Original Article
CLINICAL TRIALS: BEHAVIOR, PHARMACOTHERAPY, DEVICES, SURGERY

Obesity

Alternate Day Fasting and Endurance Exercise Combine to Reduce Body Weight and Favorably Alter Plasma Lipids in Obese Humans

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Objective: This study examined whether the combination of alternate day fasting (ADF) plus exercise produces superior changes in body composition and plasma lipid levels when compared to each intervention alone.
Design and Methods: Obese subjects ($n = 64$) were randomized to 1 of 4 groups for 12 weeks: 1) combination (ADF plus endurance exercise), 2) ADF, 3) exercise, or 4) control.
Results: Body weight was reduced ($P < 0.05$) by 6 ± 4 kg, 3 ± 1 kg, and 1 ± 0 kg in the combination, ADF, and exercise groups, respectively. Fat mass and waist circumference decreased ($P < 0.001$), while lean mass was retained in the combination group. Low-density lipoprotein (LDL) cholesterol decreased (12 ± 5%, $P < 0.05$) and high-density lipoprotein (HDL) cholesterol increased (18 ± 9%, $P < 0.05$) in the combination group only. LDL particle size increased ($P < 0.001$) by 4 ± 1 Å and 5 ± 1 Å in the combination and ADF groups, respectively. The proportion of small HDL particles decreased ($P < 0.01$) in the combination group only.
Conclusions: These findings suggest that the combination produces superior changes in body weight, body composition, and lipid indicators of heart disease risk, when compared to individual treatments.

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Introduction

Overweight and obesity are key risk factors for the development of coronary heart disease (CHD) (1). A moderate weight loss of 5-10% significantly lowers CHD risk (2). Different forms of dietary restriction, including alternate day fasting (ADF), have been shown to be effective for weight loss and improving vascular health (3,4). ADF involves consuming 25% of energy needs on the ''fast'' day and eating ad libitum on alternating ''feed'' days. Three trials of ADF that ran for 2-3 weeks have demonstrated a decrease of 3% in body weight, in conjunction with decreased triglyceride levels (5,6). Longer-term trials of ADF have shown greater weight loss (i.e., 8% from baseline) and decreased visceral fat mass (7,8). Low-density lipoprotein (LDL) cholesterol, LDL particle size, and triglyceride levels also improved significantly in this 8-week trial (7,9). Thus, ADF is effective in lowering body weight, visceral fat mass, total cholesterol, triglyceride, and LDL cholesterol levels. Despite these beneficial effects, ADF has no impact on HDL cholesterol, and does not help in the retention of lean mass (7). Endurance exercise, on the contrary, has been shown to prevent the loss of lean mass, increase HDL cholesterol, decrease the proportion of small HDL particles, and augment visceral fat loss (10). What is not yet known, however, is whether ADF combined with exercise can enhance this weight loss without the loss of lean mass. Also, it is not clear whether this combination therapy can improve all four lipid parameters (i.e., decrease total, LDL cholesterol, and triglyceride levels, in conjunction with increasing HDL cholesterol).

Accordingly, this study investigated the effect of combining ADF and endurance exercise on body weight, body composition, and CHD risk factors. Sedentary, obese males and females in the combination group (ADF plus exercise) were compared to an ADF-only group, an exercise-only group, and a control group for 12 weeks. We hypothesized that the combination of ADF and exercise would produce greater reductions in body weight, greater fat-free mass preservation, and more pronounced improvements in CHD risk factors when compared with the individual interventions.

Materials and Methods

Subjects

Independently living subjects were recruited from the University of Illinois at Chicago campus by means of flyers. Of the 146

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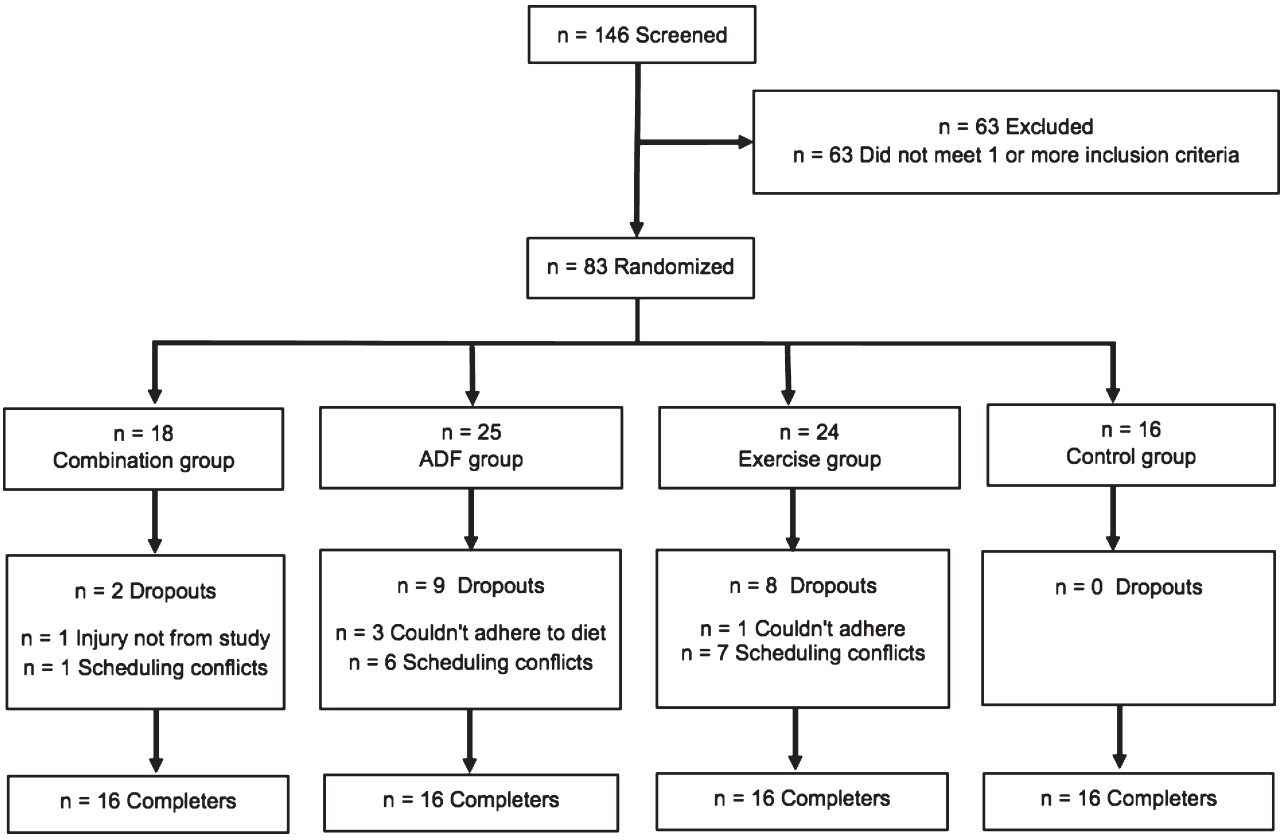

