

Alternate Day Fasting Versus Calorie Restriction for Weight Loss and Cardio-Protection

BY

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THESIS

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DEDICATION

This is for George.

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JFT

CONTRIBUTION OF AUTHORS

Chapter 1 gives a brief overview of my dissertation project that focuses on the scientific background and rationale that underlies this project, the specific aims of the project, the clinical and scientific significance of this project, and the innovative features of this project. Chapter 2 is a literature review that examines what is currently known about alternate day fasting and daily calorie restriction dietary regimens, and provides evidence that my dissertation question represents an important area of inquiry for advancing our understanding of dietary weight loss. Chapter 3 represents an unpublished clinical trial directed at answering whether adherence to alternate day fasting is greater than adherence to daily calorie restriction. I conducted the clinical trial, performed the laboratory analyses, analyzed the data, and wrote the manuscript. Cynthia Kroeger, Dr. Adrienne Barnosky, Monica Klempel, Surabhi Bhutani, and Kristin Hoddy assisted with the conduction of the clinical trial and the laboratory analyses. Drs. Jennifer Rood and Eric Ravussin conducted the doubly labeled water analysis. Dr. Krista Varady, the principal investigator of my laboratory, designed the study, assisted with the data analysis, and assisted with manuscript preparation. Chapter 4 represents an unpublished clinical trial directed at answering whether alternate day fasting attenuates visceral fat-mediated inflammation more effectively than daily calorie restriction. I conducted the clinical trial, performed the laboratory analyses, analyzed the data, and wrote the manuscript. Cynthia Kroeger, Dr. Adrienne Barnosky, Monica Klempel, Surabhi Bhutani, and Kristin Hoddy assisted with the conduction of the clinical trial and the laboratory analyses. Drs. Jennifer Rood and Eric Ravussin conducted the doubly labeled water analysis. Dr. Krista Varady, the principal investigator of my laboratory, designed the study, assisted with the data analysis, and assisted with manuscript preparation. Chapter 5 provides a discussion of how the findings from my dissertation project answer questions that arose from previous studies. Chapter 6 lists the limitations of my dissertation project. Chapter 7 outlines possible directions for future research that build on the findings

from my dissertation project. Chapter 8 summarizes the main findings from my dissertation project, and offers a conclusion regarding the scientific relevance and clinical relevance of these findings.

Chapter 1 is a literature review that places my dissertation question in the context of the larger field and highlights the significance of my research question. Chapter 2 represents a published manuscript (include complete citation) for which I was the primary author and major driver of the research. Susan Smith assisted me in the experiments shown in Figures 5 and 6. My research mentor, Dr. John Johnson contributed to the writing of the manuscript. Chapter 3 represents a published manuscript (include complete citation) for which I was the second author. I generated Figures 4, 7, 8 (3 of 7) and played a large role in the writing of the manuscript along with the first author Susan Smith and my research mentor, Dr. John Johnson. My work was critical to the conclusions of this manuscript because (fill in). Chapter 4 represents a series of my own unpublished experiments directed at answering the question (fill in). I anticipate that this line of research will be continued in the laboratory after I leave and that this work will ultimately be published as part of a coauthored manuscript. In Chapter 5 represents my synthesis of the research presented in this thesis/dissertation and my overarching conclusions. The future directions of this field and this research question are discussed.

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LIST OF ABBREVIATIONS

ADF	Alternate-day modified fasting
CHD	Coronary heart disease
CR	Calorie restriction
CRP	C-reactive protein
CT	Computed tomography
DEXA	Dual-energy X-ray absorptiometry
DLW	Doubly-labeled water
EI	Energy intake
ER	Energy restriction
ES	Energy stores
FM	Fat mass
FFM	Fat-free mass
HOMA	Homeostasis Model Assessment
IF	Intermittent fasting
IGF-1	Insulin-like growth-factor-1
IL-6	Interleukin-6
IR	Insulin resistance
MRI	Magnetic resonance imaging
SAT	Subcutaneous adipose tissue
TEE	Total energy expenditure
TNF- α	Tumor necrosis factor-alpha
VAT	Visceral adipose tissue

SUMMARY

This study examined whether alternate-day modified fasting (ADF) promotes superior dietary adherence, superior weight loss, and superior improvements in traditional and emerging biomarkers of coronary heart disease risk when compared to daily calorie restriction (CR). Following a 4-week baseline control phase, sedentary, overweight and obese individuals were randomized to 1 of 3 groups for 24 weeks: 1) an ADF group (24-h of 125% energy intake, alternated with 24-h of 25% energy intake); 2) a CR group (75% energy intake every day); or 3) a control group (100% energy intake every day). All meals were provided in the intervention groups for the first 12 weeks, and weekly dietary counseling was provided thereafter. The control group received no food or dietary counseling throughout the study. Body weight was measured weekly, circulating factors (blood lipids, glucose and insulin, and inflammatory cytokines) were measured every 4 weeks, and body composition (total fat mass and fat-free mass via dual-energy X-ray absorptiometry (DEXA); visceral fat mass via magnetic resonance imaging (MRI) and total energy expenditure (via the doubly-labeled water (DLW) method) were measured at baseline and end of study. Percent energy restriction (i.e. dietary adherence) was determined from the changes in energy expenditure, total fat mass, and fat-free mass.

Percent energy restriction, weight loss, loss of total fat mass, and reduction in circulating leptin were greater for the ADF and CR groups when compared to the control group; none of these changes were significantly different between the intervention groups. In conclusion, ADF is not superior to CR at modulating these measured parameters.

I. INTRODUCTION

A. Background and Rationale

A 10% increase in body weight is associated with a 30% greater likelihood of developing coronary heart disease (CHD) (1). Reducing body weight by means of dietary restriction greatly reduces CHD risk (2). The most common dietary restriction regimen is daily calorie restriction (CR), which involves lowering energy intake by 15 to 40% of needs every day. Another dietary restriction protocol that is prescribed for reducing CHD risk is alternate-day modified fasting (ADF) (3). ADF consists of a 24-h “feast day” where food intake is allowed ad libitum, alternated with a 24-h “fast day”, where food intake is reduced by 75% of needs. ADF and CR prescribe equivalent energy restriction over a 48-h period, but preliminary findings (4) indicate that individuals are more adherent to ADF than to CR and lose more weight on an ADF protocol over a 12-week period. These findings warrant further study to determine whether ADF can produce greater weight loss when compared to CR over a longer trial period (24 weeks).

Preliminary data (4) also indicate that “traditional” CHD risk factors improved more favorably in response to ADF when compared to CR. Traditional CHD risk factors include blood pressure, plasma lipid levels, LDL particle size, and C-reactive protein (CRP); and these parameters have been shown to improve considerably in response to weight loss (1). Recent evidence suggests that parameters related to fat cell physiology (i.e. visceral fat mass and fat cell-derived hormones) may also link weight loss to CHD risk reduction (5, 6). These fat cell parameters are therefore regarded as “emerging” CHD risk factors. Whether ADF can more favorably improve traditional and emerging CHD risk factors relative to CR over a 24-week period is an important question that has yet to be addressed. Accordingly, the specific aims of the proposed study are:

B. Specific Aims

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

Hypothesis 1: ADF subjects will be more adherent with diet than CR subjects from weeks 1 to 24, resulting in greater weight loss by ADF when compared to CR.

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

Hypothesis 2: ADF subjects will experience greater improvements in traditional CHD risk factors (blood pressure, plasma lipid levels, LDL particle size, and CRP) from weeks 1 to 24 when compared to CR subjects, due to larger reductions in body weight by ADF diets.

Specific aim 3: To establish that greater reductions in body weight by ADF over a 24-week period will result in greater improvements in *emerging* CHD risk factors in comparison to CR.

Hypothesis 3: ADF subjects will experience greater improvements in emerging CHD risk factors (visceral fat mass and fat cell-derived hormones) from weeks 1 to 24 when compared to CR subjects, due to larger reductions in body weight by ADF diets.

C. Significance

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

1. Advance clinical practice guidelines by showing that ADF is more effective than CR for weight loss and improvement in CHD risk factors.
2. Further uncover the intermediate role that adipose tissue plays in mediating CHD risk.

D. Innovation

This study will be the first to directly compare ADF to CR, and show that ADF is more effective for weight loss and CHD risk reduction. In sum, the proposed study is innovative in that it will be the first to:

1. Challenge existing dietary paradigms by proposing that ADF is more effective for weight loss than CR.
2. Employ novel markers, i.e. fat-cell derived parameters, to elucidate how dietary therapy and weight loss reduce CHD risk.

II. LITERATURE REVIEW

1. Dietary restriction regimens for weight loss

Being overweight or obese is associated with poorer cardiovascular health and higher overall mortality compared to being normal weight (7). Moderate weight loss (~5-10%) improves cardiovascular outcomes and overall mortality outcomes (8). Although body weight can also be reduced by exercise (9), pharmacotherapy (10), or surgery (11), diet is often employed as a first line of defense. The most commonly prescribed weight-loss diet is daily calorie restriction (CR), which involves reducing energy intake by a consistent amount (typically between 15-40% of energy needs) every day. While this CR is effective for many individuals, others are unable to maintain adherence to this protocol. Intermittent fasting (IF) regimens, involving alternating between a period of heavily restricted intake and a period of unrestricted intake, were designed with the goal of helping people who are unable to adhere to CR. This review compares IF and CR regimens regarding their abilities to produce weight loss and improve intermediate biomarkers of coronary heart disease (CHD) risk.

