Alternate Day Fasting with a High Fat Versus Low Fat Diet for Weight Loss and Cardio-Protection

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THESIS
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DEDICATION
This thesis is dedicated to my mother, Dr. Barbara Klempel, sister, Natalie, and husband Victor, who have never given up on me and been a source of everlasting love and support. I will be eternally grateful to you for keeping me grounded, faithful, and unbroken, even in my darkest and most difficult times.

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<tr>
<td>ABCA1</td>
<td>ATP-binding Cassette Transporter 1</td>
</tr>
<tr>
<td>ADF</td>
<td>Alternate Day Fasting</td>
</tr>
<tr>
<td>ADMA</td>
<td>Asymmetric Dimethylarginine</td>
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<tr>
<td>AMPK</td>
<td>Adenosine Monophosphate-activated Protein Kinase</td>
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<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
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<tr>
<td>BMI</td>
<td>Body Mass Index</td>
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<tr>
<td>CIMT</td>
<td>Carotid Intima Media Thickness</td>
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<tr>
<td>CETP</td>
<td>Cholesteryl Ester Transfer Protein</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary Heart Disease</td>
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<tr>
<td>CR</td>
<td>Calorie Restriction</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of Variance</td>
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<tr>
<td>DEXA</td>
<td>Dual Energy X-ray Absorptiometry</td>
</tr>
<tr>
<td>EDHF</td>
<td>Endothelium-derived Hyperpolarizing Factor</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial Nitric Oxide Synthase</td>
</tr>
<tr>
<td>FMD</td>
<td>Flow-mediated Dilation</td>
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<tr>
<td>GLUT1</td>
<td>Glucose Transporter 1</td>
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<tr>
<td>HDL-C</td>
<td>High Density Lipoprotein Cholesterol</td>
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<td>HF</td>
<td>High Fat</td>
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<tr>
<td>HL</td>
<td>Hepatic Lipase</td>
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<tr>
<td>LCATA</td>
<td>Lecithin Cholesterol Acyl-transferase Activity</td>
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<td>LDL-C</td>
<td>Low Density Lipoprotein Cholesterol</td>
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<td>LF</td>
<td>Low Fat</td>
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<td>LPLa</td>
<td>Lipoprotein Lipase Activity</td>
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<td>Acronym</td>
<td>Full Form</td>
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<tr>
<td>METS</td>
<td>Metabolic Equivalents</td>
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<tr>
<td>NHANES</td>
<td>National Health and Nutrition Examination Survey</td>
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<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric Oxide</td>
</tr>
<tr>
<td>P</td>
<td>Proportion</td>
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<tr>
<td>PAI-1</td>
<td>Plasminogen Activator Inhibitor-1</td>
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<tr>
<td>RMR</td>
<td>Resting Metabolic Rate</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard Error of the Mean</td>
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<tr>
<td>VAS</td>
<td>Visual Analog Scale</td>
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<tr>
<td>VSMC</td>
<td>Vascular Smooth Muscle Cell</td>
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<td>VLDL</td>
<td>Very Low Density Lipoprotein</td>
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SUMMARY

A randomized control trial examining the effects of alternate day fasting (ADF) with a high fat (HF) or low fat (LF) background diet on body weight and coronary heart disease (CHD) risk in obese humans was conducted over 10 weeks. Obese subjects were randomized to either an ADF-HF (45% fat) group or ADF-LF (25% fat) group. The 10-week study consisted of a 2-week baseline weight maintenance period, followed by an 8-week weight loss period. Food was provided to all subjects during the 10-week trial. During each study week, subjects came to the Human Nutrition Research Unit for body weight, body composition, waist circumference, and blood pressure measurements. At week 1, 3, and 10, vascular function, plasma lipids, and plasma adipokines were assessed.

Results reveal that the ADF-HF diet is equally as effective as an ADF-LF diet in helping obese subjects lose weight and improve CHD risk factors. Body weight reductions were comparable between the ADF-HF diet and the ADF-LF diet, with no differences between groups. Similar decreases in fat mass were also observed for the ADF-HF and ADF-LF groups, with retention of lean mass. Reductions in several key biomarkers for CHD risk, such as total cholesterol, LDL cholesterol, and triacylglycerols, were also comparable between the HF and LF diet regimens. Moreover, the ADF-HF diet was as effective as the ADF-LF intervention at increasing LDL particle size, elevating the proportion of large LDL particles, and decreasing the proportion of small LDL particles. HDL particle size and distribution were not affected by either diet. As for endothelial function, ADF-LF diets increased brachial artery flow mediated dilation (FMD) while ADF-HF diets decreased FMD. Both intervention groups demonstrated increases in adiponectin and decreases in leptin and resistin. However, only adiponectin was positively correlated with FMD in the ADF-LF group. Taken together, these data suggest that individuals who typically consume HF foods do not need to lower the fat content of their diet to experience the benefits of ADF.
I. INTRODUCTION

A. Background and rationale

Obese individuals are three times more likely to develop coronary heart disease (CHD) when compared to their normal weight counterparts [1]. Reducing energy intake by means of dietary restriction has been shown to lower the risk of CHD in obese populations [2]. Accumulating evidence suggests that alternate day fasting (ADF) is an effective diet strategy to help obese individuals lose weight and lower CHD risk [3]. ADF regimens include a “feed day” where food is consumed ad-libitum over 24 hours, alternated with a “fast day”, where food intake is limited to 25% of the individual’s needs over 24 hours. Recent findings indicate that 8 weeks of ADF with a low-fat (LF) background diet (20% kcal from fat) is able to reduce body weight by 6-8%, while lowering LDL cholesterol and triglyceride levels by 10-25% and 30-40%, respectively, in obese adults [3]. Although these data for LF diets is promising, recent evidence indicates that high-fat (HF) diets (35-45% kcal from fat) produce greater weight loss when compared to LF diets [4]. Moreover, HF diets yield greater improvements in plasma lipid levels when compared to LF diets. It should also be noted that the average obese American typically consumes a diet high in fat (i.e. 35-45% kcal from fat), so testing the effects of an ADF-HF regimen is important in terms of diet tolerability in obese subjects [5]. In view of these findings, an important question that has yet to be tested is whether an ADF-HF diet can produce more beneficial changes in body weight and plasma lipids when compared to an ADF-LF diet in obese individuals.

Endothelial dysfunction is an early hallmark and prognostic indicator of future CHD [6]. Obese individuals exhibit abnormal endothelial function marked by reduced vasodilation to increased blood flow (endothelium-dependent flow-mediated dilation; FMD) [6]. Weight loss has been shown to increase FMD, and hence, improve endothelial function [6]. The ability of an ADF-HF diet relative to an
ADF-LF diet to improve endothelial function is unknown but would contribute greatly to our understanding of the protective effects of the ADF diet.

Although the mechanisms remain unclear, evidence suggests that adipocyte physiology may link weight loss to improvements in lipid levels and FMD [7]. Adiponectin is a fat-cell derived hormone (adipokine) that exerts cardio-protective effects and is inversely related to body weight and LDL cholesterol levels [8]. Higher circulating concentrations of adiponectin are also associated with increased FMD [6]. Leptin and resistin, in contrast, are augmented in obese individuals, and are positively correlated to triglycerides and LDL cholesterol levels [9] [10]. Circulating levels of these hormones are dictated by regional fat distribution. Visceral obesity is a strong predictor of CHD, independent of body mass index (BMI) [11]. Viscerally obese individuals have higher plasma levels of leptin and resistin and lower levels adiponectin, relative to subcutaneously obese individuals [12]. Whether an ADF-HF diet can produce greater changes in these emerging indicators of CHD risk (fat-cell derived hormones and visceral fat) when compared to an ADF-LF diet, is not yet known.
The rationale for the study is as followed:

**B. Specific Aims**

**Specific Aim 1:** To determine if body weight loss and visceral fat mass loss will be greater in subjects consuming an ADF-high fat (ADF-HF) diet compared to subjects consuming an ADF-low fat (ADF-LF) diet.

**Hypothesis 1:** The ADF-HF group will lose more body weight and visceral fat mass compared to the ADF-LF group after 8 weeks of dietary intervention.
Specific Aim 2: To determine if either greater decreases in body weight and visceral fat mass by the ADF-HF diet will result in greater improvements in traditional CHD risk factors when compared to the ADF-LF diet.

Hypothesis 2: The ADF-HF group and will experience more pronounced improvements in CHD risk indicators (plasma lipids, blood pressure, and LDL particle size) when compared to the ADF-LF group after 8 weeks of dietary intervention.

Specific Aim 3: To determine if greater decreases in body weight and visceral fat mass by the ADF-HF diet will result in greater improvements in vascular endothelial function and adipokines when compared to the ADF-LF diet.

Hypothesis 3: The ADF-HF group will experience greater improvements in brachial artery flow-mediated dilation (FMD) and plasma adipokines (adiponectin, leptin and resistin) when compared to the ADF-LF diet group after 8 weeks of dietary intervention.

C. Significance

If the aims of this project are achieved, this study will:

- Advance clinical practice guidelines by showing that the ADF-HF diet is as or more beneficial than the ADF-LF diet in helping obese individuals lose larger amounts of weight, which will lead to more pronounced reductions in CHD risk in this population.
- Establish the ADF-HF diet is an effective means of improving vascular endothelial function.
- Further uncover the intermediate role that adipose tissue plays in mediating CHD risk.
D. Innovation

This study will be the first to directly compare the time-course effects of ADF-HF to that of ADF-LF on body weight and CHD risk, and will show that ADF-HF diets are more effective than ADF-LF diets for weight loss and CHD risk reduction. In sum, the proposed study is innovative in that it will be the first to:

- Challenge existing clinical paradigms by proposing that ADF-HF be implemented as an alternative to ADF-LF for weight loss and CHD risk reduction.

- Employ novel markers, i.e. plasma adipokines, to assess the impact of ADF-HF on CHD risk.
II. LITERATURE REVIEW

A. Obesity is a key risk factor for coronary heart disease (CHD)

Recent trends have shown that the creation of innovative weight loss strategies is crucial for combating the globally pervasive obesity epidemic. Obesity is a strong independent risk factor for CHD [13]. As individuals gain more body weight, the chances of CHD comorbidities rises dramatically [14]. Reduction in body weight and visceral fat mass assists in improving blood lipid parameters and has been shown to improve endothelial function as measured by brachial artery FMD [6]. Specifically, for every kilogram of body weight lost, LDL-C is lowered by 2 mg/dl [8]. In addition, 5% weight loss has been shown to decrease systolic and diastolic blood pressure by 10 mm/Hg and 5 mm/Hg, respectively, and increase brachial artery FMD [6].

Multiple dietary approaches have been tested to elicit these benefits, one of the more recently successful being alternate day fasting (ADF). ADF consists of a “fast day” in which calories are reduced by 75% or more, alternated with a “feed day” where food is unlimited [3]. On this diet, subjects are advised to eat between 12 and 2pm on the “fast day”, whereas the time frame for food intake on the “feed day” is not restricted [3]. Most often, women and men will consume a small meal of about 400-500 calories and 500-600 calories on the fast day, respectively [3]. ADF has most often been implemented with a low fat (LF) background diet to induce beneficial changes in body weight, body composition, and CHD risk factors. The effects of ADF-LF regimens on these parameters are presented in the next section.
B. Alternate day fasting (ADF) with a low-fat (LF) diet is effective for weight loss

1. ADF-LF diets: Effect on body weight

Throughout the past decade, ADF studies have moved from implementing this strategy solely in animals to employing this regimen in human subjects. To date, ADF protocols have only involved LF background diets for up to 12 weeks. Heilbronn et al. [15] executed one of the first ADF studies which followed 8 non-obese patients who decreased body weight by 2.5% after 22 days. Shortly thereafter, Johnson et al. [16] transitioned the protocol to include overweight humans and found that body weight was reduced by 8% after 8 weeks. Varady et al. [3] is one of the front-runners in human ADF research, most often implementing a LF background diet throughout each weight loss phase. The first study by Varady et al. [3] demonstrated that obese individuals could also benefit from ADF, as these individuals decreased their body weight by 6% from baseline after 8 weeks of treatment. Following this study, Bhutani et al. [17] showed that 64 obese individuals prescribed an ADF-LF regimen on feed and fast days over 12 weeks lowered body weight by 3% from baseline. Taking together, ADF-LF diets may elicit weight loss in both obese and non-obese humans, however, the effects of the diet have only been tested over a short period of time (3-12 weeks).

2. ADF-LF diets: Effect on body composition

In addition to seeing promising results for weight loss, ADF-LF studies have also shown decreases in body fat mass, and retention of fat free mass. For instance, Heilbronn et al. [15] found that normal weight participants decreased fat mass by 4% and fat free mass by 1% after 3 weeks of ADF-LF. Varady et al. [3], on the other hand, saw a 7% reduction in fat mass, and a preservation of lean mass in obese subjects after 8 weeks. Bhutani et al. [17] also showed that obese individuals reduced fat mass by 5%, with no change in lean mass, after 12 weeks of ADF-LF. In sum, ADF-LF studies in humans demonstrate fat mass reductions, while preserving fat free mass. This retention in lean mass is important, as lean
mass is a key predictor of resting metabolic rate (RMR). Keeping RMR high is essential, as this helps individuals maintain their weight loss in the long-term.

Decreases in visceral fat mass (measured by waist circumference) have also been demonstrated in two ADF-LF studies. For instance, Varady et al. [3] demonstrated a 6 cm decrease in waist circumference after 8 weeks of ADF-LF in obese males and females. In line with these findings, Bhutani et al. [17] showed a 5 cm decrease in waist circumference after 12 weeks of ADF-LF. Visceral fat mass accumulation is associated with the progression of CHD. The mechanisms that explain how visceral fat mediates CHD risk factors are well researched. For example, visceral fat is linked to an increase in small atherogenic LDL particles [18]. The high lipolytic activity of visceral fat delivers an increased load of non-esterified free fatty acids to the liver. This leads to the formation of very low-density lipoprotein (VLDL) particles, which later become LDL particles in the circulation [18]. Then, through the action of (cholesteryl ester transfer protein CETP) and hepatic lipase (HL) activity, the LDL particles become smaller and denser [18]. These small LDL particles have been shown to be easily oxidized and are therefore highly atherogenic. Visceral fat also influences blood pressure [19]. Visceral fat produces increased levels of plasminogen activator inhibitor-1 (PAI-1) [19]. PAI-1 is a major circulating inhibitor of thrombolysis, and circulating concentrations are positively related to systolic blood pressure and diastolic blood pressure values [19]. As such, visceral fat accumulation, through the action of PAI-1, may lead to increased systolic and diastolic blood pressure. Thus, the decrease in visceral fat mass by ADF-LF diets may lead to improvements in CHD risk factors.
C. Alternate day fasting (ADF) with a low-fat (LF) is effective for CHD risk reduction

1. ADF-LF diets: Effect on plasma lipids and LDL particle size

The effects of ADF-LF diets on lipid parameters and LDL particle size have been investigated in a handful of studies. For example, Heilbronn et al. [15] showed that after 22 days, non-obese humans were able to lower TG and LDL-C, while increasing HDL-C. Johnson et al. [16] also found that in his group of overweight subjects, total cholesterol (TC) and TG decreased by 9% and 43%, respectively, and HDL-C increased by 8%. Varady et al. [3] showed TC, LDL-C, and TG decreased by 21%, 25%, and 32%, respectively, after 8 weeks. Bhutani et al. [17], however, did not observe changes in TC, LDL-C, or TG in participants after 12 weeks of ADF diet therapy.

Beneficial changes in LDL particle size have also been noted in ADF-LF studies. Small dense LDL particles are independent risk factors for CHD [12]. Mechanisms linking small LDL size to atherosclerosis include long residence time in plasma and increase oxidizability [12]. After 8 weeks of ADF-LF, peak LDL particle size increased from 266 Å to 268 Å in the Varady et al. study [20]. In addition, the proportion of small LDL particles decreased from 13% to 9%, whereas the proportion of large LDL particles increased from 68% to 76% [20]. In the study by Bhutani et al. [17], LDL particle size increased after 12 weeks of treatment in ADF-LF group (5 Å). Moreover, the proportion of small atherogenic LDL particles was reduced, while the proportion of large anti-atherogenic LDL particles was increased (P<.001) at the end of the intervention [17]. Thus, ADF-LF diets may lower CHD risk by decreasing LDL-C and TG levels, while increasing LDL particle size.

2. ADF-LF diets: Effect on FMD

To date, only one ADF study has measured FMD. Bhutani et al. [17] showed that after 12 weeks, FMD score increased in by ADF-LF by 5% from baseline. The most plausible cause for the improvements FMD
is the decrease in body weight (3% from baseline) and fat mass [17]. Waist circumference also decreased by 5 cm [17]. Multiple studies that employed dietary restriction regimens have had a favorable impact on FMD mainly due to lower body weight and visceral fat mass. For example, Pierce et al. [21] performed a 12 week study in which weight loss and reduced visceral fat were correlated with improved FMD and arterial resistance in obese subjects. These subjects reduced body weight by 11% and visceral fat by 34%, while improving FMD by 30% (pre to post cuff release) [21]. Thus, reductions in body weight and fat mass may play a role in determining the improvement in FMD by ADF-LF diets.

3. ADF-LF diets: Effect on adipokines

The effect of ADF on adipose tissue-derived hormones, such as adiponectin, leptin, and resistin, has also been tested. Adiponectin decreases with obesity and increases with weight loss, and has an important role in regulating energy expenditure [22]. Adiponectin may be cardio-protective in that it leads to increased HDL-mediated cholesterol efflux, which occurs through adiponectin induced up-regulation of the expression of ATP-binding cassette transporter 1 (ABCA1) [23]. Adiponectin also inhibits the uptake of oxidized LDL into macrophages, thereby stopping the first step of atherosclerosis [24]. Adiponectin levels are positively correlated with HDL cholesterol concentrations, and negatively correlated with triglyceride levels, insulin resistance, and systemic circulating inflammatory markers [23]. In an 8 week ADF-LF study by Varady et al., adiponectin increased by 30%. In another study by Varady et al. [25], adiponectin concentrations increased by 20% in women who lost 5% to 10% of body weight. It is also worth noting that visceral and subcutaneous fat cell size was 41% and 37% smaller, respectively, in these same subjects [25]. These preliminary findings indicate that ADF-LF diets may increase circulating adiponectin concentrations.
The effects of ADF-LF diets on plasma leptin levels have also been investigated. Leptin levels are positively related to fat mass and visceral fat mass, and most importantly modulates energy balance and food intake [12]. Leptin may be pro-atherogenic in that it induces the migration, proliferation, and hypertrophy of smooth muscle cells lining the vascular wall [12]. Evidence has also shown that leptin is involved in vessel wall calcification and increased platelet aggregation, contributing to the development of atherosclerosis [26]. Thus, diet regimens that can decrease circulating leptin levels would be highly protective against CHD. In a recent ADF study, Johnson et al. [16] found that leptin decreased by 21% after 8 weeks of diet in obese patients. Bhutani et al. [27] showed that leptin decreased by 21% after a 10 week ADF-LF weight loss program. Similarly, in a different study, Bhutani et al. [17] found that after 12 weeks on an ADF-LF diet, obese individuals reduced leptin (25% from baseline). These initial findings suggest that ADF-LF may produce favorable reductions in plasma leptin.

