Modified alternate-day fasting and cardioprotection: relation to adipose tissue dynamics and dietary fat intake

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Abstract

The relation between alternate-day fasting (ADF) and cardioprotection remains uncertain. In the present study, we examined the ability of modified ADF, with a low-fat (LF) vs high-fat (HF) background diet, to modulate adipose tissue physiology in a way that may protect against coronary heart disease. In a 4-week study, male C57BL/6 mice were randomized to 1 of 3 groups: (1) ADF-85%-LF (85% energy restriction on fast day, ad libitum fed on feed day, on an LF diet), (2) ADF-85%-HF (same protocol but HF diet), and (3) control (ad libitum fed). Throughout the study, body weight did not differ between ADF and control animals. Proportion of subcutaneous fat increased ($P < .01$), whereas the proportion of visceral fat decreased ($P < .01$), in both ADF groups. Triglyceride (TG) synthesis was augmented ($P < .05$) in subcutaneous fat, but remained unchanged in visceral fat. Adiponectin concentrations were elevated ($P < .05$), whereas leptin and resistin levels decreased ($P < .05$). Aortic vascular smooth muscle cell proliferation was reduced ($P < .05$) by 60% and 76% on the LF and HF diets, respectively. Plasma total cholesterol, TG, and free fatty acid concentrations also decreased ($P < .05$). In summary, modified ADF regimens alter adipose tissue physiology (ie, body fat distribution, TG metabolism, and adipokines) in a way that may protect against coronary heart disease. These beneficial effects were noted over a wide range of fat intake, suggesting that ADF may be protective even in the presence of HF diets.

1. Introduction

The ability of alternate-day fasting (ADF) to reduce chronic disease risk in both humans and animals has been demonstrated in several recent studies [1]. Alternate-day fasting regimens generally involve a fast day, where the human or animal is fasted for a 24-hour period, alternated with a feed day, where food intake is ad libitum for 24 hours. Modified ADF regimens, which allow a certain percentage of energy needs (ie, 15%-25% of baseline requirements) to be consumed on the fast day, have also been tested [2]. Recent reports [3-5] suggest that ADF may be an effective intervention to protect against coronary heart disease (CHD). Specifically, these studies demonstrate a reduction in heart rate, blood pressure, total cholesterol, and triglyceride

(TG) levels after 4 to 12 weeks of true ADF (100% energy restriction on fast day) in rodents [3-5]. However, the ability of modified ADF to protect against vascular disease has yet to be tested. In our previous studies of ADF [2,6,7], a low-fat (LF) background diet was implemented. Because the average American consumes a diet that is high in fat (ie, >30% of energy needs as dietary fat) [8], testing the cardioprotective ability of an ADF diet that is not restricted in fat is important in terms of diet tolerability and adherence in humans. Accordingly, the goal of this study was to compare the effects of a high-fat (HF) ADF regimen to that of an isocaloric LF regimen on indicators of CHD risk. More specifically, we asked whether an HF diet prevents the beneficial effects of ADF that are observed on isocaloric LF diets.

To address this question, we explored the dynamics of vascular smooth muscle cell (VSMC) and adipose tissue, 2 target organs that might mediate dietary-induced alterations in CHD risk. We used VSMC proliferation as an outcome metric of CHD risk. Proliferation of intima-medial components of the arterial wall plays a central role in the

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atherogenic process [9,10]. We have previously developed a method for measuring the proliferation rates of VSMC by use of heavy water (^2H_2O) labeling [11]. Chu et al [11] demonstrated that HF feeding accelerates aortic VSMC proliferation and atherogenesis in this model. We also examined the role of adipose tissue in mediating these diet-induced effects. Adipose tissue physiology and regional distribution play an important role in CHD risk [12]. An accumulation of adipose tissue in visceral (intraabdominal) depots is correlated with atherosclerosis in both humans [13] and rodents [14]. One mechanism that may link fat distribution to atherosclerosis is the adipokine profile [15]. Murine visceral adipocytes release higher amounts of the proinflammatory mediator resistin when compared with subcutaneous adipocytes [16]. Resistin accelerates atherosclerosis by facilitating lipid accumulation in macrophages, thereby promoting the formation of foam cells [15]. This adipokine also indirectly down-regulates the production of the cardioprotective hormone adiponectin [15]. Recent evidence suggests that adiponectin may protect against vascular complications by promoting an antiatherogenic program of gene expression in vessels walls, which may in turn reduce plaque formation [15]. In the present study, we were interested in whether improvements in body fat distribution by ADF would result in a more favorable adipokine profile, thereby potentially protecting against the development of atherosclerosis.

Accordingly, the present study investigated the ability of modified ADF, with an LF vs HF background diet, to reduce aortic VSMC proliferation and improve CHD risk indicators. The role of adipose tissue dynamics and function, that is, body fat distribution, adipose TG metabolism, and circulating adipokines, in mediating the effect of ADF on CHD risk was also examined.