FIGURE 1 Study flow chart.

individuals who expressed interest in the study, 83 were deemed eligible to participate according to a preliminary questionnaire and body mass index (BMI) assessment (Figure 1). Key inclusion criteria were as follows: age 25-65 years; BMI between 30 and 39.9 kg/m^2; weight stable for 3 months prior to the beginning of the study (i.e., less than 5 kg weight loss or weight gain); nondiabetic; no history of cardiovascular disease; lightly active (i.e., <3 h/week of light intensity exercise at 2.5-4.0 metabolic equivalents [METs] for 3 months prior to the study); nonsmoker; no history of bariatric surgery; and not taking weight loss, lipid, or glucose lowering medications. Peri-menopausal women were excluded from the study, and post-menopausal women (absence of menses for more than 2 years) were required to maintain their current hormone replacement therapy regimen for the duration of the study. The experimental protocol was approved by the Office for the Protection of Research Subjects at the University of Illinois, Chicago, and all volunteers gave their written informed consent to participate in the trial.

Experimental design and randomization

A 12-week, randomized, controlled, parallel-arm feeding trial was implemented to test the effects of ADF, exercise, and ADF combined with exercise (combination group) on body weight, body composition, and CHD risk reduction in obese adults. Subjects were recruited and randomized by the clinical coordinator (SB). Eligible subjects were stratified on the basis of BMI, age, and sex, and then randomized into 1 of 4 groups: 1) combination group; 2) ADF group; 3) exercise group; 4) control group (Figure 2). Randomization was performed for each stratum by selecting an intervention at random from an opaque envelope. The 12-week clinical trial was run 3 times. Recruitment took place during a 4-week period before the beginning of each trial. During the second and third run of the trial, additional subjects were randomized to groups that had high dropout rates (i.e., the ADF and exercise groups). This ensured that the total number of subjects would be the same in each group at the end of the study.

Diet protocol

Only the combination and ADF groups participated in the dietary intervention, which consisted of two periods: 1) a 4-week controlled feeding period and 2) an 8-week self-selected feeding period. During the controlled feeding period (week 1-4) participants consumed 25% of their baseline energy needs on the ''fast day'' (24 h) and consumed food ad libitum on each ''feed day'' (24 h). The baseline energy requirements for the subjects were assessed by the Mifflin equation (11). The diet consisted of a 3-day rotating menu plan, and all fast day meals were prepared in the metabolic kitchen of the Human Nutrition Research Unit (HNRU). 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. The nutrient composition of the provided fast day meals is described in Table 1.

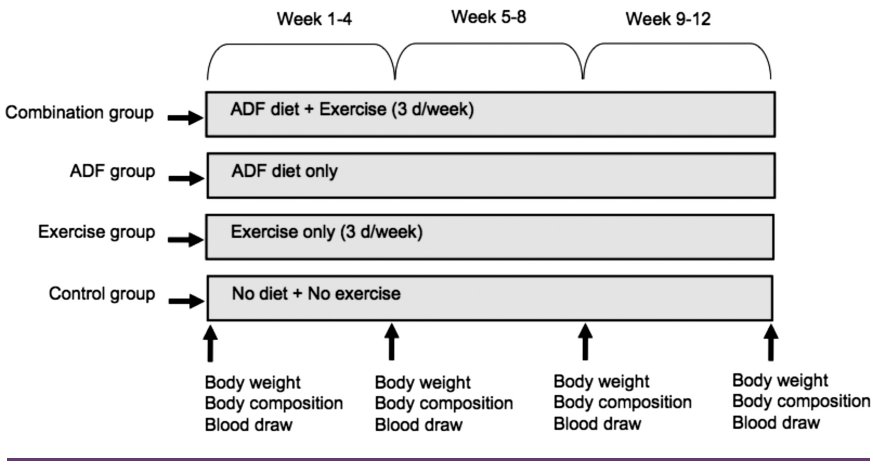

FIGURE 2 Experimental design.

