nature publishing group

Improvements in Coronary Heart Disease Risk Indicators by Alternate-Day Fasting Involve Adipose Tissue Modulations

Surabhi Bhutani[1], Monica C. Klempel[1], Reed A. Berger[2] and Krista A. Varady[1]

The ability of alternate-day fasting (ADF) to modulate adipocyte parameters in a way that is protective against coronary heart disease (CHD) has yet to be tested. Accordingly, we examined the effects of ADF on adipokine profile, body composition, and CHD risk indicators in obese adults. Sixteen obese subjects (12 women/4 men) participated in a 10-week trial with three consecutive dietary intervention phases: (i) 2-week baseline control phase, (ii) 4-week ADF controlled feeding phase, and (iii) 4-week ADF self-selected feeding phase. After 8 weeks of treatment, body weight and waist circumference were reduced ($P < 0.05$) by 5.7 ± 0.9 kg, and 4.0 ± 0.9 cm, respectively. Fat mass decreased ($P < 0.05$) by 5.4 ± 0.8 kg, whereas fat-free mass did not change. Plasma adiponectin was augmented ($P < 0.05$) by 30% from baseline. Leptin and resistin concentrations were reduced ($P < 0.05$) by 21 and 23%, respectively, post-treatment. Low-density lipoprotein cholesterol (LDL-C) and triacylglycerol concentrations were 25% and 32% lower ($P < 0.05$), respectively, after 8 weeks of ADF. High-density lipoprotein cholesterol (HDL-C), C-reactive protein, and homocysteine concentrations did not change. Decreases in LDL-C were related to increased adiponectin ($r = –0.61$, $P = 0.01$) and reduced waist circumference ($r = 0.39$, $P = 0.04$). Lower triacylglycerol concentrations were associated with augmented adiponectin ($r = –0.39$, $P = 0.04$) and reduced leptin concentrations ($r = 0.45$, $P = 0.03$) post-treatment. These findings suggest that adipose tissue parameters may play an important role in mediating the cardioprotective effects of ADF in obese humans.

Obesity (2010) **18,** 2152–2159. doi:10.1038/oby.2010.54

INTRODUCTION

Accumulating evidence from both human and animal studies suggests that alternate-day fasting (ADF) may be an effective dietary strategy to reduce the risk of coronary heart disease (CHD) (1–6). ADF regimens generally involve a fast day, where the human or animal is fasted for 24 h, alternated with a feed day, where food intake is *ad libitum* for 24 h (7). Modified ADF regimens, which allow a certain percent of energy needs (i.e., 15–25% of baseline requirements) to be consumed on the fast day, have also been tested (7). Although the cardioprotective effects of ADF in animal models have been well documented, evidence in humans is still very limited. To date, only three human trials of ADF have been performed (4–6). Results from these studies conducted in normal weight and overweight humans demonstrate reductions in several key parameters of CHD risk, including blood pressure, low-density lipoprotein cholesterol (LDL-C), and triacylglycerol concentrations after 2–8 weeks of diet (4–6). Increases in high-density lipoprotein cholesterol (HDL-C), and decreases in C-reactive protein concentrations were also reported (4–6). Findings from these preliminary studies therefore suggest that ADF may be an effective diet strategy to reduce risk of CHD in normal weight and overweight humans. An important question that has yet to be addressed, however, is whether these cardioprotective actions can be reproduced in obese individuals after a similar duration of treatment (i.e., 8 weeks of ADF). Moreover, the ability of ADF to modulate homocysteine levels, a key risk indicator of CHD, also remains unknown.

Although the mechanisms remain unclear, the antiatherogenic actions of ADF may be mediated, in part, by modulations in adipose tissue parameters (i.e., fat cell–derived hormones and body fat distribution) (8). Adiponectin, a hormone secreted primarily by adipocytes, exerts potent antiatherogenic effects as shown by its capacity to inhibit monocyte adhesion to endothelial cells and macrophage-to-foam-cell transformation *in vitro* (9). Circulating levels of adiponectin increase with weight loss, and are reduced in obese and dyslipidemic subjects (10). Leptin, another adipocyte-derived hormone, is elevated in obese individuals and is decreased when fat mass is lost (11). Although leptin is primarily known for

[1]Department of Kinesiology and Nutrition, University of Illinois at Chicago, Chicago, Illinois, USA; [2]Department of Medicine, University of Illinois at Chicago, Chicago, Illinois, USA. Correspondence: Krista A. Varady (varady@uic.edu)

Received 10 September 2009; accepted 17 February 2010; published online 18 March 2010. doi:10.1038/oby.2010.54

its role in regulating energy homeostasis, this hormone also exhibits proatherogenic actions by increasing platelet aggregation, and stimulating the proliferation and migration of endothelial cells (12). Resistin is another adipocyte-derived cytokine that may contribute to atherogenesis by increasing monocyte chemoattractant protein-1 and soluble vascular cell adhesion molecule-1 expression in vascular endothelial cells (13). Recent data has also shown that increased plasma resistin levels are exhibited in patients with premature CHD (13). Circulating concentrations of these hormones are dictated by regional fat distribution (14). Visceral obesity, as determined by an increased waist circumference, is associated with an increased incidence of metabolic disturbances, elevated risk of CHD, and premature death (14). In contrast, individuals with comparable amounts of adipose tissue stores located in the subcutaneous depots exhibit a lower morbidity and mortality risk when compared to subjects with visceral obesity (14). Viscerally obese individuals (defined as a waist circumference >88 cm for women, and >102 cm for men) have higher circulating levels of leptin and resistin and lower levels of adiponectin, relative to subcutaneously obese individuals (15). The ability of ADF to reduce visceral fat mass, and in turn, improve circulating adipokine profile has yet to be tested in human subjects.