2. Methods

2.1. Mice

Seven-week–old male C57BL/6J mice were obtained from Charles River Breeding Laboratories (Wilmington, MA). Mice were housed individually under temperature- and light-controlled conditions (12-hour light/dark cycle: lights on at 7:00 AM and off at 7:00 PM). All mice underwent a 1-week acclimation period before the 4-week treatment phase. During acclimation, mice were given free access to water and a semipurified AIN-93M diet (Bio-Serv Open Source Diets, Frenchtown, NJ). Daily food intake was recorded for each mouse.

2.2. ADF protocols

Mice were randomly allocated to 1 of 3 groups for 4 weeks: (1) ADF-85%-LF, (2) ADF-85%-HF, and (3) control. Animals in the ADF-85% groups were restricted by 85% of their baseline energy needs on each fast day and were fed ad libitum on each feed day. Control group animals were fed ad libitum each day. The ADF-85%-LF group consumed the AIN-93M diet (Bio-Serv) on both the feed and fast days throughout the study (percentage of kilocalorie distribution: 9% fat, 15% protein, 76% carbohydrate; energy density: 3.85 kcal/g). The control group also consumed the AIN-93M diet (Bio-Serv). The ADF-85%-HF group consumed the D12451 diet (Bio-Serv) throughout the study (percentage of kilocalorie distribution: 45% fat, 20% protein, 35% carbohydrate; energy density: 4.76 kcal/g). Food was provided or taken away at noon each day, and the amount of food consumed was weighed daily. Each ADF-85% animal consumed all of the fast-day food within 30 minutes. As such, the animals were fasted for approximately 24 hours each fast day. Animals were killed at 12 weeks of age by cardiac puncture under isoflurane anesthesia, followed by cervical dislocation. All procedures and protocols were approved by the University of California Berkeley Animal Use Committee.

2.3. Body weight, blood collection, and ^2H_2O labeling protocol

Body weight was measured weekly. Blood samples were collected at the time of sacrifice, the morning after a feed day. Thus, all plasma analyses were performed in fed-state mice. An intraperitoneal injection of isotonic 100% ^2H_2O (0.18 mL/10 g body weight) was administered on day 14 of the study to bring the ^2H_2O content of body water up to approximately 5%. After the injection, mice received drinking water containing 8% ^2H_2O ad libitum for the last 2 weeks of the study (days 14-28) [17].

2.4. Body fat distribution

Visceral and subcutaneous fat pads were dissected and weighed immediately after sacrifice. For the assessment of "visceral fat," epididymal and abdominal fat pads (mesenteric, perirenal, retroperitoneal, and any other fat within the abdominal cavity) were dissected. For the assessment of "subcutaneous fat," hind subcutaneous fat (pair of subcutaneous fat pads extending partway along the abdomen from the hind limbs) and interscapular subcutaneous fat pads (brown adipose tissue located deep in the interscapular space and capped by white adipose tissue) were dissected. Because these animals were lean, these depots were discrete.

For the purposes of this study, *total body fat* was defined as subcutaneous fat pad weight + visceral fat pad weight. Relative proportion of subcutaneous fat was calculated as [subcutaneous fat pad weight/(subcutaneous fat pad weight + visceral fat pad weight)]. Likewise, relative proportion of visceral fat was calculated as [visceral fat pad weight/ (subcutaneous fat pad weight + visceral fat pad weight)].

2.5. Determination of TG-glycerol synthesis and lipolysis

The measurement of TG-glycerol synthesis and net lipolysis has been described in detail previously [18].

Glycerol from each fat pad was separated from fatty acid (FA) methyl esters by a modified Folch technique [18]. Glycerol was then lyophilized by incubation with acetic anhydride-pyridine to convert glycerol to glycerol-triacetate, as described elsewhere [18]. A model 6890 GC with 5973 mass spectrometer (Agilent Technologies, Palo Alto, CA) fitted with a DB-225 fused silica column (J&W, Folsom, CA) was used for all analyses. Glycerol-triacetate was analyzed under chemical ionization conditions by selected ion monitoring of mass-to-charge ratios (m/z) 159 to 161 (representing M_0-M_2). The fraction of newly synthesized TG-glycerol (f) (ie, fractional TG synthesis) was measured as described below [18]:

$$f_{TG} = EM1_{TG-glycerol} / A_1^\infty {}_{TG-glycerol},$$

where f is the fraction of newly synthesized TG molecules present, EM1 is the measured excess mass isotopomer abundance for M_1-glycerol at time t, and A_1^∞ is the asymptotic mass isotopomer abundance for M_1-glycerol at the measured body water enrichment. Absolute synthesis rates of adipose TG were then calculated from fractional TG synthesis multiplied by adipose TG mass [18]. Net lipolysis was calculated based on the absolute TG synthesis rate combined with change in pool size [18]:

$$\text{Absolute synthesis (grams per day)} = f_{TG} \times \text{adipose TG mass (grams)}$$

$$\text{Net lipolysis (grams per day)} = [f_{TG} \times \text{adipose TG mass (grams)}] - \text{change in TG mass (grams)},$$

where change in adipose mass (expressed as gain in mass) is calculated by comparison of final mass to the measured baseline mass of each adipose depot in a subset of age-matched animals that were killed at baseline.