Resistin is another hormone derived from adipose tissue. The role of resistin in mediating CHD risk is not well understood. Circulating resistin concentrations are positively correlated to body weight and fat mass [28]. Resistin may be pro-atherogenic in that it promotes endothelial dysfunction by increasing oxidative stress and augmenting the expression of adhesion molecules [28]. Resistin may also promote foam cell formation by facilitating lipid accumulation in macrophages [28] [29]. As mentioned previously, an ADF regimen can elicit improvements in resistin levels. In the study by Bhutani et al. [27], resistin levels decreased by 23%, but did not have any relationship to reduced CHD risk. Resistin is similar to leptin in the sense that both adipokines work to deregulate nitric oxide (NO) production by impairing endothelial nitric oxide synthase (eNOS) function [30]. Taking these findings into account, more studies are needed to explain the mechanisms of resistin in the presence of weight loss.
D. Applicability of ADF-LF findings is questionable as most Americans consume a high-fat (HF) diet

From the literature reviewed in the previous section, it is evident that ADF with an LF diet is effective for weight loss and CHD risk reduction. However, modern day Americans are not accustomed to this type of LF diet, and instead gravitate towards high fat (HF) diets. Current research shows that Americans consume 35-45% of energy as dietary fat [5]. Moreover, 11% of energy is consumed as saturated fat, which is well above the 7% recommendations [31]. Both children and adults are increasingly exceeding their energy intake by filling their diet with low nutrient quality calories. These sources mainly consist of, but are not limited to, soda, fruit drinks, dairy desserts, grain desserts, pizza, and whole milk [5]. Adding to the problem, fast food restaurants and drive-through establishments are rampant, making it easier for Americans to skip the appropriate servings of fruits and vegetables, whole grains, and water required to maintain a stable and healthy weight [5]. Another potential obstacle is the lack of food security. Whereas some areas have many grocery stores, or just small corner shops, many individuals either cannot afford these healthier items or cannot physically access them [32]. All of the previously listed circumstances eventually precipitate elevated fat content of the American diet. Since the majority of Americans consume a HF diet, further research is needed to examine if ADF-HF diets can mimic the beneficial effects of ADF-LF diets. Since there are no previous studies that have investigated the effects of an ADF-HF on CHD risk, we reviewed the effects of calorie restricted-high fat (CR-HF) diets on body weight and CHD risk instead. These findings are reported in the following section.
E. Calorie restricted (CR)-HF diets may be more effective than CR-LF diets for weight loss and CHD risk reduction

Table LR.I CR-HF and CR-LF diet effects on body weight and body composition

<table>
<thead>
<tr>
<th>Reference</th>
<th>Length</th>
<th>Intervention</th>
<th>ΔBody Weight*</th>
<th>ΔFat Mass*</th>
<th>ΔFat Free Mass*</th>
<th>ΔWaist Circumference*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brinkworth, GD</td>
<td>8 w</td>
<td>30% CR-LC 30% CR-HC</td>
<td>↓8.6%</td>
<td>↓15%</td>
<td>↓4.3%</td>
<td>—</td>
</tr>
<tr>
<td>Kirk, E [34]</td>
<td>11 w</td>
<td>50% CR-LC 50% CR-HC</td>
<td>↓7.0%</td>
<td>↓12%</td>
<td>↓3.0%</td>
<td>—</td>
</tr>
<tr>
<td>Due, A [35]</td>
<td>24 w</td>
<td>Control MUFA diet LF diet</td>
<td>↓8.0%</td>
<td>↓10.0%</td>
<td>↓2.0%</td>
<td>↓3.0%</td>
</tr>
<tr>
<td>Yancy, WS Jr</td>
<td>24 w</td>
<td>HF-LC diet LF-HC diet</td>
<td>↓13%</td>
<td>↓5.8%</td>
<td>Ø</td>
<td>—</td>
</tr>
<tr>
<td>Samaha, FF [37]</td>
<td>24 w</td>
<td>25% CR-HF 25% CR-LF</td>
<td>↓4.5%</td>
<td>↓2.8%</td>
<td>Ø</td>
<td>—</td>
</tr>
<tr>
<td>Frisch, S [38]</td>
<td>52 w</td>
<td>20%CR-HF 20%CR-LF</td>
<td>↓6.2%</td>
<td>↓24%</td>
<td>Ø</td>
<td>↓6.3%</td>
</tr>
</tbody>
</table>

*Post-treatment values significantly different (P < 0.05) from baseline values within intervention groups.

Abbreviations and symbols: CR-Calorie restriction; LC-Low Carbohydrate/High Fat; HC-High Carbohydrate/Low Fat; HF-High Fat; LF-Low Fat; MUFA-Monounsaturated Fatty Acid; w-weeks; ↓decrease, Ø-no significant change.

The effects of CR-HF on body weight have been evaluated in several studies. Brinkworth et al. [33] studied 60 overweight and obese men and women on a CR-HF (30% CR, 60% fat) diet. After 8 weeks of treatment, subjects decreased their body weight by 8.6% [33]. Participants on a CR-HF (30% CR, 60% fat) diet also decreased fat mass by 15% following the dietary intervention [33]. Additionally, fat free mass was reduced by 4.3% [33]. The CR-LF (30% CR, 30% fat) group, however, saw less impressive decreases.
in body weight and fat mass (6.8% and 13%) \[33\]. Subjects also lost only 2.9% of fat free mass after this 8 week dietary treatment \[33\]. In a study by Kirk et al. \[34\], obese men and women consumed a CR-HF (50% CR, 75% fat) diet for 11 weeks, in which body weight decreased by 8.0%. Fat mass and fat free mass were reduced by 12% and 3.0%, respectively \[34\]. The CR-LF (50% CR, 25% fat) lost less fat mass (10%) and more fat free mass (4.0%) than the CR-HF (50% CR, 75% fat) group. Samaha et al. \[37\], on the other hand, compared a CR-HF (25% CR, 40% fat) versus CR-LF (25% CR, 30% fat) in obese men and women for 24 weeks. Subjects in the CR-HF (25% CR, 40% fat) group lost 4.5% of initial body weight, while subjects in the CR-LF (25% CR, 30% fat) group lost only 1.4% of body weight. In another 6 months study by Yancy et al. \[36\], participants following a HF-LC (35% CR, 35% fat) and LF-HC (35% CR, 25%) diet decreased body weight by 13% and 6.7%, respectively. This study also showed that a CR-HF (35% CR, 35% fat) diet produces greater body fat loss than a CR-LF diet (35% CR, 25% fat) with 5.8 % and 2.8% decreases in the CR-HF (35% CR, 35% fat) and CR-LF (35% CR, 25% fat) groups, respectively \[36\]. Due et al. \[35\], on the other hand, saw an 8.0% decrease in body weight in 6 months, with no differences between MUFA, LF, or control groups. All of these groups saw similar decreases in body fat, fat free mass, and waist circumference \[35\]. In a year-long study by Frisch et al. \[38\], the effect of CR-HF (20% CR, 35% fat) was tested in overweight and obese adults. Results reveal 6.2% and 7.3% reductions in body weight in CR-HF (20% CR, 35% fat) and CR-LF (20% CR, 30% fat) diets, respectively, as well as decreased waist circumference by 6.3 and 7.5 cm in the CR-HF (20% CR, 35% fat) and CR-LF (20% CR, 30% fat) diets, respectively \[38\]. Both groups also decreased fat mass by 24% \[38\]. These findings suggest that CR-HF diets may produce moderate weight loss, and in many cases may even produce greater weight loss than CF-LF diets. More research, however, is evidently warranted in this area.
Table LR.II CR-HF and CR-LF diet effects on plasma lipids and LDL particle size

<table>
<thead>
<tr>
<th>Reference</th>
<th>Length</th>
<th>Intervention</th>
<th>ΔTC*</th>
<th>ΔHDL*</th>
<th>ΔLDL*</th>
<th>ΔTG*</th>
<th>Δ LDL particles*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jenkins, DJ [39]</td>
<td>4 w</td>
<td>40%CR-LC 40%CR-HC</td>
<td>↓20%</td>
<td>↓13%</td>
<td>↓21%</td>
<td>↓40%</td>
<td></td>
</tr>
<tr>
<td>Al-Sarraj, T [40]</td>
<td>12 w</td>
<td>LC AHA</td>
<td>↓6.6%</td>
<td>↓3.7%</td>
<td>↓5.3%</td>
<td>↓28%</td>
<td>↓16% ↓12% (SM)</td>
</tr>
<tr>
<td>Frisch, S [38]</td>
<td>52 w</td>
<td>20%CR-LC 20%CR-LF</td>
<td>Ø</td>
<td>↓2.3%</td>
<td>Ø</td>
<td>Ø</td>
<td></td>
</tr>
<tr>
<td>Gardner, CD [41]</td>
<td>52 w</td>
<td>Atkins Zone LEARN Ornish (LF-plant based)</td>
<td>↑8.5%</td>
<td>↑5.2%</td>
<td>Ø</td>
<td>Ø</td>
<td></td>
</tr>
<tr>
<td>Krauss, RM [42]</td>
<td>5 w</td>
<td>CR-HSFA CR-LSFA (25% total fat)</td>
<td>↓6.0%</td>
<td>Ø</td>
<td>↓1.0%</td>
<td>Ø</td>
<td>↑5.0 Å</td>
</tr>
<tr>
<td>Hays, JH [43]</td>
<td>6 w</td>
<td>CR-HSFA (40% total fat)</td>
<td>↓4.0%</td>
<td>Ø</td>
<td>Ø</td>
<td>↓8.0%</td>
<td>↑4.0 Å</td>
</tr>
</tbody>
</table>

*Post-treatment values significantly different (P < 0.05) from baseline values within intervention groups.

Abbreviations and symbols: CR-Calorie restriction; LC-Low Carbohydrate/High Fat; HC- High Carbohydrate/Low Fat; AHA-American Heart Association recommendations; SM-Small Particle Phenotype; HF-High Fat; LF-Low Fat; MUFA-Monounsaturated Fatty Acid, HSFA-High Saturated Fat; LFSA-Low Saturated Fat; w-week; ↑-increase; ↓-decrease; Ø-no significant change; Å-Angstrom.

The lipid-lowering effects of CR-HF dietary treatments have also been examined in numerous recent studies. A ground-breaking study testing the effect of HF diets on plasma lipid levels was performed by Jenkins et al.[39] who placed 44 overweight and obese men and women on a CR-HF (40% CR, %45 fat) or CR-LF (40% CR, 25% fat) for 4 weeks. Although the study was short, total cholesterol levels were lowered by 20% and 13% in the CR-HF and CR-LF groups, respectively [39]. LDL-cholesterol was also reduced by
21% and 13% in the CR-HF (40% CR, %45 fat) and CR-LF (40% CR, 25% fat) groups, respectively.

Additionally, triglycerides levels decreased by 40% in the CR-HF (40% CR, %45 fat) group, compared to only 21% in the CR-LF (40% CR, 25% fat) group [39]. Al-Sarraj et al. [40] also completed a 12-week study in which 39 normal, overweight, and obese individuals followed either a CR-HF (50% CR, 60% fat) or CR-LF (50% CR, 30% fat) regimen. Total cholesterol levels decreased by 6.6% in the CR-HF (50% CR, 60% fat) group, and by 3.7% in the CR-LF (50% CR, 30% fat) group [40]. Participants also reduced LDL-cholesterol by 5.3% (CR-HF group) and 2.6% (CR-LF group), whereas triglycerides decreased by 28% and 20% in CR-HF (50% CR, 60% fat) and CR-LF (50% CR, 30% fat) diet groups, respectively [40]. HDL-C levels did not change in either of these studies [40]. Another interesting finding from this study included the reduction of atherogenic small LDL particles by 16% and 12% in the LC and AHA groups, respectively. Frisch et al. completed a year-long study in which a CR-HF (20% CR, %35 fat) was compared to a CR-LF (20% CR, %30 fat), however, only decreases in triglycerides in the CR-HF (20% CR, %35 fat) were markedly greater (7.6%) than the other improvements seen in total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglycerides parameters the CR-LF (20% CR, %30 fat) group. Gardner et al. [41] saw similar results in his 1 year study which compared the Atkins (HF), Zone (LC), Ornish (LF-plant based) and LEARN (LF) diets.

Although there were no significant changes in LDL cholesterol among all three diet regimens, the Atkins (HF) group decreased by 23%, compared to only 12% in the Ornish (LF-plant based) and Zone (LC) diets, and by 3.4% in the LEARN (LF) intervention. The effects of CR-HF versus CR-LF diets on LDL particle size have also been compared in studies such as Krauss et al. [42], who demonstrated that peak LDL particle size was increased by 5.0 Å in the CR-high saturated fat (50% CR, 15% saturated fat) diet. This was a slightly greater increase than was seen in the CR-low saturated fat (50% CR, 7% saturated fat) intervention (3.0 Å) after 5 weeks of treatment [42]. Similarly, in a study by Hays et al. [43], individuals increased LDL particle size by 4.0 Å after a 6 week CR-high saturated fat diet (40% fat), as well as decreasing total cholesterol and triglycerides by 4.0% and 8.0%, respectively. Taken together, these
findings suggest that CR-HF diets produce equivalent, or potentially greater, decreases in total cholesterol, LDL-cholesterol, and triglycerides, in addition to cardio-protective increases in LDL particle size, when compared to CR-LF diets.

Table LR.III CR-HF and CR-LF diet effects on FMD

<table>
<thead>
<tr>
<th>Reference</th>
<th>Length</th>
<th>Intervention</th>
<th>ΔBP*</th>
<th>ΔHR*</th>
<th>ΔGlucose*</th>
<th>ΔInsulin*</th>
<th>ΔFMD*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varady, KA [6]</td>
<td>6 w</td>
<td>25%CR-HF 25%CR-LF</td>
<td>↓9.0% S</td>
<td>0</td>
<td>↓32%</td>
<td>↓22%</td>
<td>↑19%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25%CR-HF 25%CR-LF</td>
<td>↓7.0% D</td>
<td>0</td>
<td>↓13% S</td>
<td>↓10% D</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>25%CR-HF 25%CR-LF</td>
<td>↓13% S</td>
<td>0</td>
<td>↓10% D</td>
<td>↓10% D</td>
<td></td>
</tr>
<tr>
<td>Volek, JS [44]</td>
<td>12 w</td>
<td>25%CR-HF 25%CR-LF</td>
<td>0</td>
<td>↓12%</td>
<td>↓50%</td>
<td>0</td>
<td>↑2.0%</td>
</tr>
<tr>
<td>Cortes, B [45]</td>
<td>1 w</td>
<td>40%CR-HF(N) Control</td>
<td>0</td>
<td>0</td>
<td>↓15%</td>
<td>↓10%</td>
<td>↑24%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>↑15%</td>
<td>↑10%</td>
<td>↑24%</td>
</tr>
<tr>
<td>Keogh, JB [46]</td>
<td>12 w</td>
<td>30%CR-LC 30%CR-HC</td>
<td>↓8.3% S</td>
<td>0</td>
<td>↓3.5%</td>
<td>↓3.6%</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30%CR-HC</td>
<td>↓9.5% D</td>
<td>0</td>
<td>↓28%</td>
<td>↓31%</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30%CR-HC</td>
<td>↓9.6% S</td>
<td>0</td>
<td>↓28%</td>
<td>↓31%</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30%CR-HC</td>
<td>↓9.1% D</td>
<td>0</td>
<td>↓28%</td>
<td>↓31%</td>
<td>0</td>
</tr>
</tbody>
</table>

*Post-treatment values significantly different (P < 0.05) from baseline values within intervention groups.

Abbreviations and symbols: CR-Calorie restriction; LC-Low Carbohydrate/High Fat; HC-High Carbohydrate/Low Fat; N-Walnut; S-Systolic; D-Diastolic; ↑-increase; ↓-decrease; 0-no significant change.

Several studies have examined the effect of CR-HF versus CR-LF diets on FMD. Volek et al. [44] completed a 12-week study in which FMD score increased by 2.0% in the CR-HF group (25% CR, 60% fat) and decreased by 3.0% in the CR-LF group (25% CR, 25% fat). The CR-HF (25% CR, 60% fat) also showed 12% and 50% reductions in glucose and insulin, respectively, compared to insignificant changes in the
CR-LF (25% CR, 25% fat). Another study by Cortes et al. [45] demonstrated that subjects who consumed a CR-HF diet (40% CR, 35% fat) increased FMD by 24% (pre to post cuff release) after 1 week. Since measurements were performed post-prandially, insulin greatly increased by 260% and 160%, in CR-HF (40% CR, 35% fat) and control groups, respectively [45]. Glucose also increased in these same groups by 15% and 10%, respectively [45]. On the contrary, a 6 week study by Varady et al. [6], showed FMD increased by 32% (pre to post cuff release) with a CR-LF diet (25% CR, 25% fat) and decreased by 19% with a CR-HF diet (25% CR, 60% fat) in obese humans. Additionally, favorable decreases in systolic (9.0%, 13%) and diastolic (7.0%, 10%) occurred in the CR-HF (25% CR, 60% fat) and CR-LF (25% CR, 25% fat) groups, respectively [6]. Insulin also improved by 10% more in the CR-HF (25% CR, 60% fat) group [6]. In contrast, Keogh et al. [46], who implemented a CR-HF (30% CR, 60% fat) and CR-LF diet (30% CR, 30% fat), found no effect of diet on FMD, but found favorable improvements in both groups among blood pressure, glucose, and insulin, with no differences between groups. In light of these conflicting findings, more research in this area is necessary before solid conclusions can be reached.

Table LR.IV CR-HF and CR-LF diet effects on adipokines

<table>
<thead>
<tr>
<th>Reference</th>
<th>Length</th>
<th>Intervention</th>
<th>Adiponectin Plasma*</th>
<th>Leptin Plasma*</th>
<th>Resistin Plasma*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arvidsson, E [47]</td>
<td>10 w</td>
<td>30%CR-HF 30%CR-LF</td>
<td>↑ 3.0% ↑ 7.0%</td>
<td>↓ 25%</td>
<td>↓ 29%</td>
</tr>
<tr>
<td>Cardillo, S [48]</td>
<td>24 w</td>
<td>30%CR-HF 30%CR-LF</td>
<td>Ø Ø</td>
<td>↓ 23%</td>
<td>Ø</td>
</tr>
<tr>
<td>Wycherley, TP [49]</td>
<td>52 w</td>
<td>30%CR-HF 30%CR-LF</td>
<td>↑ 15% ↑ 23%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shai, I [50]</td>
<td>104 w</td>
<td>25%CR-HF 25%CR-LF</td>
<td>↑ 11% ↑ 11%</td>
<td>↓ 21%</td>
<td>↓ 18%</td>
</tr>
</tbody>
</table>

*Post-treatment values significantly different (P < 0.05) from baseline values within intervention groups. Abbreviations and symbols: CR-Calorie restriction; LC-Low Carbohydrate/High Fat; HC-High Carbohydrate/Low Fat; N-Walnut; S-Systolic; D-Diastolic; w-week; ↑-increase; ↓-decrease; Ø-no significant change.
The effect of CR-HF and CR-LF diets on adipokine levels has also been investigated. Four studies have examined the effect of CR-HF and CR-LF diets on plasma adiponectin and leptin concentrations. In a study by Arvidsson et al. [47], obese women experienced increases in adiponectin concentrations by 3.0% and 7.0% in the CR-HF (30% CR, 45% fat) and CR-LF (30% CR, 20% fat) diets, respectively, after 10 weeks. The CR-HF (30% CR, 45% fat) and CR-LF (30% CR, 20% fat) individuals also decreased leptin by 25% and 29%, respectively [47]. Cardillo et al. [48], on the other hand, found no change in adiponectin in obese individuals following a CR-HF (30% CR, 35% fat) or CR-LF (30% CR, 30% fat) diet. Leptin levels, however, decreased by 23% only in the CR-HF (30% CR, 35% fat) group after 6 months [48]. Wycherley et al. [49] showed that after 1 year, adiponectin increased by 15% in obese adults following a CR-HF diet (30% CR, 60% fat) and by 23% in individuals on a CR-LF (30% CR, 25% LF) diet, with leptin not being measured. Shai et al. [50] completed another long-term study which included 322 overweight and obese men and women who were fed either a CR-HF diet (25% CR, 35% fat) or CR-LF (30% CR, 30% fat) diet. During the 2 year study, subjects reduced leptin levels by 21% and 18% in the CR-HF (25% CR, 35% fat) and CR-LF (25% CR, 30% fat) groups, respectively [50]. Adiponectin also increased by 11% in both groups [50]. In view of these findings, both CR-LF and CR-HF diets appear to be effective for reducing leptin levels. The effect of these diets on adiponectin levels is still not clear, however.

F. Summary

In sum, preliminary evidence suggests that ADF-LF diets may be effective for weight loss, body composition improvements, and reductions in CHD risk indicators. Drawing from the wealth of CR-HF evidence, it would appear as though HF protocols are also effective at lowering body weight and fat mass, while improving several CHD risk factors (i.e. plasma lipids, FMD and adipokines).
G. Cited literature


III. MANUSCRIPT I

Alternate day fasting (ADF) with a high-fat diet produces similar weight loss and cardio-protection as ADF with a low-fat diet*

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Running head: Alternate day fasting with a high-fat diet
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Conflict of interest: The authors have no conflicts of interest to report.

