that possibility seems unlikely, because there was not even a trend toward a difference between controls and n–3 PUFA subjects in plasma adiponectin concentrations. We therefore conclude that n–3 PUFA supplementation at a relatively high level does not increase circulating plasma total or HMW adiponectin concentrations in overweight-to-moderately obese human subjects. It appears unlikely that increased adiponectin has a role in the reported metabolic and cardiovascular effects of marine oils.

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The authors' responsibilities were as follows—MK and DSW: initiated the project and were responsible for the design and implementation of the study, collection and statistical analysis of data, and writing the first draft of the manuscript; MMS and PJH: measured total and high-molecular-weight adiponectin concentrations in plasma; HSC and CCM: calculated the diets and were responsible for the preparation of all study meals; and all authors: contributed to the preparation of the manuscript. None of the authors had a personal or financial conflict of interest.

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