Subjects picked up their food from the HNRU at the beginning of each week, and consumed the food outside of the research center. Subjects were encouraged to drink plenty of water, and were permitted to consume calorie-free foods such as black coffee, tea, water, and sugar-free gum on the fast days. During the self-selected feeding period (week 8-12), subjects continued with the ADF regimen but no fast day food was provided to them. Instead, each subject met with a dietician at the beginning of each week to learn how to maintain the ADF regimen on his or her own at home. During each counseling session, the dietician worked with the subject to develop individualized fast day meal plans. Subjects were also instructed how to make healthy food choices on the ad libitum feed days, by choosing low fat meat and dairy options and increasing fruit and vegetable intake. Throughout the 12-week study, combination and ADF group subjects consumed one meal on the fast day, and an ad libitum number of meals on the feed day. Control and exercise group subjects were asked to maintain their regular food habits and were not provided with any food or dietary counseling. Control and exercise group subjects consumed an ad libitum number of meals everyday.

TABLE 1 Nutrient composition of the fast day diet provided to the combination and ADF groups

	Fast day 1	Fast day 2	Fast day 3
Foods			
Entrée	Vegetarian pizza	Chicken enchilada	Chicken fettuccini
Fruit/Vegetable	Apple	Orange	Carrot sticks
Snack	Peanuts	Crackers	Cookie
Nutrients[a]			
Energy (kcal)	450	450	450
Fat (g)	11 (26%)[b]	12 (22%)[b]	13 (24%)[b]
Saturated fat (g)	4	5	5
Monounsaturated fat (g)	4	5	4
Polyunsaturated fat (g)	3	2	4
Trans fat (g)	0	0	0
Cholesterol (mg)	30	35	35
Protein (g)	29 (22%)[b]	27 (26%)[b]	25 (24%)[b]
Carbohydrate (g)	60 (52%)[b]	60 (52%)[b]	60 (52%)[b]
Fiber (g)	10	10	10

[a]No differences between meals for any nutrient when meals were matched for total kcal. 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.
[b]Percent of energy (kcal).

Exercise protocol

Only the combination and exercise groups participated the exercise intervention. These subjects participated in a moderate intensity exercise program three times per week under supervised conditions, for 12 weeks. Exercise was performed using stationary bikes and elliptical machines at the HNRU. Training intensity was estimated for each individual using an age-predicted heart rate maximum (HRmax) equation [209 − (0.7 × age)] (12) and a Polar Heart Rate Monitor (Polar USA, Inc, NY). Each training session began with a 5-min warm-up period, and ended with a 5-min cool-down. At the beginning of the study (weeks 1-4), each exercise session ran for a 25-min duration and corresponded to 60% of the subject's HRmax. Training duration and intensity increased incrementally at weeks 4, 7, and 10 by 5 min and 5% HRmax. As such, by week 10, each subject was exercising for a 40-min duration at an intensity of 75% HRmax. ADF and control subjects were asked to maintain their regular activity habits, and to refrain from joining an exercise class during the study.

Diet and exercise compliance

During the controlled feeding phase (week 1-4), subjects were instructed to eat only the fast day food provided and to report any extra food item consumed using an ''Extra food log.'' During the self-selected feeding phase, subjects were provided with individualized meal plans that were consistent with their food preferences and prescribed calorie levels for the fast day. Each subject was asked to report any extra food item consumed on the fast day that did not comply with their prescribed plan using the ''Extra food log.'' The log was collected and reviewed by study personnel each week. If

the log indicated that the subject ate an extra food item on a fast day, that day was labeled as ''not adherent.'' If the log revealed that the subject did not eat any extra food item, that day was labeled as ''adherent.'' Percent adherence was calculated by applying the following formula: [% ADHERENCE = # FAST DAYS ADHERENT / # OF FAST DAYS IN THE WEEK × 100]. Exercise compliance was assessed by recording attendance at each supervised exercise session. If an exercise session was missed, the subject was required to make up for the missed session that same week. Subjects were allowed to miss a maximum of 4 out of 36 total sessions.

Blood collection protocol

Twelve-hour fasting blood samples were collected between 6.00 am and 10.00 am at baseline and week 12 (Figure 2). Subjects were instructed to avoid exercise, alcohol, and coffee for 24 h before each visit. Blood was centrifuged for 10 min at 1000*g* and 4°C to separate plasma from RBC and was stored at −80°C until analyzed.

Body weight and body composition assessment

Body weight measurements were taken to the nearest 0.5 kg at the beginning of each week with subjects wearing light clothing and without shoes using a balance beam scale (HealthOMeter; Sunbeam Products, Boca Raton, FL, USA). BMI was assessed as kg/m^2. Fat mass and fat-free mass were assessed each week in triplicate using a tetra-polar bioelectrical impedance analyzer (BIA; Omron HBF-500;Omron Health Care, Bannockburn, IL, USA). Waist circumference was measured by a flexible tape to the nearest 0.1 cm, midway between the lower costal margin and super iliac crest during a period of expiration.

CHD risk indicator determination

Plasma total cholesterol, HDL cholesterol, and triglyceride concentrations were measured in duplicate using enzymatic kits (Biovision Inc, Mountainview, CA, USA) and analyzed using a microplate reader (iMark Microplate Reader; Bio-Rad Laboratories Inc, Richmond, CA, USA). The concentration of LDL cholesterol was calculated using the Friedewald equation (13).

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. Fasting plasma glucose concentrations were measured with a hexokinase reagent kit in duplicate (A-gent glucose test, Abbott, South Pasadena, CA, USA). Fasting insulin was measured as total immunoreactive insulin (Coat-A-Count Insulin, Los Angeles, CA, USA). Insulin resistance (IR) was calculated using the HOMA (Homeostasis Model Assessment) method, by applying the following formula: [HOMA-IR = Fasting insulin (μU/mL) × fasting glucose (mg/dL)/405]. C-reactive protein (CRP) was measured using enzyme-linked immunosorbent assay (Linco Inc., St Charles, MO, USA).