A. Abstract

**Background:** Alternate day fasting (ADF) with a low-fat (LF) diet is effective for weight loss and cardioprotection. However, the applicability of these findings is questionable as the majority of Americans consume a high-fat (HF) diet. **Objective:** The goal of this study was to determine if these beneficial changes in body weight and coronary heart disease (CHD) risk can be reproduced if a HF background diet is used in place of a LF diet during ADF. **Methods:** Thirty-three obese subjects were randomized to an ADF-HF (45% fat) or ADF-LF diet (25% fat), which consisted of two phases: 1) a 2-week baseline weight maintenance period, and 2) an 8-week ADF weight loss period. All food was provided during the study. **Results:** Body weight was reduced (P < 0.0001) by ADF-HF (4.9 ± 1.1%) and by ADF-LF (4.2 ± 0.8%). Fat mass decreased (P < 0.0001) by ADF-HF (5.6 ± 1.5 kg) and ADF-LF (4.2 ± 0.6 kg). Fat free mass remained unchanged. Waist circumference decreased (P < 0.001) by ADF-HF (7.5 ± 1.5 cm) and ADF-LF (7.3 ± 0.9 cm). LDL cholesterol and triacylglycerol concentrations were reduced (P < 0.001) by both interventions (ADF-HF: 19.1 ± 4.8%, 13.3 ± 4.7%; and ADF-LF: 24.8 ± 2.6%, 14.3 ± 4.4%). HDL cholesterol concentrations, blood pressure, and heart rate remained unchanged. There were no between-group differences for any parameter. **Conclusion:** These findings suggest that an ADF-HF diet is equally as effective as an ADF-LF diet in helping obese subjects lose weight and improve CHD risk factors.

**Keywords:** Calorie restriction, alternate day fasting, weight loss, coronary heart disease, high-fat diet, low-fat diet, obese adults
B. Introduction

Obesity in adulthood doubles the risk of coronary heart disease (CHD) mortality [1] [2]. Reducing energy intake by means of dietary restriction has been shown to lower the risk of CHD in obese adults [3] [4]. Evidence suggests that alternate day fasting (ADF) is an effective diet strategy to help obese individuals lose weight and lower CHD risk [5] [6]. ADF regimens include a “feed day” where food is consumed ad-libitum over 24 h, alternated with a “fast day” where intake is limited to 25% of the individual’s energy needs over 24 h. To date, only two clinical trials have been performed to evaluate the ability of ADF to facilitate weight loss and decrease CHD risk [5] [6]. Each of these trials implemented a low-fat (LF) background diet (i.e. 25% of energy from dietary fat) to test the study objectives [5] [6]. In both trials, body weight was reduced by 6-8% after 8 weeks of an ADF-LF diet in obese adults [5] [6]. Beneficial effects on CHD risk indicators were also noted. For instance, LDL cholesterol concentrations decreased by 10-25%, while triacylglycerol concentrations were lowered by 30-40% from baseline [5] [6]. In the trial by Varady et al., decreases in systolic blood pressure and heart rate were also demonstrated [5].

Although these data for ADF-LF diets are promising, the applicability of these findings is questionable as the majority of Americans consume a high-fat (HF) diet, and not a LF diet. More specifically, the most recent data from the National Health and Nutrition Examination Survey (NHANES) suggest that the average middle age American consumes 35-45% of their daily calories as dietary fat [7]. This report also indicates that 13% of energy is consumed as saturated fat [7]. In view of these findings, an important question that has yet to be tested is whether these beneficial changes in body weight and CHD risk can be reproduced if a HF background diet is used in place of a LF background diet during periods of ADF. Accordingly, the objective of the present study was to compare the effects of an ADF-HF diet to that of an ADF-LF diet on body weight, body composition, and CHD risk factors in obese adults.
C. Methods

Subjects

Subjects were recruited from the Chicago area by means of advertisements placed on and around the University of Illinois, Chicago campus. A total of 45 individuals expressed interest in the study, but only 36 were deemed eligible to participate after the preliminary questionnaire and body mass index (BMI) assessment (Figure 1). Key inclusion criteria were as follows: age 25–65 y, BMI between 30 and 39.9 kg/m\(^2\), weight stable for 3 months prior to the beginning of the study (i.e. <5 kg weight loss or gain), non-diabetic, no history of cardiovascular disease, sedentary or lightly active for 3 months prior to the beginning of the study (i.e. <3 h/week of light-intensity exercise at 2.5–4.0 metabolic equivalents (METS)), non-smoker, and not taking weight loss, lipid-lowering, or glucose-lowering medications. Perimenopausal women were excluded from the study, and postmenopausal women (defined as absence of menses for 2 y) 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. All volunteers gave written informed consent to participate in the trial.

Experimental design

Eligible subjects were randomized by way of a stratified random sample. The sample frame was divided into strata based on BMI, sex, and age. Subjects from each stratum were then randomly assigned to either the ADF-HF group or the ADF-LF group. The 10 week trial consisted of two dietary phases: 1) a 2-week baseline weight maintenance period, and 2) an 8-week weight loss ADF period. All food was provided throughout the 10-week trial to all subjects.
Baseline weight maintenance diet (Week 1-2)

Before commencing the 8-week ADF intervention, each subject participated in a 2-week baseline weight maintenance period where they consumed either the HF or LF diet (providing 100% of their energy needs). Energy requirements were calculated using the Mifflin equation [8]. Macronutrient compositions of the ADF-HF and ADF-LF diets are reported in Table 1. Diets were prepared in the metabolic kitchen of the Human Nutrition Research Unit (HNRU) at the University of Illinois, Chicago. Study diets were formulated for each participant using Nutritionist Pro Software (Axxya Systems LLC, Stafford, TX). Diets were provided as a 3-day rotating menu consisting of typical American foods. All meals were consumed outside of the research center. Participants were requested to eat only the foods provided and to bring back any leftover foods to be weighed and recorded. Subjects were also instructed to maintain their physical activity habits throughout the duration of the study.

Weight loss ADF diet (Week 3-10)

Following the baseline period, subjects participated in either an ADF-HF or ADF-LF weight loss intervention for 8 weeks. The same macronutrient composition was used during the weight loss and weight maintenance periods for the HF and LF diets (Table 1). All subjects consumed 25% of their energy needs on the fast day (24 h period), and 125% of their energy needs on the feed day (24 h period). Subjects were provided with 3 calorie-restricted meals on each feed day and one calorie restricted meal on each fast day. The feed/fast days began at midnight each day. 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.
Analyses

Adherence with ADF diets

Throughout the study, subjects were instructed to eat only the feed and fast day meals provided, and to keep track of all food items consumed using a “Food checklist”. Subjects were also asked to report any extra food item consumed using an “Extra food log”. Subjects were given a list of calorie-free foods such as sugar-free gum, mints, unsweetened tea/coffee, and water that were permitted throughout the study. The checklists and logs were collected and reviewed by study personnel each week. If the log indicated that the subject ate extra food items (totalling >50 kcal) on a feed or fast day, that day was labelled as “not adherent”. If the log revealed that the subject did not eat any extra food item, that day was labelled as “adherent”. Adherence data was assessed each week as: \[ \% \text{ Adherence} = \frac{(\# \text{ fast days adherent} / \# \text{ of fast days in the week}) + (\# \text{ feed days adherent} / \# \text{ of feed days in the week})}{2} \times 100 \]. No dietary counseling was provided to the subjects regardless of level of adherence.

Body weight and body composition assessment

Body weight measurements were taken to the nearest 0.5 kg at the beginning of every week in light clothing and without shoes using a balance beam scale (HealthOMeter; Sunbeam Products, Boca Raton, FL). BMI was assessed as kg/m^2. Fat mass and fat free mass were assessed by dual energy X-ray absorptiometry (DXA) at weeks 1, 3 and 10 (QDR 4500 W, Hologic Inc. Arlington, MA).

Blood collection protocol

Twelve-hour fasting blood samples were collected between 6.00 am and 9.00 am at baseline, week 3, 7, and 10. The subjects were instructed to avoid exercise, alcohol, and coffee for 24 h before each visit. Blood was centrifuged for 10 min at 520 \( \times \) g at 4°C to separate plasma from red blood cells and was stored at -80°C until analyzed.
Plasma lipid profile, blood pressure, and heart rate determination

Plasma total cholesterol, HDL-cholesterol, and triacylglycerol concentrations were measured in duplicate by using enzymatic kits (Biovision Inc, Mountainview, CA) and analyzed using a microplate reader (iMark Microplate Reader; Bio-Rad Laboratories Inc, Richmond, CA). The concentration of LDL cholesterol was calculated using the Friedewald, Levy, and Fredrickson equation [9]. The interassay CVs for total cholesterol, HDL-cholesterol, and triacylglycerol concentrations were 2.9%, 1.8%, and 2.4%, respectively. Blood pressure and heart rate were measured in triplicate with the subject in a seated position after a 10-min rest.

Statistics

Results are presented as mean ± SEM. Tests for normality were included in the model. An independent samples t-test was used to test baseline differences between groups. Repeated-measures ANOVA was performed (taking time as the within-subject factor and diet as the between-subject factor) to assess differences between groups over the course of the study. Post-hoc analyses were performed using the Tukey test. Differences were considered significant at P < 0.05. All data was analyzed using SPSS software (version 20.0, SPSS Inc, Chicago, IL).
D. Results

Subject dropout and baseline characteristics

Thirty-six subjects commenced the study and 33 completed the entire 10-week trial (Figure 1). Two subjects dropped out of the ADF-HF group due to an inability to comply with the ADF protocol (n = 1) and scheduling conflicts (n = 1). As for the ADF-LF group, one subject dropped out due to an inability to adhere to the diet. Baseline characteristics of the ADF-HF and ADF-LF groups are reported in Table 2. There were no differences between groups for age, sex, ethnicity, BMI, or plasma lipids.

Adherence to ADF diets

During the baseline weight maintenance period, ADF-HF and ADF-LF subjects were 96% and 94% adherent, respectively, with the provided diet. Throughout the weight loss period, the ADF-HF group had higher (P < 0.05) percent adherence (87 ± 9%) to the protocol than the ADF-LF group (77 ± 8%). There was no decline in adherence over the course of the ADF weight loss period.

Weight loss and body composition

During the baseline period (weeks 1-2), both the ADF-HF and ADF-LF groups lost weight (P < 0.001), despite being given diets that provided 100% of their energy needs (Figure 2). During the ADF weight loss period (weeks 3-10), body weight was reduced (P < 0.0001) by 4.9 ± 1.1% (4.4 ± 1.0 kg) in the ADF-HF group and by 4.2 ± 0.8% (3.7 ± 0.7 kg) in the ADF-LF group. There were no differences between groups for weight loss at any time point. BMI decreased (P < 0.0001) by 1.7 ± 0.4 and 1.5 ± 0.3 kg/m², respectively, in the ADF-HF and ADF-LF groups during the weight loss period. Changes in body composition are reported in Table 3. Fat mass and fat free mass did not change during the baseline weight maintenance period. During the weight loss period, fat mass decreased (P < 0.0001) in the ADF-HF and ADF-LF groups by 5.6 ± 1.5 kg and 4.2 ± 0.6 kg, respectively. There were no differences between
groups for fat mass at any time point. Fat free mass remained unchanged throughout the course of the trial. Waist circumference did not change during the baseline period in either intervention group. During the weight loss period, waist circumference decreased (P < 0.001) by 7.5 ± 1.5 cm and 7.3 ± 0.9 cm in the ADF-HF group and ADF-LF group, respectively.

Plasma lipids, blood pressure, and heart rate

Plasma lipids did not change during the baseline period in either the ADF-HF or ADF-LF group. During the weight loss period (week 3-10) (Figure 3), total cholesterol concentrations decreased (P < 0.0001) in both the ADF-HF group (12.5 ± 2.1%, week 3: 196 ± 10 mg/dl, week 10: 171 ± 9 mg/dl) and ADF-LF group (16.3 ± 1.7%, week 3: 193 ± 8 mg/dl, week 10: 162 ± 7 mg/dl). LDL cholesterol concentrations were also reduced (P < 0.0001) during the weight loss period by the ADF-HF diet (19.1 ± 4.8%, week 3: 110 ± 9 mg/dl, week 10: 89 ± 7 mg/dl) and ADF-LF diet (24.8 ± 2.6%, week 3: 113 ± 7 mg/dl, week 10: 85 ± 7 mg/dl). HDL cholesterol concentrations were not altered by either diet. Triacylglycerol concentrations decreased (P < 0.001) in the ADF-HF group (13.3 ± 4.7%, week 3: 121 ± 15 mg/dl, week 10: 105 ± 14 mg/dl) and ADF-LF group (14.3 ± 4.4%, week 3: 97 ± 11 mg/dl, week 10: 83 ± 10 mg/dl), during the weight loss period. There were no differences between groups for any plasma lipid parameter. Blood pressure was not altered by the ADF-HF diet (systolic: -2.8 ± 5.4 mmHg, diastolic: -2.1 ± 3.4 mmHg) or the ADF-LF diet (systolic: -2.5 ± 2.5 mmHg, diastolic: -1.9 ± 2.6 mmHg) during the weight loss period. Heart rate also remained unchanged during weeks 3 to 10 by either diet (ADF-HF: 2.8 ± 4.1 beats/min, ADF-LF: -2.2 ± 2.8 beats/min).
E. Discussion

This study is the first to show that an ADF-HF diet (45% fat) is equally as effective as an ADF-LF diet (25% fat) in helping obese subjects lose weight and improve CHD risk factors. Specifically, we show here that body weight reductions were comparable between the ADF-HF diet (4.9%) and the ADF-LF diet (4.2%). We also observed similar decreases in fat mass for the ADF-HF and ADF-LF groups, with retention of lean mass. Reductions in several key biomarkers for CHD risk, such as total cholesterol, LDL cholesterol, and triacylglycerols, were also comparable between the HF and LF diet regimens. Taken together, these data suggest that individuals who typically consume HF foods do not need to lower the fat content of their diet to experience the benefits of ADF.

The main goal of the present study was to investigate whether similar decreases in body weight could be observed if a HF diet was used in place of a LF diet during periods of ADF. Results from our trial indicate that ADF is able to decrease body weight by 4 kg in 8 weeks, independent of the background macronutrient composition of the diet. As such, an individual can consume a diet with 45% of energy as dietary fat (13% of energy as saturated fat), and still experience similar weight loss as someone consuming a diet with 25% of energy as fat (6% of energy as saturated fat). Our findings are in concordance with other calorie restriction (CR) studies that manipulate dietary fat content. For instance, in the study by Papakonstantinou et al.[10], obese subjects lost similar amounts of weight (i.e. 3 kg in both groups) after 4 weeks of either a HF (35% fat) or LF (20% fat) CR diet. Jenkins et al. [11] also showed that an energy restricted HF diet (43% fat) produced similar decreases in body weight (i.e. 4 kg in both groups) as an energy restricted LF diet (25% fat) after 4 weeks of treatment. Thus, dietary restriction protocols appear to facilitate weight loss regardless of the fat composition of the diet. In addition to body weight, we also examined dietary adherence to the ADF-HF versus ADF-LF diet. Not surprisingly, subjects were able to adhere to the HF diet to a greater extent (i.e. 87% of days adherent)
than the LF diet (i.e. 77% of days adherent). This may be related to the greater palatability of higher fat foods [12] [13]. This finding has important implications for diet tolerability, as individuals will not need to change the types of foods they eat, only the pattern of food consumption in order to experience weight loss with ADF.

Body composition was also favorably altered with both diets. To elaborate, fat mass decreased to a similar extent in the ADF-HF group (5.6 kg) and the ADF-LF group (4.2 kg). As for fat free mass, non-significant increases were noted for both the HF diet (1.2 kg) and LF diet (0.5 kg). These data suggest that the weight loss observed with ADF results from a decrease in fat mass, and not fat free mass. Our findings also indicate that this regimen may allow for the retention of lean mass during periods of dietary restriction. A similar preservation of lean mass (0.5 kg) was noted in a previous ADF study conducted by our group [4]. Interestingly, this retention in lean mass observed with ADF is not replicated with CR diets. For instance, consistent reductions of 3-5% in fat free mass are generally noted after 8 weeks of 25-40% CR [15] [16]. The reason why ADF may assist with the preservation of lean mass is not known at present, but will undoubtedly be of interest in future studies in this field. Well-documented studies have shown that in periods of prolonged fasting, levels of human growth hormone are elevated [17]. Growth hormone aids in preserving whole body nitrogen balance and regulates protein synthesis to prevent lean mass loss [17]. Another body composition parameter that was beneficially modulated by ADF was waist circumference (used as an indirect indicator of visceral fat mass). We show here that 8 weeks of ADF can decrease waist circumference by 7 cm, and that these changes can occur with either a HF or LF background diet. Other recent trials have also reported equivalent reductions in waist circumference with either HF or LF diets during dietary restriction [18] [19]. For example, in the study by Sacks et al. [19], waist circumference was decreased to the same extent (7 cm with 4 kg weight loss) when an energy restricted HF diet (40% fat) was compared to an
energy restricted LF diet (20% fat). Thus, individuals who typically consume a HF diet can continue with their usual eating habits during ADF and still observe the same reductions in visceral fat mass as seen with a LF diet.

Comparable changes in CHD risk parameters were also observed for the HF and LF diets. For instance, LDL cholesterol concentrations were reduced to a similar extent by the ADF-HF diet (19%) as the ADF-LF diet (25%). Triacylglycerol concentrations were also decreased by both the HF and LF diets (13% and 14%, respectively). It is likely that a similar degree of LDL cholesterol lowering was attained by these diets because both groups lost similar amounts of weight [20]. LDL-cholesterol has been estimated to be reduced by 2.0 mg/dl per kg of weight loss [20] based on a meta-analysis by Datillo et al. [20] Since body weight was reduced to the same extent in both groups (approximately 4 kg), it is not surprising that both diets experienced similar reductions in LDL cholesterol. These results are also in line with findings from a pilot ADF study by Varady et al. [5]. Although our subjects had greater reductions in LDL-C concentrations per kilogram body weight lost than previously mentioned studies, further studies are necessary in order to identify mechanisms behind these above average improvements. The relationship between weight loss and LDL cholesterol lowering has also been demonstrated in CR trials that compared HF to LF background diets [21] [22]. As for HDL cholesterol concentrations, no effect was noted by either the HF or LF diets. Previous studies of ADF also report no change in this lipid parameter [5, 6]. Since HDL cholesterol very rarely changes with dietary restriction [20], this result is in line with what was hypothesized. Blood pressure and heart rate also remained unchanged over the course of the trial. This lack of effect was most likely due to the high variability for these parameters between subjects. As such, it is possible that if a larger number of subjects were recruited to each intervention, the results for blood pressure and heart rate may have been significant.
This study is limited in that we were only able to recruit one male participant. Thus, whether or not these findings are generalizable to males cannot be elucidated at present. Another key weakness of the study is that both intervention groups lost weight during the baseline weight maintenance period. These reductions in body weight occurred despite distributing diets that provided 100% of each subject’s daily energy needs. To add further complication, participants reported a mean adherence rate of 94-96% with the weight maintenance diet. The reason for this drop in weight during this period is unknown. It is possible however, that upon starting the study the subjects were so eager to begin losing weight that they did not eat all the food provided, and potentially misreported their adherence rate [23]. It is also possible that the subjects may have become more physically active during these two baseline weeks to boost their weight loss. Future studies in this area should therefore aim to control for physical activity during the course of the trial by using an accelerometer to assess energy expenditure [24].

In summary, our findings demonstrate that ADF can elicit beneficial effects on body weight, body composition, and CHD risk, independent of the background fat content of the diet. Therefore, individuals who typically consume foods that are high in fat do not need to lower the fat composition of their diet to experience the benefits of ADF. Since the typical American consumes a diet high in fat (>35% fat), these findings are paramount in terms of diet tolerability. This discovery may also play a key role in determining whether individuals can comply with the ADF regimen long-term.
F. Acknowledgements

MCK designed the experiment, conducted the clinical trial, analyzed the data, and wrote the manuscript.

CMK assisted with the conduct of the clinical trial. KAV assisted with the design of the experiment, and wrote the manuscript. The authors have no conflicts of interest to report.
G. References


7. What we eat in America, NHANES 2007-2008, individuals 2 years and over, 1 day dietary intake data, weighted. In; 2010.