Accordingly, the objective of the present study was to examine the effects of ADF on CHD risk parameters in obese subjects, and to evaluate how changes in adipocyte parameters are related to modulations in CHD risk.

MATERIALS AND METHODS

Subjects

The study was conducted at the Human Nutrition Research Unit (HNRU) at the University of Illinois, Chicago. Participants underwent the study voluntarily and provided written informed consent prior to inclusion in the trial. The experimental protocol was approved by the Office for the Protection of Research Subjects at the University of Illinois, Chicago. The study comprised 16 obese adults who were recruited by means of advertisements placed in community centers in the greater Chicago area. The flow chart for subject recruitment, enrolment, and completion is displayed in **Figure 1**. The subjects were screened by a questionnaire, and BMI assessment (both performed in-person at the HNRU). Key inclusion criteria were as follows: age 35–65 years; BMI between 30 and 39.9 kg/m^2; weight stable for 3 months prior to the beginning of the study (i.e., <5 kg weight loss or weight gain); nondiabetic; no history of cardiovascular disease; normotensive (<120/<80 mm Hg) or prehypertensive (120–139/80–89 mm Hg); lightly active (i.e., <3 h/week of light intensity exercise at 2.5–4.0 metabolic equivalents for 3 months prior to the study); nonsmoker; and not taking medications that would affect study outcomes (i.e., weight loss, lipid, or glucose lowering drugs). All women were either premenopausal (regularly cycling with menses appearing every 27–32 days) or postmenopausal (absence of menses for >2 years). Pregnant women, or those trying to become pregnant were excluded. No subjects were involved in an exercise program before or during the course of the study.

Study design and experimental diets

As a means of testing the study objectives, a 10-week trial with three consecutive dietary intervention phases was implemented. The sequence of diet treatments was as follows: (i) 2-week baseline control phase, (ii) 4-week weight loss/ADF controlled feeding phase, and (iii) 4-week weight loss/ADF self-selected feeding phase. The ADF diet was administered first under clinically controlled conditions,

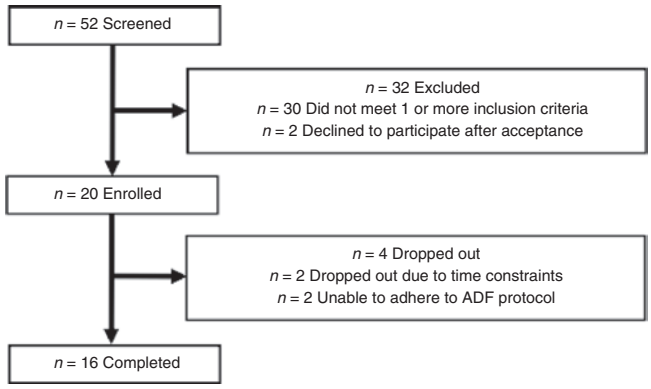

Figure 1 Study flow chart.

followed by a period of self-implementation, to test the ability of obese subjects to maintain the ADF meal pattern at home. During the baseline control phase (phase 1), each subject was required to keep their body weight stable by maintaining their usual eating and activity habits for 2 weeks. During phase 2, each subject participated in a 4-week ADF controlled feeding period. Baseline energy requirement for each subject was determined by the Mifflin equation (16). A modified ADF protocol was employed, such that subjects consumed 25% of their baseline energy needs on the fast day, and ate *ad libitum* on the feed day. All food was provided on each fast day to ensure that subjects were meeting their energy restriction goal for that day. On the feed day, subjects prepared their own meals at home. Diets were prepared in the metabolic kitchen of the HNRU. Study diets were formulated for each participant by the Research Dietician using the NDS-R Nutrition data system (Nutrition Coordinating Center, University of Minnesota). The fast day meals were provided as a 3-day rotating menu, and dietary carbohydrate, fat and protein accounted for ~55, 25, and 20% of ingested energy, respectively. The feed/fast days began at midnight each day, and all fast day meals were consumed between 12:00 PM and 2:00 PM to ensure that each subject was undergoing the same duration of fasting. Subjects were encouraged to drink plenty of water and were permitted to consume energy-free beverages such as tea and coffee on the fast days. During phase 3, subjects underwent a 4-week ADF self-selected feeding period. Throughout this last phase, subjects continued the modified ADF protocol by consuming only 25% of their baseline needs on the fast day. However, during this period, no food was provided to the subjects. Instead, each subject met with a Registered Dietician at the beginning of each week to learn how to maintain the ADF regimen on their own at home. During each counselling session, the Dietician worked with the subject to develop individualized fast day meal plans. These plans included menus, portion sizes, and food lists that were consistent with their food preferences and prescribed calorie levels for the fast day. During these sessions, subjects were also instructed how to make healthy food choices on the *ad libitum* feed days. Consistent with phase 2, subjects were asked to consume all fast day meals between 12:00 PM and 2:00 PM to ensure similar fasting durations between subjects. Adherence to diet was assessed by rate of weight loss per week.

Food intake

Subjects completed 3-day food records during the baseline phase, during each week of the controlled feeding phase, and during each week of the self-selected feeding phase (feed and fast days). Detailed instructions on how to complete the food records was provided to each subject by the study Dietician at baseline. Food records were collected at the weigh-in each week, and were reviewed by the Dietician for completeness. Dietary information from the food records was analyzed using Nutritionist Pro Software (version 4.3, Axxya Systems, Stafford, TX).