2.6. Plasma adiponectin, leptin, and resistin

Circulating concentrations of adiponectin, leptin, and resistin were measured by enzyme-linked immunosorbent assay (Linco Research, St Charles, MO) according to the manufacturer's instructions.

2.7. Aortic VSMC proliferation

The aorta (from the heart to the iliac bifurcation) and bone marrow from the femur were excised for the determination of deoxyribose (dR) enrichment or purine deoxyribonucleosides from purified DNA as described previously [11]. Briefly, the aorta was cleaned and treated with 70 U of collagenase (type 1; Sigma Chemical, St Louis, MO) for 25 minutes at 37°C to remove the endothelial and adventitial layers. The adventitia was stripped using forceps under a dissecting microscope. The remaining sleeve-like preparation represents the subendothelial intimal-medial layer and

was used for subsequent analyses. DNA was extracted from the intimal-medial layer and bone marrow cells (DNeasy tissue kit; Qiagen, Valencia, CA) and then was hydrolyzed to deoxyribonucleosides [19]. DNA was incubated overnight at 37°C with DNase, nuclease P1, snake venom phosphodiesterase, and alkaline phosphatase (Sigma Chemical). The dR group was derivatized to pentane tetraacetate [19] and analyzed by gas chromatography–mass spectrometry. Selected ion monitoring was performed on m/z of 245 and 246, representing the M_{+0} and M_{+1} ions, respectively, and chemical ionization mass spectrometry of m/z 245 (M_0) and m/z 246 (M_1). Unlabeled standards of natural abundance pentane tetraacetate were analyzed concurrently with samples. Fractional replacement (f) of cells was calculated as described previously [19], by comparison with cells from the same animal that were essentially fully turned over after 2 weeks of labeling with ^2H_2O (ie, bone marrow cells):

$$f = [(EM_1)_{sample} / (EM_1)_{bone\ marrow}] \times 100$$

2.8. Plasma lipids, free fatty acids, and C-reactive protein

Plasma TG and total cholesterol were determined by enzymatic end point measurements using enzyme reagent kits (Sigma Chemical). High-density lipoprotein (HDL) cholesterol concentration was measured directly after polyethylene glycol precipitation of apolipoprotein B–containing lipoproteins in plasma. Nonesterified free fatty acid (FFA) concentrations were measured colorimetrically according to the manufacturer's instructions (Bio Vision, Mountain View, CA). C-reactive protein (CRP) was measured using a quantitative enzyme-linked immunosorbent assay (Linco Research).

2.9. Statistical analysis

All results are presented as mean ± SEM. Differences between groups were analyzed by a 1-way analysis of variance (ANOVA). When a significant difference was noted between groups, a Tukey post hoc test was performed to determine significant differences between group means. A repeated-measures ANOVA was used to measure changes over time within an individual intervention group. Pearson correlation coefficients were calculated to assess the relationship of adipose tissue physiology and CHD risk parameters. A P value less than .05 was used as the criterion for statistical significance in all analyses. Data were analyzed by SPSS software (version 11 for Mac OS X; SPSS, Chicago, IL).

3. Results

3.1. Body weight and food intake during modified ADF regimens

At baseline, mean body weight of the ADF animals was similar to that of the control group (Table 1). Throughout the

Table 1
Body weight on ADF LF vs ADF HF diet

Body weight (g)	Day 1	Day 7	Day 14	Day 21	Day 28
Control	22.8 ± 0.4	23.6 ± 0.6	24.1 ± 0.5	25.0 ± 0.7	25.4 ± 0.5
ADF-85%-LF	23.5 ± 0.3	23.7 ± 0.5	25.5 ± 0.3	24.7 ± 0.4	25.6 ± 0.5
ADF-85%-HF	23.5 ± 0.2	23.7 ± 0.4	24.2 ± 0.5	25.8 ± 0.3	26.1 ± 0.5

Mean ± SEM; n = 6 mice per group. The ADF groups weighed the same as the controls at every time point throughout the study (1-way ANOVA).

course of the 4-week study, mice in the ADF-85%-LF and ADF-85%-HF groups gained weight at the same rate as the control group. There were no differences in body weight between the ADF groups at any point during the study. In terms of food intake, all mice ate similar amounts of food during acclimation (ADF-85%-L, 3.21 g/d; ADF-85%-HF, 3.39 g/d; control, 3.30 g/d). During the study, there were no differences in mean energy intake between ADF-85%-LF (17.9 ± 0.5 kcal/d on feed day, 1.9 ± 0.1 kcal/d on fast day) and ADF-85%-HF animals (18.0 ± 0.5 kcal/d on feed day, 2.0 ± 0.1 kcal/d on fast day), despite differences in macronutrient composition. Mean energy intake in the control group over the course of the trial was 11.0 ± 0.3 kcal/d.

