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Obesity

Meal Timing During Alternate Day Fasting: Impact on Body Weight and Cardiovascular Disease Risk in Obese Adults

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Objective: Alternate day fasting (ADF; 24-h feeding/24-h 25% energy intake at lunchtime), is effective for weight loss, but diet tolerability is questionable. Moving the fast day meal to dinnertime, or dividing it into smaller meals, may improve tolerability. Accordingly, this study compared the effects of ADF with three meal times on body weight and heart disease risk.

Methods: Obese subjects ($n = 74$) were randomized to 1 of 3 groups for 8 weeks: 1) ADF-L: lunch, 2) ADF-D: dinner, or 3) ADF-SM: small meals.

Results: Body weight decreased similarly ($P < 0.001$) in all groups (ADF-L: 3.5 ± 0.4 kg, ADF-D 4.1 ± 0.5 kg, ADF-SM 4.0 ± 0.5 kg). Reductions ($P < 0.001$) in fat mass and visceral fat were also comparable. Plasma lipids remained unchanged, and low density lipoprotein (LDL) particle size increased ($P < 0.05$) in all groups (1.3 ± 0.5 Å). Systolic blood pressure decreased ($P < 0.05$) by ADF-SM only. Fasting glucose, insulin, and HOMA-IR remained unchanged.

Conclusions: Thus, allowing individuals to consume the fast day meal at dinner or small meals produces similar weight loss and cardio-protection as consuming the meal at lunch. This flexibility in meal timing may increase tolerability and long-term adherence to ADF protocols.

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Introduction

Obesity increases an individual's risk of coronary heart disease (CHD) (1). Accumulating evidence suggests that even mild weight loss of 3-5%, can improve plasma lipid profile, decrease blood pressure, and reduce insulin resistance (IR) (2). Alternate day fasting (ADF) is a novel diet regimen that has gained considerable popularity over the past decade. In animal models, ADF consists of an ad libitum "feed day" alternated with a 100% restriction "fast day." In humans, this protocol is often modified to allow for a small amount of food consumption on the fast day (i.e., approximately 25% of the individual's energy needs). Findings from recent modified ADF trials demonstrate 4-8% reductions in body weight after 8-12 weeks (3-6). These decreases in body weight are usually accompanied by reductions in low density lipoprotein (LDL) cholesterol (4-6), triglycerides (4-6), systolic blood pressure (6), IR (3), and increases in LDL particle size (3,7,8).

Adherence and diet tolerability are essential to any weight loss protocol. Previous studies of ADF have generally required that subjects consume the fast day meal as a lunch (i.e., between 12.00 and 2.00 pm) (3-6). Unfortunately, many obese individuals are hesitant to try ADF, as they fear that they will not be able to comply with the rigid lunchtime fast day protocol. In view of these issues, it has been speculated that allowing subjects to consume the meal as either a dinner (i.e., between 6.00 and 8.00 pm) or as small meals throughout the day, may increase diet tolerability. To elaborate, the dinnertime protocol is beneficial in that it would permit subjects to eat with their families at night on the fast day, which was not possible with the previous lunchtime regimen. On the other hand, breaking up the meal into smaller meals at breakfast, lunch, and dinner could help individuals better cope with the hunger they may experience throughout the day. Whether or not these novel fast day meal schedules would produce the same degree of weight loss and cardio-protection as the traditional lunchtime protocol is an important question that has yet to be tested.

Accordingly, this study examined the effects of ADF with three different meal times (i.e., lunch, dinner, or small meals) on body weight, body composition, and CHD risk factors in obese adults. We hypothesize that the "dinnertime" and "small meals" interventions will produce the same degree of weight loss, and comparable beneficial modulations in CHD risk factors (plasma lipids, LDL particle size, blood pressure, glucose, and insulin) as the traditional "lunchtime" ADF protocol.

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Author contributions: KKH designed the experiment, ran the clinical trial, analyzed the data, and wrote the manuscript. CMK, JFT, AB, and SB assisted with the clinical trial and performed the laboratory analyses. KAV assisted with the design of the experiment, data analyses, and the preparation of the manuscript.