LDL and HDL particle size assessment

LDL and HDL particle size were measured using linear polyacrylamide gel electrophoresis (Quantimetrix Lipoprint System, Redondo Beach, CA, USA) (14). High-resolution 3% polyacrylamide gel tubes were used for electrophoresis. Briefly, 25 μL of sample was mixed with 200 μL of liquid loading gel containing Sudan black, and added to the gel tubes. After photopolymerization at room temperature for 30 min, samples were electrophoresed for 1 h (3 mA/gel tube). Lipoware computer software (Quantimetrix, Redondo Beach, CA, USA) was then used to divide LDL into small (<255 Å), medium (255-260 Å), and large (>260 Å) particles, and HDL into small (<73 Å), medium (73-88 Å), and large (>88 Å) particles (14).

Statistical analysis

Results are presented as mean ± SEM. Normality was assessed by the Kolmogorov-Smirnov test. No variables were found to be not normal. Sample size was calculated as $n = 18$ subjects per group, assuming a 5% decrease in body weight in the combination and ADF groups, with a power of 80% and an α risk of 5%. Differences between intervention groups at baseline were analyzed by a one-way ANOVA. Within-group differences were analyzed using repeated-measures ANOVA. When baseline differences were noted for a specific parameter, ANCOVA was performed with the baseline value as a covariate. An intention-to-treat analysis was performed for all variables measured. A P-value of <0.05 was used as a criterion for statistical significance in all analyses. Data were analyzed using SPSS software (version 20.0 for Mac OSX; SPSS Inc, Chicago, IL, USA).

Results

Subject baseline characteristics and dropouts

There were sixteen completers in each group at the end of the study (Figure 1). There were a greater number of dropouts in the ADF ($n = 9$) and exercise group ($n = 8$) when compared to the combination ($n = 2$) and control groups ($n = 0$). The main reason for subject dropout was scheduling conflicts. There were no between-group differences for age, sex, ethnicity, body weight, height, BMI, waist circumference, plasma lipids, or heart rate at baseline (Table 2). Systolic and diastolic blood pressure values, however, differed between groups at baseline (Table 2). Characteristics of the dropouts ($n = 19$, age: 36 ± 3 years, weight: 95 ± 3 kg, or BMI 35 ± 1 kg/m^2), were not significantly different from those of the completers (age: 42 ± 3 years, weight: 93 ± 2 kg, or BMI 35 ± 1 kg/m^2).

Diet and exercise adherence

The combination and ADF groups were adherent with 81 ± 7% and 80 ± 9% of the fast days, during the 12-week study. There were no differences in percent adherence between the combination group and ADF group ($P = 0.23$). Compliance with the exercise intervention remained high over the course of the study, with the combination group attending 95 ± 2% of sessions, and the exercise group attending 94 ± 1% of sessions. There was no difference between groups for percent adherence to the exercise intervention ($P = 0.83$).

Body weight and body composition

Changes in body weight and body composition are reported in Table 3. Body weight was reduced ($P < 0.05$) in all three intervention groups after 12 weeks. However, weight loss in the combination group (6 ± 4 kg, 7 ± 2% change from baseline) was greater than the weight loss observed in ADF group (3 ± 1 kg, 3 ± 1% change from baseline) and exercise group (1 ± 0 kg, 1 ± 0% change from baseline). BMI also decreased ($P < 0.05$) in all three intervention groups, with greater reductions occurring in the combination group (2 ± 0 kg/m^2), versus the ADF group (1 ± 0 kg/m^2) and exercise group (1 ± 0 kg/m^2). Fat mass decreased ($P < 0.01$) in the combination (5 ± 1 kg) and ADF (2 ± 1 kg) groups only, with greater decreases noted in

TABLE 2 Subject characteristics at baseline

	Combination	ADF	Exercise	Control	P-value [1]
n	18	25	24	16	
Age (y)	45 ± 5	42 ± 2	42 ± 2	49 ± 2	0.158
Sex (F/M)	18/0	24/1	23/1	15/1	0.266
Ethnicity (n)					
African American	7	12	11	11	
Caucasian	5	7	6	3	
Hispanic	6	6	4	2	
Other	0	0	3	0	
Body weight (kg)	91 ± 6	94 ± 3	93 ± 2	93 ± 5	0.904
Height (cm)	160 ± 0	163 ± 0	162 ± 0	162 ± 1	0.896
BMI (kg/m^2)	35 ± 1	35 ± 1	35 ± 1	35 ± 1	0.934
Waist circumference (cm)	96 ± 2	100 ± 2	98 ± 2	99 ± 3	0.636
Lipids (mg/dl)					
Total Cholesterol	190 ± 10	171 ± 8	181 ± 6	185 ± 7	0.394
LDL Cholesterol	125 ± 9	113 ± 8	113 ± 5	119 ± 6	0.588
HDL Cholesterol	50 ± 3	49 ± 2	51 ± 2	52 ± 3	0.831
Triglycerides	77 ± 7	81 ± 7	74 ± 6	97 ± 13	0.251
Systolic blood pressure (mmHg)	113 ± 3[a]	124 ± 3[b]	113 ± 2[a]	122 ± 5[b]	0.022
Diastolic blood pressure (mmHg)	76 ± 2[a]	82 ± 2[b]	76 ± 2[a]	86 ± 2[b]	0.004
Heart rate (bpm)	78 ± 2	75 ± 2	71 ± 2	76 ± 3	0.191

Values reported as mean ± SEM. Intention to treat analysis. BMI: Body mass index, F: Female, M: Male.
[a]P-value between groups at baseline: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test).

the combination group. Fat-free mass was retained in all the intervention groups. Waist circumference decreased ($P < 0.001$) by 8 ± 1 cm and 5 ± 1 cm in both the combination and ADF groups. Less-pronounced decreases ($P < 0.001$) in waist circumference were also noted in the exercise group (3 ± 1 cm).