Body weight and body composition assessment

Body weight was assessed at the beginning of every week between 7:00 AM and 9:00 AM at the HNRU, without shoes, in light clothing, using a balance beam scale (Health O Meter, Sunbeam Products, Boca Raton, FL). Height was measured to the nearest 1 mm with a wall-mounted stadiometer. BMI was assessed as kg/m^2. Fat mass and fat-free mass were assessed in triplicate after the weigh-in using a tetra-polar bioelectrical impedance analyzer (Omron HBF-500, Omron Healthcare, Bannockburn, IL) (17). The instrument recorded impedance from hand to foot and consequently, calculated fat mass and fat-free mass from the impedance value and the preentered personal particulars (weight, height, age, and sex). The coefficient of variation within run for percent body fat was 2.7%. Waist circumference was measured in triplicate in standing subjects as the minimum abdominal circumference between the xyphoid process and the umbilicus (18).

Blood collection protocol

The timeline for blood collection is displayed in Figure 2. Twelve-hour fasting blood samples were collected between 7:00 AM and 9:00 AM during the baseline control period (day 1 and 14), and at the end of each diet treatment period (feed day and fast day). The subjects were instructed to avoid exercise, alcohol, and coffee for 24 h before each visit. Blood was centrifuged for 15 min at 520*g* and 4 °C to separate plasma from RBCs, and was stored at −80 °C until analyzed.

Plasma adipokine determination

Plasma adiponectin, leptin, and resistin concentrations were quantified using high sensitivity ELISA kits (R&D Systems, Minneapolis, MN). Intra-assay precision of the adiponectin, leptin, and resistin ELISA kits were 4.8, 4.2, and 4.9%, respectively.

Plasma CHD risk factor determination

Plasma total cholesterol, HDL-C, and triacylglycerol concentrations were measured in duplicate using enzymatic kits, standardized reagents, and standards (Biovision, Moutainview, CA). LDL-C concentration was calculated using the Friedewald, Levy, and Fredrickson equation (19). The coefficients of variation within runs for total cholesterol, HDL-C, and triacylglycerol concentrations were 3.1, 2.6, and 2.5%, respectively. Plasma C-reactive protein concentrations were quantified in duplicate by ELISA (R&D Systems), and the coefficient of variation within run was 3.3%. Circulating homocysteine (Hcy) concentrations were measured by ELISA (Diazyme, San Diego, CA), with a within run coefficient of variation of 4.4%.

Statistical analysis

Results are presented as means ± s.e.m. Baseline differences between male and female subjects were determined by Mann–Whitney *U*-test. One-factor analysis of variance was performed to determine an overall *P* value for each variable. The Bonferroni correction was used to assess significance. Relations between continuous variables were assessed by simple regression analyses as appropriate. Residuals from the model were tested for normal distribution. Significance was set at $P < 0.05$. Data were analyzed by using SPSS software (version 17.0 for Mac OS X; SPSS, Chicago, IL).

RESULTS

Clinical and metabolic characteristics of obese subjects at baseline

Baseline characteristics of the subjects who completed the entire 10-week trial are displayed in Table 1. There were no differences between men and women on day 1 of the study for age, BMI, exercise level, plasma lipid concentrations, or blood pressure. Body weight and waist circumference of male participants was higher ($P < 0.05$) than that of female participants. Both men and women were borderline hypercholesterolemic, based on LDL-C concentrations, and prehypertensive, based on diastolic blood pressure values. Four women were postmenopausal while 8 women were premenopausal. No differences were noted between those participants who completed the trial and those who did not.

Food intake

Mean energy intake during the control phase was 1,937 ± 180 kcal/day. Mean feed day energy intake at each week of the trial was 1,801 ± 226 kcal/day. Mean energy intake on the fast day during the self-selected feeding phase was 501 ± 28 kcal/day. These data suggest that the subjects were able to follow the ADF regimen on their own at home. The macronutrient composition of the diet remained relatively constant throughout

Table 1 Clinical and metabolic characteristics of obese subjects at baseline by sex

Variables	Women (*n* = 12)	Men (*n* = 4)
Age (years)	45 ± 3	46 ± 5
Body weight (kg)	87 ± 4	124 ± 4[a]
BMI (kg/m^2)	33 ± 1	34 ± 2
Waist circumference	106 ± 3	118 ± 2[a]
Exercise level (h/week)[b]	2.4 ± 0.4	2.3 ± 0.5
Total cholesterol (mg/dl)	174 ± 8	178 ± 22
LDL-cholesterol (mg/dl)	100 ± 9	104 ± 24
HDL-cholesterol (mg/dl)	50 ± 4	43 ± 10
Triacylglycerol (mg/dl)	124 ± 18	127 ± 24
Systolic blood pressure (mm Hg)	116 ± 4	119 ± 16
Diastolic blood pressure (mm Hg)	81 ± 3	85 ± 9

All values are mean ± s.e.m.
[a]Significantly different from men, $P < 0.05$ (Mann–Whitney *U*-test). [b]Baseline exercise level defined as number of hours per week of light aerobic exercise at 2.5–4.0 METs. Activity level was self-reported.
LDL, low-density lipoprotein; HDL, high-density lipoprotein.

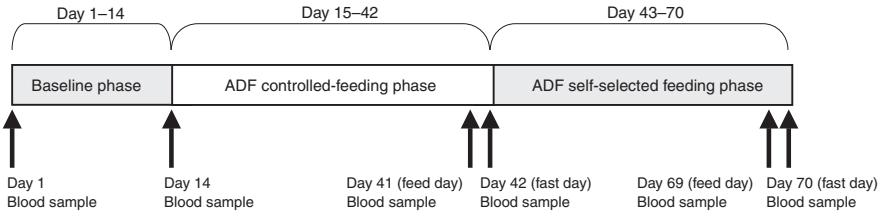

Figure 2 Timeline for experimental diets and blood sampling.