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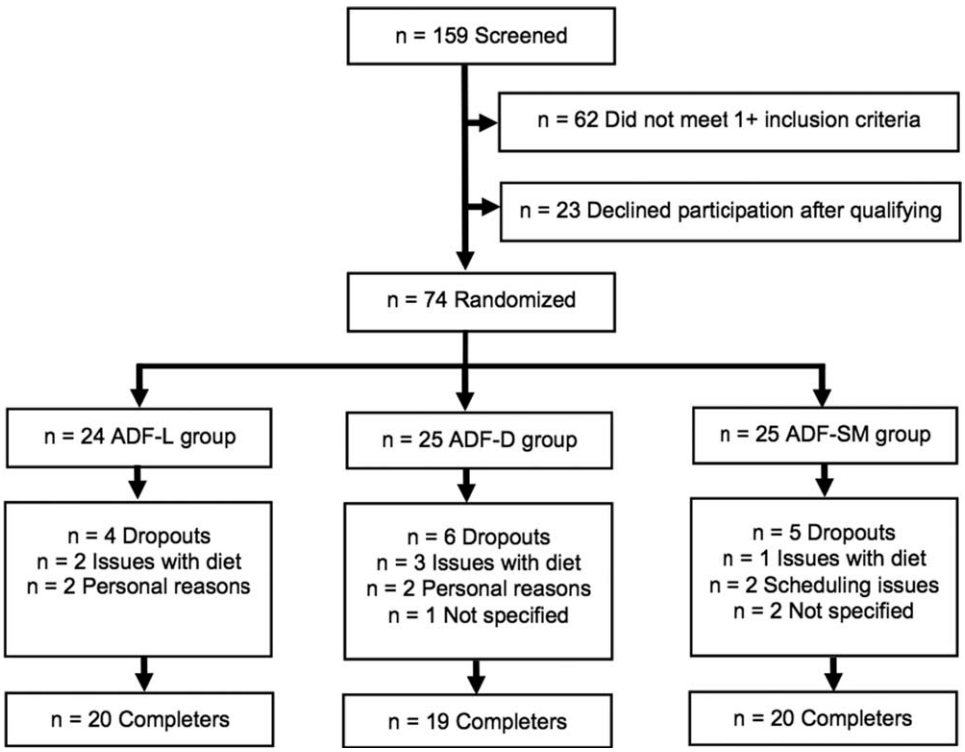

Figure 1 Study flow chart.

Methods

Subjects

Subjects were recruited from the Chicago area by means of advertisements placed around the University of Illinois, Chicago campus. A total of 159 individuals expressed interest, 97 subjects were deemed eligible to participate after screening via a preliminary questionnaire and BMI assessment, 23 subjects declined participation after qualifying, and 74 subjects were randomized to the interventions (Figure 1). Inclusion criteria were as follows: BMI between 30 and 39.9 kg/m^2; age between 25 and 65 years; pre-menopausal or post-menopausal (absence of menses for more than 2 years); lightly active (<3 h/week of light-intensity exercise at 2.5-4.0 metabolic equivalents [METs] for 3 months prior to the study); weight stable for 3 months prior to the beginning of the study (<4 kg weight loss or weight gain); non-diabetic; no history of cardiovascular disease (myocardial infarction or stroke); non-smoker; and not taking weight loss, lipid- or glucose-lowering medications. The experimental protocol was approved by the University of Illinois, Chicago, Office for the Protection of Research Subjects, and all research participants gave their written informed consent to participate in the trial.

Study design

Experimental design. A 10-week, randomized, parallel-arm feeding trial was implemented as a means of testing the study objectives. The 10-week trial consisted of two phases: 1) a 2-week baseline control period and 2) an 8-week weight loss ADF period.

Baseline control period (weeks 1-2). Before commencing the 8-week intervention, each subject participated in a 2-week baseline period, where they were requested to maintain a stable body weight and continue eating their usual diet.

Weight loss ADF period (weeks 3-10). Subjects were then randomized by a stratified random sample (based on sex, age, and BMI) into 1 of 3 interventions: 1) ADF-lunch (ADF-L), 2) ADF-dinner (ADF-D), or 3) ADF-small meals (ADF-SM). Total energy expenditure (TEE) was calculated using the Mifflin equation (9). All subjects consumed 25% of their baseline energy needs on the fast day (24 h), and ate ad libitum on each alternating feed day (24 h). The feed and fast days began at midnight each day. Subjects were asked to refrain from staying up until midnight on feed days, or getting up just after midnight following a fast day, to eat. Subjects were provided with meals on each fast day, and ate ad libitum at home on the feed day. All ADF fast day meals were prepared in the metabolic kitchen of the Human Nutrition Research Center (HNRU) at the University of Illinois, Chicago. Fast day meals were provided as a 3-day rotating menu, and were formulated based on the American Heart Association (AHA) guidelines (10) (Table 1). Subjects were permitted to consume energy-free beverages, tea, coffee, and sugar-free gum, and were encouraged to drink plenty of water on the fast day. Each intervention group consumed the fast day meal at different times throughout the day. The ADF-L group consumed their entire meal between 12.00 pm and 2.00 pm on each fast day. In contrast, the ADF-D group consumed their meal between 6.00 pm and 8.00 pm. The ADF-SM group divided their fast day meal up into three mini meals, and consumed ~100 kcal between 6.00 am and 8.00 am, ~300 kcal between 12.00 pm and 2.00 pm, and ~100 kcal between 6.00 pm and 8.00 pm.