2. Intermittent fasting

2.1 Description of studies

The IF studies reviewed here are summarized in **Table 1**. Alternate-day modified fasting (ADF) involves alternating between 24-h periods of heavily restricted intake ('fast day') and 24-h periods of unrestricted intake ('feast day'). The ADF trials by Bhutani *et al.*, Keogh *et al.*, and Klempel *et al.* (12-14) examined obese (body mass index (BMI) 30-40 kg/m²) individuals, most of whom were middle-aged, female, and African American. All ADF trials prescribed 25% of energy needs on the fast day (3, 13, 14). For the feast day, two studies prescribed *ad libitum* intake (3, 13, 14), while another prescribed 125% of energy needs (13). All ADF studies provided the fast-day meals to the participants, while only

one study (13) provided the feast day meals as well. The duration of the interventions were the following: 8 weeks for Varady and colleagues (3), 8 weeks for Klempel and colleagues (12), and 12 weeks for Bhutani and colleagues (13).

The 5:2 diet is similar to ADF, with five consecutive days of unrestricted intake alternated with two consecutive days of heavily restricted intake. The 5:2 trials (15, 16) examined overweight and obese (BMI 25-45 kg/m²) individuals, most of whom were middle-aged (approximate age of 40 years), female, and Caucasian. Prescribed energy intake was 100% of needs for the feast days, and 25% of needs for the fast days. One trial compared a "traditional" 5:2 diet to a "modified" 5:2 diet that allowed for *ad libitum* protein and unsaturated fat intake during the fast days (16). No food was provided in any of the 5:2 trials. The first 5:2 study lasted 24 weeks (15), and the second study lasted 12 weeks (16).

Less-common forms of IF have been tested as well. Arguin and colleagues (17) examined a regimen that alternated between 5-week cycles of either unrestricted intake or moderately restricted intake (i.e. 25% energy restriction per day). Keogh and colleagues (14) tested a similar regimen, but the cycles lasted 1 week at a time, and the degree of energy restriction was unspecified. Arguin and coworkers (17) enrolled obese (percentage fat 47%), post-menopausal women with an average age of 61 years. Ethnicity was not reported. Keogh and coworkers (14) enrolled overweight or obese (BMI ≥ 27) elderly women (average age of 61 years). Ethnicity was not reported. No food was provided for the trials performed by Arguin and coworkers (17) or Keogh and coworkers (14).

TABLE II.I. SUMMARY OF INTERMITTENT FASTING STUDIES DISCUSSED IN THIS LITERATURE REVIEW ¹

Reference	Subjects	Trial length	Prescribed energy restriction regimen	Measured outcomes
ADF studies				
Klempel, 2013 (12)	n = 32, F Age 43 ± 3 y BMI 35 ± 1 kg/m ²	8 weeks	Group 1: HF diet: Fast day: 75% energy restriction Feed day: 125% energy needs (food provided) Group 2: LF diet: Fast day, 75% energy restriction Feed day, 125% energy needs (food provided)	Adherence Body weight Body comp. Blood pressure Plasma lipids Adipokines
Bhutani, 2010 (42)	n = 16, MF Age 46 ± 2 y BMI 34 ± 1 kg/m ²	8 weeks	Fast day: 75 % energy restriction Feed day: <i>ad libitum</i> fed (food provided on fast day initially, then self-selected)	Adherence Body weight Body comp. Blood pressure Plasma lipids Adipokines
Bhutani, 2013 (13)	n = 16, MF Age 42 ± 2 y BMI 35 ± 1 kg/m ²	12 weeks	Fast day: 75 % energy restriction Feed day: <i>ad libitum</i> fed (food provided on fast day initially, then self-selected)	Adherence Body weight Body comp. Blood pressure Plasma lipids Adipokines
5:2 studies				
Harvie, 2013 (16)	N = 75, F Age 47 ± 1 y BMI 31 ± 1 kg/m ²	12 weeks	Traditional: 2 fast days (75% energy restriction) Alternated with 5 feed days (<i>ad libitum</i> fed) Modified: same as traditional, but with <i>ad libitum</i> lean meat, MUFA, and PUFA (food not provided)	Adherence Body weight Body comp. Blood pressure Plasma lipids Adipokines
Harvie, 2011 (15)	n = 42, F Age 40 ± 4 y BMI 31 ± 5 kg/m ²	24 weeks	2 fast days: 75% energy restriction Alternated with 5 feed days: <i>ad libitum</i> fed (food not provided)	Adherence Body weight Body comp. Blood pressure Plasma lipids Adipokines
Other intermittent fasting studies				
Keogh, 2014 (14)	N = 19, F Age 60 ± 9 y BMI 33 ± 4 kg/m ²	8 weeks	1-week period of energy restriction (5,500 kJ) alternated with 1-week period of 100% energy needs (food not provided)	Body weight Body comp.
Arguin, 2012 (17)	n = 12, F Age 61 ± 7 y Body weight 81 ± 11 kg	30 weeks	5-week period of <i>ad libitum</i> intake alternated with 5-week period of unspecified energy restriction (food not provided)	Body weight Body comp. Plasma lipids

¹ F: Female, HF: High-fat, LF: Low-fat, M: Male

2.2 Effect of intermittent fasting on body weight

Varady and coworkers (3) found that ADF reduced body weight by 6%. Klempel and colleagues went on to examine whether weight loss in response to ADF was modulated by dietary macronutrient composition (12). The researchers compared a high-fat (45% of energy) diet to a low-fat (25%) diet, resulting in statistically similar weight loss of 5% and 4%, respectively (12). Bhutani and coworkers examined a low-fat (25% of energy) ADF diet, which was found to reduce body weight by 3% (13); however, Bhutani and colleagues (13) used a last-observation-carried-forward analysis, which may explain the lower weight loss observed in that study. Harvie *et al.* (15) reported a body weight reduction of 7%. The same research group also reported a body weight reduction of 6% for both the traditional and modified 5:2 diets (16). Two studies have tested IF regimens that alternated between 7 consecutive days or more of restricted intake with a similar duration of unrestricted intake. For Keogh and coworkers (14), the restricted and unrestricted periods consisted of 1-week cycles repeated for a total of 8 weeks, yielding weight loss of 2%. For Arguin and colleagues (17), the restricted and unrestricted periods consisted of 5-week cycles repeated for a total of 30 weeks, yielding a body weight reduction of 13%. Arguin *et al.* (17) may have observed a much greater amount of weight loss due to a much higher prescribed average daily energy restriction; although the energy reduction amount was not specified, the authors noted that it was formulated to reduce body weight by 1% per week.

2.3 Adherence to intermittent fasting protocols

Each of the aforementioned studies measured dietary compliance to the days of restricted intake in their dietary regimens. The studies performed by Bhutani and coworkers used “extra food logs” completed by the research participant to document his or her noncompliance. Based on the extra food logs, compliance was reported to be 87% by Varady *et al.* (3), 87% for the high-fat diet and 78% for the low-

fat diet by Klempel et al. (12), and 80% by Bhutani et al. (13). The studies performed by Harvie and coworkers used questionnaires that asked participants whether they were completely compliant to the 2-day restricted periods of the 5:2 diet. Complete adherence was reported by 44% of the participants in their first study (15), and by 76% and 74% of the participants in the traditional and modified 5:2 diets (respectively) in their second study (16). Keogh and colleagues (14) reported that 37% of their participants were completely adherent. Arguin and coworkers (17) did not report adherence data.

Each of the aforementioned studies used inadequate methods for reporting dietary adherence, because they all relied on self-reporting methods (18). Underreporting of energy intake is consistently observed, (19-21), and obese individuals exhibit the greatest bias in this regard; one review of nine trials (20) found that underreporting in this population ranged between 35-50%. Objective methods that do not rely on self-report are needed to accurately measure compliance to IF protocols. The doubly labeled water (DLW) method is the gold standard for measuring dietary compliance (22); however, this method has a unique limitation when applied to IF research, because while it can measure compliance over week-long periods, it cannot do so day-to-day. Thus the results from DLW pertaining to compliance to the restricted periods of an IF protocol would be confounded by the compliance to the unrestricted periods. Therefore, new methods are needed (e.g. remote sensing devices (23, 24) and remote food photography (25)) to measure compliance specifically to the restricted periods of IF.

2.4 Effect of intermittent fasting on body composition

2.4.1 Fat mass

ADF consistently reduces fat mass, although the magnitude of this reduction is variable among the published studies to date. Varady and colleagues (3) observed a 5-kg reduction, while Bhutani and colleagues (13) observed only a 2-kg reduction. Klempel and coworkers (12) found a 5-kg reduction in

the high-fat diet group and a 4-kg reduction in the low-fat diet group, although the between-group difference was not statistically significant. The 5:2 diet studies observed more-consistent reductions in fat mass: 5 kg in one study (15) and 4 kg for both diet groups (traditional and modified) in the other study (16). Arguin *et al.* (17) reported the largest lowering of fat mass of any of the IF trials (9 kg).