Table 2 Body weight and body composition at baseline and at the end of each phase of the trial

	Baseline control phase		Weight loss/ADF controlled feeding phase		Weight loss/ADF self-selected feeding phase	
	Day 1	Day 14	Day 41 Feed day	Day 42 Fast day	Day 69 Feed day	Day 70 Fast day
Body weight (kg)	96.4 ± 5.3	96.5 ± 5.2	93.8 ± 5.0*	93.7 ± 5.0*	92.8 ± 4.8*	90.8 ± 4.8*
BMI (kg/m^2)	33.7 ± 1.0	33.7 ± 1.0	32.8 ± 1.0	32.8 ± 0.9	32.1 ± 0.8*	31.4 ± 0.9*
Fat mass (kg)	43.0 ± 2.2	43.5 ± 2.5	41.8 ± 2.7	41.3 ± 2.7	38.1 ± 2.6*	38.1 ± 1.8*
Fat-free mass (kg)	52.0 ± 3.6	51.4 ± 3.4	51.8 ± 3.8	51.1 ± 3.2	52.8 ± 3.3	51.9 ± 3.7
Waist circumference (cm)	109 ± 2	109 ± 3	106 ± 3	106 ± 3	105 ± 3*	105 ± 3*

All values are mean ± s.e.m. Body weight and body composition variables did not change during the baseline period (day 1–14).
*Significantly different from baseline (day 14), $P < 0.05$ (one-factor ANOVA with Bonferroni analysis).

the 10-week trial with respect to protein (baseline: 18 ± 1% of kcal; post-treatment: 20 ± 1% of kcal), and carbohydrates (baseline: 46 ± 3% of kcal; post-treatment: 51 ± 3% of kcal). Total fat consumption decreased ($P < 0.05$) from 36 ± 5% of kcal to 29 ± 1% of kcal from baseline to the end of the trial. Thus, subjects were consuming a diet that complied with the American Heart Association guidelines. Caffeine consumed during the baseline phase (156 ± 35 mg/day) was similar to that of the controlled feeding phase (feed day: 144 ± 51 mg/day; fast day: 148 ± 63 mg/day), and the self-selected feeding phase (feed day: 154 ± 60 mg/day; fast day: 139 ± 42 mg/day).

Weight loss and improvements in body composition by ADF

Body weight of the subjects remained stable during the baseline control phase (Table 2). However, by the end of the controlled feeding phase (i.e., after 4 weeks of ADF), body weight decreased ($P < 0.05$) by 2.8 ± 0.5 kg. By the end of the self-selected feeding phase (i.e., after 8 weeks of ADF), body weight was reduced by 5.7 ± 0.9 kg, relative to baseline. There were no differences in body weight when feed day values were compared to fast day values at the end of either phase. BMI was reduced ($P < 0.05$) by 2.3 ± 0.2 kg/m^2 after 8 weeks of ADF. Fat mass decreased ($P < 0.05$) by 5.4 ± 0.8 kg after 8 weeks of diet. Fat-free mass did not change over the 8-week intervention period. Fat mass and fat-free mass did not differ when feed day values were compared to fast day values. Waist circumference (indicator of visceral fat mass) decreased ($P < 0.05$) by 4.0 ± 0.9 cm after 8 weeks of ADF.

Beneficial modulations in adipokines by ADF

Mean plasma adiponectin, leptin, and resistin concentrations over the 10-week trial are presented in Figure 3. Circulating adiponectin was unaltered after 4 weeks of ADF. However, after 8 weeks of treatment, plasma adiponectin increased ($P < 0.05$) by ~30% from baseline on the feed and fast day. Increased adiponectin was related to decreased body weight ($r = -0.56$, $P = 0.03$), BMI ($r = -0.75$, $P = 0.002$), and waist circumference ($r = -0.42$, $P = 0.04$) post-treatment. When data were analyzed separately based on sex and menstrual status, adiponectin was not significantly altered in any group (men: 46 ± 22%; premenopausal women: 23 ± 7%; postmenopausal women: 29 ± 23%). Circulating leptin concentrations were reduced ($P < 0.05$) by