TABLE 1 Nutrient composition of the provided fast day meals

	Fast day 1	Fast day 2	Fast day 3
Foods			
Entrée	Chicken enchilada	Lasagna w/meat sauce	Roasted turkey
Fruit/vegetable	Grapes	Carrot sticks	Grapes
Dessert/snack	Peanuts	Cookie	Crackers
Dairy	Yogurt	Yogurt	Yogurt
Nutrients[a]			
Energy (kcal)	500	500	500
Fat (g)	15 (27%)[b]	13 (24%)[b]	12 (22%)[b]
Saturated fat (g)	5	5	4
Monounsaturated fat (g)	6	5	4
Polyunsaturated fat (g)	4	3	4
Trans fat (g)	0	0	0
Cholesterol (mg)	32	36	33
Protein (g)	20 (16%)[b]	23 (18%)[b]	22 (17%)[b]
Carbohydrate (g)	72 (57%)[b]	73 (58%)[b]	78 (61%)[b]
Fiber (g)	11	11	11

[a]No differences between meals for any nutrient when meals matched for total kcal. ADF-lunch (ADF-L) group consumed entire fast day meal between 12.00 pm and 2.00 pm. ADF-dinner (ADF-D) group consumed entire fast day meal between 6.00 pm and 8.00 pm. ADF-small meals (ADF-SM) group divided the fast day meal during the day and consumed 100 kcal between 6.00 am and 8.00 am, 300 kcal between 12.00 pm and 2.00 pm, and 100 kcal between 6.00 pm and 8.00 pm.
[b]Percent of energy (kcal).

Blood collection protocol. Twelve-hour fasting blood samples were collected between 6.00 am and 9.00 am at weeks 1, 3, and 10 (after a feed day). The subjects were instructed to avoid exercise, alcohol, and coffee for 24 h before each visit. Blood was centrifuged for 10 min at 520*g* at 4°C to separate plasma from red blood cells and was stored at −80°C until analyzed.

Analyses

Adherence to the ADF diet. Subjects were instructed to eat only the foods provided on each fast day, and to report any extra food items consumed using an "Extra food log." If the log indicated that the subject ate extra food items (totalling >75 kcal) on a fast day, that day was labeled as "not adherent." If the log revealed that the subject did not eat an extra food item, that day was labeled as "adherent." Adherence data were assessed each week as: % adherence to kcal goal = (no. of fast days adherent/no. of fast days in the week) × 100.

Compliance to the timing of the ADF meal was assessed using a "Fast day food checklist" (specific for each intervention group). The checklist contained a list of food items for that particular fast day, and clearly indicated the time range in which each food item was to be consumed. The checklist also contained a column where the subject was to report the specific time of day that each food item was eaten. If the checklist indicated that the subject ate one or more food items outside of the specified time range, that day was labeled as "not adherent." If the checklist revealed that the subject ate all the food items during the correct time range, that day was labeled as "adherent." Adherence data were assessed each week as: % adherence to meal timing = (no. fast days adherent/no. of fast days in the week) × 100.

Physical activity maintenance assessment. All subjects were asked to maintain their physical activity habits during the trial. Physical activity was quantified by the use of a validated (11) pattern recognition monitor (Sense Wear Mini, Bodymedia, Pittsburg, PA). Subjects wore the lightweight monitor on their upper arm for 7 days (~23 h/day) at weeks 3 and 10. The data were processed using Bodymedia Software V.7.0 (11).

Weight loss and body composition. Body weight was assessed to the nearest 0.25 kg at the beginning of every week without shoes and in light clothing using a balance beam scale at the research center (HealthOMeter, Boca Raton, FL). BMI was assessed as kg/m^2. Body composition (fat mass, lean mass, visceral fat mass) was measured using dual X-ray absorptiometry (12) (DXA; iDXA, General Electric Inc).