2.4.2 Fat-free mass

Fat-free mass is generally well preserved under an IF protocol. Both Varady and colleagues (3) as well as Klempel and colleagues (12) reported no change in fat-free mass. Bhutani *et al.* (13) was the only ADF study to observe a reduction in this parameter (1 kg). The 5:2 diet reduced fat-free mass by 1 kg in the first published study (15) as well as in both of the groups examined in the second study (16). Arguin *et al.* (17) also reported the largest lowering of fat-free mass of any of the IF trials (2 kg).

2.4.3 Visceral fat mass

Visceral fat is an important mediator of the relationship between obesity and cardio-metabolic disease (26). Two main factors involved in this relationship are atherogenic dyslipidemia and insulin resistance, which are caused by excess non-esterified fatty acids (27) and inflammatory cytokines (28), respectively, being drained from visceral fat directly to the liver (29). None of the IF trials to date have measured visceral fat mass via an imaging technique, making the measurement of waist circumference the best available surrogate. The ADF papers (3, 13) reported reductions of 4 cm, 5 cm, and 7 cm (in both the high-fat and low-fat diet groups), respectively. The traditional 5:2 diet resulted in reductions of 6 cm in both studies that examined it (15, 16), while the modified 5:2 diet resulted in a 5 cm reduction (that was not significantly different when compared to the reduction caused by the traditional 5:2 diet). Arguin and colleagues (17) observed the largest reduction of the IF trials (11 cm), and Keogh and colleagues (14) observed the lowest reduction (3 cm).

2.5 Effect of intermittent fasting on coronary heart disease risk factors

2.5.1 Blood pressure

The findings for ADF and blood pressure reduction are inconsistent. For instance, while Varady *et al.* (3) reported a reduction in blood pressure of 6 mm Hg, Bhutani *et al.* (13) and Klempel *et al.* (12) reported no change to this parameter. No ADF study found a reduction in diastolic blood pressure. It may be the case that the Varady *et al.* (3) study found greater reductions in blood pressure because this study did not include a control group. The first study of the 5:2 diet reported reductions of 4 mm Hg for both systolic and diastolic pressure (15). In their second study, Harvie and coworkers (16) reported that systolic pressure was reduced by 3 mm Hg in the traditional diet group and by 17 mm Hg in the modified diet group. Substantially different systolic pressures at baseline (15 mm Hg higher in the modified diet group) likely explain much of this discrepancy. Although Harvie and coworkers (16) reported that diastolic pressure was measured in their study, the authors did not report on their findings. Neither Arguin *et al.* (17) nor Keogh *et al.* (14) measured blood pressure in their studies. In terms of the clinical relevance of these findings, the 6 mm Hg reduction in systolic blood pressure observed by Varady *et al.* (3) has been shown to reduce the likelihood of experiencing a cardiovascular event, and this risk reduction appears to be independent of baseline BMI and baseline blood pressure (30, 31). Reductions in systolic blood pressure less than this amount do not appear to alter the risk of a cardiovascular event (31).

2.5.2 Plasma lipids

The main function of LDL-C is to transport cholesterol from the liver and intestines to areas of need (32). However, LDL-C does not always reach its intended target and instead becomes trapped in the arterial intima, an early step in the development of atherosclerosis (33). Atherosclerosis eventually leads

to the formation of a thrombus, which can result in the development of several cardiovascular events, including myocardial infarction and stroke (34). The ability of HDL-C to protect against atherosclerosis is mainly mediated through its role in reverse cholesterol transport. Through this process, HDL-C travels throughout the bloodstream and gathers cholesterol from areas of overabundance, including atherogenic foam cells (35). HDL-C then transports this cholesterol to the liver, where it is converted into bile and excreted (36). In addition to its role in reverse cholesterol transport, HDL-C helps protect against inflammation, LDL oxidation, and thrombosis (36). Also, HDL-C increases nitric oxide production and inhibits the expression and migration of atherosclerotic adhesion molecules (35). Elevated TAG increases the risk of CHD by partnering with VLDL-C and cholesteryl ester transfer protein to make LDL-C more atherogenic and HDL-C less anti-atherogenic (37). Specifically, VLDL (with the aid of cholesteryl ester transfer protein) exchanges its TAG-rich lipoprotein particles for cholesterol esters from LDL-C and HDL-C. Once these lipoproteins encounter hepatic lipase, they are stripped of their TAG particles, rendering them much smaller than normal. This removal of TAG makes it easier for LDL-C to enter the arterial wall and lowers the amount of cholesterol that HDL-C can transport back to the liver. LDL-C is typically within the normal range in viscerally obese individuals (26), and therefore weight loss is not expected to appreciably alter circulating levels. In contrast, weight loss increases HDL-C and decreases TAG (38).

Regarding plasma lipids, the only consistent finding among the ADF studies is that HDL-C does not change (3, 13). Regarding total cholesterol, Varady *et al.* (3) and Klempel *et al.* (12) reported impressive reductions of this parameter (37 mg/dL for Varady and colleagues, and 26 mg/dL and 31 mg/dL for the high-fat and low-fat diet groups, respectively, for Klempel and colleagues). On the other hand, Bhutani *et al.* (13) reported that total cholesterol did not change. Similar findings related to the lowering of LDL-C were observed in the ADF studies: Varady *et al.* (3) and Klempel *et al.* (12) reported

impressive reductions (33 mg/dL for Varady and colleagues, and 19 mg/dL and 28 mg/dL for the high-fat and low-fat diet groups, respectively, for Klempel and colleagues), but this parameter did not change in the Bhutani *et al.* (13) study. Similar findings related to the lowering of triacylglycerol (TAG) were observed in the ADF studies: Varady and coworkers (3) found a 41 mg/dL reduction, Klempel and coworkers (12) found reductions of 15 mg/dL and 14 mg/dL (for the high-fat diet group and the low-fat diet group, respectively), and Bhutani and coworkers (13) found no change.

The findings pertaining to plasma lipid changes in response to a 5:2 diet are fairly consistent. Total cholesterol was lowered by 0.3 mmol/L (~12 mg/dL) in the first study (15), and by 0.2 mmol/L (~8 mg/dL) for both the traditional and modified diets in the second study (16). LDL-C was lowered by 0.3 mmol/L in the first study (15), and by 0.1 mmol/L (~4 mg/dL) and 0.2 mmol/L for the traditional and modified diets, respectively, of the second study (16). HDL-C did not change in any group examined in either study (15, 16). TAG was reduced by 0.2 mmol/L (~18 mg/dL) in the first study (15), and by 0.1 mmol/L (~9 mg/dL) for the traditional diet group and 0.2 mmol/L for the modified diet group in the second study (16).

Arguin and coworkers (17) reported a 1.1 mmol/L (~43 mg/dL) lowering of total cholesterol, a 0.8 mmol/L (~71 mg/dL) lowering of TAG, with no changes in either LDL-C or HDL-C. Keogh and coworkers (14) did not measure plasma lipids.

2.5.3 LDL particle size

LDL particle size is linked to CHD risk through several mechanisms. Smaller size makes LDL particles less able to be cleared (via binding to LDL receptors), more likely to enter the vascular wall, and more likely to undergo oxidative modification (26). Of the IF studies, only those that investigated ADF

measured LDL particle size. Their findings are inconsistent. For instance, Varady and colleagues (39) reported no changes in any parameter related to LDL size (peak size, integrated size, or proportion of small-, medium-, or large LDL particles). In contrast, Bhutani and colleagues (13) observed a 5Å increase in mean size, a 15% increase in the proportion of large particles (> 260Å), and a 12% lowering in the proportion of small particles (< 255Å). Klempel and colleagues (40) reported a 3Å increase in peak size, a 6% increase in the proportion of large particles, and an 8% lowering in the proportion of small particles in the high-fat diet group. They also found a 3Å increase in peak size, a 3% increase in the proportion of large particles, an 8% increase in the proportion of medium particles (255-260Å), and an 11% lowering in the proportion of small particles in the low-fat diet group (40).

2.5.4 C-reactive protein and homocysteine

C-reactive protein (CRP) is believed to have little causal effect on atherogenesis (41). However, CRP promotes plaque vulnerability and thrombus formation (41). While the degree of reduction of CRP that is clinically significant has not yet been agreed upon, a treatment goal of < 2 mg/l has been espoused (42). CRP was reduced (by 0.5 mg/l) in only one 5:2 trial by Harvie and coworkers (15). Two other IF trials by Bhutani and colleagues and Varady and colleagues reported no change in this parameter (3, 13). Elevated homocysteine levels are associated with increased cardiovascular disease risk (43). Proposed mechanisms to explain this association include vascular smooth muscle growth, endothelial dysfunction, reduced synthesis of HDL-cholesterol, and accumulation of inflammatory monocytes in atherosclerotic lesions (44-47). Varady and coworkers were the only IF study to measure homocysteine (3) and found no change.