CHD risk indicators

Plasma lipids, blood pressure, heart rate, glucose, insulin, and CRP concentrations over the 12-week trial are presented in Table 4. Total cholesterol was not affected by any intervention throughout the course of the trial. LDL cholesterol decreased (12 ± 5%, $P < 0.05$) and HDL cholesterol increased (18 ± 9%, $P < 0.05$) in the combination group only. Triglyceride levels were not altered by any intervention. Systolic and diastolic blood pressure was reduced ($P < 0.05$) in the ADF group only by 3 ± 1% and 2 ± 2%, respectively. Heart rate was not affected by any intervention. Fasting glucose was lower ($P < 0.05$) in the combination (92 ± 3 mg/dL) and ADF group (95 ± 5 mg/dL) when compared to the control group (111 ± 6 mg/dL) at week 12. No changes were observed for fasting insulin, HOMA-IR, or CRP values in any intervention group.

LDL and HDL particle size

Changes in LDL particle size are reported in Table 5. An increased proportion of small, dense LDL and HDL particles is associated with increased risk of CHD (15,16). In the present trial, LDL particle size increased ($P < 0.001$) after 12 weeks of treatment in both the combination (4 ± 1 Å) and ADF groups (5 ± 1 Å). The proportion of small atherogenic LDL particles was reduced ($P < 0.01$) in the combination and ADF groups, whereas the proportion of large anti-atherogenic LDL particles was increased ($P < 0.001$) in the ADF group only. The proportion of medium LDL particles remained unchanged in all groups. The exercise intervention had no effect on any parameter of LDL particle size. Changes in HDL particle size are presented in Table 6. A decrease ($P < 0.01$) in the proportion of small HDL particles was noted in the combination group only (week 1: 15 ± 2%, week 12: 11 ± 1%). No other parameters of HDL particle size were affected by the combination, ADF, or exercise interventions.

Discussion

This study is the first to show that the combination of ADF and exercise produces superior changes in body weight, body composition, and lipid indicators of CHD risk, when compared to ADF or exercise alone. More specifically, we report here that the combination group lost more weight (6 kg), and experienced greater decreases in fat mass (5 kg), when compared to individual interventions after 12 weeks. The combination therapy also elicited retention in lean mass; however, this preservation of lean mass was also noted in the ADF and exercise groups. As for CHD risk indicators, the combination of ADF plus exercise decreased LDL cholesterol (12% from baseline)

TABLE 3 Body weight and body composition during the 12-week trial

	Intervention	Week 1	Week 12	P-value[a]	P-value[b]	Change[c]	P-value[d]
Body weight (kg)	Combination	91 ± 6	85 ± 6	<0.001	0.393	−6 ± 4[a]	<0.001
	ADF	94 ± 3	91 ± 3	<0.001		−3 ± 1[b]	
	Exercise	93 ± 2	92 ± 2	0.027		−1 ± 0[b]	
	Control	93 ± 5	93 ± 5	0.577		0 ± 0[c]	
Body mass index (kg/m^2)	Combination	35 ± 1	33 ± 1	<0.001	0.334	−2 ± 0[a]	<0.001
	ADF	35 ± 1	34 ± 1	<0.001		−1 ± 0[b]	
	Exercise	35 ± 1	34 ± 1	0.030		−1 ± 0[b]	
	Control	35 ± 1	35 ± 1	0.707		0 ± 0[c]	
Fat mass (kg)	Combination	45 ± 2	40 ± 2	<0.001	0.054	−5 ± 1[a]	<0.001
	ADF	43 ± 2	41 ± 2	0.008		−2 ± 1[b]	
	Exercise	46 ± 2	45 ± 2	0.182		−1 ± 0[b]	
	Control	43 ± 4	43 ± 4	0.570		0 ± 1[b]	
Fat free mass (kg)	Combination	46 ± 2	46 ± 2	0.221	0.299	0 ± 1	0.527
	ADF	51 ± 2	50 ± 2	0.031		−1 ± 1	
	Exercise	48 ± 1	47 ± 1	0.321		−1 ± 0	
	Control	50 ± 2	49 ± 2	0.693		−1 ± 1	
Waist circumference (cm)	Combination	96 ± 2	88 ± 1	<0.001	0.310	−8 ± 1[a]	<0.001
	ADF	100 ± 2	95 ± 2	<0.001		−5 ± 1[b]	
	Exercise	98 ± 2	95 ± 2	<0.001		−3 ± 1[b]	
	Control	98 ± 3	97 ± 2	0.640		−1 ± 1[b]	

Value reported as mean ± SEM. Intention to treat analysis. ADF: Alternate day fasting.
[a]P-value between week 1 and week 12: Repeated-measures ANOVA.
[b]P-value between groups at week 12: One-way ANOVA.
[c]Absolute change between week 1 and week 12 values.
[d]P-value between groups for absolute change: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test).

while increasing HDL cholesterol (18% from baseline), a change that was not noted for any other intervention. The combination group also experienced an increase in LDL particle size, and a reduction in the proportion of small LDL and HDL particles.