### Table I. Nutrient composition of the ADF-HF and ADF-LF diets

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>ADF-HF</th>
<th>ADF-LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total fat (g)</td>
<td>100 (45%) 3</td>
<td>55 (25%) 3</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>30</td>
<td>13</td>
</tr>
<tr>
<td>Monounsaturated fat (g)</td>
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<td>30</td>
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<tr>
<td>Polyunsaturated fat (g)</td>
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<td>12</td>
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<tr>
<td>Trans fat (g)</td>
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<td>0</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>328</td>
<td>112</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>75 (15%) 3</td>
<td>75 (15%) 3</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>200 (40%) 3</td>
<td>300 (60%) 3</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>27</td>
<td>27</td>
</tr>
<tr>
<td>Sodium (mg)</td>
<td>780 (39%)</td>
<td>600 (30%)</td>
</tr>
</tbody>
</table>

1 Values reported as mean ± SEM. Alternate day fasting high-fat diet (ADF-HF); alternate day fasting low-fat diet (ADF-LF).
2 Values for nutrients based on a 2000 kcal diet.
3 Percent of daily kcal.
### Table I. Subject characteristics at baseline $^{1,2}$

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ADF-HF</th>
<th>ADF-LF</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>16</td>
<td>17</td>
</tr>
<tr>
<td>Age (y)</td>
<td>$41.3 \pm 3.0$</td>
<td>$43.2 \pm 2.3$</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
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<tr>
<td>Male</td>
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<td>0</td>
</tr>
<tr>
<td>Female</td>
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<td>17</td>
</tr>
<tr>
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<td>Caucasian</td>
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<td>0</td>
</tr>
<tr>
<td>Hispanic</td>
<td>6</td>
<td>3</td>
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<tr>
<td>Body weight (kg)</td>
<td>$91.6 \pm 5.3$</td>
<td>$91.5 \pm 2.9$</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>$161.7 \pm 1.6$</td>
<td>$160.2 \pm 1.4$</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>$35.1 \pm 0.7$</td>
<td>$35.5 \pm 0.7$</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>$204 \pm 11$</td>
<td>$201 \pm 6$</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>$118 \pm 9$</td>
<td>$124 \pm 6$</td>
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<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>$62 \pm 4$</td>
<td>$60 \pm 4$</td>
</tr>
<tr>
<td>Triacylglycerols (mg/dl)</td>
<td>$122 \pm 16$</td>
<td>$108 \pm 13$</td>
</tr>
</tbody>
</table>

$^1$ Values reported as mean $\pm$ SEM. Alternate day fasting high-fat diet (ADF-HF); alternate day fasting low-fat diet (ADF-LF). No differences between groups for any parameter (Independent samples t-test).
Table I.III Body composition changes during the weight loss period

<table>
<thead>
<tr>
<th></th>
<th>ADF-HF</th>
<th>ADF-LF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 1</td>
<td>Week 3</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>91.4 ± 2.7</td>
<td>89.3 ± 2.6</td>
</tr>
<tr>
<td>BMI</td>
<td>35.1 ± 0.7</td>
<td>34.3 ± 0.7</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>44.5 ± 1.9</td>
<td>43.1 ± 1.9</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>46.8 ± 1.1</td>
<td>46.3 ± 1.6</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>100.8 ± 1.8</td>
<td>98.3 ± 1.6</td>
</tr>
</tbody>
</table>

1 Values reported as mean ± SEM. Alternate day fasting high-fat diet (ADF-HF), n = 16; alternate day fasting low-fat diet (ADF-LF), n = 17.
2 Change expressed as the difference between week 3 and week 10 values. No differences between the ADF-HF and ADF-LF groups for absolute change in any body composition parameter (Independent samples t-test).
3 Significantly different from baseline (week 3), P < .005 (Repeated-measures ANOVA).
I. Figures

Figure 1.1 Study flow chart
Figure 1.2 Body weight during the 10-week trial

Mean body weight of ADF-LF (alternate day fasting–low fat) subjects (n = 17) and ADF-HF (alternate day fasting-high fat) subjects (n = 16) at each week. *Week 10 values significantly different (P = 0.0001) from week 3 values (Paired t-test). No differences between groups at week 10 (Independent samples t-test).
Figure 3. Body composition changes during the 10-week trial

1.3A Mean change in fat mass and fat free mass of ADF-LF (alternate day fasting–low fat) subjects (n = 17) and ADF-HF (alternate day fasting–high fat) subjects (n = 16) between week 3 and week 10.

*Significantly different (P = 0.0001) within group (Paired t-test).

1.3B Mean waist circumference of ADF-LF subjects (n = 17) and ADF-HF subjects (n = 16) between week 3 and week 10. *Week 10 values significantly different (P = 0.0001) from week 3 values (Paired t-test). No differences between groups at week 10 (Independent samples t-test).
Figure 1.4 Plasma lipid changes during the 10-week trial.

Mean change in plasma lipids of ADF-LF (alternate day fasting–low fat) subjects (n = 17) and ADF-HF (alternate day fasting–high fat) subjects (n = 16) between week 3 and week 10. *Significantly different (P = 0.0001) within group (Paired t-test).
IV. MANUSCRIPT II

Alternate day fasting increases LDL particle size independently of dietary fat content in obese humans*

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Running title: Alternate day fasting and LDL particle size

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Conflicts of interest: The authors have no conflicts of interest to report.

A. Abstract

Alternate day fasting (ADF) with a low-fat (LF) diet increases LDL particle size. Whether these beneficial effects can be reproduced by a high-fat (HF) ADF diet is unclear. This study compared an ADF-HF to an ADF-LF diet for plasma lipids, LDL size, and HDL size. Thirty-five obese adults were randomized to an ADF-HF or ADF-LF diet for 10 weeks. Body weight decreased (P < 0.0001) by 4.3 ± 1.0 kg (4.8 ± 1.1%) and 3.7 ± 0.7 kg (4.2 ± 0.8%) in the ADF-HF and ADF-LF group, respectively. LDL cholesterol was reduced (P < 0.0001) by 19 ± 8 mg/dl (18 ± 5%) by ADF-HF and 28 ± 7 mg/dl (25 ± 3%) by ADF-LF. LDL particle size increased (P < 0.005) by 3 ± 1 Å in both groups. The proportion of atherogenic small LDL particles decreased (P < 0.005) by 8 ± 2% and 11 ± 3% in the ADF-HF and ADF-LF groups, respectively, compared to baseline levels. HDL cholesterol and HDL size remained unchanged. Thus, our results suggest that the ADF-HF diet is equally as effective as the ADF-LF diet in improving LDL particle size and distribution.

**Keywords:** Alternate day fasting, calorie restriction, dietary fat, weight loss, LDL particle size, HDL particle size
B. Introduction

LDL and HDL particle size are important predictors of cardiovascular events and progression [1]. Small LDL particles are detrimental to vascular health due to an increased propensity for oxidation [1]. Small HDL particles, on the other hand, may increase coronary heart disease (CHD) risk by altering the activity of lipases involved with the maturation and transformation of lipoproteins [1]. Dietary restriction regimens have shown promise for increasing LDL and HDL particle size. Alternate day fasting (ADF) is an effective diet regimen that has helped obese individuals to improve LDL and HDL size and distribution [2] [3]. ADF regimens include a “feed day” where food is consumed ad-libitum over 24 h (which generally equates to 125% of energy needs consumed ([2] [3]), followed by a “fast day” where intake is limited to 25% of the individual’s energy needs over 24 h. Two recent clinical trials of ADF, with a low-fat (LF) background diet, demonstrate that LDL and HDL particle size increased, while the proportion of small particles decreased, after 8-12 weeks of treatment [2] [3]. These favorable changes were accompanied by LDL cholesterol and triglyceride reductions of 10-25% and 30-40% respectively, from baseline [2] [3].

Although ADF-LF diets have shown promising results, recent data from the National Health and Nutrition Examination Survey (NHANES) suggest that the average middle age American consumes 35-45% of their daily calories as dietary fat [4]. In view of the high fat content of the American diet, it is important to investigate whether the improvements in LDL and HDL particle size by ADF can be reproduced if a high-fat (HF) background diet is used in place of a LF diet. Accordingly, the objective of the present study was to compare the effects of an ADF-HF diet to that of an ADF-LF diet on HDL and LDL particle size and distribution in obese adults.
C. Methods

Subject selection

Obese subjects were recruited from the Chicago area by advertisements. Key inclusion criteria were as follows: females, age 25–65 y, BMI between 30 and 39.9 kg/m², weight stable, previously sedentary, and no history of cardiovascular disease. Eligible subjects were randomized by a stratified random sample based on BMI and age to either the ADF-HF group or the ADF-LF group. The experimental protocol was approved by the Office for the Protection of Research Subjects at the University of Illinois, Chicago, and all volunteers gave written informed consent.

Study design and diet protocol

The trial ran for 10 weeks and involved a 2-week baseline weight maintenance period followed by an 8-week weight loss period. During the baseline period, subjects in the ADF-HF group consumed 100% of their energy needs as the HF diet, while subjects in the ADF-LF group consumed 100% of their energy needs as the LF diet. During the 8-week weight loss period, subjects consumed either the HF or LF diet while undergoing ADF. The ADF protocol involved consuming 25% of energy needs on the fast day (24 h period), and 125% of energy needs on the feed day (24 h period). Fast day meals were consumed between 12.00 pm and 2.00 pm. All meals were provided during the entire 10-week trial. Energy requirements were calculated using the Mifflin equation [5]. Macronutrient distributions of the two diets were as follows: ADF-HF (45% fat, 40% carbohydrate, 15% protein), ADF-LF (25% fat, 60% carbohydrate, 15% protein). Fat content of the diets were as follows: ADF-HF (14% saturated fat, 20% monounsaturated fat, 11% polyunsaturated fat, 0% trans fat), and ADF-LF (6% saturated fat, 13% monounsaturated fat, 6% polyunsaturated fat, 0% trans fat). Body weight was measured weekly using a balance beam scale (HealthOMeter; Sunbeam Products, Boca Raton, FL). Fat mass and fat free mass
were assessed by dual energy X-ray absorptiometry (DXA) at weeks 1, 3 and 10 (QDR 4500 W, Hologic, Arlington, MA).

**Plasma lipids and particle size determination**

Twelve-hour fasting blood samples were collected at week 1, 3 and 10. Plasma total cholesterol, direct LDL-cholesterol, HDL-cholesterol, and triglyceride concentrations were measured in duplicate using enzymatic kits (Biovision Inc., Mountainview, CA, USA). LDL and HDL particle size were measured by linear polyacrylamide gel electrophoresis (Quantimetrix Lipoprint System, Redondo Beach, CA), as described previously [6]. 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 HDL into small (<73 Å), medium (73-88 Å), and large (>88 Å) particles [6], which were then used to calculate single size values. The interassay coefficients of variation (CV) for total cholesterol, HDL-cholesterol, triacylglycerol concentrations, and LDL particle size were 2.9%, 1.8%, 2.4%, and 1.9% respectively.

**Statistics**

Results are presented as mean ± SEM. Normality was assessed by the Kolmogorov-Smirnov test. No variables were found to be not normal. An independent samples t-test was used to test baseline differences between groups. Mixed model ANOVA using time x treatment was performed (taking time as the within-subject factor and diet as the between-subject factor) to assess differences between groups over the course of the study. Post-hoc analyses were performed using the Tukey test. Power was based on the primary outcome of weight loss, and calculated to be 80%. Differences were considered significant at P < 0.05. All data was analyzed using SPSS software (version 20.0, SPSS Inc, Chicago, IL).
D. Results

Baseline characteristics and weight loss

Thirty-five subjects began the study (ADF-HF: n = 17, ADF-LF: n = 18). Two subjects dropped out of the ADF-HF group, and one subject dropped out of the ADF-LF group due to an inability to comply with the diet. Baseline body weight (ADF-HF: 91.5 ± 2.6 kg, ADF-LF: 91.5 ± 2.9 kg), percent body fat (ADF-HF: 48.5 ± 0.9%, ADF-LF: 47.8 ± 1.1%), age (ADF-HF: 42.4 ± 3.0 y, ADF-LF: 43.2 ± 2.3 y) and BMI (ADF-HF: 35.3 ± 0.7 kg/m², ADF-LF: 35.5 ± 0.7 kg/m²) did not differ between groups. During the weight loss period (weeks 3-10), body weight was reduced (P < 0.0001) by 4.3 ± 1.0 kg and 3.7 ± 0.7 kg (4.8 ± 1.1% and 4.2 ± 0.8%), fat mass decreased by (P < 0.0001) 5.4 ± 1.5 kg and 4.2 ± 0.6 kg, while fat free mass remained unchanged (ADF-HF: 1.1 ± 1.3 kg; ADF-LF: 0.5 ± 0.7 kg).

Plasma lipids

Total cholesterol decreased by 13 ± 2 and 16 ± 2% (P < 0.0001) in the ADF-HF and ADF-LF groups, respectively (Table 1). LDL cholesterol concentrations were also reduced by 18 ± 5 and 25 ± 3% (P < 0.0001) by the ADF-HF and ADF-LF diet, respectively. Triglycerides decreased by 14 ± 5 and 14 ± 4 % (P < 0.001) in the ADF-HF and ADF-LF groups, respectively. HDL cholesterol did not change in either group. There were no between-group differences for any plasma lipid parameter.

LDL and HDL particle size

Peak LDL particle size increased by 3 ± 1 Å (P < 0.005) in both groups (Table 2). The proportion of large LDL particles increased by 6 ± 2 and 3 ± 1% (P < 0.005) in the ADF-HF and ADF-LF groups respectively, while the proportion of small particles decreased (P < 0.005) by 8 ± 2 and 11 ± 3% (P < 0.005) in the ADF-HF and ADF-LF groups, respectively. Medium LDL particles increased by 8 ± 3 % (P < 0.005) in the ADF-LF group only. HDL particle size was not changed in either group.
E. Discussion

This study is the first to show that ADF with a HF background diet (45% fat) elicits the same beneficial effects on LDL particle size as ADF with an LF diet (25% fat). Specifically, the ADF-HF diet was as effective as the ADF-LF intervention at increasing LDL particle size, elevating the proportion of large LDL particles, and decreasing the proportion of small LDL particles. HDL particle size and distribution were not affected by either diet.

These findings are in concordance with previous studies examining the effects of calorie restricted-high fat (CR-HF) diets on LDL particle size [1] [7]. For instance, Krauss et al. demonstrated that peak LDL particle size was increased to a similar extent by a CR-HF (5 ± 1 Å) and a CR-LF intervention (3 ± 1 Å), after 5 weeks of treatment [1]. Similarly, in a study by Hays et al., obese individuals were observed to have increases in LDL particle size (4 ± 1 Å) after 6 weeks on an energy restricted HF diet (40% fat) [7]. Recent evidence indicates that diets high in saturated fat, such as in our study, and cholesterol cause progressive elevations in LDL particle size because of an increase in cholesterol ester content (8). As for HDL particle size, the present study indicates that ADF-HF and ADF-LF diets have no effect on this lipid parameter. This is not surprising as HDL particle size is rarely improved through changing dietary patterns alone. Although one recent study demonstrated increases in HDL particle size (2 ± 1 Å) after 6 weeks of a CR-HF diet (40% fat) [1], others have shown no effect [9]. To date, the most effective intervention to improve HDL size and distribution is endurance exercise training [10]. Exercise-induced mechanisms for this change may involve increases in lipoprotein lipase activity (LPLa) and lecithin cholesterol acyl-transferase activity (LCATa) [11]. As such, it is possible that if this intervention were combined with exercise, beneficial changes in HDL size would be noted.
This study is limited in that the sample size of the two intervention groups (n = 15-17) was quite small. This may have limited our ability to detect significant differences in HDL particle size. Our study is also limited in that we used polyacrylamide gel electrophoresis, instead of a more robust measure, such as nuclear magnetic resonance (NMR). A key strength of our study is the controlled feeding component. Since we provided all the food, we can be certain that the dietary fat intake of the subjects was close to 45% in the HF group and 25% in the LF group.

Taken together, ADF can improve certain CHD risk factors, such as LDL particle size, regardless of dietary fat content. Whether these effects persist long-term warrants further investigation. From a clinical standpoint, these findings suggest that individuals will not need to change the types of foods they eat, only the pattern of food consumption, to experience the cardio-protective benefits of ADF.
F. Acknowledgements

We would like to thank the research participants for their enthusiasm and commitment to the study.
G. References:


### H. Tables

#### Table II.1 Plasma lipids at baseline and post-treatment

<table>
<thead>
<tr>
<th></th>
<th>ADF-HF</th>
<th></th>
<th></th>
<th>ADF-LF</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 3</td>
<td>Week 10</td>
<td>% Change</td>
<td>Week 3</td>
<td>Week 10</td>
<td>% Change</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>198 ± 11</td>
<td>172 ± 9 (^3)</td>
<td>-13 ± 2</td>
<td>193 ± 8</td>
<td>162 ± 7 (^3)</td>
<td>-16 ± 2</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>109 ± 9</td>
<td>90 ± 7 (^3)</td>
<td>-18 ± 5</td>
<td>113 ± 7</td>
<td>85 ± 7 (^3)</td>
<td>-25 ± 3</td>
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<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>63 ± 4</td>
<td>63 ± 4</td>
<td>0 ± 0</td>
<td>58 ± 4</td>
<td>60 ± 3</td>
<td>3 ± 3</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>123 ± 15</td>
<td>108 ± 15 (^3)</td>
<td>-14 ± 5</td>
<td>97 ± 11</td>
<td>83 ± 10 (^3)</td>
<td>-14 ± 4</td>
</tr>
</tbody>
</table>

\(^1\) Values reported as mean ± SEM. Alternate day fasting high-fat diet (ADF-HF), n = 15; alternate day fasting low-fat diet (ADF-LF), n = 17.

\(^2\) Change expressed as the percent difference between week 3 and week 10 values.

\(^3\) Significantly different from baseline (week 3), P < .005 (Repeated-measures ANOVA).

There were no differences between groups for any plasma lipid parameter.
Table II.II LDL/HDL particle size and distribution at baseline and post-treatment

<table>
<thead>
<tr>
<th></th>
<th>ADF-HF</th>
<th>ADF-LF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 3</td>
<td>Week 10</td>
</tr>
<tr>
<td>LDL size (Å)</td>
<td>258 ± 1</td>
<td>261 ± 1 ³</td>
</tr>
<tr>
<td>% Large LDL particles</td>
<td>38 ± 1</td>
<td>44 ± 2 ³</td>
</tr>
<tr>
<td>% Medium LDL particles</td>
<td>29 ± 1</td>
<td>31 ± 4</td>
</tr>
<tr>
<td>% Small LDL particles</td>
<td>33 ± 1</td>
<td>25 ± 2 ³</td>
</tr>
<tr>
<td>% Large HDL particles</td>
<td>46 ± 2</td>
<td>44 ± 3</td>
</tr>
<tr>
<td>% Medium HDL particles</td>
<td>35 ± 3</td>
<td>37 ± 3</td>
</tr>
<tr>
<td>% Small HDL particles</td>
<td>19 ± 2</td>
<td>19 ± 3</td>
</tr>
</tbody>
</table>

¹Values reported as mean ± SEM. Alternate day fasting high-fat diet (ADF-HF), n = 15; alternate day fasting low-fat diet (ADF-LF), n = 17.
²Change expressed as the difference between week 3 and week 10 values.
³Significantly different from baseline (week 3), P < .005 (Repeated-measures ANOVA).
There were no differences between groups for any particle size parameter.
V. MANUSCRIPT III

Benefit of a low-fat over high-fat diet on vascular health during alternate day fasting*

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Running head: Alternate day fasting, dietary fat and endothelial function

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Conflict of interest: The authors have no conflicts of interest to report.

A. Abstract

Background: Alternate day fasting (ADF) with a low-fat (LF) diet improves brachial artery flow-mediated dilation (FMD). Whether these beneficial effects can be reproduced with a high-fat (HF) diet remains unclear. Objective: This study compared the effects of ADF-HF to ADF-LF regimens on FMD. The role that adipokines play in mediating this effect was also investigated. Methods: Thirty-two obese subjects were randomized to an ADF-HF (45% fat) or ADF-LF diet (25% fat), consisting of two phases: 1) a 2-week baseline weight maintenance period, and 2) an 8-week ADF weight loss period. Food was provided throughout the study. Results: Body weight was reduced (P < 0.0001) in the ADF-HF (4.4 ± 1.0 kg) and ADF-LF group (3.7 ± 0.7 kg). FMD decreased (P < 0.05) by ADF-HF relative to baseline (7 ± 1% to 5 ± 2%) and increased (P < 0.05) by ADF-LF (5 ± 1% to 7 ± 2%). Blood pressure remained unchanged in both groups. Adiponectin increased (P < 0.05) in the ADF-HF (43 ± 7%) and ADF-LF group (51 ± 7%). Leptin and resistin decreased (P < 0.05) in the ADF-HF (32 ± 5%; 23 ± 5%) and ADF-LF group (30 ± 3%; 27 ± 4%). Increases in adiponectin were associated with augmented FMD in the ADF-LF group only (r = 0.34, P = 0.03). Conclusion: Thus, improvements in FMD with ADF may only occur with LF diets and not with HF diets, and adipokines may not play a significant role in mediating this effect.

Keywords: Alternate day fasting, calorie restriction, dietary fat, endothelial function, flow-mediated dilation, adipokines, weight loss, obese humans
B. Introduction

Obesity is a well-established risk factor for coronary heart disease (CHD). Obese individuals often exhibit increased LDL cholesterol and triglyceride concentrations, along with elevated blood pressure [1]. Excessive fat mass is also linked to abnormal endothelial function, marked by reduced vasodilation to an increased blood flow (endothelium-dependent flow-mediated dilation; FMD) [2]. Endothelial dysfunction is an early predictor of future vascular events, and most CHD risk factors are correlated with reduced FMD [3].