Plasma lipids and LDL particle size. Plasma total cholesterol, direct LDL cholesterol, high density lipoprotein (HDL)-cholesterol, and triacylglycerol concentrations were measured in duplicate using enzymatic kits (Biovision Inc., Moutainview, CA) at weeks 1, 3, and 10. LDL particle size was measured by linear polyacrylamide gel electrophoresis (Quantimetrix Lipoprint System, Redondo Beach, CA) (13). Lipoware computer software (Quantimetrix, Redondo Beach, CA) was then used to divide LDL into small (<255 Å), medium (255-260 Å), and large (>260 Å) particles, and to assess mean LDL particle size (8).

Metabolic disease risk factors. All measurements were taken at weeks 1, 3, and 10 (after a feed day). Blood pressure and heart rate were measured in triplicate using a digital automatic blood pressure/heart rate monitor (Omron HEM 705 LP, Kyoto, Japan) with the subject in a seated position after a 10-min rest. Resting metabolic rate (RMR) was measured by a handheld open circuit indirect calorimeter (14) (MedGem Indirect Calorimeter, Microlife, USA). RMR measurements were taken between 6.00 am and 9.00 am. Subjects were instructed to abstain from food, drink, and exercise for 12 h

TABLE 2 Subject characteristics at baseline

	ADF-L	ADF-D	ADF-SM	*P*-value[a]
N	20	19	20	
Age (y)	45 ± 3	45 ± 3	46 ± 2	0.88
Sex (F/M)	17/3	15/4	18/2	0.58
Body weight (kg)	94 ± 2	97 ± 3	90 ± 2	0.16
Height (cm)	165 ± 2	168 ± 2	163 ± 2	0.08
Body mass index (kg/m^2)	35± 1	34 ± 1	34 ± 1	0.90
Lean mass (kg)	48 ± 1	51 ± 2	47 ± 2	0.27
Fat mass (kg)	42 ± 1	42 ± 2	40 ± 1	0.49
Visceral fat mass (kg)	1.2 ± 0.1	1.4 ± 0.2	1.1 ± 0.1	0.61
Lipids (mg/dl)				
Total cholesterol	190 ± 11	199 ± 8	185 ± 8	0.57
LDL cholesterol	114 ± 11	120 ± 6	110 ± 7	0.66
HDL cholesterol	55 ± 3	57 ± 4	57 ± 3	0.95
Triglycerides	104 ± 15	108 ± 14	94 ± 9	0.69
Systolic blood pressure (mm Hg)	115 ± 3	117 ± 3	119 ± 2	0.70
Diastolic blood pressure (mm Hg)	78 ± 2	79 ± 2	83 ± 1	0.10
Heart rate (bpm)	69 ± 3	71 ± 2	72 ± 3	0.63

Values reported as means ± SEM. ADF-L: alternate day fasting-lunch, ADF-D: alternate day fasting-dinner, ADF-SM: alternate day fasting-small meals, F: female, M: male.
[a]*P*-value between groups at baseline (week 1): one-way ANOVA.

prior to the visit, and the timing since the last meal (12 h) was standardized for each subject prior the RMR measurement. Subjects first rested in a dark room in the supine position for 25 min, then a mouthpiece/nose clip were placed on the subject, and oxygen consumption was measured until it reached a stable flow (∼10 min). Fasting glucose concentrations were measured with a hexokinase reagent kit (Abbott, South Pasadena, CA). Fasting insulin was measured as total immunoreactive insulin (Coat-A-Count Insulin, Los Angeles, CA). IR was calculated using the HOMA (Homeostasis Model Assessment) method, by applying the following formula: [HOMA-IR = Fasting insulin (μlU/ml) × Fasting glucose (mg/dl)/405].

Statistics

Results are presented as means ± standard error of the mean (SEM). Tests for normality were included in the model. No variables were found to be not normal. Differences between groups at baseline and post-treatment were tested by one-factor analysis of variance (ANOVA) and a Tukey's *post hoc* test. Within-group changes from weeks 3 to 10 were tested by a paired *t*-test. *P*-values of <0.05 were considered significant. Data were analyzed by using SPSS software (v.21, SPSS Inc., Chicago, IL).

Results

Subject baseline characteristics and dropouts

There were 20 completers in both the ADF-L and ADF-SM group, and 19 completers in the ADF-D group (Figure 1). Dropout rates were similar for each intervention (ADF-L: $n = 4$, ADF-D: $n = 6$, ADF-SM: $n = 5$). Dropouts were primarily due to issues with adhering to the diet, scheduling conflicts, and personal reasons. At baseline, there were no between-group differences for age, sex, body weight, height, BMI, body composition, lipids, blood pressure, or heart rate (Table 2).