Although this is the first trial to test the effects of ADF combined with exercise, several other investigations have been performed to evaluate the effects of daily calorie restriction (CR) combined with endurance exercise on body weight (17-19). In a recent study by Redman et al. (18), obese men and women participated in an intervention that combined daily CR (25% reduction in energy intake) with a supervised endurance exercise program (5 d/week, 60-min duration, moderate intensity). After 12 weeks of treatment, body weight was reduced by 6% from baseline (18). Interestingly, subjects in the Redman et al. (18) study lost a similar amount of weight (6%) after 12 weeks when compared with the subjects in this study (7%), despite exercising more frequently per week and for longer durations. One possible explanation for the similar weight loss in both trials, despite the different exercise prescriptions, may be the degree to which the subjects were supervised. Although the Redman et al. (18) intervention required that the subjects exercise 5 d/week, only 3 of these sessions were performed under supervised conditions. In contrast, our trial only required that the subjects exercise 3 d/week, but all of these sessions were supervised. Thus, it is possible that the subjects in the Redman et al. (18) study did not perform the exercise as stringently on their own, as they would under supervision. This would result in less total energy expended during exercise per week, which would result in a total weight loss similar to what was observed in the present trial. As for the effect of ADF alone on body weight, the present findings differ slightly with what has been reported previously (7,8). For instance, in two recent trials of ADF that ran for 8 weeks, body weight was reduced by 8% (8) and 6% (7) from baseline, which is greater than the weight loss experienced in the present trial (3%). The less-pronounced weight loss in the present trial may partly be explained by the use of an intention-to-treat analysis, which may have diluted the effect. As for the impact of exercise alone on body weight, the majority of previous studies report very minimal reductions (1-2 kg) after 12 weeks of training (20,21). In a 12-week study by Christiansen et al., a supervised exercise program (3 d/week, 60-75 min duration, 70% HRmax, with an estimated energy expenditure 500-600 kcal/session) reduced body weight by 3.5 kg (22). This minimal effect of exercise on body weight may be explained by the compensatory eating that generally occurs post-exercise training (23). Although exercise blunts hunger acutely for 30 min post-training, most individuals experience a spike in hunger 45-60 min

TABLE 4 Coronary heart disease risk indicators during the 12-week trial

	Intervention	Week 1	Week 12	P-value[a]	P-value[b]	Change (%)[c]	P-value[d]
Total cholesterol (mg/dl)	Combination	190 ± 10	186 ± 12	0.658	0.975	−2 ± 5	0.247
	ADF	171 ± 8	183 ± 11	0.053		7 ± 4	
	Exercise	181 ± 6	181 ± 8	0.921		0 ± 3	
	Control	185 ± 7	187 ± 10	0.784		1 ± 4	
LDL cholesterol (mg/dl)	Combination	125 ± 9	109 ± 11	0.043	0.660	−12 ± 5	0.216
	ADF	113 ± 8	112 ± 9	0.917		−1 ± 6	
	Exercise	113 ± 5	113 ± 7	0.947		0 ± 5	
	Control	119 ± 6	123 ± 8	0.586		3 ± 5	
HDL cholesterol (mg/dl)	Combination	50 ± 3	59 ± 4	0.041	0.194	18 ± 9 [a]	0.016
	ADF	49 ± 2	49 ± 3	0.807		0 ± 4 [b]	
	Exercise	51 ± 2	52 ± 3	0.457		2 ± 3 [b]	
	Control	52 ± 3	56 ± 3	0.166		8 ± 5 [b]	
Triglycerides (mg/dl)	Combination	77 ± 7	87 ± 8	0.161	0.267	13 ± 11	0.759
	ADF	81 ± 7	86 ± 8	0.341		6 ± 6	
	Exercise	74 ± 6	79 ± 5	0.290		7 ± 6	
	Control	97 ± 13	102 ± 11	0.452		5 ± 7	
Systolic BP (mm Hg)[e]	Combination	113 ± 3	111 ± 3	0.262	0.176	−2 ± 2	0.254
	ADF	124 ± 3	120 ± 3	0.007		−3 ± 1	
	Exercise	113 ± 2	115 ± 3	0.284		2 ± 2	
	Control	122 ± 5	120 ± 6	0.603		−2 ± 3	
Diastolic BP (mm Hg)[e]	Combination	76 ± 2	76 ± 2	0.939	0.123	0 ± 3	0.570
	ADF	82 ± 2	80 ± 2	0.034		−2 ± 2	
	Exercise	76 ± 2	76 ± 2	0.976		0 ± 2	
	Control	86 ± 2	84 ± 4	0.480		−2 ± 3	
Heart rate (bpm)	Combination	78 ± 2	76 ± 2	0.384	0.198	−2 ± 2	0.660
	ADF	75 ± 2	75 ± 2	0.711		0 ± 1	
	Exercise	71 ± 2	71 ± 2	0.925		0 ± 2	
	Control	76 ± 3	77 ± 3	0.763		1 ± 5	
Fasting glucose (mg/dl)	Combination	94 ± 2	92 ± 3 [a]	0.589	0.021	−2 ± 4	0.461
	ADF	98 ± 5	95 ± 5 [a]	0.146		−3 ± 2	
	Exercise	92 ± 2	91 ± 2 [a,b]	0.862		−1 ± 2	
	Control	109 ± 7	111 ± 6 [b]	0.637		2 ± 4	
Fasting insulin (μIU/ml)	Combination	14 ± 2	11 ± 2	0.305	0.436	−21 ± 15	0.559
	ADF	23 ± 8	21 ± 8	0.050		−7 ± 6	
	Exercise	11 ± 1	11 ± 1	0.666		0 ± 8	
	Control	25 ± 4	21 ± 4	0.178		−16 ± 9	
HOMA-IR	Combination	3 ± 1	3 ± 0	0.296	0.396	0 ± 17	0.589
	ADF	7 ± 3	7 ± 3	0.092		0 ± 7	
	Exercise	3 ± 0	3 ± 0	0.782		0 ± 10	
	Control	7 ± 2	7 ± 2	0.165		0 ± 11	
C-reactive protein (mg/dl)	Combination	0.5 ± 0.2	0.5 ± 0.2	0.488	0.943	0 ± 28	0.859
	ADF	0.5 ± 0.1	0.5 ± 0.1	0.344		0 ± 12	
	Exercise	0.3 ± 0.1	0.3 ± 0.3	0.349		0 ± 21	
	Control	0.8 ± 0.4	0.8 ± 0.2	0.823		0 ± 25	