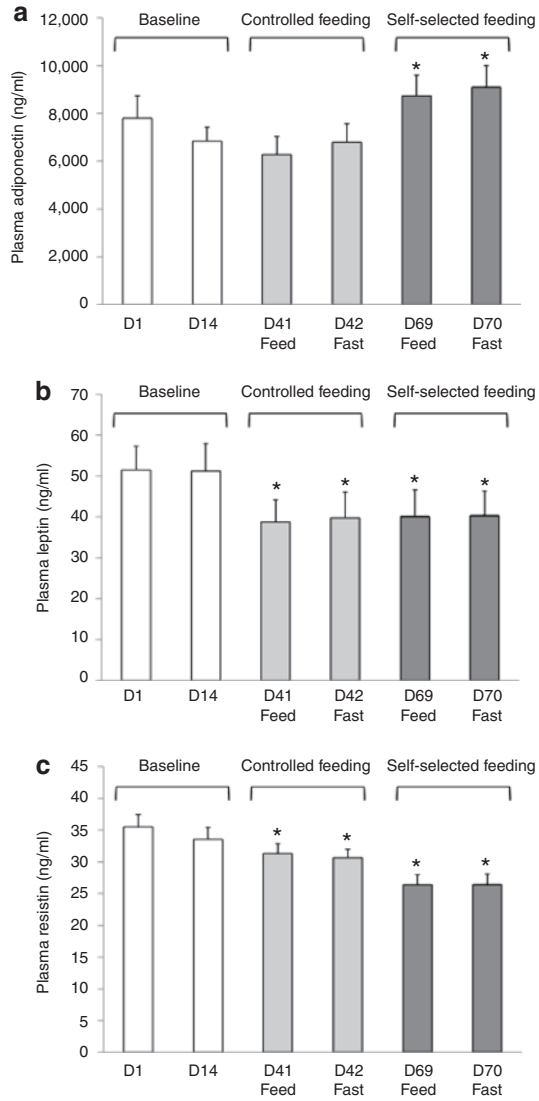

Figure 3 Plasma adipokines at baseline and at the end of each phase of the trial. All values are mean ± s.e.m. *Significantly different from baseline (day 14), $P < 0.05$ (one-factor ANOVA with Bonferroni analysis). (**a**) Plasma adiponectin increased ($P < 0.05$) after 8 weeks of alternate-day fasting (ADF). (**b**) Plasma leptin decreased ($P < 0.05$) after 4 and 8 weeks of ADF. (**c**) Plasma resistin decreased ($P < 0.05$) after 4 and 8 weeks of ADF.

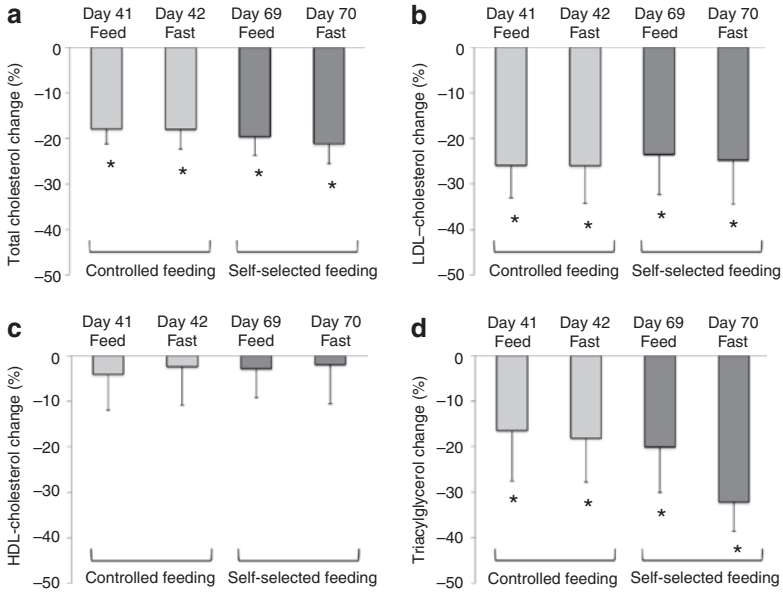

Figure 4 Change in plasma lipids from baseline to the end of each diet phase. All values are mean ± s.e.m. *Significantly different from baseline (day 14), $P < 0.05$ (one-factor ANOVA with Bonferroni analysis). (**a**) Total cholesterol decreased ($P < 0.05$) after 4 and 8 weeks of alternate-day fasting (ADF). (**b**) Low-density lipoprotein cholesterol decreased ($P < 0.05$) after 4 and 8 weeks of ADF. (**c**) High-density lipoprotein did not change during the trial. (**d**) Plasma triacylglycerols decreased ($P < 0.05$) after 4 and 8 weeks of ADF.

22 ± 8% after 4 weeks of diet, and by 21 ± 6% after 8 weeks of diet. Lower leptin at the end of the study was related to decreased body weight ($r = 0.52$, $P = 0.01$) and fat mass ($r = 0.48$, $P = 0.02$). When data were analyzed separately, plasma leptin was not significantly altered in any group post-treatment (men: −4 ± 9%; premenopausal women: −27 ± 8%; postmenopausal women: −30 ± 15%). Concentrations of plasma resistin decreased ($P < 0.05$) by 19 ± 4% at the end of the ADF controlled feeding phase, and by 23 ± 6% at the end of the self-selected feeding period. Reductions in plasma resistin were associated with decreased body weight ($r = 0.53$, $P = 0.008$), and BMI ($r = 0.48$, $P = 0.01$). When data were analyzed separately, resistin was not significantly altered in any group post-treatment (men: −20 ± 10%; premenopausal women: −23 ± 7%; postmenopausal women: −10 ± 10%). There were no differences between feed day and fast day values for any adipokine.

Improvements in CHD risk factors by ADF and relation to adipose tissue parameters

The effect of ADF on plasma lipid concentrations was also assessed (Figure 4). Total cholesterol concentrations decreased ($P < 0.05$) to 140 ± 7 mg/dl after the controlled feeding period, and to 138 ± 8 mg/dl after the self-selected feeding period, relative to baseline (175 ± 8 mg/dl). Likewise, LDL-C was reduced ($P < 0.05$) to 73 ± 9 mg/dl after 4 weeks of ADF, and to 72 ± 8 mg/dl after 8 weeks of diet, from baseline (106 ± 10 mg/dl). HDL-C concentrations did not change over the course of the trial. Pronounced reductions ($P < 0.05$) in triacylglycerol concentrations from 136 ± 17 mg/dl (baseline) to 110 ± 17 mg/dl (after 4 weeks of diet), and 88 ± 15 mg/dl (after 8 weeks of diet), were also observed. Plasma lipid levels did not differ when feed day values were compared to fast day values at the end of either phase.