3.2. Redistribution of body fat by modified ADF regimens

After 4 weeks of diet, subcutaneous fat pad weight was greater ($P < .01$) in the ADF-85%-LF and ADF-85%-HF groups relative to the control group (Fig. 1A). Visceral fat pad weight decreased ($P < .01$) only in the ADF-85%-LF group when compared with the control group. In both ADF groups, the relative proportion of subcutaneous fat was augmented ($P < 0.01$), whereas the proportion of visceral fat was reduced ($P < .01$), relative to ad libitum–fed animals (Fig. 1B). The magnitude of these favorable alterations in fat distribution was similar in the ADF-85%-LF and the ADF-85%-HF groups.

3.3. Modulations in adipose TG metabolism in relation to changes in fat distribution

Changes in adipose tissue TG metabolism after 4 weeks of ADF are shown in Table 2. Fractional and absolute TG syntheses were augmented ($P < .05$) in the subcutaneous fat pad in both the ADF-85%-LF and ADF-85%-HF groups. In contrast, no changes in TG synthesis in the visceral fat pad were observed. In both ADF groups, net lipolysis increased ($P < .05$) in subcutaneous fat, but remained unchanged in visceral fat. The increases in absolute TG synthesis, exceeding the rate of lipolysis, may explain why more fat accumulated in the subcutaneous depot of the ADF animals.

3.4. Changes in adipokine levels in relation to alterations in body fat distribution

Posttreatment concentrations of adiponectin, leptin, and resistin are shown in Fig. 2. Circulating adiponectin concentrations were elevated ($P < .05$) by 85% in the

ADF-85%-HF group relative to the control group. Animals in the ADF-85%-LF group also exhibited a trend of increased adiponectin levels, but this effect was not statistically significant. Leptin levels were reduced ($P < .05$) to a similar extent in both the ADF-85%-LF (62%
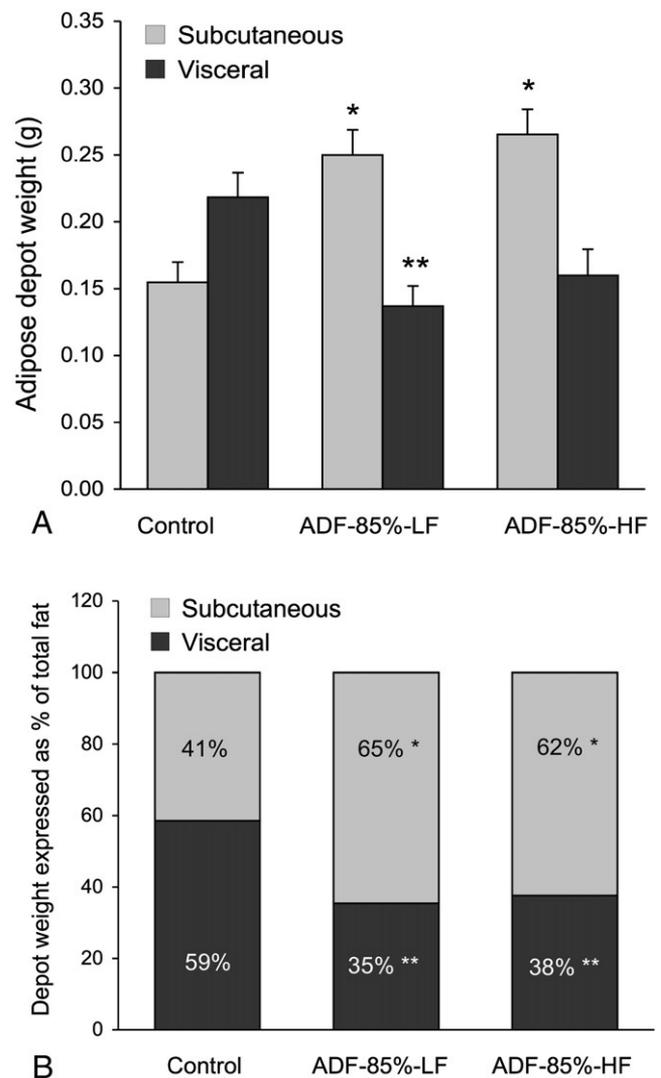

Fig. 1. Body fat distribution on ADF LF vs ADF HF diet. Mean ± SEM; n = 6 mice per group. A, Subcutaneous and visceral fat pad weights. B, Relative proportion of subcutaneous and visceral fat. *Subcutaneous fat pad in ADF group was significantly different ($P < .01$) from control group. **Visceral fat pad in ADF group was significantly different ($P < .01$) from control group (1-way ANOVA). Total fat: sum of subcutaneous and visceral fat compartments.

