FULL PROTOCOL

Alternate day fasting for weight loss, weight maintenance and cardio-protection

Abstract

Overweight and obese individuals are at increased risk for coronary heart disease (CHD). Losing weight by means of dietary restriction greatly reduces vascular disease risk. Daily calorie restriction is the most commonly implemented dietary restriction protocol. Another dietary restriction regimen employed, although far less commonly, is alternate day fasting (ADF). ADF involves a "feed day" where food is consumed ad-libitum, alternated with a "fast day", where food intake is partially reduced. Preliminary data suggests that adherence to ADF exceeds that of CR after 8 weeks of treatment. This increased adherence to ADF results in greater weight loss, which produces more pronounced improvements in CHD risk. What has yet to be determined is whether adherence to ADF remains near maximal for longer treatment durations (24 weeks), and if this improved adherence results in greater reductions in body weight and CHD risk, versus CR. Once weight loss is achieved, weight maintenance is extremely important as CHD risk can increase if weight is regained. Whether ADF is an effective strategy for weight maintenance remains unknown. Accordingly, the aims of this proposal are: Aim 1: To establish that adherence to ADF is greater than that of CR during a 24-week intervention period and to determine if increased adherence to ADF results in greater weight loss; Aim 2: To establish that greater reductions in body weight by ADF over a 24-week period will result in greater improvements in *traditional* CHD risk parameters (blood pressure, plasma lipid levels, and CRP) and *emerging* CHD risk parameters (fat cell-derived hormones and body fat distribution) in comparison to CR; and Aim 3: To establish that ADF is an effective diet therapy to maintain weight loss and sustain improvements in CHD risk indicators. A 52-week randomized, controlled, parallel-arm feeding trial will be implemented to test these objectives. The trial will be divided into 3 consecutive intervention periods: (1) 4-week baseline; (2) 24-week weight loss with food provided for 12-weeks; and (3) 24-week weight maintenance with no food provided. Overweight and obese subjects (n = 90) will be randomized to 1 of 3 groups: (1) ADF, 75% energy restriction on the "fast day" and 125% energy intake on the "feed day"; (2) CR, 25% restriction everyday; or 3) control, 100% energy intake everyday. During the weight maintenance phase, ADF subjects will consume 50% of their energy needs on the "fast day" and 150% of energy needs on the "feed day", while CR and control subjects will consume 100% of their needs everyday. Our findings will show that ADF can be implemented as an *alternative* to CR to help overweight and obese individuals lose weight, maintain weight loss, and sustain reductions in CHD risk.

1. Specific aims

Evidence suggests that a 10% increase in body weight is associated with a 30% increase in coronary heart disease (CHD) risk [1]. Losing weight by means of dietary restriction greatly reduces an individual's risk of CHD [2]. The most common dietary restriction protocol implemented is daily calorie restriction (CR), which involves reducing energy intake by 15 to 40% of needs daily. Another dietary restriction regimen employed, although far less commonly, is alternate day fasting (ADF) [3]. ADF regimens include a "feed day" where food is consumed ad-libitum over 24-h, alternated with a "fast day", where food intake is reduced by 75% for 24-h. The degree of energy restriction achieved by ADF is equivalent to that of CR over a 48-h period. These two dietary restriction regimens differ, however, in that adherence to ADF exceeds that of CR. It is well established that adherence to CR begins to diminish after *8 weeks* of treatment [4-6]. As for ADF, preliminary findings indicate that adherence to ADF remains steady for up to 12 weeks of diet. Preliminary data also show that this increased adherence to ADF results in a greater degree of weight loss, when compared to CR. Whether improved adherence to ADF results in substantially more weight loss over a longer trial period (24 weeks), when compared to CR, has yet to be elucidated.