Decreases in LDL-C concentrations were related to increased plasma adiponectin ($r = −0.61$, $P = 0.01$) post-treatment. Reductions in LDL were also correlated to decreased body weight ($r = 0.33$, $P = 0.02$) and waist circumference ($r = 0.39$, $P = 0.04$). Lower triacylglycerol concentrations at the end of the study were related to augmented adiponectin ($r = −0.39$, $P = 0.04$) and reduced leptin concentrations ($r = 0.45$, $P = 0.03$). These changes in triacylglycerol levels were also related to decreased body weight ($r = 0.54$, $P = 0.006$) and waist circumference ($r = 0.33$, $P = 0.01$) post-treatment. There were no differences in CRP concentrations after 4 weeks of ADF (feed day: 2.8 ± 0.3 mg/l; fast day: 2.5 ± 0.2 mg/l), or 8 weeks of ADF (feed day: 2.6 ± 0.2 mg/l; fast day: 2.7 ± 0.2 mg/l), relative to baseline (2.5 ± 0.3 mg/l). Likewise, homocysteine concentrations remained unchanged after 4 weeks of diet (feed day: 8.6 ± 0.6 μmol/l; fast day: 8.2 ± 0.6 μmol/l), and after 8 weeks of diet (feed day: 7.9 ± 0.5 μmol/l; fast day: 7.9 ± 0.6 μmol/l), when compared to baseline (8.4 ± 0.6 μmol/l). No relationship between CRP/homocysteine and adipokines was observed at any time point.

DISCUSSION

This study is the first to show that ADF can produce potent decreases in key CHD risk parameters in obese subjects, and that these effects are mediated in part by improvements in adipose tissue parameters. More specifically, our data show that ADF can decrease total cholesterol, LDL-C, and triacylglycerol concentrations after only 8 weeks of diet in obese men and women. These beneficial modulations in CHD risk were related to reduced body weight and waist circumference (visceral fat mass), in conjunction with improvements in adipokine profile (i.e., decreases in leptin and resistin, and increases in adiponectin). We also show here that fat-free mass did not change

over the course of the trial suggesting a conservation of lean mass by ADF.

A wide variety of observational and experimental evidence strongly implies that LDL is the most atherogenic lipoprotein (20). Findings from the present trial indicate that ADF is an effective diet therapy to reduce LDL-C concentrations (25% from baseline) in obese, borderline hypercholesterolemic men and women. Additionally, we also observed decreases in total cholesterol (21% from baseline) and triacylglycerol concentrations (32% from baseline) post-treatment. Our findings are complementary to those of Johnson *et al.* (6) who demonstrate 10% and 42% reductions in total cholesterol and triacylglycerol concentrations, respectively, after 8 weeks of ADF in overweight subjects. Beneficial modulations in LDL-C and triacylglycerol concentrations have also been shown in normal weight subjects undergoing 3 weeks of ADF (4). The mechanism whereby ADF alters lipid levels is not yet clear. However, findings from calorie restriction studies indicate that oxidation of circulating free fatty acids (FFA) is augmented during periods of weight loss, whereas FFA synthesis is decreased (21). This decreased availability of precursor FFA results in a reduction in hepatic synthesis and secretion of very LDL (VLDL) into plasma, leading to lower circulating levels of LDL-C (22). Although this process has only been shown for calorie restriction, it possible that ADF may alter lipid levels by a similar mechanism. Future research which explores the process by which ADF improves lipid profile is well warranted. Increased caffeine intake has been shown to slightly increase circulating LDL-C levels (23). Because caffeine intake remained constant throughout the study, we speculate that there was no effect of this dietary constituent on LDL-C concentrations. As for HDL-C concentrations, no changes were observed over the course of the trial. HDL-C levels generally only increase in response to exercise training (24). Because the activity level of the subjects remained stable throughout the trial (weekly exercise level self-reported), this may explain why HDL-C did not increase. The effects of ADF on other circulating parameters of CHD, such as CRP and homocysteine, was also assessed. We show here that short-term ADF had no effect on either of these parameters. This lack of effect of ADF on CRP concentrations was also noted in the study by Johnson *et al.* (6). Evidence from weight loss trials indicate that CRP and homocysteine concentrations only decrease in response to a significant reduction in body weight (i.e., at least 15% weight loss from baseline) (25). Because the subjects in the present study only lost 6% of their initial body weight, this may explain why CRP and homocysteine remained unchanged post-treatment. These changes in CHD risk factors by ADF are similar to that of calorie restriction (26). Whether one of these regimens confers greater protection against heart disease is not yet known. A trial directly comparing the effects of ADF vs. calorie restriction on CHD risk parameters is clearly warranted.

Improvements in several parameters of body composition were also noted in response to this ADF regimen. After 8 weeks of diet, body weight of obese subjects declined by 6% (5.7 kg) from baseline. A consistent rate of weight loss of (0.7 kg/week) was noted during both the controlled feeding period (fast day meals provided), and the self-selected feeding period (all meals prepared at home). These data indicate that obese subjects were able to self-implement the ADF meal pattern at home in a way that achieves consistent, healthy weight loss. It should be noted, however, that there was a large discrepancy between body weight values for day 69 (92.8 kg) vs. day 70 (90.8 kg). This disparity may have been due to the weight of food contained in the gastrointestinal tract of the subjects after feeding. Another possible reason may be that, by the end of the trial, subjects started to consume more water/liquids on the fast days to deal with their hunger. As such, if more water was consumed on day 68 (i.e., the night before the feed day blood draw), body weight would be increased on day 69. Nonetheless, our inability to identify the precise reason for this discrepancy in body weight values is an evident limitation to the study. Total fat mass and waist circumference were also significantly reduced. It was estimated that subjects would decrease their fat mass by 4.5 kg after 8 weeks, based on a 75% decrease in energy intake on the fast day, with no change in energy intake on the feed day. In actuality, subjects lost 5.4 kg of fat mass, which exceed our predictions. Interestingly, fat-free mass did not change during the study, indicating that ADF may preserve lean mass at the expense of fat mass during weight loss. Since this study is the first to examine the effects of ADF on body composition in humans, there are no data to which to compare the present findings. We did, however, observe similar changes in body composition by ADF in rodents (i.e., reductions in visceral fat mass after 4-weeks of ADF in mice) (1). Results from the present study also show that decreased waist circumference was related to reductions in LDL-C levels. The link between augmented visceral fat mass and dyslipidemia is well established. Adipose tissue lipolysis in visceral adipocytes is higher than that of subcutaneous adipocytes, leading to augmented efflux of FFA from visceral depots (14). FFA released from visceral fat are collected by the portal vein and reach the liver at much higher concentrations than they do the systemic circulation (14). In the liver, these FFA trigger increased production of glucose and triacylglycerols, as well as elevated secretion of VLDL (14). Increased secretion of VLDL contributes to higher circulating levels of LDL, because VLDL is rapidly converted to LDL in plasma. In view of these relationships, it is possible that the reductions in visceral fat mass by ADF played a key role in the decreases in LDL-C observed here.