Table 2
Adipose tissue TG metabolism in subcutaneous and visceral fat depots

	Subcutaneous fat			Visceral fat		
	Control	ADF-85%-LF	ADF-85%-HF	Control	ADF-85%-LF	ADF-85%-HF
TG synthesis f (%)	0.41 ± 0.02	0.54 ± 0.02*	0.47 ± 0.02*	0.41 ± 0.06	0.41 ± 0.01	0.40 ± 0.02
TG synthesis (g/d)	0.12 ± 0.01	0.25 ± 0.02*	0.23 ± 0.03*	0.16 ± 0.02	0.10 ± 0.01	0.12 ± 0.02
Net lipolysis (g/d)	0.08 ± 0.01	0.20 ± 0.02*	0.18 ± 0.03*	0.10 ± 0.02	0.08 ± 0.01	0.09 ± 0.01

Mean ± SEM; n = 6 mice per group.
 * Significantly different ($P < .05$) from control group within 1 fat pad (1-way ANOVA).

reduction relative to the control group) and ADF-85%-HF group (58% reduction relative to the control group), despite differences in dietary macronutrient composition. Resistin concentrations also declined ($P < .05$) in the ADF-85%-LF (51% reduction) and ADF-85%-HF group (22% reduction) when compared with the control group. A significant association between resistin concentrations and visceral fat pad weight ($r = 0.47$, $P = .04$) was observed.

3.5. Reduction in aortic VSMC proliferation by modified ADF regimens

Proliferation rates of VSMC were markedly reduced ($P < .05$) in both ADF groups after 4 weeks of diet (Fig. 3). Equivalent reductions ($P < .05$) in VSMC proliferation were noted on both the LF (60% reduction relative to control) and HF diets (76% reduction relative to control). Reductions in VSMC proliferation rates were significantly associated with increased adiponectin concentrations ($r = -0.46$, $P = .04$).

3.6. Changes in circulating CHD risk parameters by modified ADF regimens

The effects of 4 weeks of diet on plasma indicators of CHD risk are displayed in Fig. 4. Total cholesterol levels were reduced ($P < .05$) by 27% in the ADF-85%-LF group, but remained unchanged in the ADF-85%-HF group. Low-density lipoprotein (LDL) cholesterol and HDL cholesterol levels were not affected by either ADF diet. Triglyceride concentrations decreased ($P < .05$) on both the LF (55% reduction) and HF (31% reduction) ADF diets relative to the control group. Circulating FFA was also significantly decreased ($P < .01$) after 4 weeks of treatment in both ADF groups. Plasma CRP concentrations were not affected by either intervention. Lower resistin levels were associated with decreased TG ($r = 0.45$, $P = .04$) and FFA ($r = 0.59$, $P = .01$) concentrations. There were no associations between adiponectin or leptin levels and any CHD risk parameter.

4. Discussion

We show here, for the first time, a decrease in CHD risk by modified ADF regimens, which may be associated with ADF-induced improvements in adipose tissue physiology. Specifically, we observed a favorable redistribution in body fat, that is, decreased proportion of visceral fat and increased proportion of subcutaneous fat, after only 4 weeks of modified ADF. We also demonstrate an increase in circulating adiponectin levels and a decrease in leptin and resistin levels, which may be linked to the decrease in visceral fat mass observed. This increase in plasma adiponectin concentrations was correlated with the marked decrease in aortic VSMC proliferation rates, whereas the decrease in resistin levels was associated with improvements in TG and FFA concentrations. Interestingly, these improvements in adipose physiology and CHD risk occurred independently of dietary fat content of the background diet.