Preliminary findings also show that greater weight reduction by ADF results in more pronounced improvements in "traditional" CHD risk factors, when compared to CR. Traditional CHD risk factors (blood pressure, plasma lipid levels, and C-reactive protein (CRP)) improve considerably in response to weight loss [1]. Although the mechanisms remain unclear, evidence suggests that fat cell physiology (i.e. fat cell-derived hormones and body fat distribution) may link weight loss to vascular disease risk reduction [7,8]. As such, these fat cell parameters are now considered "emerging" indicators of CHD risk. Whether greater reductions in body weight after 24 weeks of ADF can produce more pronounced improvements in each of these CHD risk indicators, relative to CR, is not yet known. Once weight loss is achieved, maintaining post-reduction body weight is extremely important as CHD risk can increase if weight is regained. Whether ADF is an effective strategy for weight maintenance is an important question that has yet to be addressed. Accordingly, the specific aims of the proposed study are:

Specific aim 1: To establish that adherence to ADF is greater than that of CR during a 24-week intervention period and to determine if increased adherence to ADF results in greater weight loss.

> **Hypothesis 1:** ADF subjects will be more adherent with diet than CR subjects, resulting in greater energy restriction and weight loss by ADF when compared to CR.

Specific aim 2: To establish that greater reductions in body weight by ADF over a 24-week period will result in greater improvements in *traditional* and *emerging* CHD risk indicators in comparison to CR.

> **Hypothesis 2:** ADF subjects will experience greater improvements in traditional CHD risk indicators (blood pressure, plasma lipid levels, and CRP) and emerging CHD risk indicators (fat cell-derived hormones and body fat distribution) over 24 weeks when compared to CR subjects, due to larger reductions in body weight by ADF diets.

Specific aim 3: To establish that ADF is an effective diet therapy to maintain weight loss and sustain improvements in CHD risk indicators.

> **Hypothesis 3:** ADF subjects will maintain their post-reduction body weight and sustain their improvements in CHD risk, while CR subjects (on a weight maintenance diet) will regain weight and regress to their baseline level of CHD risk, from weeks 25 to 48 of the trial.

To test the study objectives, a 52-week randomized, controlled, parallel-arm feeding trial, divided into 3 consecutive periods: (1) 4-week baseline; (2) 24-week weight loss with food provided for 12 weeks; and (3) 24-week weight maintenance with no food provided, will be implemented. Overweight and obese subjects (n = 90) will be randomized to 1 of 3 groups: (1) ADF (n = 30), 75% energy restriction on the fast day and 125% energy intake on the feed day; (2) CR (n = 30), 25% restriction everyday; or 3) control (n = 30), 100% energy intake everyday.

2. Background

Dietary restriction for weight loss and prevention of CHD. The ability of calorie restriction (CR) to facilitate weight loss and protect against coronary heart disease (CHD) in humans is well documented [9,10]. In contrast, only 4 human trials have been performed for alternate day fasting (ADF) [11-14], and only 2 of those [13,14] have examined the effects of ADF on weight loss and CHD risk reduction. Results from these weight loss trials indicate that 8 weeks of ADF (25% energy intake on fast day, ad libitum intake on feed day) reduced body weight by 6-8%, while lowering LDL cholesterol and triglyceride levels by 10-25% and 30-40%, respectively, in overweight and obese adults [13,14]. Although these short-term trials show that ADF is effective for weight loss and cardio-protection, long-term effects of ADF remain unknown.

Weight loss by CR or ADF is directly related to dietary adherence (Specific aim 1). Although CR is more frequently implemented than ADF, many obese patients find it difficult to adhere to CR since food intake must be limited *every day*. As such, adherence to CR begins to diminish at 8 weeks of treatment, and continues to decline for the remainder of the treatment period [4-6]. This lack of adherence equates to less total weight loss per week. ADF, on the other hand, allows individuals to eat ad libitum *every other day*, which greatly increases adherence to these protocols. However, no trial to date, other than our pilot study, has demonstrated that ADF does in fact augment adherence.

Weight loss improves <u>traditional</u> CHD risk indicators (Specific aim 2). Weight loss plays a key role in modifying traditional CHD risk factors. However, in order for beneficial modulations to occur, a certain degree of weight loss must be achieved, and varies for each parameter. For instance, a 10% reduction in body weight is required to lower systolic and diastolic blood pressure [15,16]. As for plasma LDL cholesterol, and triglycerides, a 10% weight loss is needed [17-20]. In order to increase HDL cholesterol and decrease CRP, a 15% weight loss is required [17,21,22]. Thus, in order to improve all of these traditional risk factors, *15% weight loss is optimal*.