Value reported as mean ± SEM. Intention to treat analysis. ADF: Alternate day fasting, BP: Blood pressure, HOMA-IR: Homeostatic model assessment-Insulin resistance.
[a]P-value between week 1 and week 12: Repeated-measures ANOVA.
[b]P-value between groups at week 12: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test).
[c]Percent change between week 1 and week 12 values.
[d]P-value between groups for percent change: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test).
[e]ANCOVA was performed with baseline values as a covariate.

TABLE 5 LDL particle size during the 12-week trial

	Intervention	Week 1	Week 12	P-value[a]	P-value[b]	Change[c]	P-value[d]
LDL particle size (Å)	Combination	260 ± 1	264 ± 2 [a]	<0.001	0.031	4 ± 1 [a]	0.010
	ADF	261 ± 1	266 ± 1 [a]	<0.001		5 ± 1 [a]	
	Exercise	261 ± 2	262 ± 2 [b]	0.426		1 ± 1 [b]	
	Control	259 ± 1	260 ± 2 [b]	0.884		0 ± 1 [b]	
Large LDL particles (%)	Combination	38 ± 4	45 ± 5 [a]	0.142	0.014	7 ± 5	0.064
	ADF	36 ± 3	51 ± 4[a]	<0.001		15 ± 3	
	Exercise	39 ± 3	40 ± 4[b]	0.792		1 ± 5	
	Control	30 ± 3	31 ± 4[b]	0.883		1 ± 4	
Medium LDL particles (%)	Combination	37 ± 2	38 ± 2	0.845	0.301	1 ± 3	0.817
	ADF	37 ± 1	35 ± 1	0.288		−2 ± 2	
	Exercise	41 ± 3	40 ± 3	0.453		−1 ± 2	
	Control	41 ± 2	40 ± 2	0.717		−1 ± 2	
Small LDL particles (%)	Combination	25 ± 3	18 ± 3[a]	0.010		−7 ± 2[a]	0.007
	ADF	27 ± 3	15 ± 3[a]	<0.001	0.023	−12 ± 3[a]	
	Exercise	21 ± 3	20 ± 4[b]	0.972		−1 ± 4[b]	
	Control	29 ± 3	30 ± 3[b]	0.776		1 ± 3[b]	

Value reported as mean ± SEM. Intention to treat analysis. ADF: Alternate day fasting. Large LDL particles (>260 Å), medium LDL particles (255-260 Å), and small LDL particles (<255 Å).
[a]P-value between week 1 and week 12: Repeated-measures ANOVA.
[b]P-value between groups at week 12: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test).
[c]Absolute change between week 1 and week 12 values.
[d]P-value between groups for absolute change: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test).

TABLE 6 HDL particle size during the 12-week trial

	Intervention	Week 1	Week 12	P-value[a]	P-value[b]	Change[c]	P-value[d]
Large HDL particles (%)	Combination	32 ± 3	34 ± 3	0.102	0.783	2 ± 1	0.106
	ADF	34 ± 2	32 ± 2	0.157		−2 ± 1	
	Exercise	32 ± 2	31 ± 1	0.818		−1 ± 1	
	Control	34 ± 3	34 ± 3	0.777		0 ± 1	
Medium HDL particles (%)	Combination	53 ± 2	54 ± 2	0.191	0.231	1 ± 1	0.904
	ADF	52 ± 2	53 ± 1	0.885		1 ± 1	
	Exercise	55 ± 2	55 ± 2	0.747		0 ± 1	
	Control	49 ± 2	50 ± 2	0.423		1 ± 1	
Small HDL particles (%)	Combination	15 ± 2	11 ± 1	0.007	0.134	−4 ± 1[a]	0.006
	ADF	13 ± 1	15 ± 2	0.136		2 ± 1[b]	
	Exercise	13 ± 1	14 ± 1	0.709		1 ± 1[b]	
	Control	16 ± 2	16 ± 2	0.663		0 ± 1[b]	

Value reported as mean ± SEM. Intention to treat analysis. ADF: Alternate day fasting. Large HDL particles (>88 Å), medium HDL particles (73-88 Å), and small HDL particles (<73 Å).
[a]P-value between week 1 and week 12: Repeated-measures ANOVA.
[b]P-value between groups at week 12: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test).
[c]Absolute change between week 1 and week 12 values.
[d]P-value between groups for absolute change: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test).

post-exercise (24). This surge of hunger generally results in compensatory energy consumption that negates the extra energy expended with exercise (23). As such, energy intake balances out with energy expenditure, and little weight is lost as a result.