An accumulation of adipose tissue in visceral compartments is associated with increased CHD risk [12-14]. In the present study, we observed a marked decrease in the proportion of visceral fat (both ADF-85% groups) and visceral fat mass (ADF-85%-LF group only) in the absence of body weight loss. After 4 weeks of treatment, visceral fat accounted for approximately 40% of total body fat in the ADF groups, whereas it accounted for approximately 60% in the ad libitum–fed control group. Accompanying this reduction in visceral adiposity was a distinct increase in subcutaneous fat mass. These beneficial alterations in body fat distribution occurred with both the HF and LF background diets. As such, ADF may improve body fat distribution over a wide range of percentage fat intake, which (if translatable to humans) might enhance subject tolerability and compliance with this fasting regimen. We also show here that this redistribution in fat was explained kinetically by an increase in TG synthesis in the subcutaneous fat pad. Although lipolysis was also augmented in this compartment, the TG mass synthesized (~24 g/d) exceeded that which was broken down (~19 g/d), thus resulting in net fat accumulation in subcutaneous sites. Other dietary restriction protocols, such as daily calorie restriction, also result in decreased visceral fat mass in both humans [20,21] and rodents [22]. It should be noted however that these decreases in visceral adiposity by calorie restriction generally occur as a result of overall body fat loss [20-22]. To our knowledge, modified ADF is the only dietary restriction regimen that has been shown to favorably *redistribute* fat to subcutaneous compartments in the absence of weight loss. If these findings can be replicated in humans, ADF may therefore benefit normal or slightly overweight individuals who wish to reduce vascular disease risk by

redistributing fat from visceral to subcutaneous sites, without substantial weight loss.

We also examined whether this shift in body fat distribution was related to changes in circulating adipokine
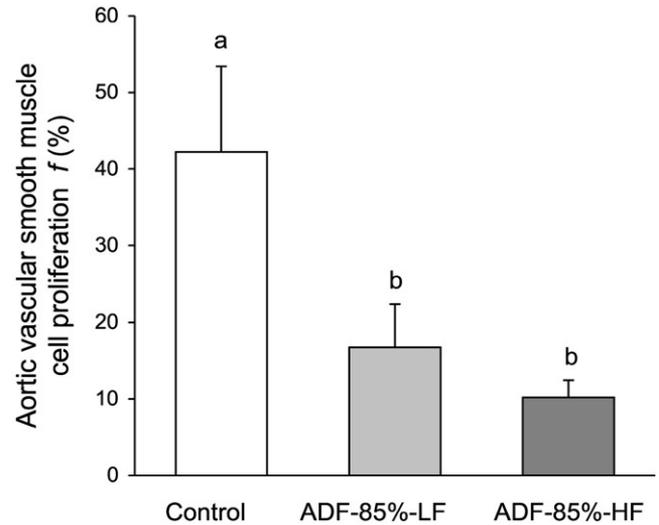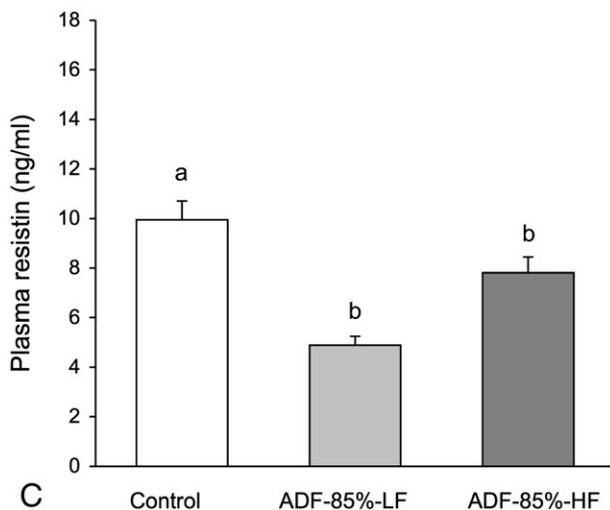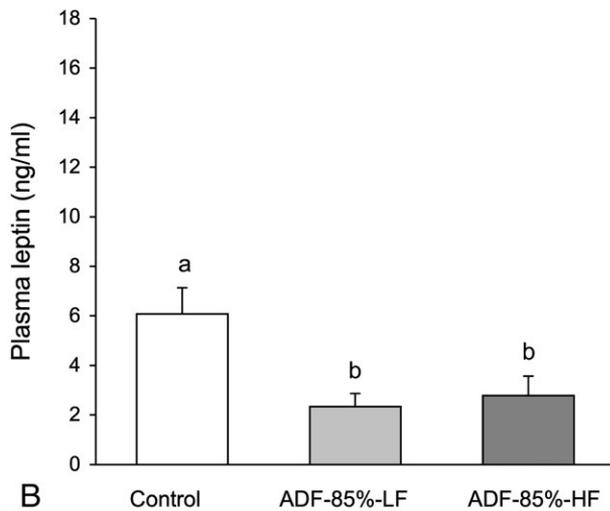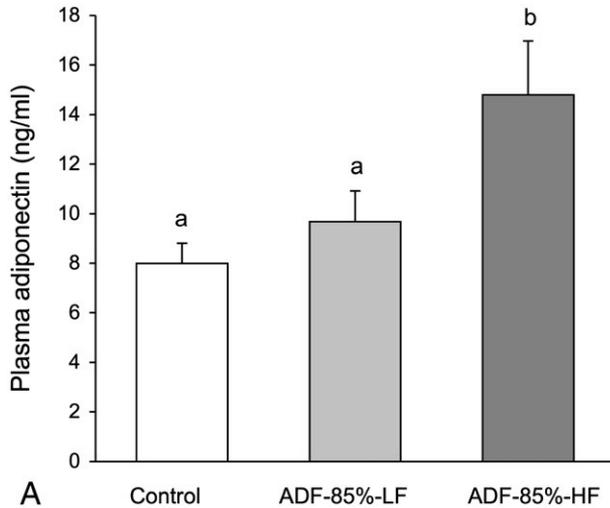