Degree of weight loss required to reduce CHD risk and role of ADF versus CR (Specific aim 2). The majority of CHD risk indicators only improve with 15% weight loss. In view of our pilot findings, we hypothesize that ADF subjects will be more adherent with diet, and will experience a 15% weight loss by 24 weeks. Based on the literature [4-6], the CR group will not achieve a 15% weight loss by week 24. This enhanced weight loss by ADF will improve all of the above-mentioned CHD risk factors, while CR will only improve those indicators requiring a 10% reduction in weight.

Alternate day fasting for weight maintenance (Specific aim 3). Reports of long-term weight loss success are generally discouraging [23]. Individuals who are successful in maintaining significant amounts of weight loss over time report regular self-monitoring of weight and food intake, and increased cognitive restrained eating behavior [23,24]. We hypothesize that preserving the ADF meal pattern without restricting energy, will help these subjects self-monitor energy intake and augment dietary restraint. These behavioral changes that occur with ADF will assist these individuals in preventing weight regain.

3. Significance. If the aims of this application are achieved, this study will:

1. Advance clinical practice guidelines by showing that ADF can be implemented as an *alternative* to CR to help overweight and obese individuals lose larger amounts of weight, which will lead to more pronounced reductions in CHD risk in this population
2. Further uncover the intermediate role that adipose tissue plays in mediating CHD risk
3. Demonstrate that ADF is effective for weight maintenance and sustained cardio-protection

4. Innovation. This study will be the first to directly compare the time-course effects of ADF to that of CR on physiological variables, and show that ADF is more effective than CR for weight loss, weight maintenance, and CHD risk reduction. In sum, the proposed study is innovative in that it will be the first to:

1. Challenge existing clinical paradigms by proposing that ADF be implemented as an alternative to CR for weight loss and weight maintenance in overweight and obese individuals at risk for CHD
2. Employ novel markers, i.e. fat cell parameters, to assess the impact of ADF on CHD risk

5. Approach

5.1 Overview: A 52-week randomized, controlled, parallel-arm feeding trial will be implemented to test the effects of ADF versus CR on weight loss, CHD risk reduction, and weight maintenance in overweight and obese adults. Subjects (n = 90) will be randomized to 1 of 3 groups: 1) ADF, 2) CR, or 3) control.

5.2 Inclusion criteria. Adult subjects meeting the following criteria will be eligible to participate:

- Age between 18-65 years old
- BMI between 25.0 and 39.9 kg/m^2
- Previously sedentary (<60 minutes/week of light activity corresponding to 2.5 to 4.0 metabolic equivalents (METs) for the 3 months prior to the study)

Rationale for inclusion. Overweight or obese adults who are inactive will be included as they are: 1) candidates for weight loss therapy, and 2) at risk for developing CHD, thus any influence of diet on CHD risk should be readily observable.

5.3 Exclusion criteria. Subjects excluded from participating in the study include those who:

- Have a history cardiovascular disease (prior angina, myocardial infarction or stroke)
- Are diabetic (fasting blood glucose > 126 mg/dl)
- Are taking anti-depressant or anti-anxiety medications
- Are taking drugs that affect study outcomes (weight loss medications)
- Are not weight stable for 3 months prior to the beginning of study (weight gain or loss > 4 kg)
- Are not able to keep a food diary or activity log for 7 consecutive days during screening
- Are perimenopausal or have an irregular menstrual cycle (menses that does not appear every 27-32 days)
- Are claustrophobic or have implanted electrical devices (cardiac pacemaker or a neurostimulator)
- Are pregnant, or trying to become pregnant
- Are smokers