Adherence to the ADF diet and physical activity maintenance

Adherence to the fast day energy goal was similar ($P = 0.475$) for each group throughout the course of the trial (ADF-L: 91 ± 1%, ADF-D: 92 ± 2%, ADF-SM: 89 ± 2%). Compliance to the timing of the ADF meal was high in each intervention group (ADF-L: 98 ± 1%, ADF-D: 99 ± 1%, ADF-SM: 98 ± 1%). Maintenance of physical activity habits was measured using an activity monitor. There were no differences in activity from baseline to post-treatment in any of the groups (ADF-L week 3: 5,663 ± 650 steps/day, week 10: 5,995 ± 633 steps/day; ADF-D week 3: 5,733 ± 1,304 steps/day, week 10: 5,847 ± 627 steps/day; ADF-SM week 3: 7,113 ± 938 steps/day, week 10: 6,457 ± 812 steps/day). Moreover, there were no differences between groups for activity level at week 3 ($P = 0.361$) or week 10 ($P = 0.326$).

Weight loss and body composition

There were no changes in body weight or body composition during the baseline period (Figure 2). Body weight was reduced ($P < 0.001$) by 3.5 ± 0.4 kg (3.8 ± 0.5%) in the ADF-L group, 4.1 ± 0.5 kg (4.2 ± 0.5%) in the ADF-D group, and 4.0 ± 0.5 kg (4.6 ± 0.6%) in the ADF-SM group, with no differences between groups post-treatment. Fat mass and lean mass decreased ($P < 0.001$) similarly by all interventions. Visceral fat mass also declined ($P < 0.001$) in all groups (ADF-L: 0.075 ± 0.027 kg, ADF-D: 0.135 ± 0.042 kg, ADF-SM: 0.135 ± 0.032 kg), with no differences between groups at week 10. BMI was reduced ($P < 0.05$) by ADF-L (1.3 ± 0.2 kg/m^2),

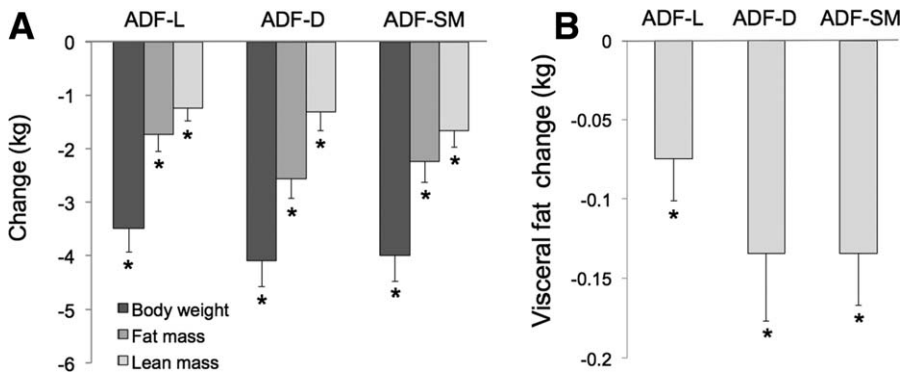

Figure 2 Changes in body weight and body composition during the weight loss period. (**A**) Absolute change in body weight, fat mass, and lean mass from weeks 3 to 10 in the ADF-L, ADF-D, and ADF-SM groups. (**B**) Absolute change in visceral fat mass from weeks 3 to 10 of the trial for each intervention group. Values reported as means ± SEM. *Week 3 value significantly ($P < 0.001$) different from week 10 values within group (paired *t*-test). No differences between groups for absolute change in body weight, fat mass, lean mass, or visceral fat mass (one-way ANOVA).

ADF-D (1.4 ± 0.2 kg/m^2), and ADF-SM (1.5 ± 0.2 kg/m^2) after 8 weeks of treatment.

Plasma lipids and LDL particles

Total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride concentrations remained unchanged during the control phase, and during the weight loss phase in all groups (Table 3). Mean LDL particle size increased ($P < 0.05$) after 8 weeks of treatment in the ADF-L (1.3 ± 0.5 Å), ADF-D (1.3 ± 0.5 Å), and ADF-SM (1.3 ± 0.6 Å) group, with no differences between groups.