2.6 Effect of intermittent fasting on adipokines

2.6.1 Adiponectin, leptin, resistin

Adiponectin protects against CHD via several mechanisms, including: 1) lowering the expression of adhesion molecules on endothelial cells; 2) reducing the proliferation of vascular smooth muscle cells; 3) inhibiting foam cell formation; and 4) acting as an antioxidant (48). Evidence regarding the effect of IF on circulating adiponectin is inconclusive. Two ADF trials reported increases in this parameter: Bhutani *et al.* (49) observed a 30% increase, while Klempel *et al.* (12) observed increases of 43% and 51% for the high-fat ADF group and low-fat ADF group respectively. On the other hand, Bhutani *et al.* (13) found no change in this parameter. Regarding the 5:2 diet, the first study reported a 10% increase in plasma adiponectin (15), while the second study did not observe a change in either of the two 5:2 diet groups (16).

Leptin's relationship to CHD risk is mostly indirect. Specifically, leptin contributes to expanded fat mass (48), which is expected to disrupt the secretion of other adipokines, thereby promoting CHD. Leptin levels consistently decrease in response to IF. The following reductions have been observed: 21% (49), 24% (13), 40% (15), 43% and 36% for the traditional and modified 5:2 diets, respectively (16), and 32% and 30% for the ADF high-fat and low-fat diet groups, respectively (12).

The role of resistin in promoting cardio-metabolic disease is controversial. While resistin is known to promote hepatic insulin resistance in rodents, the data in humans is contradictory (50). Only two IF trials have measured resistin. Both Bhutani and coworkers (21% decrease) (49) and Klempel and coworkers (23% decrease and 27% decrease for the high-fat and low-fat ADF groups, respectively) (12) observed reductions.

2.6.2 Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF- α)

Interleukin-6 promotes CHD indirectly by propagating inflammation. Indeed, portal vein levels of IL-6 correlate highly with circulating CRP (28). Harvie and colleagues (16) found that both a traditional 5:2 diet and a modified 5:2 diet reduced IL-6 (by 13% and 14%, respectively), while neither diet affected TNF- α . This was the only IF trial to date to examine these parameters.

2.7 Effect of intermittent fasting on insulin sensitivity

Insulin resistance appears to be the fundamental link between obesity and CVD. Important CVD risk factors such as systolic blood pressure, diastolic blood pressure, TAG level, HDL-C level, fasting plasma glucose level and 2-h plasma glucose level during an OGTT are all worse in insulin-resistant obesity compared to insulin-sensitive obesity when participants are matched for BMI, gender and age (51-57). Furthermore, whereas insulin-resistant obese individuals experience improvement in insulin sensitivity and CVD risk factors with weight loss, insulin-sensitive obese individuals do not see improvements in these variables for the same amount of weight loss (51-57).

Insulin resistance increases the risk of CVD through many mechanisms (58). An inability of insulin to suppress lipolysis in adipose tissue leads to an influx of non-esterified fatty acids to the liver, skeletal muscle, and other organs (59). In skeletal muscle, insulin resistance manifests as a diminished ability to uptake glucose (59). Hepatic insulin resistance manifests as an increased rate of gluconeogenesis and a decreased rate of glycogen synthesis, which results in fasting hyperglycemia (60). Excessive non-esterified fatty acid delivery to the liver, along with hepatic insulin resistance, lead to increased secretion of apolipoprotein B-containing particles, particularly VLDL 1 (61). This contributes to an atherogenic dyslipidaemia in insulin resistance that is characterized by elevated serum TAG levels, low HDL-C levels, and a preponderance of small-dense LDL particles (62). Insulin resistance in endothelial cells

leads to diminished endothelial nitric oxide synthesis and release (63), thereby increasing the risk of hypertension. Risk of hypertension is also elevated by the effects of compensatory hyperinsulinemia on sympathetic drive, smooth muscle growth, and sodium–fluid retention (64).

Surprisingly, only a few IF studies to date have measured HOMA-IR, and no studies have measured more robust indicators of insulin sensitivity. Bhutani et al. did not observe a reduction in HOMA-IR in response to ADF (13). Harvie *et al.* observed reductions in HOMA-IR from 1.5 au to 1.1 au in their first study (15), and from 1.6 au to 1.2 au in their second study (16).

3. Calorie restriction

3.1 Description of studies

The CR studies reviewed here are summarized in **Table 2**. Inclusion criteria were the following: (i) randomized controlled trial; (ii) primary endpoint of weight loss or change in body composition; (iii) study duration of 8-30 weeks; (iv) overweight or obese, nondiabetic participants; (v) publication date no earlier than year 2000; and (vi) total fat mass measured with dual-energy X-ray absorptiometry (DEXA), hydrodensitometry, air displacement plethysmography, computed tomography (CT), or magnetic resonance imaging (MRI).

Haugaard and coworkers (65) examined 60% CR for 8 weeks in men and women with an average age of 50 years and an average BMI of 36; all meals were provided in this study. Luscombe and coworkers (66) examined 25% CR for 8 weeks in men and women with an average age of 50 years and an average BMI of 34; all meals were provided in this study. Kirk and coworkers (67) examined 50% CR for 11 weeks in men and women with an average age of 43 years and an average BMI of 37. This study

compared a high-carbohydrate (65% of daily energy) diet to a low-carbohydrate (10%) one, and all meals were provided. Coker and coworkers (68) examined 60% CR for 8 weeks in men and women with an average age of 58 years and an average BMI of 30; all meals were provided in this study. Pierce and coworkers (69) examined CR (unspecified level of restriction) for 12 weeks in men and women with an average age of 50 years and an average BMI of 29; food was provided initially, and then the diet was self-selected. Redman and coworkers (22) examined 25% CR for 12 weeks in men and women with an average age of 39 years and an average BMI of 28; food was provided initially, and then the diet was self-selected. Ross and coworkers (70) examined 25% CR for 8 weeks in women with an average age of 44 years and an average BMI of 32; the diet was self-selected. Arguin and coworkers (17) examined CR (unspecified level of restriction) for 15 weeks in women with an average age of 61 years and an average body weight of 78 kg; the diet was self-selected. Nicklas and coworkers (71) examined 25% CR for 20 weeks in women with an average age of 58 years and an average BMI of 34; the diet was self-selected. Brochu and coworkers (72) examined 35% CR for 24 weeks in women with an average age of 58 years and an average BMI of 32; the diet was self-selected. Harvie and coworkers (15) examined 25% CR for 24 weeks in women with an average age of 40 years and an average BMI of 31; the diet was self-selected.

TABLE II.II. SUMMARY OF CALORIE RESTRICTION STUDIES DISCUSSED IN THIS LITERATURE REVIEW ¹

Reference	Subjects	Trial length	Prescribed CR regimen	Measured outcomes
Haugaard, 2007 (44)	n = 13, MF Age 50 ± 3 y BMI 36 ± 1 kg/m ²	8 weeks	60% energy restriction daily (food provided)	Body comp. Body weight
Keogh, 2014 (14)	n = 17, F Age 61 ± 13 y BMI 33 ± 8 kg/m ²	8 weeks	5,500 kJ/week energy restriction (food not provided)	Body comp. Body weight
Luscombe, 2006 (45)	n = 11, MF Age 50 ± 3 y BMI 34 ± 2 kg/m ²	8 weeks	25% energy restriction daily (food provided)	Adherence Body comp. Body weight
Kirk, 2009 (46)	n = 22, MF Age 43 ± 4 y BMI 37 ± 1 kg/m ²	11 weeks	50% energy restriction daily + LC diet (food provided) 50% energy restriction daily + HC diet (food provided)	Adherence Body comp. Body weight
Coker, 2009 (47)	n = 9, MF Age 58 ± 2 y BMI 30 ± 0 kg/m ²	12 weeks	15% energy restriction daily (food provided)	Body comp. Body weight
Harvie, 2013 (16)	n = 40, F Age 46 ± 1 y BMI 30 ± 1 kg/m ²	12 weeks	25% energy restriction daily (food not provided)	Adpokines Body comp. Blood lipids Blood pressure Body weight
Pierce, 2008 (48)	n = 26, MF Age 50 ± 3 y BMI 29 ± 1 kg/m ²	12 weeks	Unspecified (food provided initially, then self-selected)	Adherence Adpokines Body comp. Blood lipids Blood pressure Body weight
LeFevre, 2009 (56)	n = 12, MF Age 39 ± 5 y BMI 28 ± 1 kg/m ²	12 weeks	25% energy restriction daily (food provided)	Adherence Body comp. Blood lipids Blood pressure Body weight
Ross, 2004 (49)	n = 15, F Age 44 ± 5 y BMI 32 ± 3 kg/m ²	14 weeks	25% energy restriction daily (food not provided)	Adherence Body comp. Body weight
Arguin, 2012 (17)	n = 10, F Age 61 ± 7 y Body weight 78 ± 10 kg	15 weeks	Unspecified (food not provided)	Body comp. Blood lipids Body weight
Nicklas, 2009 (50)	n = 29, F Age 58 ± 6 y BMI 34 ± 4 kg/m ²	20 weeks	25% energy restriction daily (food provided)	Adherence Blood lipids Body comp. Body weight
Brochu, 2009 (51)	n = 71, F Age 58 ± 5 y BMI 32 ± 5 kg/m ²	24 weeks	34% energy restriction daily (food not provided)	Adpokines Body comp. Blood lipids Blood pressure Body weight
Harvie, 2011 (15)	n = 47, F Age 40 ± 4 y BMI 31 ± 5 kg/m ²	24 weeks	25% energy restriction daily (food not provided)	Adherence Adpokines Body comp. Blood lipids Blood pressure Body weight