Body composition was also preferentially altered in the combination group when compared with the individual interventions. We report here that the combination group lost a greater degree of fat mass (5 kg), when compared with the ADF group (2 kg). The combination therapy also resulted in retention of lean mass. These results suggest that combining ADF plus exercise may preserve lean mass at the expense of fat mass during period of energy restriction. Retention of lean mass is highly beneficial as it maintains resting metabolic rate (RMR) (25). More specifically, maintaining lean mass while dieting ensures that RMR will be kept high, which can allow for a higher hourly energy burning capacity and greater weight loss. This preservation of lean mass was also noted in the ADF and exercise groups. This finding is in accordance with previous 12-week endurance exercise trials (21,26). On the contrary, the effects of ADF on lean mass are equivocal. Although one study indicates a possible retention in lean mass with 8 weeks of ADF (27), another trial indicates a reduction in lean mass (8). More work in this area is evidently required before solid conclusions can be reached regarding the efficacy of ADF for lean mass retention. Visceral fat mass (measured indirectly by waist circumference) was decreased in the combination (8 cm) and ADF groups (5 cm). Reductions in waist circumference were also noted in the exercise group (3 cm). These findings for waist circumference are in line with what has been reported previously for ADF (27) and short-term exercise interventions (22,28).

Previous studies of ADF consistently demonstrate reductions in LDL cholesterol and triglyceride concentrations (6,8,27). Short-term endurance exercise interventions ($\leq$12 weeks), on the contrary, have been shown to increase HDL cholesterol levels (29,30), although results are not consistent (31,32). In this study, only the combination intervention observed decreases in LDL cholesterol and increases in HDL cholesterol concentrations. No interventions lowered triglyceride levels. The reason why LDL cholesterol and triglycerides were not reduced in the ADF-only group, and why HDL cholesterol did not increase in the exercise-only group, is not clear. LDL cholesterol has been estimated to decrease by 2.0 mg/dL/kg of weight loss (33). As such, it is possible that the decrease in body weight by the ADF group was not substantial enough to produce significant reductions in LDL cholesterol. In addition, it should be noted that the varying effects of these interventions on LDL cholesterol may be attributable to baseline LDL cholesterol levels. For instance, LDL cholesterol of ADF group at baseline (113 mg/dL) was lower (though not significantly) than that of the combination group (125 mg/dL). As the LDL cholesterol concentration of the ADF group was already near optimal (34), this could possibly explain why less of a LDL cholesterol-lowering effect was noted in this group.

Remarkably, both the combination and ADF groups experienced increases in LDL particle size and reductions in the proportion of small LDL particles post-treatment. An increased proportion of small, dense LDL particles is strongly associated with the development of CHD (35). Potential mechanisms that link small LDL particles to increased risk of vascular events include augmented oxidizability (36) and increased permeability through the endothelial barrier (37). In view of this, our findings suggest that both the combination and ADF groups may confer cardio-protection by increasing LDL particle size and lowering the proportion of small particles. This increase in particle size in the absence of reduced LDL cholesterol concentrations (as noted in the ADF group) is not common, but has been reported previously (38). The combination group also experienced decreases in the proportion of small HDL particles. The mechanisms that link small HDL particles to increased CHD risk have yet to be firmly established, but may involve the altered activity of lipases involved with the maturation and transformation of lipoproteins (39). This beneficial effect of the combination therapy on HDL particle size further strengthens our key finding that this lifestyle therapy is more cardio-protective than ADF or exercise alone.

There are some limitations that should be considered when interpreting these results. First, our trial duration (12 weeks) may have not been long enough to observe changes in plasma lipids (10). Evidence suggests that HDL cholesterol may require $>$16 weeks to be altered with endurance training (10). Second, the exercise intensity (60-75% HRmax) and frequency (3 d/week) may have not been sufficient to alter CHD risk indicators (40). HDL cholesterol concentrations generally only show consistent improvements with an exercise intensity exceeding 75% HRmax at a frequency of 5 d/week (40). Third, we used BIA to measure fat mass and fat free mass instead of a more robust method, such as dual-energy X-ray absorptiometry (DXA). BIA is limited in that it may underestimate fat mass and overestimate fat-free mass in obese individuals. Fourth, we recruited subjects solely from the University of Illinois, Chicago campus, which undoubtedly impacts the generalizability of our findings. More specifically, the subjects that partook in the study may differ from the general population in terms of race, education, and income. Fifth, the large number of dropouts in the ADF and exercise groups is also a limitation. Nevertheless, as the majority of these dropouts were because of scheduling conflicts and not adherence issues, these lifestyle therapies can still be considered viable options for weight loss and CHD risk reduction. Sixth, our randomization procedure may be flawed in that we chose to randomize additional subjects into groups that had high dropout rates. This uneven allocation of subjects to the intervention groups may have negatively impacted the internal validity of the study. These factors should be considered when interpreting the study findings.

In summary, our results suggest that the combination of ADF plus endurance training results in greater body composition and lipid-altering effects than that of each intervention alone. For instance, the combination therapy produced greater weight loss and fat loss than the ADF-only and exercise-only groups, while eliciting retention of lean mass. This lifestyle regimen also favorably altered LDL and HDL cholesterol concentrations, and decreased the proportion of small LDL and HDL particles. Thus, the combination of ADF plus exercise may be implemented as a viable lifestyle intervention to help obese individuals lose weight, retain lean mass, and lower their risk of CHD.O

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SB designed the experiment, conducted the clinical trial, analyzed the data, and wrote the manuscript. MCK and CMK assisted with the conduction of the clinical trial. JFT assisted with the preparation of the manuscript and the analysis of the data. KAV assisted with the design of the experiment and wrote the manuscript.

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