Metabolic disease risk factors

There were no changes in any metabolic disease risk factors during baseline. Systolic blood pressure was reduced ($P < 0.05$) in the ADF-SM group only (6 ± 3 mm Hg) (Table 4). Diastolic blood pressure remained unchanged in all groups. Heart rate decreased ($P < 0.001$) in the ADF-L group only (7 ± 2 bpm). RMR was reduced ($P < 0.001$) from baseline to post-treatment in the ADF-D group only (198 ± 48 kcals/day). Fasting glucose, insulin, and HOMA-IR remained unchanged in all groups after 8 weeks of diet.

TABLE 3 Changes in plasma lipids and LDL particle size during the weight loss period

	Intervention	Week 3	Week 10	*P*-value[a]	*P*-value[b]	Change[c]	*P*-value[d]
Total cholesterol (mg/dl)	ADF-L	185 ± 12	184 ± 9	0.92	0.75	−1 ± 7	0.73
	ADF-D	195 ± 8	190 ± 8	0.19		−5 ± 4	
	ADF-SM	182 ± 8	181 ± 7	0.82		−1 ± 4	
LDL cholesterol (mg/dl)	ADF-L	115 ± 9	113 ± 9	0.54	0.75	−2 ± 3	0.87
	ADF-D	119 ± 6	119 ± 8	0.98		0 ± 6	
	ADF-SM	109 ± 7	110 ± 7	0.79		1 ± 3	
HDL cholesterol (mg/dl)	ADF-L	54 ± 3	52 ± 3	0.22	0.80	−2 ± 1	0.91
	ADF-D	54 ± 4	54 ± 3	0.70		0 ± 2	
	ADF-SM	56 ± 3	55 ± 3	0.69		−1 ± 2	
Triglycerides (mg/dl)	ADF-L	102 ± 12	96 ± 14	0.38	0.54	−6 ± 7	0.70
	ADF-D	111 ± 14	102 ± 12	0.10		−9 ± 5	
	ADF-SM	85 ± 8	84 ± 9	0.91		−1 ± 8	
LDL particle size (Å)	ADF-L	257.6 ± 0.8	258.9 ± 0.8	0.02	0.95	1.3 ± 0.5	0.96
	ADF-D	257.3 ± 0.9	258.6 ± 1.0	0.01		1.3 ± 0.5	
	ADF-SM	257.7 ± 0.7	259.0 ± 0.7	0.02		1.3 ± 0.6	

Values reported as means ± SEM. ADF-L: alternate day fasting-lunch, ADF-D: alternate day fasting-dinner, ADF-SM: alternate day fasting-small meals.
[a]*P*-value between week 3 and week 10: paired *t*-test.
[b]*P*-value between groups at week 10: one-way ANOVA.
[c]Absolute change between week 3 and week 10 values.
[d]*P*-value between groups for absolute change: one-way ANOVA.

TABLE 4 Changes in metabolic disease risk factors during the weight loss period

	Intervention	Week 3	Week 10	*P*-value[a]	*P*-value[b]	Change[c]	*P*-value[d]
Systolic blood pressure (mm Hg)	ADF-L	114 ± 2	112 ± 3	0.57	0.99	−2 ± 2	0.52
	ADF-D	117 ± 5	112 ± 2	0.07		−5 ± 3	
	ADF-SM	118 ± 3	112 ± 3	0.04		−6 ± 3	
Diastolic blood pressure (mm Hg)	ADF-L	77 ± 2	76 ± 2	0.56	0.25	−1 ± 2	0.68
	ADF-D	79 ± 3	76 ± 1	0.15		−3 ± 2	
	ADF-SM	80 ± 2	79 ± 1	0.60		−1 ± 2	
Heart rate (bpm)	ADF-L	71 ± 2	64 ± 2	<0.001	0.63	−7 ± 2a	0.03
	ADF-D	71 ± 2	68 ± 2	0.05		−3 ± 2a,b	
	ADF-SM	72 ± 3	72 ± 3	0.80		0 ± 2b	
Resting metabolic rate (kcal/day)	ADF-L	1423 ± 48	1336 ± 51	0.06	0.22	−87 ± 42	0.22
	ADF-D	1557 ± 62	1359 ± 74	<0.001		−198 ± 48	
	ADF-SM	1356 ± 52	1324 ± 48	0.52		−32 ± 47	
Fasting glucose (mg/dl)	ADF-L	96 ± 2	94 ± 2	0.28	0.23	−2 ± 2	0.87
	ADF-D	100 ± 3	99 ± 2	0.77		−1 ± 3	
	ADF-SM	101 ± 3	99 ± 2	0.20		−2 ± 2	
Fasting insulin (μIU/ml)	ADF-L	12 ± 1	12 ± 1	0.49	0.14	0 ± 1	0.66
	ADF-D	11 ± 1	9 ± 2	0.09		−2 ± 1	
	ADF-SM	16 ± 2	14 ± 2	0.07		−2 ± 7	
HOMA-IR	ADF-L	3.0 ± 0.2	2.7 ± 0.3	0.44	0.10	−0.3 ± 0.4	0.68
	ADF-D	3.0 ± 0.4	2.2 ± 0.4	0.09		−0.8 ± 0.3	
	ADF-SM	4.2 ± 0.6	3.4 ± 0.5	0.09		−0.8 ± 0.4	