¹ BMI: Body mass index (kg/m²), F: Female, HC: High-carbohydrate, LC: Low-carbohydrate, M: Male

3.2 Effect of calorie restriction on body weight

Body weight reduction ranged between 4-12% in short-term (12 weeks or fewer) trials. Haugaard and coworkers (65) observed a 9% reduction. Luscombe and coworkers (66) reported a 12% reduction. Kirk et al. (67) observed a 7% and 8% lowering in response to a high-carbohydrate diet and a low-carbohydrate one, respectively. Coker et al. (68) reported a 6% lowering. Pierce and colleagues (69) reported a 12% reduction. Redman and colleagues (22) found a 7% reduction. Keogh et al. (14) observed the smallest body weight reduction (4%) of the short-term trials. Moderate-term (13-24 weeks) weight loss ranged between 4-13%. Ross and colleagues (70) reported a reduction of 8%. Arguin and colleagues (17) reported weight loss of 12%. Nicklas et al. (71) observed a 13% reduction in body weight. Brochu et al. (72) found a 6% reduction. Harvie and colleagues (15) reported a 5% reduction. The same research group reported a 4% reduction in a similar study (16). Prescribed percent energy reduction surprisingly does not appear to be related to weight loss in the above-mentioned trials. For example, weight loss was greater on a 25% CR regimen (12% for Luscombe et al.) (66) than on a 60% CR regimen (9% for Haugaard and colleagues) (65). Also, intervention duration does not appear to be related to weight loss, but this observation is confounded by the fact that most CR trials in this review lasting longer than 12 weeks did not provide food, while food was provided in most short-term trials.

3.3 Adherence to calorie restriction protocols

Adherence metrics serve a few purposes. First, they provide context to a trial's results, specifically discriminating between the effects of prescribing an intervention versus the effects of (near-) perfectly completing the intervention. Second, they identify areas of improvement for future research aimed at enhancing adherence and weight-loss outcomes (73, 74). Harvie and coworkers (15) reported that 32% of subjects self-reported that they completely adhered to the prescribed 25% daily energy restriction protocol. Kirk and colleagues (67) measured plasma 3-hydroxybutyrate concentrations to determine

dietary adherence. The researchers found that subjects were adherent to both the high-carbohydrate and low-carbohydrate diets (67). Redman and colleagues (22) reported that subjects who were prescribed a 25% daily energy restriction actually achieved an 18% restriction (measured via DLW). However, the calculated actual energy restriction was based on the (incorrect) assumption that energy expenditure does not change during weight loss. When actual energy restriction was recalculated to reflect the weight-loss-induced decrease in energy expenditure, the subjects were found to achieve a degree of energy restriction that was "very close" (authors' words) to the prescribed 25% (22). Ross *et al.* (70), using dietary records, found that energy intake was reduced by 400 kcal/d, which was slightly less than the prescribed 500 kcal/d restriction. The remaining studies either provided all food to the subjects throughout the intervention (65, 66, 68, 69, 71) or did not measure dietary adherence (15, 17, 72, 75).

3.4 Effect of calorie restriction on body composition

3.4.1 Fat mass

Fat mass reduction ranged between 4-8 kg in the short-term CR trials. Haugaard and colleagues (65) reported a 6 kg lowering. Luscombe and coworkers (66) reported an 8 kg reduction. Kirk *et al.* (67) observed a 4 kg reduction for both diet groups. Coker *et al.* (68) also observed a 4 kg reduction. Pierce and coworkers (69) reported a 6 kg reduction. Redman and colleagues (22) found an 8 kg reduction. In moderate-term trials, fat mass reduction ranged between 2-9 kg. Ross and coworkers found a 4 kg lowering (70). Arguin *et al.* (17) reported the largest reduction at 9 kg. Nicklas *et al.* (71) reported a 7 kg reduction. Brochu and colleagues (72) observed a 4-kg reduction. Harvie and coworkers reported a 4 kg reduction in their first study of 25% CR (15), and a 2 kg reduction in their second trial (16). As with body weight reduction, fat mass reduction appeared to be unrelated to percent energy restriction or intervention length in the aforementioned trials. Between-study differences in subject compliance likely underlie these paradoxical findings.

3.4.2 Fat-free mass

Fat-free mass reduction ranged between 1-4 kg in short-term trials. Haugaard *et al.* (65) observed a 3 kg decrease. Luscombe *et al.* (66) reported a 4 kg reduction. Kirk and coworkers (67) found a 2 kg reduction in both diet groups. Coker and coworkers (68) reported a 1 kg reduction. Pierce and colleagues (69) found a 2 kg reduction. Redman *et al.* (22) also found a 2 kg reduction. The reduction in fat-free mass also ranged between 1-4 kg in the moderate-term trials. Ross and colleagues (70) reported a 1 kg lowering. Arguin and coworkers (17) reported a 1 kg reduction. Nicklas and colleagues (71) observed a 4 kg reduction. Brochu and coworkers (72) observed a 1 kg lowering. Harvie *et al.* reported a 1 kg lowering in each of their two studies (15, 16). As with body weight and fat mass, change in fat-free mass did not appear to depend on intervention length or prescribed percent energy reduction.

3.4.3 Visceral fat mass

Although the CR trials in this review used different methodology for measuring visceral fat, they consistently observed a reduction in response to diet intervention. Here are the findings regarding short-term CR: Using DEXA, Luscombe and coworkers (66) observed a 2 kg reduction. Kirk and colleagues (67) found that both diet groups reduced visceral fat by 12% as measured by magnetic resonance imaging (MRI). Coker *et al.* (68) reported a 15% lowering as measured by CT. Also using CT, Pierce and colleagues (69) reported a 33% reduction, while Redman and coworkers (22) found a 27% reduction. Haugaard and coworkers (65) estimated visceral fat via waist circumference and observed a 7 cm lowering. The moderate-term CR trials also consistently observed reductions in visceral fat. Using CT, Brochu and coworkers (72) reported a 23 cm² reduction. Also using CT, Nicklas *et al.* (71) reported a 612 cm³ lowering. Ross and colleagues (70) used MRI to measure total abdominal fat (subcutaneous fat + visceral fat), observing a 1 kg reduction. Finally, Harvie *et al.* (15), Harvie *et al.* (16), and Arguin

et al. (17) observed reductions in waist circumference of 4 cm, 3 cm, and 10 cm, respectively. In general, the CR studies that produced greater weight loss also produced greater visceral fat reduction, an observation that is in line with the findings of a recent meta-analysis (76). However, different methods of measuring or estimating visceral fat make it difficult to draw firm conclusions from the above studies.

3.5 Effect of calorie restriction on coronary heart disease risk factors

3.5.1 Blood pressure

The results pertaining to the blood pressure response to CR are mixed. Harvie and colleagues (15) reported a systolic pressure reduction of 8 mm Hg, and a diastolic pressure reduction of 6 mm Hg. The same research group noted a 10 mm Hg reduction in systolic pressure in their second study (16). Diastolic pressure was not reported. No change in blood pressure was reported by Brochu *et al.*, (72) Pierce *et al.*, (69) and Lefevre *et al.* (77).

3.5.2 Plasma lipids

Most CR studies report lowering of TC. Indeed, reductions of 0.15 mmol/l (6 mg/dL) (16), 0.71 mmol/L (27 mg/dL) (17), 0.5 mmol/L (19 mg/dL) (15), and 23 mg/dL (69) have been reported. On the other hand, one study reported no change to TC (72). There are mixed findings pertaining to LDL-C reduction. Reductions of 9 mg/dL (71), 0.4 mmol/L (15 mg/dL) (17), and 0.3 mmol/L (12 mg/dL) (15) have been reported, but other studies have observed no change in this parameter (16, 69, 72, 77). There are also heterogeneous findings pertaining to HDL-C. Reductions of 3 mg/dL (71), 4 mg/dL (77), and 0.1 mmol/L (4 mg/dL) (15) were reported, but an absence of change was reported in other research (16, 17, 69, 72). The findings regarding TAG lowering are more consistent, with reductions of 26 mg/dL (71), 0.08 mmol/L (7 mg/dL) (16), 0.5 mmol/L (44 mg/dL) (17), 0.3 mmol/L (27 mg/dL) (15), and 0.23

mmol/L (20 mg/dL) (72) being reported. Only one trial (69) found that TAG did not change in response to CR.

3.5.3 C-reactive protein and homocysteine

Three studies have reported changes in CRP in response to CR, with one study observing a 0.8 mg/L reduction (15), and the other two reporting no change (69, 72). The only study to examine homocysteine (69) observed no change.