Rationale for exclusion. The goal of this study is to examine the efficacy of ADF versus CR for the prevention of CHD, thus, individuals who have already experienced an atherothrombotic event will be excluded. Diabetic individuals will be excluded since key outcome measures (i.e. plasma lipids) respond differently in diabetics versus non-diabetic subjects [25]. Taking anti-depressant or anti-anxiety medication is an indicator of a diagnosed psychiatric disorder, thus these individuals will be excluded. Moreover, individuals taking drugs to control body weight will be excluded, as these medications may confound the effect of diet on the key outcome measure. Subjects who are not weight stable will be excluded since a weight stable baseline must be set in order to attribute physiological effects to the intervention. Individuals who are unable to adequately report dietary intake or physical activity will also be excluded. Being perimenopausal, or having long or irregular menstrual cycles may be associated with altered lipid metabolism and metabolic disturbances [26], thus these women will be excluded. Restricting energy during pregnancy can harm the developing foetus, therefore pregnant women will be excluded [27]. Smokers will be excluded since smoking alters lipid metabolism [28].

5.4 Recruitment: Independently living subjects from Chicago and greater area will be recruited at the Human Nutrition Research Unit at the University of Illinois at Chicago. **Screening:** Screening will be performed by the Study Manager, John Trepanowski, at the Human Nutrition Research Unit. Each subject will attend 2 screening visits. During **Visit 1** subjects will be screened by a questionnaire, which will assess eligibility based on the requirements listed above. The following parameters will also be assessed: body weight and height (for BMI) and a pregnancy test. The Study Manager will also distribute a 7-d food and physical activity record, and provide detailed instructions on how to complete the records. Since this is a very time-intensive trial, the purpose of **Visit 2** (scheduled 10 d after the first screening) is to evaluate each subject's motivation to participate in the study. As such, subjects who do not attend the second visit, or who do not adequately complete 7-d food and physical activity records, will be excluded. Written informed consent will be obtained prior to screening.

Randomization: Subjects will be randomized by the Biostatistician, Dr. Sally Freels, Ph.D., by way of a stratified random sample. The sample frame will be divided into strata based on BMI, sex, age. Subjects from each stratum, will then be randomized to 1 of 3 groups: 1) ADF, 2) CR, or 3) control.

5.5 Sample size and power

Sample size needed for ADF to achieve greater weight loss than CR:									
Outcome	ADF At wk 24	CR At wk 24	Dif	SD	α	1-β	n/group	Dropout rate (estimated)	n/group with dropouts
Body weight	-15% (estimated)	-10% Ref [29]	5%	6.1 Ref [29]	0.05	0.80	26	12%	30

Dif: Significant difference between CR and ADF group at week 24. **SD:** Used for both ADF and CR. **Dropout rate:** Based on dropout data from previous 24-week CR trial. Calculations are for two-tailed independent samples t-tests.

Calculations are based on hypothesis 1 (weight loss) as this is the primary outcome measurement for the study. Calculations were performed using Power Analysis and Sample Size (NCSS Statistical software, PASS, 2005). We estimate that the ADF group will reduce their initial body weight by 15% by week 24. As for the CR group, Redman et al. [29] investigated the efficacy of 24 weeks of CR on weight loss in overweight and obese men and women. They report a weight loss of 9.8%, with an SD of 6.1. Using the SD from Redman et al. for both the ADF and CR group, we calculated that n = 26 subjects per group will provide 80% power to detect a significant difference of 5% in body weight *between* the ADF and CR group at week 24, using a two-tailed independent-samples t-tests with $\alpha = 0.05$. Based on dropout data from the literature [29], we anticipate a drop out rate of 12%. Thus, an initial recruitment sample of 90 (n = 30 per group) will yield 78 subjects (n = 26 per group) that complete the trial.

5.6 Study design

Baseline period (4 weeks): Before commencing the 24-week diet intervention, each subject will participate in a 4-week baseline weight maintenance period. During this period, subjects will be requested to maintain a stable weight and continue eating their usual diet. Baseline weight-maintenance energy requirements (assumed to be equal to total energy expenditure (TEE)) will be measured by the doubly labeled water (DLW) technique. Clinical parameters (body weight, circulating CHD risk factors) will be assessed at the beginning and end of this period.