Values reported as means ± SEM. ADF-L: alternate day fasting-lunch, ADF-D: alternate day fasting-dinner, ADF-SM: alternate day fasting-small meals, HOMA-IR: homeostatic model assessment-insulin resistance.
[a]*P*-value between week 3 and week 10: paired *t*-test.
[b]*P*-value between groups at week 10: one-way ANOVA.
[c]Absolute change between week 3 and week 10 values.
[d]*P*-value between groups for absolute change: one-way ANOVA.
Means not sharing a common letter are significantly different (Tukey *post hoc* test).

Discussion

This study is the first to show that subjects can consume the ADF fast day meal as either a dinner or small meals, and experience similar weight loss and cardio-protection as consuming the meal as a lunch (traditional protocol). We show here that changing the timing of the fast day meal does not negatively impact adherence to ADF. Since adherence remained high throughout the trial in each intervention (lunch, dinner, and small meals), similar weight loss, visceral fat mass loss, and LDL particle size increases were noted in each of the groups.

A key objective of this study was to determine whether adherence would be altered if the timing of the fast day meal shifted from lunch to dinner or small staggered meals. Results reveal that adherence remained high (90%) in all groups, and that dropout rates were similar for each intervention ($n = 4$-6 per group). All in all, these findings suggest that individuals do not need to stick to the rigid lunchtime protocol to gain the benefits of ADF. Subjects may now choose the protocol that best suits their needs, and implement particular protocol to achieve long-term success with ADF.

Body weight and body composition improved to the same extent in each group, due to similar levels of adherence. Specifically, body weight, fat mass, and lean mass decreased by approximately 4 kg, 3 kg, and 1 kg, respectively, by each intervention. Previous studies have reported similar modulations in body weight and fat mass after 8 weeks of ADF (4-6). However, the present trial differs from previous studies (5,6) in terms of lean mass findings. For instance, in a study by Klempel et al. (5), obese subjects were provided with either a high-fat or low-fat diet during 8 weeks of ADF. This study demonstrated impressive reductions in body weight (4 kg) and fat mass (4 kg), with a complete retention in lean mass (5). Similarly, Varady et al. (6) reported a 5 kg reduction in body weight/fat mass, with no change in lean mass. Johnson et al. (4) demonstrated the greatest decreases in body weight (8 kg) after 8 weeks of ADF, but failed to report changes in body composition. The percentage of body weight lost as fat mass versus lean mass, also differed considerably between present and previous trials (5,6). While previous ADF studies suggests that 90% of weight is lost as fat and 10% is lost as lean (5,6), the present study demonstrates a shift in this ratio to 75% lost as fat and 25% lost as lean. The reason for these contradictory findings is not clear, but may be related to the instruments used to measure body composition. For example, previous work used bioelectrical impedance analysis (BIA) (5,6), while the present study used DXA. Results from validation studies demonstrate that BIA routinely overestimates lean mass by 5-10% (15,16), when compared to DXA. These systematic errors in accuracy may therefore partly explain why lean mass findings differed between studies. Visceral fat mass was also assessed. Results from DXA analysis reveal comparable modest reductions (0.1 kg) by all three mealtime protocols. Previous short-term ADF studies also report reductions in visceral fat (5,6).

However, it is difficult to compare findings, as past studies (5,6) used an indirect measure (i.e., waist circumference reported in cm), while the present study used DXA (i.e., reported as kg).