Weight loss, by means of dietary restriction, has been shown to improve several CHD risk factors, including FMD. Alternate day fasting (ADF) is a novel diet restriction strategy that has gained considerable popularity over the past decade. ADF consists of a 24-h period of ad libitum food consumption termed the “feed day”, alternated with a 24-h period of 75% energy restriction, termed the “fast day” [4] [5]. Recent reports indicate that ADF, with a low-fat (LF) diet background (25% of energy as fat), decreases body weight by 5% after 8 weeks of treatment in obese subjects [6]. FMD was also increased in this study [6]. Although these findings are promising, it is still unclear if the same beneficial increases in FMD would be seen if a high-fat (HF) diet (45% of energy as fat) was used in place of a LF diet during ADF. This question is of importance as the majority of Americans consume 35-45% of their daily calories as dietary fat [7].

The underlying mechanisms that link weight loss to improved endothelial function are still uncertain. Accumulating evidence demonstrates that adipokines may have an impact on vascular function. Adiponectin is a fat-cell-derived hormone that increases with weight loss, and protects the endothelium by decreasing oxidative stress [8] [9]. Leptin and resistin, in contrast, are adipokines that are positively correlated to body weight and visceral fat mass [10] [11]. Leptin and resistin have been shown to cause
endothelial dysfunction by promoting oxidative stress [10] [11]. In view of this, it can be hypothesized that weight loss strategies, such as ADF, would increase adiponectin and decrease leptin and resistin concentrations. In turn, these improvements in adipokine profiles may have a protective effect on the vascular endothelium resulting in increased FMD [12]. The effects of ADF-LF versus ADF-HF diets on FMD and adipokines have yet to be tested, however.

Accordingly, the objective of this study was to compare the effects of ADF-HF to ADF-LF regimens on FMD in obese adults. The role that adipokines play in mediating this effect was also investigated.
C. Methods

Subjects and study design

As previously described [13], obese subjects were recruited from the Chicago area based on the following inclusion criteria: female, age 25–65 y, BMI between 30 and 39.9 kg/m², weight stable for 3 months prior to the beginning of the study (i.e. <5 kg weight loss or gain), non-diabetic, no history of cardiovascular disease, sedentary or lightly active for 3 months prior to the beginning of the study (i.e. <3 h/week of light-intensity exercise at 2.5–4.0 metabolic equivalents (METS)), non-smoker, and not taking weight loss, lipid-lowering, or glucose-lowering medications. The experimental protocol was approved by the Office for the Protection of Research Subjects at the University of Illinois, Chicago, and all volunteers gave written informed consent. Eligible subjects were randomized by way of a stratified random sample based on BMI and age. Subjects from each stratum were then randomized to 1 of 2 intervention groups: 1) ADF-HF group (n = 17), or 2) ADF-LF group (n = 18). The 10 week trial consisted of two dietary phases: 1) a 2-week baseline weight maintenance period, and 2) an 8-week weight loss ADF period.

Diet protocol

All food was provided throughout the 10-week trial to all subjects. During the 2-week baseline weight maintenance period, subjects consumed either the HF or LF diet (providing 100% of their energy needs). Following the baseline period, subjects participated in either an ADF-HF or ADF-LF weight loss intervention for 8 weeks. During the weight loss period, subjects consumed 25% of their energy needs on the fast day (24 h period), and 125% of their energy needs on the feed day (24 h period). The feed/fast days began at midnight each day. Fast day meals were consumed between 12.00 pm and 2.00 pm. The same macronutrient composition was used during the weight loss and weight maintenance periods for each group: ADF-HF (45% fat, 40% carbohydrate, 15% protein), ADF-LF (25% fat, 60%...
carbohydrate, 15% protein), as described previously [13]. Fat content of the diets were as follows: ADF-HF (14% saturated fat, 20% monounsaturated fat, 11% polyunsaturated fat, 0% trans fat), and ADF-LF (6% saturated fat, 13% monounsaturated fat, 6% polyunsaturated fat, 0% trans fat). Energy requirements were calculated using the Mifflin-St. Jeor equation [14]. Study diets were formulated for each participant using Nutritionist Pro Software (Axxya Systems LLC, Stafford, TX). Diets were provided as a 3-day rotating menu consisting of typical American foods. All meals were consumed outside of the research center. Participants were requested to eat only the foods provided and to bring back any leftover foods to be weighed and recorded. Subjects were also instructed to maintain their physical activity habits throughout the duration of the study.

Analyses

**Diet compliance, hunger, and physical activity maintenance**

Subjects were instructed to keep track of all food items consumed using a “Food checklist”, and to report any extra food item consumed using an “Extra food log”. If the log indicated that the subject ate extra food items (totalling >50 kcal) on a feed or fast day, that day was labelled as “not adherent”. To measure changes in hunger, satisfaction, and fullness, subjects completed a validated visual analog scale (VAS) on each fast day [15]. The form was completed in the evening, approximately 5 min before going to bed. In brief, the VAS consisted of 100-mm lines, and subjects were asked to make a vertical mark across the line corresponding to their feelings from 0 (not at all) to 100 (extremely) for hunger, satisfaction, and fullness. The VAS was collected each week and reviewed for completeness. Quantification was performed by measuring the distance from the left end of the line to the vertical mark [15]. Free-living physical activity was assessed by a pedometer (Digiwalker SW-200, Yamax Corporation, Tokyo, Japan SW). Subjects wore the pedometer each day throughout the 10-week trial.
Number of daily steps were recorded in a pedometer log provided, and the log was collected by study personnel at the weigh-in each week.

**Body weight and body composition assessment**

Body weight measurements were taken weekly to the nearest 0.25 kg using a balance beam scale (HealthOMeter; Sunbeam Products, Boca Raton, FL). Height was assessed using a wall-mounted stadiometer to the nearest 0.1 cm. BMI was assessed as kg/m². Fat mass and fat free mass were assessed by dual energy X-ray absorptiometry (DXA) at weeks 1, 3 and 10 (QDR 4500 W, Hologic Inc. Arlington, MA). 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.

**Brachial artery measurements of flow mediated dilation (FMD)**

Brachial artery FMD was assessed at week 3 and 10. Ultrasound imaging of the brachial artery (MicroMaxx, Sonosite, Seattle, WA) was performed in a longitudinal plane at a site 1-3 cm proximal to the antecubital fossa, with the arm abducted approximately 80° from the body and the forearm supinated. The ultrasound probe (11 MHz) was positioned to visualize the anterior and posterior lumen-intima interfaces to measure diameter or central flow velocity (pulsed Doppler). After baseline images were recorded, a blood pressure cuff on the forearm was inflated to 200 mm Hg for 5 min. To assess FMD, 10 seconds of images were captured at a rate of 10 images/second, 30 seconds, one min and 2 min after cuff release. Baseline brachial flow velocity and peak velocity after cuff release were recorded. Images were digitally recorded using Brachial Imager (Medical Imaging, Iowa City, IA) and analyzed. Percent FMD was calculated using the averaged minimum mean brachial artery diameter at baseline compared to the largest mean values obtained after release of the forearm occlusion. Blood pressure was assessed in triplicate after a 10-min rest each week.
**Plasma adipokine determination**

Twelve-hour fasting blood samples were collected between 6.00 am and 10.00 am at week 1, 3 and week 10. Subjects were instructed to avoid exercise, alcohol and coffee for 24 h before each visit. Blood was centrifuged for 10 minutes at 1000 g and 4°C to separate plasma from RBC and was stored at -80°C until analyzed. Plasma adiponectin, leptin and resistin were measured in triplicate using high sensitivity enzymatic kits (R&D Systems, Minneapolis, MN).

**Statistics**

Values are presented as mean ± SEM. An independent samples t-test was used to test baseline differences between groups. Repeated-measures ANOVA was performed (taking time as the within-subject factor and diet as the between-subject factor) to assess differences between groups over the course of the study. Post-hoc analyses were performed using the Tukey test. Power was based on the primary outcome of weight loss, and calculated to be 80%. Differences were considered significant at P < 0.05. All data was analyzed using SPSS software (version 20.0 for Mac, SPSS Inc, Chicago, IL).
D. Results

Subject dropout and baseline characteristics

Thirty-two out of the initial 35 subjects completed the 10-week study. Two subjects in the ADF-HF group dropped out due to an inability to comply with the ADF protocol (n = 1) and scheduling conflicts (n = 1). One subject dropped out of the ADF-LF group due to an inability to follow the diet protocol. Thus, there were n = 15 completers in the ADF-HF group, and n = 17 completers in the ADF-LF group. Baseline characteristics of the ADF-HF and ADF-LF groups are reported in Table 1. Subjects were all female, with no differences between groups for age, weight, BMI, body composition, or adipokines.

Diet compliance, hunger, and physical activity maintenance

ADF-HF and ADF-LF subjects were 96% and 94% compliant, respectively, with the prescribed diet during the baseline weight maintenance period. However, during the weight loss period, the ADF-HF group had higher (P < 0.05) percent adherence (87 ± 9%) versus the ADF-LF group (77 ± 8%). Changes in hunger and physical activity are reported in Table 2. Hunger decreased (P < 0.05) by 44 ± 5 mm and 52 ± 2 mm in the ADF-HF and ADF-LF groups, respectively, from the beginning to the end of the study. Satisfaction with the diet remained elevated throughout the trial in the ADF-HF diet. Satisfaction in the ADF-LF group started out low (35 ± 5 mm), but gradually increased (P < 0.05) by week 10 (61 ± 5 mm). Fullness decreased (P < 0.05) in the ADF-HF group by 17 ± 6 mm, and increased in the ADF-LF group by 20 ± 10 mm during the weight loss phase. Physical activity, measured in steps/d, remained unchanged throughout the course of the study in both groups, although there was a slight insignificant trend in decreased steps (11%) in the ADF-HF group.
Weight loss and body composition

Changes in body weight and body composition are displayed in Figure 1. Subjects in the ADF-HF and ADF-LF group reduced body weight (P < 0.0001) by 4.4 ± 1.0 kg and 3.7 ± 0.7 kg, respectively, from weeks 3 to 10, with no differences between groups. Fat mass also decreased (P < 0.0001) in the ADF-HF and ADF-LF groups by 5.4 ± 1.5 kg and 4.2 ± 0.6 kg, respectively. Fat free mass did not change during the course of the trial. Subjects in the ADF-HF and ADF-LF groups decreased waist circumference (P < 0.001) by 7.2 ± 1.5 cm and 7.3 ± 0.9 cm, respectively, from week 3 to 10.

Brachial artery flow mediated dilation (FMD)

There were no differences in FMD between groups at baseline (Figure 2). At the end of the trial, FMD decreased (P < 0.05) in the ADF-HF group relative to baseline (7 ± 1% to 5 ± 2%; absolute change: -2% decrease), and increased (P < 0.05) in the ADF-LF group (5 ± 1% to 7 ± 2%; absolute change: +2% increase). Post-treatment values were higher (P < 0.05) in the ADF-LF group compared to the ADF-HF group. Systolic blood pressure remained unchanged in the ADF-HF group (week 3: 111 ± 2 mmHg, week 10: 109 ± 2 mmHg) and the ADF-LF group (week 3: 116 ± 3 mmHg, week 10: 118 ± 3 mmHg). Similarly, diastolic blood pressure was not affected by the ADF-HF diet (week 3: 77 ± 3 mmHg, week 10: 75 ± 2 mmHg) or the ADF-LF diet (week 3: 79 ± 3 mmHg, week 10: 81 ± 3 mmHg).

Plasma adipokines

Adiponectin increased (P < 0.05) by 43 ± 7% and 51 ± 7% in the ADF-HF and ADF-LF group, respectively. Leptin decreased (P < 0.05) in the ADF-HF and ADF-LF group by 32 ± 5% and 30 ± 3%, respectively. Similarly, resistin (P < 0.05) decreased by 23 ± 5% and 27 ± 4% in the ADF-HF and ADF-LF groups, respectively (Figure 3). Increases in adiponectin were associated with augmented FMD post-treatment
in the ADF-LF group only ($r = 0.34$, $P = 0.03$). Leptin and resistin were not correlated with changes in FMD.
E. Discussion

In the present study, we observed an improvement in brachial artery FMD with an ADF-LF (25% fat) diet after 8 weeks of weight loss. In contrast, FMD was impaired with an ADF-HF diet (45% fat). Both intervention groups experienced increases in adiponectin and decreases in leptin and resistin. However, only adiponectin was correlated with FMD in the ADF-LF group.

The primary goal of this study was to determine how dietary fat composition affects endothelial function during periods of ADF. Previous work in this field indicates that ADF with a LF background diet increases FMD [6]. Though these results are encouraging, it was still unclear if these favorable effects could be reproduced if a HF diet was used in place of a LF diet. Results from the present study demonstrate that implementing a HF background diet impairs FMD (-2%) while employing a LF diet improves FMD (+2%). Although these findings are not great as those seen in Phillips et al. [17], they do mimic some of the results observed by Volek et al. [18]. These results are considered clinically significant, particularly in the obese population. These effects on FMD occurred despite similar weight loss (4 kg) and waist circumference reductions (7 cm) in both groups. These findings are also similar to what has been reported previously for calorie restriction (CR) diets [16] [17]. For instance, obese patients following a CR-LF diet (25% fat) increased FMD after 8 weeks of weight loss in a study by Khoo et al. [16]. Similarly, Phillips et al. [17] report increases in FMD after 6 weeks of a CR-LF diet (25% fat) and decreases in FMD with a CR-HF diet (60% fat). In contrast, Volek et al. [18], reported increases in FMD with a CR-HF (60% fat) diet, and decreases with a CR-LF diet (20% fat), while Keogh et al. [19] [20] demonstrated no effect of either diet on vascular endothelial function during weight loss. The reason why our findings differ from those of Volek et al. [18] and Keogh et al. [19] [20] is not clear. However, it should be noted that the studies showing beneficial effects of LF diets and deleterious effects of HF diets [16] [17], all provided food to subjects to ensure that the macronutrient composition of the diets were
well controlled. On the other hand, the studies that demonstrate opposite [18], or no effect [19] [20], did not provide food to subjects. Thus, the lack of diet standardization in the Volek et al. (18) and Keogh et al. [19] [20] studies may partly explain these inconsistent findings. The reason why HF diets impair FMD is not clear, but may involve increased intake of saturated fat. In the present study, subjects in the ADF-HF group consumed 14% of total energy as saturated fat. High intakes of saturated fat have been shown to directly impair arterial endothelial function by reducing the anti-inflammatory potential of HDL [21]. Therefore, the high saturated fat intake may have contributed to the decreased FMD observed in the ADF-HF group.

Our secondary objective was to determine the role that adipokines play in mediating these changes in FMD. Adiponectin increased in both ADF-LF (43%) and ADF-HF (51%) groups after 8 weeks of weight loss. However, increases in adiponectin were only correlated to augmented FMD in the ADF-LF group. Although the precise mechanism by which adiponectin may improve FMD has yet to be established, we speculate that modulations in nitric oxide (NO) may be involved [22]. Nitric oxide, released from the endothelium, is a powerful vasodilator that is important in regulating vascular tone. Plasma adiponectin can stimulate the phosphorylation of endothelial nitric oxide synthase (eNOS), thereby increasing NO-dependent endothelial vasodilation [22]. As such, increased plasma adiponectin in the LF group may contribute to enhanced endothelial function. The reason why the augmented adiponectin in the HF did not contribute to improvements in FMD is not clear.

Leptin and resistin were also improved during the ADF-LF and ADF-HF regimen. Leptin decreased by 32% and 30% in the ADF-HF and ADF-LF group, respectively, while resistin decreased by 23% and 27%, respectively. These reductions in leptin and resistin were not correlated to changes in FMD in either group, however. The role that leptin and resistin play in mediating FMD most likely involves changes in
the production of NO. More specifically, leptin and resistin blunt the production of NO, which likely occurs through the stimulation of reactive oxygen species that scavenge NO and impair eNOS function [23]. Since concentrations of these adipokines were reduced in the present trial, we would assume that there would be less leptin and resistin in the circulation to inhibit NO [23]. This would lead to a higher production of NO, resulting in an enhancement in endothelium-dependent vasodilation. The reason why these decreases in leptin and resistin did not contribute to increases in FMD is unclear. However, it is possible that greater decreases in leptin (>50%) would be necessary to improve FMD [24].

Results from the hunger questionnaire indicate that the ADF-HF and ADF-LF diets were well tolerated. For instance, hunger was elevated at the beginning of the trial in both groups, but quickly decreased within the first 2 weeks (data not shown). Satisfaction and fullness remained relatively high in the ADF-HF group throughout the trial, which is not surprising as HF diets are generally more palatable. In contrast, satisfaction and fullness started out low in the LF group, but gradually increased over 10 weeks. We also show here that physical activity remained constant throughout the trial, suggesting that the weight loss is primarily due to the decrease in energy intake with the ADF diet.

This study has several limitations. First, the sample size of the study was small (n = 15-17 per group). This small sample size may have impacted our ability to detect a significant relationship between adipokine concentrations and changes in FMD. Secondly, we did not assess NO-independent dilations. As such, we cannot confirm that the effects of the diets were specific to the endothelium or altered the smooth muscle sensitivity to NO. Thirdly, the study duration was quite short (10 weeks). It will be of interest in future studies to see if these changes in FMD persist long-term (>24 weeks) with ADF-HF and AD-LF diets. Finally, there was a decrease in steps per day in the ADF-HF group, although not statistically
significant. This may have had an influence on FMD response, and would be beneficial to monitor more closely via more accurate devices such as an accelerometer.

This study suggests that the improvements in endothelial function observed with ADF may only occur with LF background diets and not with HF diets. We also show here that although ADF-LF and ADF-HF diets improved adipokine profile, these improvements may not necessarily impact FMD. Previous reports suggest that an ADF-HF diet has the same cardio-protective effects as an ADF-LF diet, in terms of LDL-cholesterol and visceral fat mass reduction [13]. Taken together, ADF-HF diets may have favorable lipid-lowering effects, but these benefits may be counteracted by impairments in FMD, a composite physiological marker of cardiovascular risk. As such, the results of this study may suggest that individuals undergoing ADF should be cautioned against the long-term use of HF background diets (45% fat) as this may eventually have harmful effects on cardiovascular health.
F. Acknowledgements

MCK designed the experiment, conducted the clinical trial, analyzed the data, and wrote the manuscript.