Weight loss/ dietary intervention period (24 weeks): After this period, subjects will be randomized into 1 of 3 groups. Food will be provided from weeks 1-12 in the ADF and CR groups. All diets will be prepared in the metabolic kitchen of the Human Nutrition Research Center. Study diets will be formulated for each participant using Nutritionist Pro (Axxya Systems). The diets will be provided as a 3-day rotating menu, and will be formulated based on the American Heart Association (AHA) guidelines (30% kcal from fat, 15% from protein, 55% from carbohydrate). All meals will be consumed outside of the research center. Participants will be requested to eat only the foods provided on calorie-restriction days and to bring back any leftover foods. Subjects will be advised to drink plenty of water. Calorie-free foods (i.e. black coffee, diet sodas) will be permitted as desired. During weeks 13-24, food will no longer be provided. Instead, ADF and CR subjects will attend weekly dietary counseling sessions where they will learn how to follow their diet on their own at home.

- **ADF weight loss protocol:** Food will be provided on the feed and fast days during weeks 1-12. ADF subjects will be energy restricted by 75% of their TEE on the fast day and eat 125% of needs on the feed day. Fast day meals will be consumed between 12.00 pm and 2.00 pm to ensure that each ADF subject is fasting for the same duration. For the following 12-weeks (weeks 13-24), only dietary counseling will be provided, and ADF subjects will be energy restricted by 75% of their TEE on the fast day and eat food ad libitum on the feed day.
- **CR weight loss protocol:** CR subjects will be restricted by 25% of their TEE every day, and will be provided with all calorie-restricted meals.
- **Control diet protocol:** Control subjects will eat ad libitum at home every day, but will visit the research center at the same frequency as the treatment groups to alleviate investigator-interaction bias between groups.

Visits to the research center. From week 1 to 24, subjects will visit the research center once per week for meal pickup and clinical measurements (total of 24 visits). All study activities will take place on 1 day per week to minimize subject burden. The trial will be staggered so that subjects will be recruited in 9 overlapping stages. At each stage, n = 3-4 ADF, n = 3-4 CR, and n = 3-4 control subjects will be recruited (n = 10 subjects will begin at the same time at each stage). These 10 participants will then be randomly assigned either a standing Monday, Tuesday, or Wednesday appointment day. This staggering protocol will divide this large sample in manageable sizes. Subjects will pick up his or her food from the research center in the morning on their pre-assigned day. All meals for the week will be packaged in a rolling insulated cooler that can be conveniently transported. Subjects will be given reminder phone calls the night prior to their appointment. If a subject misses their appointment, they will be required to reschedule their appointment for the following day. If a subject completely misses their weekly appointment (i.e. does not pick up their food for the week), food will be delivered to the subjects, and study personnel will reinforce the importance of attending these visits.

Project timeline

Study activity / Study months			Year 1		Year 2		Year 3		Year 4	
			1-6	7-12	13-17	18-24	25-30	31-36	37-42	43-48
1. Coordination of study personnel			--							
2. Subject recruitment			----------							
3. Subject screening			----------							
Subjects	n /group	Total n								
4. Main study:	4 ADF	4	Stage 1 ----------							
	3 CR	7	----------							
Trial length: 52-weeks	3 CON	10	----------							
	3 ADF	13	Stage 2	----------						
	4 CR	17		----------						
Subjects staggered into 9 overlapping stages	3 CON	20		----------						
	3 ADF	23	Stage 3		----------					
	3 CR	26			----------					
	4 CON	30			----------					
n = 3-4 /group recruited at each stage	4 ADF	34	Stage 4			----------				
	3 CR	37				----------				
	3 CON	40				----------				
	3 ADF	43	Stage 5				----------			
New stage begins every 3 months	4 CR	47				----------				
	3 CON	50				----------				
	3 ADF	53	Stage 6				----------			
	3 CR	56					----------			
	4 CON	60					----------			
	4 ADF	64	Stage 7					----------		
	3 CR	67						----------		
	3 CON	70						----------		
	3 ADF	73	Stage 8						----------	
	4 CR	77							----------	
	3 CON	80							----------	
	3 ADF	83	Stage 9							----------
	3 CR	86								----------
	4 CON	90								----------
5. Prep/submission: Abstracts										----------
Manuscripts										----------