Plasma lipids remained unchanged in all intervention groups after 8 weeks of treatment. These findings are inconsistent with previous trials (4-6). For instance, other 8-week ADF studies have demonstrated fairly reliable reductions in total cholesterol (9-21%), LDL cholesterol (12-31%), and triglyceride concentrations (14-42%) (4-6). Only one other ADF study reported no effect on these lipid parameters (3). The reason for this lack of effect is not clear, as weight loss and baseline lipid values were similar between present and previous studies (4-6). No ADF trial to date has demonstrated increases in HDL cholesterol levels. This is not surprising as HDL cholesterol concentrations are generally only augmented with exercise (17,18). The effect of these interventions on LDL particle size was also investigated. Small dense LDL particles are more atherogenic than their larger counterparts, due to increased oxidizability and permeability through the endothelial barrier (19). We show here that LDL particle size increased by 1 Å by all interventions, despite no change in HDL cholesterol or triglyceride concentrations (20). Thus, each of these mealtime protocols produces similar cardiovascular benefits by increasing mean LDL particle size.

The impact of these interventions on metabolic disease risk factors was also assessed. Systolic blood pressure was reduced in the ADF-SM group only (6 mm Hg) after 8 weeks of diet. The reason for this selective effect is not clear, as all groups lost comparable amounts of body weight, fat mass, and visceral fat mass. However, it should be noted that the ADF-SM group had the highest systolic values at baseline (though not significantly). Since these subjects were closer to being hypertensive than the other groups, this may explain why their systolic blood pressure was more responsive to the treatment. None of the interventions had any effect on diastolic blood pressure. The minimal effect on blood pressure is most likely due to the normotensive status of the subjects at baseline (<120 mm Hg systolic, <80 mm Hg diastolic). Previous ADF studies that demonstrated reductions in systolic (4-6 mm Hg) and diastolic blood pressure (2 mm Hg) recruited individuals with borderline hypertension (3,6). Thus, the hypertensive status of the sample may be a key determinant of blood pressure change by ADF. Heart rate was also evaluated. Heart rate was reduced by ADF-L only. The reason why the lunchtime protocol produced this specific effect is uncertain, as all groups had similar heart rates at baseline (71-72 bpm) and the activity levels of the ADF-L subjects did not increase to a greater extent than the other groups (21). This finding is also somewhat surprising, as ADF does not generally improve heart rate (3,5). As for glucose, insulin, and IR, no changes were observed for any intervention. Seeing as baseline levels of glucose and insulin were within the normal range for each group, it is not surprising that these diets had little effect on these parameters. The effect of ADF on RMR was also evaluated. Interestingly, RMR was maintained in the ADF-L and ADF-SM group, but declined considerably by ADF-D (200 kcal/day from baseline). The reason for this is unclear as all groups lost the same amount of lean mass, and lean mass is a key predictor of RMR (22). Whether or not these RMR findings can be reproduced using a more robust technique for measuring energy expenditure, such as doubly labeled water (23), is well warranted.

This study has several limitations. First and foremost, this trial did not include a "breakfast" arm to test the effects of consuming the fast day meal between 6.00 am and 8.00 am. Thus, it is still uncertain whether consuming the meal as breakfast produces similar adherence and weight loss as the other mealtimes. Second, activity data were collected only for 80% of the subjects in each intervention group due to malfunctioning activity monitors. As such, the study may not be powered adequately to identify changes in physical activity from baseline to post-treatment. Third, adherence to the fast day protocol was determined using self-reports. It is well known that obese subjects underestimate energy intake by 10-20% (24,25); thus the reliability of our adherence data is questionable. Fourth, due to the nature of the "small meals" intervention, this group was not technically taking part in an ADF protocol. Instead, this group was following a very low calorie diet, which should be considered when interpreting the present findings. Fifth, our study lacked a control group, so whether or not the present findings can truly be attributed to these treatments, remains unknown. Sixth, since the ADF-SM group consumed the majority of their calories (~300 kcal) between 12.00 pm and 2.00 pm on the fast day, this intervention may not be that different from the traditional lunchtime approach. In retrospect, the ~500 kcal meal for the ADF-SM group should have been divided evenly over the three meals, to more effectively test our hypotheses.

In summary, these findings demonstrate that there is considerable flexibility in the timing of the fast day meal during ADF. Obese subjects may consume the meal at dinner or as small meals throughout the day, and experience similar weight loss, body composition, and cardiovascular benefits as the traditional lunchtime approach. These data have important clinical implications in terms of diet tolerability. More specifically, obese individuals who may have previously been deterred from trying ADF due to the rigid lunchtime meal schedule may now be more likely to try the diet. As a result, a greater percentage of the obese population may now be able to reap the benefits of ADF. O

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