CMK, MG and EN assisted with the conduction of the clinical trial and data analysis. SAP assisted with the data analysis and helped prepare the manuscript. KAV assisted with the design of the experiment, and wrote the manuscript.
References


7. What we eat in America, NHANES 2007-2008, individuals 2 years and over 1 day dietary intake data, weighted. 2010.


### H. Tables

**Table III. I Subject baseline characteristics**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ADF-HF</th>
<th>ADF-LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Age (y)</td>
<td>43 ± 3</td>
<td>43 ± 2</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>92 ± 3</td>
<td>92 ± 3</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161 ± 2</td>
<td>160 ± 1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>35 ± 1</td>
<td>35 ± 1</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>44 ± 2</td>
<td>42 ± 2</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>46 ± 1</td>
<td>46 ± 2</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>98 ± 2</td>
<td>99 ± 2</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>7319 ± 2312</td>
<td>10259 ± 3478</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>41 ± 9</td>
<td>41 ± 7</td>
</tr>
<tr>
<td>Resistin (ng/ml)</td>
<td>14 ± 4</td>
<td>14 ± 2</td>
</tr>
</tbody>
</table>

1 Values reported as mean ± SEM. Alternate day fasting high-fat diet (ADF-HF); alternate day fasting low-fat diet (ADF-LF). No differences between groups for any parameter (Independent samples t-test).
### Table III.II Hunger and physical activity maintenance

<table>
<thead>
<tr>
<th></th>
<th>ADF-HF</th>
<th>ADF-LF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Week 3</td>
<td>Week 10</td>
</tr>
<tr>
<td>Hunger (mm)</td>
<td>69 ± 3</td>
<td>25 ± 4</td>
</tr>
<tr>
<td>Satisfaction (mm)</td>
<td>68 ± 3</td>
<td>69 ± 4</td>
</tr>
<tr>
<td>Fullness (mm)</td>
<td>72 ± 3</td>
<td>55 ± 5</td>
</tr>
<tr>
<td>Steps/d</td>
<td>5698 ± 502</td>
<td>5034 + 307</td>
</tr>
</tbody>
</table>

1 Values reported as mean ± SEM. Alternate day fasting high-fat diet (ADF-HF), n = 15; alternate day fasting low-fat diet (ADF-LF), n = 17.
2 Change expressed as the difference between week 3 and week 10 absolute values.
3 Significantly different from baseline (week 3), P < 0.05 (Repeated-measures ANOVA).
4 Significantly different between groups for absolute change, P < 0.05 (Repeated-measures ANOVA).
**Table III.III. Brachial-artery flow-mediated dilation (FMD)**

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Week 8</th>
<th>FMD change score (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Max dilation (%)</td>
<td>Max dilation (%)</td>
<td></td>
</tr>
<tr>
<td>ADF-HF</td>
<td>6.5 ± 1.3 (-2.4 – 11.8)</td>
<td>4.7 ± 1.6 (-2.4 – 15.2)</td>
<td>-1.8 ± 1.7 (-8.6 – 6.8)</td>
</tr>
<tr>
<td>ADF-LF</td>
<td>4.6 ± 0.7 (0 – 9.7)</td>
<td>6.7 ± 0.1 (0 – 13.4)</td>
<td>2.1 ± 1.0 (-4.7 – 8.2)</td>
</tr>
</tbody>
</table>

1 Values reported as mean ± SEM (range). Alternate day fasting high-fat diet (ADF-HF), n = 15; alternate day fasting low-fat diet (ADF-LF), n = 17.
2 Change expressed as the difference between baseline and week 8 absolute values.
3 Significantly different from baseline, P < 0.05 (Paired t-test).
4 Significantly different between groups for absolute change, P < 0.05 (Independent samples t-test).
Figure 3.1 Body weight and composition changes during the weight loss period

Values reported as mean ± SEM. Alternate day fasting high-fat diet (ADF-HF), n = 15; alternate day fasting low-fat diet (ADF-LF), n = 17. BW: Body weight, FM: Fat mass, FFM: Fat free mass. *Week 10 values significantly different (P < 0.0001) from week 3 values (Repeated-measures ANOVA). No differences between groups at any time point (Repeated-measures ANOVA).
Figure 3.2 Brachial artery flow-mediated dilation (FMD) during the weight loss period

Values reported as mean ± SEM. Alternate day fasting high-fat diet (ADF-HF), n = 15; alternate day fasting low-fat diet (ADF-LF), n = 17. *Week 10 value significantly different (P < 0.05) from week 3 values (Repeated-measures ANOVA). Week 10 values significantly higher in the ADF-LF group compared to the ADF-HF group (Repeated-measures ANOVA).
Figure 3.3 Plasma adipokines during the weight loss period

Values reported as mean ± SEM. Alternate day fasting high-fat diet (ADF-HF), n = 15; alternate day fasting low-fat diet (ADF-LF), n = 17. *Week 10 values significantly different (P < 0.05) from week 3 values (Repeated-measures ANOVA). No differences between groups at any time point (Repeated-measures ANOVA).
VI. DISCUSSION

A. Hypotheses in relation to our findings

1. Hypothesis 1

*The ADF-HF group will lose more body weight and visceral fat mass compared to the ADF-LF group after 8 weeks of dietary intervention.*

Contrary to our initial hypothesis, which predicted greater decreases in body weight by the ADF-HF group, we found similar improvements in the ADF-HF and ADF-LF groups for weight loss and visceral fat mass loss. Our results show that during the ADF weight loss period (weeks 3-10), body weight was reduced by 4.9 ± 1.1% (4.4 ± 1.0 kg) in the ADF-HF group and by 4.2 ± 0.8% (3.7 ± 0.7 kg) in the ADF-LF group. There were no differences between groups for weight loss at any time point. Fat mass decreased in the ADF-HF and ADF-LF groups by 5.6 ± 1.5 kg and 4.2 ± 0.6 kg, respectively, with no differences between groups. As for fat free mass, increases of 1.1 ± 1.3 kg and 0.5 ± 0.7 kg were noted in the ADF-HF and ADF-LF groups, respectively. During the weight loss period, waist circumference decreased by 7.5 ± 1.5 cm and 7.3 ± 0.9 cm in the ADF-HF group and ADF-LF group, respectively. Some of our findings were unexpected, particularly in the early baseline weeks. Although we fed all of our participants 100% of their individual baseline needs, subjects still lost weight (though not significantly) during the initial maintenance phase. The most plausible reason for the premature weight loss was most likely due to subjects starting the diets earlier than directed. Underreporting during this time may also have been an issue, as participants were very eager to begin losing weight right away.
Hypothesis 2

The ADF-HF group will experience more pronounced improvements in CHD risk indicators (plasma lipids, LDL particle size, and adipokines) when compared to the ADF-LF group after 8 weeks of dietary intervention.

We hypothesized that the ADF-HF group would experience greater improvements in circulating plasma lipids, adipokines, and LDL particle size when compared to the ADF-LF after 10 weeks. Instead, we found that modulations in each of these CHD risk indicators were similar in both groups, again with no significant differences between groups. More specifically, total cholesterol concentrations decreased in both the ADF-HF group (12.5 ± 2.1%, week 3: 196 ± 10 mg/dl, week 10: 171 ± 9 mg/dl) and ADF-LF group (16.3 ± 1.7%, week 3: 193 ± 8 mg/dl, week 10: 162 ± 7 mg/dl). LDL cholesterol concentrations were also lowered during the weight loss period by the ADF-HF diet (19.1 ± 4.8%, week 3: 110 ± 9 mg/dl, week 10: 89 ± 7 mg/dl) and ADF-LF diet (24.8 ± 2.6%, week 3: 113 ± 7 mg/dl, week 10: 85 ± 7 mg/dl). HDL cholesterol concentrations were not altered by either diet. Triglyceride concentrations decreased in the ADF-HF group (13.3 ± 4.7%, week 3: 121 ± 15 mg/dl, week 10: 105 ± 14 mg/dl) and ADF-LF group (14.3 ± 4.4%, week 3: 97 ± 11 mg/dl, week 10: 83 ± 10 mg/dl), during the weight loss period. There were no differences between groups for any plasma lipid parameter. The proportion of large LDL particles increased by 6 ± 2 and 3 ± 1% in the ADF-HF and ADF-LF groups respectively, while the proportion of small particles decreased by 8 ± 2 and 11 ± 3% in the ADF-HF and ADF-LF groups, respectively. Medium LDL particles increased by 8 ± 3% in the ADF-LF group only. Adiponectin increased in the ADF-HF (43 ± 7%) and ADF-LF group (51 ± 7%). Leptin and resistin decreased in the ADF-HF (32 ± 5%; 23 ± 5%) and ADF-LF group (30 ± 3%; 27 ± 4%). The beneficial changes in both groups were similar to our pilot ADF trial, as well as other short terms CR-HF studies that are reported in the Literature Review section.
3. **Hypothesis 3**

The ADF-HF group and will experience greater increases in FMD compared to the ADF-LF group after 8 weeks of dietary intervention.

We originally hypothesized that the ADF-HF subjects would show greater improvements in FMD than the ADF-LF subjects following the 8-week diet intervention. However, our results differed from our hypotheses in that the LF group experienced increases in FMD, while the HF group experienced decreases in FMD. Specifically, we showed that FMD decreased by ADF-HF relative to baseline (7 ± 1% to 5 ± 2%) and increased by ADF-LF (5 ± 1% to 7 ± 2%). Blood pressure remained unchanged in both groups. Increases in adiponectin were associated with augmented FMD in the ADF-LF group only (r = 0.34, P = 0.03). These results coincide with the findings of Phillips et al., which observed a 34% increase and 14% decrease in CR-LF and CR-HF subjects, respectively [51]. FMD can be influenced by certain factors such as blood pressure, glucose, and sodium intake. The role that each of variables may have played in modulating FMD is discussed in the next section.

B. **Effects and mechanisms of blood pressure on FMD**

Our findings demonstrate that FMD was increased by the ADF-LF diet and decreased by the ADF-HF diet. No changes in blood pressure were noted throughout the trial, and FMD was not related to blood pressure at any time point. Contrary to our findings, mounting evidence suggests that blood pressure may indeed be related to FMD. For example, Diaz et al. [52] found visit-to-visit blood pressure variability was inversely associated with FMD normalized by smooth muscle function [52]. Moreover, Kocaman et al. [53] showed that FMD also correlated negatively to age, ambulatory systolic, and diastolic blood pressure. Multivariate linear regression analyses, carried out to identify predictors of both carotid intima-media thickness (CIMT) and FMD, showed that only age and mean ambulatory blood pressure were independent predictors of CIMT and FMD [53]. Possible mechanisms that link blood pressure to
FMD could be the structural abnormalities in vessels of obese and hypertensive patients which include decreased luminal diameter in larger arterioles and capillaries [55] [56]. Functional abnormalities of microvessels include reduced capillary blood flow in the basal state and reduced vasodilatation [57, 58]. The structural change, which may occur in the presence of obesity and hypertension, may therefore result in increased resistance in vessels, which may spread throughout macro and microvasculature.

C. Effects and mechanisms of glucose on FMD

In the present study, neither the ADF-LF nor ADF-HF diet had any effect on fasting glucose levels. Nevertheless, it is still of interest to examine how changes in glucose may potentially mediated FMD. In a study by Wang et al. [60], glucose loading lowered FMD and NO. It has also been shown that a combined glucose and fat load impairs endothelial function [61]. More specifically, this study [61] showed that 1-hour FMD in the combined oral glucose and fat loading group was significantly reduced compared to that in the oral glucose loading group (5.5 ± .75% versus 9.5 ± 1.32%). The 4-hour FMD response in the combined glucose and fat group was also significantly reduced compared with the oral glucose loading group (8.6 ± 1.1% versus 9.8 ± 2.0% [61]. In another study by Bigornia et al. [62] FMD increased significantly from 6.8 ± 4.2 to 10.0 ± 4.7% in subjects who lost weight, and decreased from 6.5 ± 4.0 to 5.7 ± 4.1% in subjects who did not lose weight. Most interestingly, these vascular improvements correlated most strongly with glucose levels and were independent of weight change [62]. Although the literature is limited, there have been a few mechanisms proposed for explaining the effects of glucose on endothelial function, particularly in the case of vascular smooth muscle cells (VSMC) [63]. VSMC have both biosynthetic and proliferative roles within the endothelium [63]. For example, following vascular injury, increased levels of glucose transporter 1 (GLUT1) are observed in VSMC, which suggest a potential relationship between glucose uptake and VSMC proliferation [63]. Additionally, changes in mitochondrial function can be inhibited by dysregulation of mitofusin-2, a small GTPase associated with
mitochondrial fusion, which is of importance because exacerbated proliferation has been observed in arterial VSMC arteries with hyperpolarized mitochondria and enhanced glycolysis/glucose oxidation ratio [63]. Therefore, dysregulated glucose metabolism can have adverse effects on endothelial function.
### D. Effects and mechanisms of sodium intake on FMD

Table D.1 Sodium content of the ADF-HF and ADF-LF frozen meals \(^1,2\)

<table>
<thead>
<tr>
<th>Name of Product</th>
<th>Total Calories (kcal)</th>
<th>Total Fat (g)</th>
<th>Saturated Fat (g)</th>
<th>Sodium (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ADF-HF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spaghetti with Meat Sauce</td>
<td>350</td>
<td>12</td>
<td>4</td>
<td>660 (33%) (^3)</td>
</tr>
<tr>
<td>Meat Loaf</td>
<td>450</td>
<td>17</td>
<td>7</td>
<td>810 (41%) (^3)</td>
</tr>
<tr>
<td>Chicken Quesadilla</td>
<td>370</td>
<td>15</td>
<td>6</td>
<td>640 (32%) (^3)</td>
</tr>
<tr>
<td>Vegetable Lasagna</td>
<td>400</td>
<td>19</td>
<td>7</td>
<td>680 (34%) (^3)</td>
</tr>
<tr>
<td>Tuna Noodle Casserole</td>
<td>450</td>
<td>20</td>
<td>6</td>
<td>990 (49%) (^3)</td>
</tr>
<tr>
<td>Chicken and Rice</td>
<td>380</td>
<td>12</td>
<td>5</td>
<td>900 (45%) (^3)</td>
</tr>
<tr>
<td><strong>ADF-LF</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orange Chicken</td>
<td>310</td>
<td>8</td>
<td>1</td>
<td>640 (32%) (^3)</td>
</tr>
<tr>
<td>Macaroni and Cheese</td>
<td>250</td>
<td>4</td>
<td>2</td>
<td>540 (27%) (^3)</td>
</tr>
<tr>
<td>Chicken Parmesan</td>
<td>300</td>
<td>8</td>
<td>2</td>
<td>660 (33%) (^3)</td>
</tr>
<tr>
<td>Chicken Poblano</td>
<td>310</td>
<td>6</td>
<td>2.5</td>
<td>640 (32%) (^3)</td>
</tr>
<tr>
<td>Philly Panini</td>
<td>330</td>
<td>9</td>
<td>3.5</td>
<td>540 (27%) (^3)</td>
</tr>
<tr>
<td>4 Cheese Pizza</td>
<td>350</td>
<td>6</td>
<td>2</td>
<td>580 (29%) (^3)</td>
</tr>
</tbody>
</table>

\(^1\) Values reported as mean ± SEM. Alternate day fasting high-fat diet (ADF-HF); alternate day fasting low-fat diet (ADF-LF).

\(^2\) Values for nutrients based on a 2000 kcal diet.

\(^3\) Percent of daily kcal.
Due to the convenience of frozen meals, we chose to feed our subjects pre-packaged entrees. These meals were selected based on total calories and fat content, i.e. high-fat entrees for the HF group, and low-fat entrees for the LF group. The ADF-HF group was fed mainly Stouffer’s and Marie Callendar brands, whereas the ADF-LF group received Lean Cuisine brands. Prior to designing each of the menus, we did not take into account the amount of sodium in each entree. Subjects in the ADF-HF group consumed an average of 780 mg (39%) of sodium from the individual entrees, not including the additional snacks and breakfast we provided them throughout the day. The ADF-LF group, on the other hand, ate on average 600 mg (30%) of sodium from frozen meals alone. Therefore, the improvements in FMD in the ADF-LF group may potentially have been due to the lower dietary sodium intake. Many studies have shown that dietary sodium intake plays a role in modifying FMD response. Jablonski et al. [64] studied the effects of 4 weeks of low versus normal sodium intake on FMD. Results reveal that urinary sodium excretion was reduced by 50% and FMD was 68% higher following the low sodium diet [64]. The low sodium intervention also enhanced NO-mediated endothelium-dependent dilation without changing endothelial NO synthase expression/activation [64]. Similarly, DuPont et al. [65] showed that participants following either a 7-day high sodium (300-350 mmol/day) or 7-day low sodium (20 mmol/day) diet increased their sodium excretion during the high-sodium diet, and experienced a 3% lower FMD than with the low-sodium diet (low: 10.3 ± 0.9%, high: 7.3 ± 0.7%). The effect of the DASH diet on FMD has also been evaluated [66]. Blumenthal et al. demonstrated that the DASH diet intervention improved FMD by 4.0 ± 1.0% FMD, compared to only 2.8 ± 1.0% with normal salt intake [66]. A few mechanisms may explain how dietary salt intake affects FMD. These include impaired eNOS activation, decrease in eNOS expression, and an increase in oxidative stress and asymmetric dimethylarginine (ADMA) [67]. Decreased production of nitric oxide and elevated plasma levels of ADMA lead to a deficiency of nitric oxide which may contribute to salt-induced hypertension and diminished vascular elasticity [67]. The imbalance between nitric oxide and angiotensin II may also
prevent the upregulation of nitric oxide in response to high levels of salt intake, which may contribute to endothelial dysfunction [68]. Avoiding excess dietary sodium, therefore, may have a protective effect on endothelial function.

**VII. FUTURE DIRECTIONS**

In future studies, it would be of interest to test the longer-term effects (>24 weeks) of ADF-HF versus ADF-LF diets on body weight and CHD risk. This would allow us to see if these improvements in body weight, visceral fat mass, cholesterol levels, LDL particle size, and adipokines are maintained in the HF versus LF groups over longer periods of time. It would also be of interest to conduct a study that employs a weight maintenance period, following the 24-week weight loss period. This would allow us to see if these diets are not only effective for weight loss, but for weight loss maintenance as well. Whether or not improvements in CHD risk factors can be maintained during the maintenance period, is also of interest.
VIII. SUMMARY OF FINDINGS AND GENERAL CONCLUSION

A. Summary of findings

Collectively, this study is the first to show that an ADF-HF diet (45% fat) is equally as effective as an ADF-LF diet (25% fat) in helping obese subjects lose weight and improve CHD risk factors. Specifically, we show here that body weight reductions were comparable between the ADF-HF diet (4.9%) and the ADF-LF diet (4.2%). We also observed similar decreases fat mass for the ADF-HF and ADF-LF groups, with retention of lean mass. Reductions in several key biomarkers for CHD risk, such as total cholesterol, LDL cholesterol, and triacylglycerols, were also comparable between the HF and LF diet regimens. This study also demonstrates that an ADF-HF diet elicits the same beneficial effects on LDL particle size as ADF with an LF diet. Specifically, the ADF-HF diet was as effective as the ADF-LF intervention at increasing LDL particle size, elevating the proportion of large LDL particles, and decreasing the proportion of small LDL particles. HDL particle size and distribution were not affected by either diet. We also show here that ADF-LF diets increase FMD while ADF-HF diets decrease FMD. We demonstrated that improved brachial artery FMD only occurred in the ADF-LF group, and was mediated by favorable changes in adipokines. Both intervention groups saw increases in adiponectin and decreases in leptin and resistin. However, only adiponectin was positively correlated with FMD in the ADF-LF group.
B. Conclusion

In summary, our findings demonstrate that ADF can elicit beneficial effects on body weight, body composition, and CHD risk, independent of the background fat content of the diet. These results have several clinical implications. First and foremost, obese individuals will not need to change the types of foods they eat, when initially starting the diet, only the pattern of food consumption in order to experience the benefits of ADF. This may increase adherence to the diet. Once the individual has adjusted to the ADF eating pattern, we would then recommend gradually switching to a LF diet (<35% kcal from fat, <7% kcal from saturated fat, and <200 mg/d of dietary cholesterol). This eventual switch to a LF diet is important as consuming a HF/high cholesterol diet for long periods of time has been associated with increased CHD risk. Individuals wishing to try this diet are in no way advised to increase their level of fat intake to conform to this protocol. For those who consume 35-44% of their energy as fat, we recommend staying at this level during the first 8 weeks of ADF, and then gradually reducing intake to <35% of energy as fat. Secondly, the ADF diet only requires that an individual restrict energy every other day, instead of daily, as with CR diets. The freedom to consume food ad libitum every other day may decrease the sense of deprivation often associated with energy restriction. In turn, this decrease deprivation may result in increased dietary compliance, and thus, greater weight loss and CHD risk reduction in obese populations.
University of Illinois at Chicago  
Research Information and Consent for Participation in Biomedical Research  

Effects of High Fat versus Low Fat Intake in Obese Adults Following An Alternate Day Fasting Diet  

You are being asked to participate in a research study. Researchers are required to provide a consent form such as this one to tell you about the research, to explain that taking part is voluntary, to describe the risks and benefits of participation, and to help you to make an informed decision. You should feel free to ask the researchers any questions you may have.

Principal Investigator Name and Title: Dr. Krista Varady, Ph.D., Assistant Professor  
Department: Kinesiology and Nutrition  
Address and Contact Information: 1919 West Taylor Street, Room 506F, Phone: 312-996-7897  
Emergency Contact Name and Information: Karen Vuckovic, A.P.N., Phone: 312-996-1042  
Sponsor: Department of Kinesiology and Nutrition at UIC  

Why am I being asked?  

You are being asked to be a subject in a research study about alternate day fasting combined with high fat or low fat diet for weight loss. You have been asked to participate in the research because you responded to our ad and may be eligible to participate. We ask that you read this form and ask any questions you may have before agreeing to be in the research.

Your participation in this research is voluntary. Your decision whether or not to participate will not affect your current or future dealings with the University of Illinois at Chicago. If you decide to participate, you are free to withdraw at any time without affecting that relationship.

Approximately 60 subjects may be involved in this research at UIC.

What is the purpose of this research?  

This research is being done to better understand whether a high fat or low fat diet using alternate day reductions in calorie intake can help people lose more weight and reduce their risk of heart disease.
What procedures are involved?

This research will be performed at Human Nutrition Research Unit (HNRU), 1919 W Taylor St., First floor, Room 121C, Applied Health Sciences Building at UIC.

You will need to come to the study site 12 times over the next 10 weeks. Each of those visits will take about 20 minutes. However the first and last visit may take up to 45 minutes.

The study procedures are:

Before you begin the main part of the study you will need to have the following “screening” tests or procedures to find out if you can be in the main part of the study.

- **Body weight assessment**: You will be weighed during the screening visit, and if you do not fall in the range of obese, you will not be eligible to participate.
- **Pregnancy Screening**: If you are a premenopausal woman, you will be asked if you may be pregnant, and undergo a urine pregnancy test.