Weight maintenance period (24 weeks): During this phase, all subjects will visit the research center at 4-week intervals. ADF and CR subjects will maintain their post-reduction body weight by attending dietary counseling sessions (no food will be provided). Weight-maintenance energy requirements (TEE) will be reassessed by the DLW at the beginning of this period. The Dietician will meet with ADF and CR subjects to develop individualized weight maintenance meal plans. These plans will include menus, portion sizes, and food lists that are consistent with their food preferences and prescribed calorie levels for weight maintenance. Food scales will be provided to help with appropriate portioning. Participants will meet with the Dietician every 4 weeks throughout this period to review and modify the meal plans. During

these sessions, subjects will also be instructed how to make general healthy food choices that conform to AHA guidelines and learn behavioral weight maintenance techniques.

- **ADF weight maintenance protocol:** ADF subjects will consume 50% of their post-reduction TEE on the fast day and eat ad libitum on the feed day to maintain body weight. Fast day meals will be consumed between 12.00pm and 2.00 pm to ensure similar fasting durations between subjects.
- **CR weight maintenance protocol:** CR subjects will eat 100% of their TEE to maintain body weight.
- **Control diet protocol:** Control subjects will eat ad libitum at home every day, but will visit the research center at the same frequency as the treatment groups to alleviate investigator-interaction bias between groups.

Monitoring of daily physical activity habits. Changes in daily energy expenditure associated with alterations in physical activity habits will be quantified by the use of a validated [30] pattern recognition monitor (Sense Wear Mini (SWM), Bodymedia, Pittsburg, PA). Subjects will wear the lightweight monitor on their upper arm for 7 d at week 1, 24, and 48 of the trial. The SWM is a wireless multi-sensor activity monitor that integrates motion data from a triaxial accelerometer along with several other physiological sensors (heat flux, skin temperature and galvanic skin response). Participants will be asked to keep a diary of non-wearing periods (i.e. showering), and these gaps in data will be manually filled with a corresponding MET equivalent for "self care activities" [31]. The data will be processed using Bodymedia Software V.7.0, algorithm V.2.2.4 [30].

Blood sampling protocol. Twelve-hour fasting blood samples will be collected in each group to measure CHD risk parameters. Blood will be centrifuged for 15 min at 520 x g and 4°C to separate plasma from RBC, and will be stored at -80°C.

Subject compensation. Each subject will receive $1000 upon completion of the trial.

5.7 Measurement of outcomes

Time points for outcome measurements

Dietary intervention period (week 1 to 24)																								
Outcome measured	**Study week**																							
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**
Aim 1																								
DLW/adherence	•																							•
Body weight	•				•				•				•				•				•			•
Aim 2																								
Blood pressure	•												•											•
Plasma lipids	•												•											•
CRP	•												•											•
Adipokines	•												•											•
Fat distribution	•																							•

Weight maintenance period (week 25 to 48)																								
Outcome measured	**Study week**																							
	25	**26**	**27**	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**
Aim 3																								
Body weight				•				•				•				•				•				•
Blood pressure												•												•
Plasma lipids												•												•
CRP												•												•
Adipokines												•												•
Fat distribution																								•

Specific aim 1: To establish that adherence to ADF is greater than that of CR during a 24-week intervention period and to determine if increased adherence to ADF results in greater weight loss.