Fig. 3. Aortic VSMC proliferation on ADF LF vs ADF HF diet. Mean ± SEM; n = 6 mice per group. Proliferation of VSMC cells was reduced ($P <$ .05) in both ADF groups relative to control. Means not sharing a common superscript letter are significantly different between groups (1-way ANOVA).

concentrations. After 4 weeks of treatment, adiponectin plasma levels were significantly increased, whereas leptin and resistin were decreased. Also observed was a correlation between reduced visceral fat mass and decreased plasma resistin concentrations. Resistin is highly expressed in macrophages embedded in visceral adipocytes in rodents [16,23]. Resistin stimulates the synthesis of proinflammatory cytokines, such as interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) [24]. Both of these proinflammatory mediators suppress the production of adiponectin [25]. As such, it is possible that the decrease in visceral adiposity observed here may have resulted in blunted production of resistin, IL-6, and TNF-α, thus allowing for more adiponectin to be produced. This interaction among adipokines is speculative, however, because concentrations of IL-6 and TNF-α were not measured here.

Proliferation rates of aortic VSMC are a central feature of atherosclerosis [11]. Our laboratory has developed a method for measuring in vivo proliferation rates of VSMC by the incorporation of ^2H_2O into the dR moiety of newly synthesized DNA in VSMC of mouse aorta [11]. The goal of the present study was to determine whether an HF background diet prevents the beneficial effects of modified ADF on indicators of CHD risk. We show here that a potent reduction in aortic VSMC proliferation rates as a result of

Fig. 2. Plasma adipokine levels on ADF LF vs ADF HF diet. Mean ± SEM; n = 6 mice per group. A, Plasma adiponectin levels increased ($P <$ .05) in the ADF-85%-HF group only relative to controls. B, Leptin levels decreased ($P <$ .05) in both ADF groups relative to controls. C, Circulating resistin levels decreased ($P <$ .05) in both ADF groups relative to controls. Means not sharing a common superscript letter are significantly different between groups (1-way ANOVA).
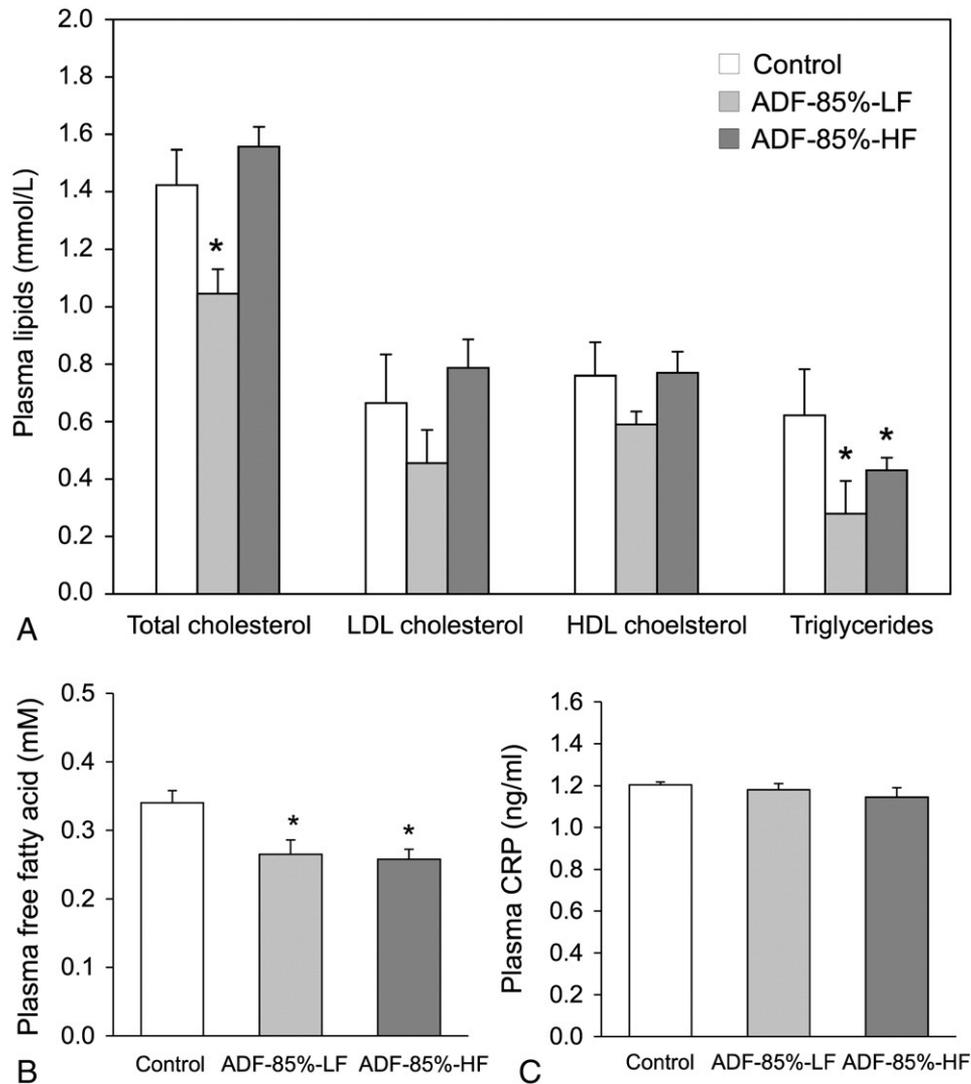
Fig. 4. Plasma lipids, FFA, and CRP levels on ADF LF vs ADF HF diet. Mean ± SEM; n = 6 mice per group. A, Total cholesterol levels decreased ($P < .05$) in the ADF-85%-LF, whereas TG levels decreased in both ADF groups, relative to control. B, Circulating FFA levels decreased ($P < .01$) in both ADF groups relative to control. C, C-reactive protein levels were not changed in either ADF group (1-way ANOVA).