If the screening exam shows that you can continue to be in the study, and you choose to take part, then you will be "randomized" into one of the study groups described below. Randomization means that you are put into a group by chance. A computer program will place you in one of the groups. Neither you nor your doctor can choose the group you will be in. You will have an equal chance of being placed in any group.

1. **Alternate day fasting-High fat diet group**: If you fall in this category you will be asked to consume three meals that are higher in fat, which we provide to you, on the "feed day". You will be instructed to eat only one meal, which we also provide to you, on each "fast day". You will not need to change your exercise habits throughout the study.

2. **Alternate day fasting-Low fat diet group**: If you fall in this category you will be asked to consume three meals that are lower in fat, which we provide to you, on the "feed day". You will be instructed to eat only one meal, which we also provide to you, on each "fast day". You will not need to change your exercise habits throughout the study.

You will take part in a study that is 10 weeks long, and at each visit you will be asked to do the following:

**Week 1 visit**: During your first visit, you will go to Human Nutrition Research Unit to have a blood draw (about 4 tablespoons of blood). You will also have your blood pressure taken and your body weight / percent fat measured. You will also have an ultrasound done on your arm, and a full-body x-ray to determine the distribution of fat in your body. You will be given a pedometer (little counter that measures the amount of steps you take per day) and instructed how and when to wear it during the study. At this visit, you will also be given 3 questionnaires to fill out, which will determine your level of hunger on feed and fast days and your general eating behaviors. Whether you are in the alternate day fasting high fat group or alternate day fasting low fat group, you will be given your daily “fast day” meal (1) and “feed day” meals (3) for the rest of the week.
If you are a premenopausal women, at every visit you will be asked if you are using acceptable method of birth control (e.g. low dose contraceptives, Nuvaring, Mirena IUD, Paragard IUD, barrier methods (such as condoms) combined with spermicide or diaphragm) and if you might be pregnant (i.e. if you have missed a period). If you have a reason to believe that you might be pregnant, a pregnancy test will be performed. If you become pregnant during the study, you will not be allowed to continue.

**Week 2 visit:** During this visit you will have your blood pressure taken, and your body weight/percent fat measured. You will also be given hunger questionnaires to complete that week. Whether you are in the alternate day fasting high fat group or alternate day fasting low fat group, you will be given your daily “fast day” meal (1) and “feed day” meals (3) for the rest of the week.

**Week 3 visits (2 visits at the beginning of the week):** During this visit you will have your blood drawn (about 4 tablespoons), your blood pressure taken, and your body weight/percent fat measured. You will also be given hunger questionnaires to complete that week. You will also have an ultrasound done on your arm, and a full-body x-ray to determine the distribution of fat in your body. Whether you are in the alternate day fasting high fat group or alternate day fasting low fat group, you will be given your daily “fast day” meal (1) and “feed day” meals (3) for the rest of the week.

**Week 4 visit:** During this visit you will have your blood pressure taken, and your body weight/percent fat measured. You will also be given food records and hunger questionnaires to complete that week. Whether you are in the alternate day fasting high fat group or alternate day fasting low fat group, you will be given your daily “fast day” meal (1) and “feed day” meals (3) for the rest of the week.

**Week 5 visit:** During this visit you will have your blood pressure taken, and your body weight/percent fat measured. You will also be given hunger questionnaires to complete that week. Whether you are in the alternate day fasting high fat group or alternate day fasting low fat group, you will be given your daily “fast day” meal (1) and “feed day” meals (3) for the rest of the week.

**Week 6 visit:** During this visit you will have your blood pressure taken, and your body weight/percent fat measured. You will also be given hunger questionnaires to complete that week. Whether you are in the alternate day fasting high fat group or alternate day fasting low fat group, you will be given your daily “fast day” meal (1) and “feed day” meals (3) for the rest of the week.

**Week 7 visits: (2 visits at the beginning of the week):** During these visits you will have your blood drawn (about 4 tablespoons), your blood pressure taken, and your body weight/percent fat measured. You will also be given hunger questionnaires to complete that week. Whether you are in the alternate day fasting high fat group or alternate day fasting low fat group, you will be given your daily “fast day” meal (1) and “feed day” meals (3) for the rest of the week.
**Week 8 visit:** During this visit you will have your blood pressure taken, and your body weight/percent fat measured. You will also be given food records and hunger questionnaires to complete that week. Whether you are in the alternate day fasting high fat group or alternate day fasting low fat group, you will be given your daily “fast day” meal (1) and “feed day” meals (3) for the rest of the week.

**Week 9 visit:** During this visit you will have your blood pressure taken, and your body weight/percent fat measured. You will also be given hunger questionnaires to complete that week. Whether you are in the alternate day fasting high fat group or alternate day fasting low fat group, you will be given your daily “fast day” meal (1) and “feed day” meals (3) for the rest of the week.

**Week 10 visits (2 visits at the end of the week):** During these visits you will have your blood drawn (about 4 tablespoons), your blood pressure taken, and your body weight/percent fat measured. You will also be given hunger and eating behavior questionnaires to complete during the visit. You will also have an ultrasound done on your arm, and a full-body x-ray to determine the distribution of fat in your body. Whether you are in the alternate day fasting high fat group or alternate day fasting low fat group, you will be given your daily “fast day” meal (1) and “feed day” meals (3) for the rest of the week.

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**What are the potential risks and discomforts?**

The likely risks and discomforts expected in this study are:

1. **Blood draw risk:** Drawing blood may cause local pain, bruising, and more rarely, infection, light-headedness or fainting. A total of 120 ml of blood will be drawn during the study.

2. **Alternate day fasting/reducing energy intake:** Reducing daily energy intake has been shown to have beneficial effects on health. Studies of calorie restriction in people for 3 to 6 months have shown that it is generally well tolerated and has no harmful effects. You may feel hungry, however, which may be unpleasant.

3. **Radiation exposure with X-ray scanning:** The amount of radiation you will be exposed to during X-ray scanning is relatively small. Such doses may be potentially harmful, but the risks are so small that they are difficult to measure. If you have already had many x-rays, you should discuss this with the researchers before agreeing to be in the study.

4. **Ultrasound:** Risks associated with the ultrasound include occasional heat generation at the location where the measurement is being taken, and/or a mild irritation of the skin from the ultrasound gel, but these are very rare.

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**What are the reproductive risks?**

If you are a woman: Participating in this research may involve risks to pregnant women and/or an unborn baby which are currently unforeseeable. To protect against possible side effects, if you are pregnant or nursing a child you may not take part in this study. If you are a woman of childbearing ability, you and the study doctor must either agree on a method of birth control to
use or you must agree to be abstinent (i.e., not have sex) throughout the study. At every visit you will be asked if you are using acceptable method of birth control (e.g. low dose contraceptives, Nuvaring, Mirena IUD, Paragard IUD, barrier methods (such as condoms) combined with spermicide or diaphragm).

If you think that you have become pregnant during the study, you must tell the doctor immediately. If you become pregnant, your participation will be stopped.

**Will I be told about new information that may affect my decision to participate?**

During the course of the study, you will be informed of any new findings (either good or bad), such as changes in the risks or benefits resulting from participation in the research or new alternatives to participation, that might cause you to change your mind about continuing in the study. If new information is provided to you, your consent to continue participating in this study may be re-obtained.

**Are there benefits to taking part in the research?**

Previous studies of alternate day fasting and exercise show that these interventions may help people lose weight and lower heart disease risk. However, because individuals respond differently to therapy, no one can know in advance if it will be helpful in your particular case.

**What other options are there?**

If you decide not to enter this study, there is other care available to you, such as losing weight by reducing your daily energy intake on your own. The study coordinator will discuss these with you.

**What about privacy and confidentiality?**

The people who will know that you are a research subject are members of the research team, and if appropriate, your physicians and nurses. No information about you, or provided by you, during the research, will be disclosed to others without your written permission, except if necessary to protect your rights or welfare (for example, if you are injured and need emergency care or when the UIC Office for the Protection of Research Subjects monitors the research or consent process) or if required by law.

Study information which identifies you and the consent form signed by you will be looked at and/or copied for examining the research by:

- Authorized Representatives of the Sponsor (Department of Kinesiology and Nutrition at UIC)
- UIC Office for the Protection of Research Subjects, State of Illinois Auditors

A possible risk of the research is that your participation in the research or information about you and your health might become known to individuals outside the research. Your personal information and results will be kept as confidential as possible. To maintain confidentiality, code numbers only will identify all lab specimens, evaluation forms, reports and other records. All
records will be kept in locked files; code sheets linking your name to your identification number will be stored separately in a locked cabinet. Only study personnel will have access to the files. When the results of the research are published or discussed in conferences, no information will be included that would reveal your identity.

**What if I am injured as a result of my participation?**

You may have medical problems or side effects from taking part in this research study. If you believe that you have become ill or been injured from taking part in this study, treatment may be obtained through:

- The UIC Medical Center OR
- Your regular doctor OR
- The treatment center or clinic of your choice.

If you do seek medical treatment, please take a copy of this document with you because it may help the doctors where you seek treatment. It will also provide the doctors where you seek treatment with information they may need if they want to contact the research doctors.

You may contact the researcher: Dr. Krista Varady at 312-996-7897 to talk to her about your illness or injury. You can also contact the study clinician: Karen Vuckovic, A.P.N. at 312-996-1042

You or your insurance company will be billed for this medical care. Your insurance company may not pay for some or all of this medical care because you are participating in a research study. There are no plans for the University to provide free medical care or to pay for research-related illnesses or injuries, or for the University to provide other forms of compensation (such as lost wages or pain and suffering) to you for research related illnesses or injuries. By signing this form you will not give up any legal rights.

**What are the costs for participating in this research?**

There are no costs to you for participating in this research.

**Will I be reimbursed for any of my expenses or paid for my participation in this research?**

You will not be offered payment for being in this study.

**Can I withdraw or be removed from the study?**

If you decide to participate, you are free to withdraw your consent and discontinue participation at any time without affecting your future care at UIC.

You have the right to leave a study at any time without penalty. For your safety, however, you should consider the investigator’s advice about how to leave the study. If you leave the study before the final planned study visit, the investigator may ask you to complete the final steps.
The researchers and sponsor also have the right to stop your participation in this study without your consent if:

- They believe it is in your best interest.
- You were to object to any future changes that may be made in the study plan.
- If you become ill during the research or you develop certain conditions during the study.
- If you don’t follow the prescribed procedure.

**Who should I contact if I have questions?**

Contact the researchers Dr. Krista Varady (312-996-7897) or Monica Klempel (312-355-0542)

- If you have any questions about this study or your part in it,
- If you feel you have had a research-related injury (or a bad reaction to the study treatment), and/or
- If you have questions, concerns or complaints about the research.

**What are my rights as a research subject?**

If you have questions about your rights as a research subject or concerns, complaints, or to offer input you may call the Office for the Protection of Research Subjects (OPRS) at 312-996-1711 or 1-866-789-6215 (toll-free) or e-mail OPRS at uicirb@uic.edu.

**What if I am a UIC student?**

You may choose not to participate or to stop your participation in this research at any time. This will not affect your class standing or grades at UIC. The investigator may also end your participation in the research. If this happens, your class standing or grades will not be affected. You will not be offered or receive any special consideration if you participate in this research.

**What if I am a UIC employee?**

Your participation in this research is in no way a part of your university duties, and your refusal to participate will not in any ways affect your employment with the university, or the benefits, privileges, or opportunities associated with your employment at UIC. You will not be offered or receive any special consideration if you participate in this research.

**Remember:** Your participation in this research is voluntary. Your decision whether or not to participate will not affect your current or future relations with the University. If you decide to participate, you are free to withdraw at any time without affecting that relationship.

**Signature of Subject or Legally Authorized Representative**

I have read (or someone has read to me) the above information. I have been given an opportunity to ask questions and my questions have been answered to my satisfaction. I agree to participate in this research. I will be given a copy of this signed and dated form.
Signature __________________________ Date ______

Printed Name ________________________

Signature of Person Obtaining Consent __________________ Date (must be same as subject’s) ______

Printed Name of Person Obtaining Consent __________________________
B. Poster for recruitment
Volunteers needed for a

Weight Loss Study

Volunteers are needed for a 10-week research study of the effects of alternate-day food restriction for weight loss and heart disease prevention.

The study is open to men and women who are:
Between the ages of 25 and 65 •
Obese, not diabetic, sedentary or moderately active •

For more information, please call: 312-355-0542

University of Illinois at Chicago
Department of Kinesiology and Nutrition
1919 West Taylor Street, Chicago, IL
Krista Voccola, Ph.D., Principal Investigator
C. Eligibility questionnaire for recruitment

Screening questionnaire

Date of screening: ___________________

First name: ________________________  Middle initial: ____
Last: ______________________________

Phone: ______________________________
Email: __________________________________________

Age: ______________ (Must be 25-65 yrs)  DOB: ________________  Sex: □ Female  □ Male

Weight: ___________  Height: _________  BMI: ______________________ (BMI must be 30-39.9kg/m²)

Do you smoke?  □ yes  □ no (If yes, disqualify)
Currently Dieting?  □ yes  □ no (If yes, disqualify, not wt stable)

Weight gain/loss in past 3 months (>10 lb)?  □ yes  □ no (If yes, disqualify)
If yes, explain: ____________________________________________

Undergone weight loss surgery in the past?  □ yes  □ no (If yes, disqualify)
Vegan or vegetarian?  □ yes  □ no (If yes, disqualify)
Can you commit to alternate day fasting for 8 wks?  □ yes  □ no (If no, disqualify)
Food allergies? (Nuts, soy, etc) __________________________________________

Do you exercise?  □ yes  □ no
Kind of exercise: ____________________  Total hours/week: ______________

Do you have any health problems:  □ yes  □ no
If yes, explain: ___________________________________________________________
(Disqualify if diabetic, history of heart disease or stroke, or cancer)

Are you on any medications?  □ yes  □ no
What kind: ________________________  Taken for: __________________________
Dosage: ____________________________  Started meds
when: ______________________________

What kind: ____________________________  Taken
for: ________________________________

Dosage: ____________________________  Started meds
when: ______________________________

(Must not be taking lipid lowering, glucose lowering, weight loss, or psychotropic medications)

Peri-menopausal (3-6 missed periods in 12 mo)?  □ yes  □ no (If yes, disqualify)
Pregnant or trying to become pregnant?  □ yes  □ no (If yes, disqualify)
D. ADF diet instructions

Instructions for ADF and Food Menu Checklist: You will get two papers in your cooler; one will be the nutrient breakdown of your meals with the dates each meal is to be eaten, and the other will be a simplified checklist of the meals in your plan.

1) On the food checklist, there is a column with “EATEN” written at the top. If you eat this item, please put a checkmark in this column. Since the meals are repeated every three days, some food items may be repeated twice a week. Therefore it is fine to put two checkmarks in the column.

2) Each food item, including frozen meals and snacks are labeled with the date they are to be eaten, though on FEED days these items may be eaten at any time. On FAST days, please consume these food items between 12pm and 2pm. A checkmark must still be placed in the EATEN column if the food was eaten.

3) If food is not consumed, either on the FEED or FAST day, please bring the item back to the HNRC the following week of your clinical visit. Please DO NOT place a checkmark in the EATEN column if the food was not consumed.

4) If only part of the food in the menu plan for the week was eaten, next to the checkmark in the EATEN column, please specify approximately how much was consumed and how much was left.

5) Daily completion of the checklist can be done after every meal or snack, or at the end of the day.

6) BE HONEST….although it is ideal to follow the plan exactly, there is no penalty for small mistakes.

7) Zero calorie foods such as diet soda, unsweetened tea, black coffee, sugarfree gum, and mints are acceptable.

Instructions for Pedometer: You will be given a pedometer which calculates how many steps you take per day.

1) Please make sure to clip this into the waistband of your pants/skirt, otherwise the steps will be inaccurate. Please do not let it hang loosely off a jacket because of risk of damage, loss, and wrong calculation of steps taken.

2) If you are moderately active, either through your job or on your personal time, please list the type of activity you have done in addition to walking throughout the day.

3) Please specify what group you are in, the date, and whether you are on a FEED or FAST day.

Instructions for Questionnaires: You will be given 6 questionnaires to fill out during the first week. You may complete them during your first clinical visit or bring them back the following week. For the rest of the study you will not have to complete all of the questionnaires.

1) Appetite assessment (Hunger VAS) should be done every week

2) Eating habits questionnaire will be done on weeks 1, 3, and 10

3) Eating behavior questionnaire will be done on weeks 1, 3, and 10

4) Happy Healthy Kids questionnaire will be done on weeks 1, 3, and 10

5) Social support scale will be done on weeks 1 and 10

6) Demographics questionnaire will be completed during week 1 only
E. Journal waiver for manuscript I

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Alternate day fasting (ADF) with a high-fat diet produces similar weight loss and cardio-protection as ADF with a low-fat diet

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Alternate day fasting (ADF) with a high-fat diet produces similar weight loss and cardio-protection as ADF with a low-fat diet

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ABSTRACT
Alternate day fasting (ADF) with a low-fat (LF) diet is effective for weight loss and cardioprotection. However, the applicability of those findings is questionable as the majority of Americans consume a high-fat (HF) diet.

Objective: The goal of this study was to determine if those beneficial changes in body weight and coronary heart disease (CHD) risk can be reproduced if an HF background diet is used in place of an LF diet during ADF.

Methods: Thirty-two obese subjects were randomized to an ADF-HF (45% fat) or ADF-LF diet (25% fat), which consisted of two phases: 1) a 2-week baseline weight maintenance period, and 2) an 8-week ADF weight loss period. All food was provided during the study.

Results: Body weight was reduced (P<0.001) by ADF-HF (4.8±1.1%) and by ADF-LF (4.2±0.8%). Fat mass decreased (P<0.001) by ADF-HF (5.6±1.3%) and ADF-LF (4.2±0.6%). Fat mass remained unchanged. Waist circumference decreased (P<0.001) by ADF-HF (7.2±1.5 cm) and ADF-LF (3.3±1.0 cm). LDL cholesterol and triglyceride concentrations were reduced (P<0.001) by both interventions (ADF-HF: 13.9±6.0% and ADF-LF: 24.0±2.6%, 14.7±4.4%). HDL cholesterol, blood pressure, and heart rate remained unchanged. There were no between-group differences for any parameter.

Conclusion: These findings suggest that an ADF-HF diet is equally as effective as an ADF-LF diet in helping obese subjects lose weight and improve CHD risk factors.

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1. Introduction
Obesity in adulthood doubles the risk of coronary heart disease (CHD) mortality [12]. Reducing energy intake by means of dietary restriction has been shown to lower the risk of CHD in obese adults [3,4]. Evidence suggests that alternate day fasting (ADF) is an effective diet strategy to help obese individuals lose weight and lower CHD risk [5,6]. ADF regimens include a "fast day" where food is consumed ad libitum over 24 h, alternated with a "feed day" where intake is limited to 25% of the individual's energy needs over 24 h. To date, only two clinical trials have been performed to evaluate the ability of ADF to facilitate weight loss and decrease CHD risk [5,6]. Each of these trials implemented a low-fat (LF) background diet (i.e., 25% of energy from dietary fat) to test the study objectives [5,6]. In both trials, body weight was reduced by 6%–8% after 8 weeks of an ADF-LF diet in obese adults [5,6]. Beneficial effects on CHD risk indicators were also noted. For instance, LDL cholesterol concentrations decreased by 10%–25%, while triglyceride concentrations were lowered by...
30%-40% from baseline [15]. In the trial by Varady et al., decreases in systolic blood pressure and heart rate were also demonstrated [5].

Although these data for ADF-LF diets are promising, the applicability of these findings is questionable as the majority of Americans consume a high-fat (HF) diet, and not an LF diet. More specifically, the most recent data from the National Health and Nutrition Examination Survey (NHANES) suggest that the average middle age American consumes 30%-40% of their daily calories as dietary fat [7]. This report also indicates that 13% of energy is consumed as saturated fat [7]. This level of fat consumption (45% total as fat) also corresponds to the highest level of fat intake reported in the Women's Health Initiative trial [8]. In view of these findings, an important question has yet to be tested is whether these beneficial changes in body weight and CMR can be reproduced if an HF (45% fat) background diet is used in place of an LF (25% fat) background diet during periods of ADF. Accordingly, the objective of the present study was to compare the effects of an ADF-HF diet to those of an ADF-LF diet on body weight, body composition, and CMR risk factors in obese adults. We chose 40% as the level of fat intake to see if the beneficial effects of ADF could still be reproduced during periods of extremely high fat consumption.