Doubly labeled water protocol: The TEE of the subjects will be measured over a 14-d period at baseline and week 24. At the start of each TEE measurement, the subject will be fasted overnight and given an oral dose of DLW ($^2H_2{}^{18}O$) containing 0.22 g $H_2{}^{18}O$/kg estimated total body water and 0.115 g ^2H_2O/kg total body water after collection of 2 independent baseline urine specimens. The subjects will then required to remain fairly sedentary and not to consume any food or water while urine samples are collected from complete voids made at 3, 4.5, and 6 h after dose administration. After completion of urine collections, the subjects will be discharged from the unit and carry out their usual daily activities for 14 d, with supervised urine specimen collection on days 7 and 14. This standard, nonradioactive isotopic method has been extensively validated and is described elsewhere [32,33]. Abundances of $H_2{}^{18}O$ and ^2H_2O in dilutions of the isotope doses and in urine specimens will then be measured in duplicate using IRMS [34]. Isotope elimination rates (kd and ko) will be calculated by using linear regression of logged values, and carbon dioxide production will be calculated by using the equations of Schoeller [35], as modified by Racette et al [36]. TEE will then be calculated on the basis of an assumed respiratory quotient of 0.86.
Dietary adherence: Measurements of TEE obtained at baseline and week 24 will be used to calculate the actual energy intake of the subjects at these time periods. Because energy intake is equal to TEE plus the change in energy stores (when a subject is not in neutral energy balance), TEE data can be used to calculate a value for energy intake unbiased by subject reporting, by correcting for the estimated change in body energy stores during the same period based on weight change [37]. Individual values for weight change during the DLW period will be calculated from the regression of daily measurements of body weight made during the period of TEE measurements. The energy content of weight change will then be calculated assuming energy content of weight loss of 7.4 kcal/g [38]. **Weight loss:** Body weight will be measured at 4-week intervals, in the morning, in the fasted state, without shoes, and in a hospital gown. Measurements will be taken to the nearest 0.25 lb using a balance beam scale.

Specific aim 2: To establish that greater reductions in body weight by ADF over a 24-week period will result in greater improvements in *traditional* and *emerging* CHD risk indicators in comparison to CR.

Measurement of traditional CHD risk factors

Plasma lipid levels and blood pressure: Plasma total, HDL cholesterol, and triglyceride concentrations will be determined by enzymatic kits (Roche Diagnostics, Indianapolis, IN). Samples will be assessed in duplicate. LDL cholesterol will be determined by the Friedewald equation [39]. Blood pressure will be assessed every week after the weigh-in following a 10 min rest. **C-reactive protein**: Plasma CRP concentrations will be measured by a commercially available, highly sensitive assay (Behring Diagnosis, Westwood, Mass).

Measurement of emerging CHD risk factors

Adipokine plasma levels: Plasma adiponectin, leptin, and resistin concentrations will be measured by commercially available ELISA kits (R&D Systems, Minneapolis, MN). Samples will be assessed in duplicate. **Body fat distribution:** Subcutaneous and visceral fat mass will be assessed at 24-week intervals by magnetic resonance imaging (MRI). MRI scans will be performed at the Center for Magnetic Resonance Research (located 3 blocks from the research center). Images will be obtained using a 3.0 Tesla MRI scanner. Briefly, the subjects will be placed in a prone position, and the scan will be conducted in two parts: 1) upper body scan, and 2) lower body scan, as described previously [40]. The 4th and 5th lumbar (L_4-L_5) vertebrae will act as the dividing point between the upper and lower body regions. All MRI data will be analyzed using the Hippo Fat program.

Specific aim 3: To establish that ADF is an effective diet therapy to maintain weight loss and sustain improvements in CHD risk indicators.

Weight maintenance: Body weight will be measured at 4-week intervals at the research center in the morning, in the fasted state, without shoes, and in a hospital gown.
CHD risk factors: During the weight maintenance period, traditional and emerging CHD risk factors will be assessed at the same frequency and by the same methods described for Specific Aim 2.

5.8 Statistical analysis

All continuous variables will be examined for distributions and the presence of outliers. Variables that are not normally distributed will be transformed and if normality cannot be achieved, will be analyzed using non-parametric tests. Standardized descriptive statistics including measures of means, median, standard deviations, ranges, and standard errors for continuous variables within each group will be calculated to describe the groups at fixed time points. Differences in outcome variables between ADF, CR, and control groups will be assessed by a one-way ANOVA. Post hoc comparisons between groups will be performed by Tukey's tests. Changes in variables over the course of the trial will be assessed by repeated-measures ANOVA. Data will be analyzed using SAS software (version 9.2; SAS Inc., Cary, NC).

The intention-to-treat analysis will include data from all participants who underwent randomization, and the last recorded value will be carried forward in the case of missing data for all variables. The completion analysis will include all participants for whom data will be available from the time of randomization to the end of the trial (month 12).

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