3.6 Effect of calorie restriction on adipokines

3.6.1 Adiponectin, leptin, resistin

Three studies reported on adiponectin, with one noting a 9% reduction (16), and the other two studies observing no change (15, 69). Leptin is consistently reduced in response to CR: reductions of 44% (16), 57% (15), and 40% (69) have been reported. None of the CR trials mentioned in this review measured resistin.

3.6.2 Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-alpha)

IL-6 and TNF- α were reduced by 7% and 6%, respectively, in one trial (15). The only other investigation to measure these parameters reported no change for each (69).

3.7 Effect of calorie restriction on insulin sensitivity

With some exceptions, the methods for assessing insulin sensitivity in CR studies were generally far more robust than those employed in IF studies. Haugaard *et al.* (65) reported a 44% lowering of HOMA-IR in response to CR. The glucose disposal rate during a euglycemic hyperinsulinemic clamp increased in response to CR in the studies by Kirk *et al.* (67) and Coker *et al.* (68), but did not change in

the study by Ross *et al.* (70) or the study by Brochu *et al.* (72). Pierce *et al.* (69) found no change in insulin sensitivity as assessed by an intravenous glucose tolerance test. Nicklas *et al.* (71) reported no change in 2-h glucose during an OGTT. Harvie *et al.* observed a reduction in HOMA-IR from 1.6 au to 1.3 au in their first study (15), and no change in their second study (16).

4. Comparison between intermittent fasting and daily calorie restriction

Comparisons between IF and CR dietary regimens are difficult to make, because the overwhelming majority of clinical trials have assessed one dietary regimen but not the other. This means that indirect comparisons between the diets are confounded by numerous factors, such as differences in participant characteristics (e.g. age, BMI, ethnicity, baseline metabolic health), dietary regimen (e.g. macronutrient distribution, degree of prescribed energy restriction), factors in study design that may promote greater or lesser dietary adherence (e.g. provision of food, dietary counseling, aspects of enrollment criteria that pertain to perceived ability to comply with dietary instructions) and trial length. With these caveats in mind, presented below are comparisons between the effects of IF and CR dietary regimens on selected cardiometabolic parameters.

4.1 Dietary adherence, body weight and body composition

Unfortunately, all IF studies to date have measured dietary adherence with self-report, which is not only invalid in obesity research (18) but also incomparable with the more objective measurements of dietary adherence performed in the reviewed CR studies. Fortunately, measuring the change in body weight is an effective method for estimating dietary compliance (78). Calorie restriction appears to decrease body weight (range of response: 4-12%) slightly more than IF (3-7%). As a consequence, CR also produces slightly greater reductions in fat mass (4-8 kg) and fat-free mass (1-4 kg) than ADF (2-5 kg and 0-1 kg, respectively). Change in visceral fat mass cannot be appropriately compared between the IF and CR regimens included in this review, because the IF studies used waist circumference as a surrogate for visceral fat mass, while the CR studies were more likely to use imaging techniques. However, a recent review of IF and CR studies concluded that the two dietary regimens were equally effective at reducing waist circumference for up to 12 weeks of dieting, while IF regimens were more effective when tested for durations longer than 12 weeks (79).

4.2 Blood pressure and plasma lipids

Comparisons in blood pressure reduction between IF and CR regimens are difficult to make because the findings have been highly variable. For instance systolic blood pressure response has ranged from no change to a 17 mm Hg reduction in IF studies, and from no change to a 10 mm Hg reduction in CR studies. Similarly, diastolic blood pressure response has ranged from no change to a 4 mm Hg reduction in IF studies, and from no change to a 6 mm Hg reduction in CR studies. In general, higher blood pressure at baseline appears to be associated with a greater blood pressure reduction with weight loss.

As with blood pressure, comparisons in plasma lipid response between IF and CR regimens are difficult to make due to highly heterogeneous findings. Intermittent fasting studies have noted reductions in TC, LDL-C, and TAG as high as 37 mg/dL, 33 mg/dL, and 41 mg/dL, respectively; but other studies have reported no change to any of these parameters. Similarly, CR studies have noted reductions in TC, LDL-C, and TAG as high as 27 mg/dL, 15 mg/dL, and 44 mg/dL, respectively; but other studies have reported no change to any of these parameters. HDL-C consistently does not change in response to IF, while decreasing by as much as 4 mg/dl in response to CR.

4.3 Circulating inflammatory molecules and insulin sensitivity

The changes to circulating inflammatory molecules have generally been similar between IF and CR. Examples include CRP (range of response: reduction of 0-0.5 mg/L for IF and 0-0.8 mg/L for CR), homocysteine (no change in response to either diet), TNF- α (no change in response to IF, and a reduction ranging between 0-6% in response to CR), and IL-6 (range of response: reduction of 13-14% for IF and 0-7% for CR). Leptin has generally decreased more in response to CR (40-57%) than IF (21-43%), presumably due to a greater reduction in fat mass in response to CR. None of the reviewed CR

studies measured resistin, thus precluding a comparison. The response of adiponectin to IF has been highly variable, ranging from no change to a 51% increase. In contrast the adiponectin response to CR has been more consistent, ranging from no change to a 9% decrease. At this time a proper comparison of the two diets' abilities to improve insulin sensitivity cannot be made due to the different methods across studies for assessing insulin sensitivity, along with the small number of IF studies that have assessed this variable.

III. MANUSCRIPT 1

Effect of 6 months of alternate day fasting versus daily calorie restriction on body weight, body composition and metabolic disease risk in obese adults

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A. ABSTRACT

Background: Alternate-day modified fasting (ADF) has been shown to be effective for reducing body weight when tested for a short duration (8-12 weeks). However, no trial to date has examined adherence to ADF for longer periods, or compared ADF to daily calorie restriction (CR) for weight loss and improvement in cardiometabolic parameters.

Objective: Accordingly, this study compared the effects of ADF versus CR on dietary adherence, weight loss, and cardiometabolic disease risk factors.

Design: In a controlled feeding trial, overweight and obese participants (n = 102) were randomized to 1 of 3 groups: 1) ADF (24-h feast day = 125% of needs, alternated with 24-h fast day = 25% of needs); 2) CR (75% of needs every day); or 3) control (100% of needs every day). In this way, the prescribed average daily energy restriction (25%) was the same for both intervention groups. The intervention period was 6 months, and consisted of a 3-month controlled feeding period followed by a 3-month self-selected feeding period. Dietary adherence, body weight, body composition, and cardiometabolic parameters were measured at month 0, 3, and 6.

Results: Actual energy restriction (ADF: $21 \pm 4\%$; CR: $24 \pm 4\%$, $P = 0.89$) was similar between the intervention groups and greater when compared to the control group ($8 \pm 5\%$, $P = 0.03$). Weight loss (ADF: -6.9 ± 0.8 kg; CR: -7.6 ± 1.0 kg, $P = 0.79$) and total fat mass reduction (ADF: -4.5 ± 0.6 kg; CR: -5.6 ± 0.7 kg, $P = 0.33$) were also similar between groups and greater than the respective reductions in the control group (-0.7 ± 0.5 kg, $P < 0.001$; -0.6 ± 0.3 kg, $P < 0.001$). ADF demonstrated within-group reductions in total cholesterol, systolic blood pressure, heart rate, fasting insulin, and insulin resistance by month 6. CR experienced decreases in total cholesterol, LDL cholesterol, systolic blood pressure, and fasting insulin, post-treatment. No differences were noted between intervention groups or versus control for any of these vascular endpoints ($P > 0.05$ for all comparisons).

Conclusion: Dietary adherence and weight loss are similar between ADF and CR; however neither intervention alters other measured cardiometabolic parameters versus controls.

B. INTRODUCTION

Individuals who are overweight or obese are more likely to develop coronary heart disease (CHD) (80). Weight loss of 5-10% has been shown to reduce CHD risk (81). Alternate-day modified fasting (ADF) is a dietary strategy for reducing energy intake and eliciting weight loss. Rather than reducing energy intake by a moderate amount every day, as do calorie restriction (CR) regimens, ADF alternates between a 24-h period of heavy restriction ('fast days') and a 24-h period of no restriction ('feast days'). In trials lasting 8-12 weeks, ADF elicits moderate weight loss (4-8%) (3, 4, 12, 13, 82, 83). Moreover, some studies have noted reductions in key CHD risk factors such as LDL-cholesterol levels, triacylglycerol levels, heart rate, and blood pressure (3, 4, 12, 13, 82, 83). These findings suggest that ADF might eventually be recommended clinically as a method for weight loss. Before such a recommendation can be issued, however, more evidence is needed, especially with regards to the following: 1) the longer-term effects of ADF on body weight, blood lipids, and blood pressure relative to the standard-care dietary method for weight loss, i.e. CR; and 2) adherence to an ADF protocol over a longer timeframe than in previous research.

Accordingly, this study compared the effects of ADF versus CR on dietary adherence, weight loss, and CHD risk factors in a 6-month clinical feeding trial. We hypothesized that ADF would elicit greater dietary adherence and weight loss – and concomitantly greater improvements in key CHD risk factors – when compared to CR.