In addition to these favorable modulations in body composition, we also observed increases in plasma adiponectin by ADF. Circulating levels of the cardioprotective adipokine increased by 30% from baseline after only 8 weeks of treatment. These data are consistent with findings from previous ADF studies (1,5). For instance, in the study by Halberg *et al.* (5), 37% increases in adiponectin concentrations after 2 weeks of ADF in normal weight men and women were reported. Moreover, in a study conducted by our group in rodents (1), adiponectin concentrations in the ADF group were 42% higher than those of the control group after 4 weeks of treatment. In the present study, blood sampling

for adipokines was performed between 7:00 AM and 9:00 AM at each time point. Because circulating concentrations of adiponectin are constant during the day (27), the elevation in adiponectin observed post-treatment can be contributed to the intervention rather than nocturnal variations. We also observed a relationship between augmented adiponectin concentrations and decreased LDL-C and triacylglycerol levels post-treatment. Although the precise mechanism underlying this relationship is still unclear, it has been hypothesized that adiponectin may decrease the supply of FFA to the liver for gluconeogenesis, thereby decreasing triacylglycerol synthesis (28,29). This decrease in triacylglycerol synthesis results in blunted VLDL secretion, which would in turn decrease LDL-C concentrations in plasma. It was also noted that increased adiponectin was related to decreased waist circumference post-treatment. It is well established that plasma adiponectin is reduced in individuals with visceral fat accumulation, and that hypoadiponectinemia caused by visceral obesity may be related to the development of metabolic syndrome (30). Taken together, these findings suggest that ADF may decrease visceral fat in a way that leads to increased adiponectin thereby improving lipid concentrations.

Decreases in circulating concentrations of resistin and leptin were also observed. This study is the first to test the effects of ADF on plasma resistin. Results reveal that after 8 weeks of ADF, resistin levels were reduced by 23% from baseline. The physiologic role of resistin in mediating obesity-related disorders has yet to be clarified. While some studies demonstrate an association between increased resistin and determinants of the metabolic syndrome (31,32), other have failed to show such relationships (33). No relationship between decreased resistin and a reduction in CHD risk was observed here. Thus, the role of resistin in mediating the beneficial effects of ADF is still unclear. As for plasma leptin, 21% decreases from baseline were noted for this adipokine. Our findings are complementary to those of Halberg *et al.* (5). A correlation between lower plasma leptin and reduced triacylglycerol concentrations was also observed. *In vitro* studies demonstrate that leptin is a potent stimulator of lipolysis and fatty acid oxidation in adipocytes and other cell types (34). Consequently, leptin is also a regulator of circulating triacylglycerol concentrations (34). It is likely that these decreases in leptin were mediated by reductions in body weight and fat mass, because positive correlations were noted between these parameters. Taken together, we hypothesize that the decrease in fat mass by ADF stimulated a reduction in leptin which in turn contributed to the lipid profile improvements observed here. It is important to note, however, that adipokine profile may be independently related to sex and menstrual status. Due to the heterogeneity of our sample, we performed additional statistical analyses to determine if changes in adipokines varied according to these determinants (i.e., men vs. women, premenopausal women vs. postmenopausal women). No significant differences between these groups were noted, and as such, we decided to combine the data into a single group. The heterogeneity of our sample is indeed a limitation of the trial.

Taken together, these findings indicate that reducing energy intake by implementing ADF may decrease the risk of CHD in obese individuals. Our results also show that these beneficial modulations in vascular disease risk by ADF may be mediated, in part, by improvements in adipose tissue parameters, i.e., body composition and adipokine profile. This work is an important first step towards understanding the role that ADF may play in inducing an energy deficit leading to weight loss, which may have an impact on the lipid and metabolic risk profile.

ACKNOWLEDGMENTS

We are grateful for the help of Judy Henry, and Kristen Hoody during the human trial component of the study. University of Illinois at Chicago, Department of Kinesiology and Nutrition, Departmental funding.

DISCLOSURE

The authors declared no conflict of interest.