modified ADF occurs independently of fat content of the background diet. Specifically, the modified ADF regimen reduced VSMC proliferation by 60% on an LF diet and 76% on an HF diet relative to the ad libitum–fed control group. We also show here that this decrease in VSMC proliferation was significantly related to increased circulating adiponectin levels. An antiatherosclerotic effect of adiponectin is consistent with recent findings in rodents [26,27]. The ability of ADF to improve vascular health has also been demonstrated by other groups [3,4,28]. After 4 weeks of ADF, Mager et al [3] and Wan et al [4] demonstrated significant declines in heart rate and blood pressure in rodents. In a study by Ahmet et al [28], myocardial infarction (MI) was induced by coronary artery ligation after 12 weeks of ADF in rats. Twenty-four hours after the induction of MI, the MI size in the ADF group was 2-fold smaller than that of the ad libitum–fed control group. Findings from the present

study complement this body of evidence supporting the cardioprotective effects of short-term ADF in animal models. A mechanism by which ADF may decrease CHD risk is modulation of oxidative stress. Oxidative damage to lipids has been shown to play a key role in the development of CHD [29]. In a recent study by Johnson et al [30], it was demonstrated that 8 weeks of ADF in overweight humans dramatically lowered markers of oxidative stress (ie, 8-isoprostane, nitrotyrosine, and protein carbonyls). Although oxidative stress was not directly measured in the present study, it may be a factor contributing to the reduction in CHD risk experienced by these mice on ADF diets.

The effect of ADF on other indicators of cardiometabolic risk was also assessed. We show here a significant decline in circulating TG and FFA concentrations by both ADF interventions. Total cholesterol levels were also reduced (ADF-85%-LF group only), whereas LDL cholesterol, HDL

cholesterol, and CRP concentrations remained unchanged, after 4 weeks of treatment. The lack of effect for HDL cholesterol is not surprising, as this lipoprotein is generally only augmented in response to increased activity levels. The lack of effect for LDL cholesterol is not clear. Similar decreases in total cholesterol and TG concentrations have been noted after 8 weeks of true ADF (ADF-100%) on an LF diet in C17/B1-10 mice [5]. The improvements in TG and FFA concentration in the present study were correlated with the lower resistin levels observed. In the fed state, resistin induces the expression of FA synthesis enzymes and activates de novo lipogenesis in the liver [31]. In view of these actions, it is possible that the ADF-induced reduction in resistin levels may have blunted hepatic de novo lipogenesis, thereby deceasing circulating TG concentrations and helping in the maintenance of vessel wall health [32].

In summary, our findings indicate that modified ADF regimens alter adipose tissue physiology (ie, body fat distribution, adipocyte TG metabolism, and adipokine levels) in a way that may protect against the development of atherosclerosis. These beneficial effects were noted over a wide range of percentage fat intake in the modified ADF diets, suggesting that ADF may protect against CHD even in the presence of HF background diets. Because most Americans consume a diet that is high in fat, these results could improve adherence to ADF in individuals at risk for developing CHD if confirmed in human subjects.

Acknowledgments

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