Given the large number of African American participants that were recruited, we were also interested in examining this group's weight loss trajectory over the course of the trial. African American women have been shown to lose less weight than Caucasian women in trials of behavioral intervention (84).

Therefore, we performed a post hoc analysis to determine if weight loss in either intervention group was affected by whether the participant was African American or Caucasian.

C. SUBJECTS AND METHODS

Participants

Men and women aged 18-65 y with a body mass index (BMI; in kg/m^2) between 25-40 were recruited from Chicago and the greater area by means of posters placed in community centers, libraries, cafes, and the University of Illinois, Chicago campus. Individuals excluded from participating in the study constituted those who had a prior history of cardiovascular disease, were diabetic (fasting blood glucose ≥ 126 mg/dL), were not weight stable (weight gain or loss > 4 kg during the 3 months prior to study enrollment), were physically active (≥ 3 h/week of self-reported structured exercise) were perimenopausal or had an irregular menstrual cycle, were pregnant or trying to become pregnant, or were smoking cigarettes. Also, because magnetic resonance imaging (MRI) was employed to measure visceral fat mass, individuals who were claustrophobic or who had metal implants were also excluded. Individuals were also excluded if they took weight-loss or lipid-lowering medications. However, following medications were taken by enrolled participants: angiotensin 2 receptor blocker, angiotensin converting-enzyme inhibitor (x2), antihistamine, beta blocker (x2), calcium channel blocker (x2), corticosteroid, levothyroxine (x2), nonsteroidal anti-inflammatory drug, proton-pump inhibitor (x2), selective serotonin reuptake inhibitor, and tricyclic antidepressant. All participants provided written informed consent to participate in this study. The protocol was approved by the Office for the Protection of Research Subjects at the University of Illinois, Chicago.

Experimental design

Baseline period (1 month): This study included a 1-month baseline period, during which time the participants were requested to maintain a stable weight and continue eating their usual diet. During this period, total energy expenditure was quantified for each subject by the doubly labeled water (DLW) method (described in detail below).

Intervention period (6 months): Following this, the participants were randomly assigned (stratified by age, sex, and BMI) to 1 of 3 groups in a 1:1:1 ratio for 6 months: 1) ADF group (n = 34); 2) CR group (n = 34); or 3) control group (n = 34) (**Figure 3.1**). The first 3 months of the 6-month intervention consisted of a “controlled feeding period” where the ADF and CR groups were provided with all meals. The second 3-month period consisted of a “self-selected feeding period”, where ADF and CR subjects were no longer provided with meals. Instead, subjects received weekly dietary counselling to learn how to continue with their intervention assignments on their own. Control subjects consumed their usual diet throughout the entire trial and received no dietary counselling. All subjects visited the research center weekly throughout the study for meal pickups/dietary counselling, and outcome testing. The control group visited the center at the same frequency as the intervention groups to alleviate any investigator-interaction bias. This group participated in all outcome testing but did not receive any form of intervention.

Study diets during the controlled feeding period

For the first 3 months of the intervention, all meals were provided as a 3-day rotating menu, based on the American Heart Association macronutrient guidelines (30% kcal from fat, 15% from protein, 55% from carbohydrate) (85). The study diets were formulated for each participant using the NDS-R Nutrition data system (Nutrition Coordinating Center, University of Minnesota), with the goal of achieving an average energy restriction (ER) of 25% over a 48-h period. More specifically, the CR group consumed 75% of energy needs every day. The ADF group, on the other hand, consumed 25% of energy needs during fast days, and 125% of energy needs during feast days. Fast day meals were consumed between 12:00 pm and 2:00 pm in order to standardize the duration of food abstinence among participants in the ADF group. All of the meals were consumed outside of the research center, and participants were requested to consume only the meals provided with the exception of calorie-free foods

(i.e. black coffee, black tea and diet sodas), which were permitted as desired. In addition, participants were advised to drink $\geq 2\text{L/d}$ of water throughout the trial.

Physical activity

Participants were instructed to maintain their physical activity levels throughout the study. To confirm this, physical activity was measured for 7 consecutive d at baseline and end of study using a validated (86) pattern recognition monitor (Sense Wear Mini, Bodymedia, Pittsburgh, PA). The Sense Wear Mini is a wireless multi-sensor activity monitor that integrates motion data from a triaxial accelerometer along with several other physiological sensors (heat flux, skin temperature and galvanic skin response). The data were processed using Bodymedia Software V.7.0, algorithm V.2.2.4 (86).

Body weight, height, and body composition

Height was measured at the research center at the beginning of the study with a wall-mounted stadiometer to the nearest 0.25 in. Body weight was measured to the nearest 0.1 kg every week using a digital scale (Omron HBF-500; Omron Health Care, Bannockburn, IL), after the participants had fasted overnight, while the participants were wearing only underwear and a hospital gown. Total fat mass and fat-free mass were assessed at baseline and end of study by dual energy X-ray absorptiometry (DEXA) (QDR 4500W, Hologic Inc. Arlington, MA). Abdominal subcutaneous and visceral fat mass were assessed at baseline and end of study by MRI. MR images were collected at the Center for Magnetic Resonance Research at the University of Illinois, Chicago. Subjects were instructed to lie in the magnet in a supine position. Images were obtained with a Siemens Vision 1.5 Tesla scanner using a T1-weighted fast-spin echo pulse sequence. Subjects were instructed to hold their breath for approximately 25 seconds per acquisition to minimize the effects of respiratory motion on the images. Slice thickness was 1 cm and contiguous slices were obtained every 1 cm from the 9th thoracic vertebra (T9) to the first

sacral vertebra (S1). Thus, 20-35 images were obtained for each subject, depending on subject height. MR images were retrieved from the scanner using the DICOM® (Digital Imaging and Communications in Medicine, Rosslyn, VA). All images were analyzed by Hippo Fat program, as described previously (87, 88). We did not assess intra- or inter-observer reliability, which is a limitation of our work. However, the Hippo Fat program has been shown previously to have excellent intra- and inter-observer reliability in the measurement of visceral fat mass and subcutaneous fat mass (intra-class correlation coefficients ranging between 0.975-0.998) in a group of overweight participants (89).

Energy expenditure

Total energy expenditure (TEE) was measured over a 14-d period at baseline and end of the study. At the start of each TEE measurement, the subject was given an oral dose of DLW ($^2\text{H}_2^{18}\text{O}$) containing 0.22 g H_2^{18}O /kg estimated total body water and 0.115 g $^2\text{H}_2\text{O}$ /kg total body water after collection of 2 independent baseline urine specimens. The subjects were then required to remain sedentary and not to consume any food or water while urine samples were collected from complete voids made at 3, 4.5, and 6 h after dose administration. After completion of urine collections, the subjects were discharged from the research center and carried out their usual daily activities for 14 d, with supervised urine specimen collection on days 7 and 14. Abundances of H_2^{18}O and $^2\text{H}_2\text{O}$ in dilutions of the isotope doses and in urine specimens were measured in duplicate using isotope-ratio mass spectrometry (90). Isotope elimination rates (k_h and k_o) were calculated using linear regression of logged values, and CO_2 production was calculated by using the equations of Schoeller (91), as modified by Racette *et al.* (92). TEE was then calculated on the basis of an assumed respiratory quotient of 0.86.

Dietary adherence

Dietary adherence (i.e. the degree of achieved ER) was calculated by comparing actual energy intake (EI) during the intervention phase to prescribed EI:

$$ER (\%) = (EI_{\text{prescribed}} - EI_{\text{actual}}) / EI_{\text{prescribed}} \quad (1)$$

$EI_{\text{prescribed}}$ was 75% of EI at baseline, and EI at baseline was assumed to equal TEE at baseline because participants were weight stable during this period. EI_{actual} was calculated based on TEE at end of study plus change in energy stores (ES) during the intervention period:

$$EI_{\text{actual}} (\text{kcal/d}) = \text{TEE} + \Delta\text{ES} \quad (2)$$

and change in ES was calculated based on changes in total fat mass (FM) and fat-free mass (FFM) during the intervention (22):

$$\Delta\text{ES} = (9.3 \times \Delta\text{FM, g/d}) + (1.1 \times \Delta\text{FFM, g/d}) \quad (3)$$

Perfect adherence was regarded as 25% ER.

As a secondary measure of adherence, intervention participants recorded missed study foods and non-study foods eaten during the first 12 weeks of the intervention.

Circulating parameters, blood pressure, and heart rate

Twelve-h fasting blood samples were collected at month 0, 3, and 6. Participants were instructed to refrain from strenuous physical activity for 48 h prior to each blood collection. Blood was centrifuged for 10 min at 1000g and 4°C to separate plasma from red blood cells and analyzed off-site (Alverno Clinical Laboratories, LLC; Hammond, IN). Total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides were measured using spectrophotometric assays with intra-assay variances of 1.4, 2.5, 3.0, and 2.0%, respectively. Glucose concentration was measured using the glucose oxidase procedure (Beckman Autoanalyser II; Beckman Coulter Inc.; Fullerton, CA) with intra-assay variance of 1.5%.