2. Methods

2.1. Subjects

Subjects were recruited from the Chicago area by means of advertisements placed on and around the University of Illinois, Chicago campus. A total of 44 individuals expressed interest in the study, but only 35 were deemed eligible to participate after the preliminary questionnaire and body mass index (BMI) assessment (Fig. 1). Key inclusion criteria were as follows: female, age 25-65y, BMI between 30 and 39.9, light weight for 3 months prior to the beginning of the study (i.e. <5 kg weight loss or gain), non-diabetic, no history of cardiovascular disease, sedentary or lightly active for 3 months prior to the beginning of the study (i.e. <1h/week of light-intensity exercise at 2.5-4.0 metabolic equivalents (METs)), non-smoker, and not taking weight loss, lipid-lowering, or glucose-lowering medications. Postmenopausal women were excluded from the study, and premenopausal women (defined as absence of menses for 2y) 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. All volunteers gave written informed consent to participate in the trial.

2.2. Experimental design

Eligible subjects were randomized by way of a stratified random sample. The sample frame was divided into strata based on BMI and age. Subjects from each stratum were then randomly assigned to either the ADF-HF group or the ADF-LF group. The 10-week trial consisted of three dietary phases: 1) a 2-week baseline weight maintenance period, and 2) an 8-week weight loss ADF period. All food was provided throughout the 10-week trial to all subjects.

2.2.1. Baseline weight maintenance diet (Week 1-2)

Before commencing the 8-week ADF intervention, each subject participated in a 2-week baseline weight maintenance period where they consumed either the HF or LF diet (providing 100% of their energy needs). Energy requirements were calculated using the Mifflin-St. Jeor equation [9].

Fig. 1 - Study flow chart.
Table 1—Nutrient composition of the ADF-1F and ADF-LF diets. 

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<th>Nutrients</th>
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<th>ADF-LF</th>
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<tr>
<td>Total fat (g)</td>
<td>100(4%)</td>
<td>55(0.5%)</td>
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<tr>
<td>Saturated fat (g)</td>
<td>40(0.5%)</td>
<td>18(0.5%)</td>
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<td>10(0.5%)</td>
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<td>Polysaturated fat (g)</td>
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<td>Trans fat (g)</td>
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<td>0</td>
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<td>Cholesterol (mg)</td>
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<td>12</td>
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<tr>
<td>Protein (g)</td>
<td>35(1.5%)</td>
<td>75(0.5%)</td>
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<tr>
<td>Carbohydrates (g)</td>
<td>200(4%)</td>
<td>100(0.5%)</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>21</td>
<td>27</td>
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</table>

*Values reported as mean±SEM. Values for saturated fat were not calculated due to the low fat content of the diets.

2.2.2. Weight loss ADF diet (Week 3–12)

Following the baseline period, subjects participated in a 12-week weight loss program. Each week, participants were instructed to consume a fixed daily calorie intake of 1200 kcal and to maintain a daily fiber intake of 25 g. The subjects were encouraged to consume a diet rich in fiber foods that were high in soluble fiber (e.g., oat bran, beans, peas, rice bran, barley, and other fiber sources) and low in insoluble fiber (e.g., whole-grain bread, wheat cereals, wheat bran, rice, corn, and potato skin). All meals were consumed outside of the laboratory. Participants were instructed to eat only the food provided and to drink only water. Participants were allowed to consume 100 kcal of fluid each day. Subjects were also instructed to maintain their physical activity levels through the duration of the study.

2.3. Analysis

2.3.1. Adherence with ADF diets

Throughout the study, subjects were instructed to eat only the food items provided and to keep track of all food items consumed using a "Food check list." Subjects were asked to report any extra food items consumed using an "Extra food log." The check lists and logs were collected and reviewed by study personnel each week. The log indicated that the subject ate extra food items (totaling >50 kcal) on a feed or fast day, that day was labeled as "not adherent." If the mean indicated that the subject did not eat any extra food items, that day was labeled as "adherent." Adherence rates were assessed based on the number of days adherent, the number of feed days, and the number of fast days. Adherence rates were assessed weekly as:

\[ \text{Adherence rate} = \frac{\text{# feed days adherent} + \text{# fast days adherent}}{\text{# feed days in the week}} \times 100 \]

2.3.2. Body weight and body composition assessment

Body weight measurements were taken at the nearest 0.5 kg at the beginning of each week in light clothing and without shoes using a balance beam scale (Besa HR 80, Sencor, USA). Height was measured using a wall-mounted stadiometer to the nearest 0.1 cm. BMI was calculated as kg/m². Fat mass and fat-free mass were assessed by dual energy X-rays an electromagnetic (DXA) at weeks 1, 3, and 10 (QDR 4500W, Hologic Inc., Bedford, MA). Body mass index was measured using a stadiometer at the nearest 0.1 cm. Waist circumference was measured at the midpoint between the lower costal margin and the top of the iliac crest during a period of expiration.

2.3.3. Blood collection protocol

Twelve-hour fasting blood samples were collected between 6:00 am and 9:00 am at baseline, weeks 3 and 10. The subjects were instructed to avoid eating, drinking, and smoking for 24 h before each visit. Blood was centrifuged at 10 min at 3200 × g at 4°C to separate plasma from red blood cells and was stored at -80°C until analysis.

2.3.4. Plasma lipid profile, blood pressure, and heart rate determination

Plasma total cholesterol, HDL-cholesterol, and triglyceride concentrations were measured in duplicate by using enzymatic kits (Kabi, Stockholm, Sweden) and analyzed using a microplate reader (Bio-Rad Laboratories, Richmond, CA). The measurement of LDL and HDL cholesterol was calculated using the Friedewald, Levy, and Fredrickson equation [10]. The hematocrit was determined at baseline using a centrifugal hematocrit analyzer (Micronova EM 705, Micronova, Japan) with the subject in a seated position after a 10-min rest.

2.3.5. Statistics

Results are presented as mean±SEM. Normality was assessed by the Kolmogorov-Smirnov test. No variables were found to be normally distributed. An independent samples t-test was used to test baseline differences between groups. Repeated-measures ANOVA was performed using SPSS software (version 20.0, SPSS Inc., Chicago, IL).
3. Results

3.1. Subject dropout and baseline characteristics

Thirty-five subjects commenced the study and 32 completed the entire 10-week trial (Fig. 1). Two subjects dropped out of the ADF-1F group due to an inability to comply with the ADF protocol (n=1) and aching using conflicts (n=1). As for the ADF-LF group, one subject dropped out due to an inability to adhere to the diet. Baseline characteristics of the ADF-1F and ADF-LF groups are reported in Table 2. There were no differences between groups for age, ethnicity, BMI, or plasma lipids.

3.2. Adherence to ADF diets

During the baseline weight maintenance period, ADF-1F and ADF-LF subjects were 90% and 93% adherent, respectively, with the provided diet. Throughout the weight loss period, the ADF-1F group had higher (P<0.05) percent adherence (87% vs 92%) than the ADF-LF group (78% vs 88%). There was no decline in adherence over the course of the ADF weight loss period. All subjects in the ADF-1F and ADF-LF groups were considered "adequate," as all subjects adhered to the diet for at least 70% of the days during the weight loss period.

3.3. Weight loss and body composition

During the baseline period (weeks 1–2), both the ADF-1F and ADF-LF groups lost weight (P<0.001), despite being given more than 100% of their energy needs (Fig. 2). During the ADF weight loss period (weeks 3–10), body weight was reduced (P<0.0001) by 4.0%±1.1% (3.3±1.0 kg) in the ADF-1F group and by 4.2%±0.8% (3.7±0.9 kg) in the ADF-LF group. There were no differences between groups for weight loss at any time point. BMI decreased (P<0.0001) by 1.6±0.4 and 1.5±0.3 kg/m², respectively, in the ADF-1F and ADF-LF groups during the weight loss period. Changes in body composition are reported in Table 3. Fat mass and fat-free mass did not change during

![Fig. 2](https://example.com/fig2.png)

**Fig. 2.** Body weight changes throughout the 10-week trial. Mean body weight of ADF-1F (alternate-day fasting high-fat diet) subjects (n=15) and ADF-LF (alternate-day fasting low-fat diet) subjects (n=17) at each week. "Week 10 values were significantly different (P<0.0001) from week 3 values (Repeated-measures ANOVA). No differences between groups at any time point (Repeated-measures ANOVA)."

Table 2 - Subject characteristics at baseline.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>ADF-1F</th>
<th>ADF-LF</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>Age (y)</td>
<td>42.4±3.0</td>
<td>42.2±3.3</td>
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<td>Ethnicity</td>
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<tr>
<td>African American</td>
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<td>Hispanic</td>
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<tr>
<td>Body weight (kg)</td>
<td>91.5±2.6</td>
<td>91.5±2.9</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>161±8.16</td>
<td>160±8.16</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>36.5±6.7</td>
<td>35.5±6.7</td>
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<tr>
<td>Total cholesterol (mg/dl)</td>
<td>206±11</td>
<td>203±11</td>
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<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>126±34</td>
<td>126±34</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>62±16</td>
<td>62±16</td>
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<tr>
<td>Triglycerides (mg/dl)</td>
<td>120±49</td>
<td>120±49</td>
</tr>
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</table>

*Values reported as mean±SD. Alternate day fasting high-fat diet (ADF-1F). No differences between groups for any parameter (independent samples t-test).

3.4. Plasma lipids, blood pressure, and heart rate

Plasma lipids did not change during the baseline period in either the ADF-1F or ADF-LF group. During the weight loss period (week 3–10) (Fig. 3), total cholesterol concentrations decreased (P<0.0001) in both the ADF-1F group (13.0%±1.8%), week 3: 196±11 mg/dl, week 10: 172±9 mg/dl) and ADF-LF group (6.5%±1.7%, week 3: 197±13 mg/dl, week 10: 182±7 mg/dl). LDL cholesterol concentrations were also reduced (P<0.0001) in the ADF-1F group (11.7%±4.8%, week 3: 120±15 mg/dl, week 10: 105±7 mg/dl) and ADF-LF group (14.3%±4.4%, week 3: 127±11 mg/dl, week 10: 103±10 mg/dl) during the weight loss period. There were no differences between groups for any plasma lipid parameter.

**Fig. 3.** Blood pressure changes throughout the 10-week trial. Mean blood pressure of ADF-1F (alternate-day fasting high-fat diet) subjects (n=15) and ADF-LF (alternate-day fasting low-fat diet) subjects (n=17) at each week. "Week 10 values were significantly different (P<0.0001) from week 3 values (Repeated-measures ANOVA). No differences between groups at any time point (Repeated-measures ANOVA)."

Systolic blood pressure was not altered by the ADF-1F diet (week 3: 111±2 mmHg, week 10: 109±2 mmHg) or the ADF-LF diet (week 3: 116±3 mmHg, week 10: 118±3 mmHg). Similarly, diastolic blood pressure did not change in either the ADF-1F group (week 3: 77±3 mmHg, week 10: 75±2 mmHg) or the ADF-LF group (week 3: 79±3 mmHg, week 10: 81±3 mmHg). Heart rate also remained unchanged in the ADF-1F group (week 3: 75±3 beats/min, week 10: 77±3 beats/min) and ADF-LF group (week 3: 74±3 beats/min, week 10: 73±2 beats/min).
4. Discussion

This study is the first to show that an ADF-HF diet (40% fat) is equally as effective as an ADF-LF diet (25% fat) in helping obese subjects lose weight and improve CHD risk factors. Specifically, we show here that body weight reductions were comparable between the ADF-HF diet (4.9%) and the ADF-LF diet (4.2%). We also observed similar decreases in fat mass for the ADF-HF and ADF-LF groups, with a retention of lean mass. Reductions in several key biomarkers for CHD risk, such as total cholesterol, LDL cholesterol, and triacylglycerols, were also comparable between the HF and LF diet regimens.

Results from our trial indicate that ADF is as effective as body weight by 4 kg in 6 weeks, independent of the background macronutrient composition of the diet. As such, an individual can maintain a diet with 45% of energy as dietary fat (15% of energy as saturated fat), and still experience similar weight loss as those consuming a diet with 25% of energy as fat (10% of energy as saturated fat). Our findings are in concurrence with other calorie restriction (CR) studies that manipulate dietary fat consumption [11,12]. For example, Jenkins et al. [12] showed that an energy restricted HF diet (43% fat) produced similar reductions in body weight (1.4 kg) in both groups as an energy restricted LF diet (25% fat) after 4 weeks of treatment.

Thus, dietary restriction protocols appear to facilitate weight loss in a manner of the fat composition of the diet. In addition to body weight, we also examined dietary adherence to the ADF-HF versus ADF-LF diet. Not surprisingly, subjects were able to adhere to the HF diet to a greater extent (i.e., 87% of days adherent) than the LF diet (i.e., 78% of days adherent). This may be related to the greater palatability of higher fat foods [13,14].

Body composition was also favorably altered with both diets. Total body fat decreased to a similar extent in the ADF-HF group (-4.4 kg) and the ADF-LF group (-4 kg). As for fat mass, non-significant increases were noted for both the HF diet (0.6 kg) and LF diet (0.5 kg). These data suggest that the weight loss observed with ADF results from a decrease in fat mass, and not fat-free mass. A similar representation of lean mass (0.5 kg) was noted in a previous ADF study conducted by our group [15]. Interestingly, this retention in lean mass observed with ADF is not replicated with CR diets. For instance, consistent reductions of 3%-5% in fat mass are generally noted after 4 weeks of 20%-30% CR [16,17]. The reason why ADF may assist with the preservation of lean mass is not known at present, but will undoubtedly be of interest in future studies in this field. Another body composition parameter that was beneficially modulated by ADF was waist circumference (used as an indirect indicator of visceral fat mass). We show here that weeks of ADF can decrease waist circumference by 7 cm, and that these changes can occur with either an HF or LF background diet. Other recent trials have also reported equivalent reductions in waist circumference with either HF or LF diets during dietary restriction [18,19]. For example, in the study by Sacks et al. [19], waist circumference was decreased to the same extent (7 cm with 4 kg weight loss) when an energy restricted HF diet (60% fat) was compared to an energy restricted LF diet (20% fat). Thus, individuals who typically consume an HF diet can continue with their usual eating habits during ADF and still observe the same reductions in visceral fat mass as seen with an LF diet.

Comparable changes in CHD risk were also observed for the HF and LF diets. For instance, LDL cholesterol concentrations were reduced to a similar extent by the ADF-HF diet (18%) as the ADF-LF diet (20%). Triglyceride concentrations also decreased by both the HF and LF diets (14% and 14%, respectively). It is likely that a similar degree of LDL cholesterol lowering was attained by these diets because both groups lost similar amounts of weight [20]. LDL-cholesterol has been estimated to be reduced by 2.0 mg/dl
per kg of weight loss [20]. Since body weight was reduced to the same extent in both groups (approximately 4 kg), it is not surprising that both diets experienced similar reductions in LDL cholesterol. This relationship between weight loss and LDL cholesterol lowering has also been demonstrated in CR trials that compared HF to LF background diets [2, 22]. It is also possible that the ADF regimen may maintain the duration and metabolic responses in a way that prevents HF diet-induced disruption of the normal lipid metabolic pathways [23]. As for HDL cholesterol concentrations, no effect was noted by either the HF or LF diets. Previous studies of ADF also report no change in this lipid parameter [5, 6]. Since HDL cholesterol very readily changes with dietary restriction [20], this result is in line with what was hypothesized. Blood pressure and heart rate also remained unchanged over the course of the trial. This lack of effect was most likely due to the high variability of these parameters between subjects. Since our power calculation was based on body weight, and not heart rate or blood pressure, this may explain why an insufficient number of subjects were recruited to see changes in these particular parameters.

This study is limited in that the intervention groups lost weight during the baseline weight maintenance period. These reductions in body weight occurred despite a dieting protocol that provided 100% of each subject's daily energy needs. To add further complexity, participants reported a mean adherence rate of 90% with the weight maintenance diet. The reason for this drop in weight during the period is unknown. It is possible that, however, that upon starting the study the subjects were eager to begin losing weight that they did not eat all the food provided, and potentially misrepresented their adherence rate [24]. It is also possible that the subjects may have become more physically active and thus spent less energy during two baseline weeks to boost their weight loss. Future studies in this area should therefore aim to control for physical activity during the course of the trial by using an accelerometer to measure energy expenditure [25].

In summary, our findings demonstrate that ADF can elicit beneficial effects on body weight, body composition, and CHD risk, independent of the background fat content of the diet. These results have several clinical implications. First and foremost, obese individuals will not need to change the types of foods they eat, when initially starting the diet, only the pattern of food consumption in order to experience the benefits of ADF. This may increase adherence to the diet. Once the individual has adjusted to the ADF eating pattern, we would then recommend gradually switching an HF diet (<35% kcal from fat, <6% kcal from saturated fat, and <200 mg of dietary cholesterol). This gradual switch to an LF diet is important as consuming an HF/high cholesterol diet for long periods of time has been associated with increased CHD risk [26]. Individuals wishing to try this diet are in no way advised to increase their level of fat intake to conform to this protocol. For those who consume 35%-44% of their energy as fat, we recommend staying at this level during the first weeks of ADF, and then gradually reducing intake to <35% of energy as fat. Secondly, the ADF diet only requires that an individual restrict energy every other day, instead of daily, as with the CR diet. The freedom to consume food ad libitum every other day may decrease the sense of deprivation often associated with energy restriction. In turn, this decreased deprivation may result in increased dietary compliance, and thus, greater weight loss in obese populations.

Author contributions

MCX designed the experiment, conducted the clinical trial, analyzed the data, and wrote the manuscript. CMK assisted with the conduct of the clinical trial. KAV assisted with the design of the experiment, and wrote the manuscript.

Funding

University of Illinois at Chicago, Department of Kinesiology and Nutrition, Departmental funding.

Conflict of interest

The authors have no conflicts of interest to report.

REFERENCES


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(312) 996-0354 • mcklempel@gmail.com

EDUCATION

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<td>Ph.D.</td>
<td>University of Illinois at Chicago – Kinesiology and Nutrition</td>
<td>Human Nutrition</td>
<td>August 2009 – May 2013</td>
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<tr>
<td>B.S.</td>
<td>Loyola University Chicago</td>
<td>Biology &amp; Bioethics</td>
<td>August 2004 – May 2008</td>
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ACADEMIC AND RESEARCH APPOINTMENTS

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<tr>
<td>Clinical Coordinator</td>
<td>University of Illinois of Chicago – Kinesiology and Nutrition</td>
<td>Human Nutrition Research Center</td>
<td>January 2012-present</td>
</tr>
<tr>
<td>Research Assistant</td>
<td>University of Illinois at Chicago – Kinesiology and Nutrition</td>
<td>Human Nutrition Research Center</td>
<td>August 2008 – August 2010, June 2012-present</td>
</tr>
<tr>
<td>Teaching Assistant</td>
<td>University of Illinois at Chicago – Kinesiology and Nutrition</td>
<td>Nutrition Throughout the Life Cycle, Nutritional Assessment, Clinical Nutrition I</td>
<td>August 2010 – May 2012</td>
</tr>
<tr>
<td>Research Assistant</td>
<td>Northwestern University – Developmental Biology</td>
<td>Children’s Memorial Research Center</td>
<td>April 2008 – August 2008</td>
</tr>
<tr>
<td>Clinical Coordinator</td>
<td>Children’s Memorial Hospital – Department of Psychiatry</td>
<td>Laboratory of Dr. Kathleen McKenna</td>
<td>June 2007 – August 2007</td>
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HONORS AND AWARDS

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<td>2013</td>
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<td>2012</td>
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<tr>
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<td>John F. Grant Medical Endowment for Bioethics</td>
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<td>2004-2008</td>
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INVITED TALKS

2011 **Loyola University Chicago Health Fair.**
“Figure Facts”. Chicago, Illinois, USA.

2010 **St. Paul of the Cross Annual Youth Conference.**
“Peer Influence of Eating Disorders Among Pre-Teens”. Park Ridge, Illinois, USA.

2009 **Nutrition and Wellness Expo of Oak Park.**
“Balancing Dietary Intake with Physical Activity”. Oak Park, Illinois, USA.

PROFESSIONAL AFFILIATIONS

Member of the American Heart Association since 2012
Member of the American Dietetic Association since 2011
Member of the Obesity Society since 2009
Member of the American Society for Nutritional Sciences since 2009

PUBLICATIONS


7. **Klempel MC, Kroeger C, Varady KA.** Alternate day fasting (ADF) with a high fat background diet produces similar weight loss and cardio-protection when compared to ADF with a low fat background diet. Federation of American Societies for Experimental Biology (FASEB) Annual Meeting. San Diego, CA, USA, 2012. [Poster presentation].


10. Varady KA, Bhutani S, **Klempel MC.** Weight loss, coronary heart disease risk reduction, and adipokine profile improvement by alternate day fasting. The Obesity Society. San Diego, CA, USA, 2010. [Poster presentation].