REFERENCES

1. Varady KA, Hudak CS, Hellerstein MK. Modified alternate-day fasting and cardioprotection: relation to adipose tissue dynamics and dietary fat intake. *Metab Clin Exp* 2009;58:803–811.
2. Mager DE, Wan R, Brown M *et al*. Caloric restriction and intermittent fasting alter spectral measures of heart rate and blood pressure variability in rats. *FASEB J* 2006;20:631–637.
3. Wan R, Camandola S, Mattson MP. Intermittent fasting and dietary supplementation with 2-deoxy-D-glucose improve functional and metabolic cardiovascular risk factors in rats. *FASEB J* 2003;17:1133–1134.
4. Heilbronn LK, Smith SR, Martin CK, Anton SD, Ravussin E. Alternate-day fasting in nonobese subjects: effects on body weight, body composition, and energy metabolism. *Am J Clin Nutr* 2005;81:69–73.
5. Halberg N, Henriksen M, Söderhamn N *et al*. Effect of intermittent fasting and refeeding on insulin action in healthy men. *J Appl Physiol* 2005;99:2128–2136.
6. Johnson JB, Summer W, Cutler RG *et al*. Alternate day calorie restriction improves clinical findings and reduces markers of oxidative stress and inflammation in overweight adults with moderate asthma. *Free Radic Biol Med* 2007;42:665–674.
7. Varady KA, Hellerstein MK. Alternate-day fasting and chronic disease prevention: a review of human and animal trials. *Am J Clin Nutr* 2007;86:7–13.
8. Bays HE. "Sick fat," metabolic disease, and atherosclerosis. *Am J Med* 2009;122:S26–S37.
9. Ouchi N, Kihara S, Arita Y *et al*. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 1999;100:2473–2476.
10. Kawano J, Arora R. The role of adiponectin in obesity, diabetes, and cardiovascular disease. *J Cardiometab Syndr* 2009;4:44–49.
11. Sattar N, Wannamethee G, Sarwar N *et al*. Leptin and coronary heart disease: prospective study and systematic review. *J Am Coll Cardiol* 2009;53:167–175.
12. Bowman JD, Bowman CD, Bush JE *et al*. Parity nonconservation for neutron resonances in 238U. *Phys Rev Lett* 1990;65:1192–1195.
13. Burnett MS, Lee CW, Kinnaird TD *et al*. The potential role of resistin in atherogenesis. *Atherosclerosis* 2005;182:241–248.
14. Rodríguez A, Catalán V, Gómez-Ambrosi J, Frühbeck G. Visceral and subcutaneous adiposity: are both potential therapeutic targets for tackling the metabolic syndrome? *Curr Pharm Des* 2007;13:2169–2175.
15. Schober F, Neumeier M, Weigert J *et al*. Low molecular weight adiponectin negatively correlates with the waist circumference and monocytic IL-6 release. *Biochem Biophys Res Commun* 2007;361:968–973.
16. Mifflin MD, St Jeor ST, Hill LA *et al*. A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr* 1990;51:241–247.
17. Bosy-Westphal A, Later W, Hitze B *et al*. Accuracy of bioelectrical impedance consumer devices for measurement of body composition

in comparison to whole body magnetic resonance imaging and dual X-ray absorptiometry. *Obes Facts* 2008;1:319–324.
18. Wang J, Thornton JC, Bari S *et al*. Comparisons of waist circumferences measured at 4 sites. *Am J Clin Nutr* 2003;77:379–384.
19. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502.
20. Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP III Final Report). 2002. NHLBI Publication # 02-5215. National Institutes of Health.: 1–280.
21. Poynten AM, Markovic TP, Maclean EL *et al*. Fat oxidation, body composition and insulin sensitivity in diabetic and normoglycaemic obese adults 5 years after weight loss. *Int J Obes Relat Metab Disord* 2003;27:1212–1218.
22. Kudchodkar BJ, Sodhi HS, Mason DT, Borhani NO. Effects of acute caloric restriction on cholesterol metabolism in man. *Am J Clin Nutr* 1977;30:1135–1146.
23. Riksen NP, Rongen GA, Smits P. Acute and long-term cardiovascular effects of coffee: implications for coronary heart disease. *Pharmacol Ther* 2009;121:185–191.
24. Hausenloy DJ, Yellon DM. Targeting residual cardiovascular risk: raising high-density lipoprotein cholesterol levels. *Postgrad Med J* 2008;84:590–598.
25. Hakeam HA, O'Regan PJ, Salem AM, Bamehriz FY, Jomaa LF. Inhibition of C-reactive protein in morbidly obese patients after laparoscopic sleeve gastrectomy. *Obes Surg* 2009;19:456–460.
26. Fontana L. Calorie restriction and cardiometabolic health. *Eur J Cardiovasc Prev Rehabil* 2008;15:3–9.
27. Gavrila A, Peng CK, Chan JL *et al*. Diurnal and ultradian dynamics of serum adiponectin in healthy men: comparison with leptin, circulating soluble leptin receptor, and cortisol patterns. *J Clin Endocrinol Metab* 2003;88:2838–2843.
28. Yamauchi T, Kamon J, Waki H *et al*. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001;7:941–946.
29. Chan DC, Watts GF, Ng TW *et al*. Adiponectin and other adipocytokines as predictors of markers of triglyceride-rich lipoprotein metabolism. *Clin Chem* 2005;51:578–585.
30. Matsuzawa Y. The role of fat topology in the risk of disease. *Int J Obes (Lond)* 2008;32 Suppl 7:S83–S92.
31. Norata GD, Ongari M, Garlaschelli K *et al*. Plasma resistin levels correlate with determinants of the metabolic syndrome. *Eur J Endocrinol* 2007;156:279–284.
32. Shin HJ, Park S, Yoon SJ *et al*. Association between serum resistin and carotid intima media thickness in hypertension patients. *Int J Cardiol* 2008;125:79–84.
33. Iqbal N, Seshadri P, Stern L *et al*. Serum resistin is not associated with obesity or insulin resistance in humans. *Eur Rev Med Pharmacol Sci* 2005;9:161–165.
34. Reidy SP, Weber J. Leptin: an essential regulator of lipid metabolism. *Comp Biochem Physiol, Part A Mol Integr Physiol* 2000;125:285–298.