Insulin was measured using an electrochemiluminescence assay with an intra-assay variance of 3.1%. Insulin resistance (IR) was calculated using the Homeostasis Model Assessment (HOMA) method (93) by applying the following formula: $\text{HOMA-IR} = \text{fasting insulin } (\mu\text{U/ml}) \times \text{fasting glucose (mg/dL)} / 405$. Blood pressure and heart rate were measured in triplicate using a digital automatic blood pressure/heart rate monitor (Omron HEM 705 LP, Kyoto, Japan) with the subject in a seated position after a 5 min rest.

Power and sample size

Based on our previous findings, we estimated that the ADF group would reduce their initial body weight by 15% by month 6. As for the CR group, Lefevre et al. (77) investigated the effect of 6 months of CR on body weight in obese men and women. They reported a weight loss of 10.4%, with an SD of 6.1. Using the SD from Lefevre et al. (77) for both the ADF and CR group, we calculated that $n = 26$ subjects per group would provide 80% power to detect a significant difference of 5% in body weight between the ADF and CR group at month 6, using a two-tailed independent-samples t-tests with $\alpha = 0.05$. This sample size would also provide 80% power to detect a significant difference of 13% in LDL-cholesterol reduction, 14% in systolic blood pressure reduction, and 23% in adiponectin increase between the ADF and CR group at month 6. Based on dropout data from our previous studies, we anticipated a dropout rate of 12%. Thus, we assumed an initial recruitment sample of 90 ($n = 30$ per group) would yield 78 subjects ($n = 26$ per group) to complete the trial. It should be noted however, that more individuals were recruited in each group (i.e. $n = 34$) to accommodate for the greater than expected dropout rate.

Statistics

Normality was assessed by the Kolmogorov-Smirnov test, and no variables were found to be not normal. Baseline differences between the ADF, CR, and control groups were assessed by a one-way ANOVA.

Post hoc comparisons between groups were performed by Tukey's tests as appropriate. Changes in variables over the course of the trial were assessed by a split-plot ANOVA. To examine the effects of ethnicity on the results, an initial model with a linear effect of time and including interactions between time and ethnicity as well as time by ethnicity by group was performed. Data from Asian and Hispanic participants were not included in this analysis due to the low number of participants. To examine whether baseline BMI, baseline HOMA-IR, age, or change in body weight during the first two weeks of the intervention could predict change in body weight at the end of the intervention, a stepwise linear regression was performed. Data were analyzed using SPSS software (version 20; SPSS Inc.). Alpha was set at a P -value = 0.05. Results are presented as mean $\pm$ SEM.

D. RESULTS

Participants

The flow of participants throughout the trial is displayed in **Figure 3.1**. A total of $n = 102$ subjects were randomized to the three intervention groups ($n = 34$ per group). After 6 months of intervention, $n = 6$, $n = 6$, and $n = 10$ subjects dropped out the ADF, CR, and control groups, respectively. The main reason for subject dropout was dissatisfaction with study diets or scheduling conflicts. The number of completers in each group was as follows: ADF ($n = 28$); CR ($n = 28$), control ($n = 24$). Baseline characteristics for the participants who completed the study are presented in **Table III.I**. There were no statistically significant differences among the groups for age, ethnicity, body weight, height, energy intake, physical activity, or cardiometabolic parameters at baseline.

Energy restriction and physical activity

According to DLW-based estimates, percent energy restriction in both intervention groups approached the prescribed 25% and did not differ significantly from each other (ADF: $21 \pm 4\%$; CR: $24 \pm 4\%$, $P = 0.89$) (**Figure 3.2**). Physical activity, defined as the number of steps taken per d, changed numerically from baseline (ADF: 7422 ± 699 steps/d; CR: 6895 ± 563 steps/d; control: 7363 ± 833 steps/d) to end of study (ADF: 7911 ± 516 steps/d; CR: 8591 ± 898 steps/d; control: 6948 ± 625 steps/d), but the changes did not reach significance within or between any groups (all $P > 0.05$).

Body weight

There was a diet-by-time interaction ($P < 0.01$) for weight loss, with post-hoc testing revealing that the ADF group and CR group lost more weight (ADF: -6.9 ± 0.8 kg; CR: -7.6 ± 1.0 kg) than the control group but a similar amount of weight as each other (**Figure 3.3**). Within each group, the variability in

body weight change was considerable (**Figure 3.4**). A stepwise linear regression found that older age (**Figure 3.5**) and greater weight loss during the first two weeks of the intervention (**Figure 3.6**) each independently albeit weakly predicted change in body weight at the end of the intervention. Neither baseline BMI nor baseline HOMA-IR predicted change in body weight at the end of the intervention.

Ethnicity and body weight

At baseline, body weight and BMI were similar between African American and Caucasian participants. During the intervention, no statistically significant ethnicity-by-time interactions or ethnicity-by-intervention group-by-time interactions were observed for change in body weight. Nonetheless, the numerical difference in weight loss between Caucasians (10.0 ± 2.3 kg) and African Americans (5.6 ± 0.6 kg) in the two intervention groups was substantial (**Figure 3.7**).

Body composition

The intervention groups lost more fat mass (ADF: -4.5 ± 0.6 kg; CR: -5.6 ± 0.7 kg) compared to the control group ($P < 0.001$ for both comparisons) (**Figure 3.8**), but the amount of fat-mass reduction was similar between the intervention groups. There were numerical reductions in fat-free mass for both intervention groups, but these changes were not significant within group or compared to the control group. Visceral fat mass decreased within both intervention groups (ADF: -0.5 ± 0.1 kg; CR: -0.6 ± 0.1 kg; both $P < 0.001$), but these changes were not significant when compared to the control group.

Blood lipids

There was no diet-by-time interaction or main effect of treatment for total cholesterol, but a main effect of time was statistically significant ($P < 0.001$), with values decreasing over the course of the study (**Figure 3.9**). There was no diet-by-time interaction or main effect of treatment for LDL-cholesterol,

but a main effect of time was statistically significant ($P < 0.001$), with values decreasing over the course of the study. There was an interaction effect for HDL-cholesterol ($P < 0.05$), with post-hoc testing revealing that this plasma lipid increased in the ADF group (5 ± 4 mg/dL) relative to the change in the CR group (0 ± 1 mg/dL) ($P < 0.05$). There was no diet-by-time interaction or main effect of treatment for serum triglycerides, but a main effect of time was statistically significant ($P < 0.001$), with values decreasing over the course of the study.

Blood pressure and heart rate

There was no diet-by-time interaction or main effect of treatment for systolic blood pressure, but a main effect of time was statistically significant ($P < 0.05$), with values decreasing over the course of the study (**Table III.II**). There was no diet-by-time interaction or main effect of treatment for diastolic blood pressure, but a main effect of time was statistically significant ($P < 0.001$), with values decreasing over the course of the study. There was no diet-by-time interaction or main effect of treatment for heart rate, but a main effect of time was statistically significant ($P < 0.001$), with values decreasing over the course of the study.

Glucose and insulin

There was no diet-by-time interaction or main effect of treatment for glucose, but a main effect of time was statistically significant ($P < 0.05$), with values increasing over the course of the study (**Table III.III**). There was no diet-by-time interaction or main effect of treatment for insulin, but a main effect of time was statistically significant ($P < 0.05$), with values decreasing over the course of the study. There was no diet-by-time interaction or main effect of treatment for HOMA-IR, but a main effect of time was statistically significant ($P < 0.05$), with values decreasing over the course of the study.

Self-reported dietary adherence

The percentage of participants who did not consume 4 or fewer study foods in any given week during the first 12 weeks of the intervention averaged 40% in the ADF group and 52% in the CR group. The percentage of participants who consumed all study foods in any given week during the first 12 weeks of the intervention averaged 12% in the ADF group and 28% in the CR group. The percentage of participants who did not consume any non-study foods in any given week during the first 12 weeks of the intervention averaged 27% in the ADF group and 33% in the CR group.

E. DISCUSSION

Contrary to our hypothesis, dietary adherence and reductions in body weight and total fat mass were similar between ADF and CR after 6 months of treatment. As for cardiometabolic improvements, HDL-cholesterol increased in the ADF group relative to the CR group. With the exception of glucose, the other measured cardiometabolic parameters improved similarly with time irrespective of group assignment.

This is the first study to objectively measure energy intake on an ADF regimen. We found that percent energy restriction was similar between ADF and CR, and because of this, weight loss was also comparable between interventions. The percent energy restriction in our study is similar to or greater than those of other trials that examined adherence to 6 mo of CR using the DLW method. Redman and colleagues prescribed 25% CR to overweight (BMI between 25-30 kg/m²) men and women aged 25-50 y, and observed the actual percent energy restriction was “very close to the (prescribed) 25% energy deficit”, although an actual value was not reported (94). Weiss and colleagues prescribed 20% CR to overweight (BMI between 24-30 kg/m²) men and women aged 50-60 y, and although actual percent energy restriction was not reported, this value appears to be close to 13% based on published data (95). However, the validity of this data is questionable when considering that a control group in this study achieved similar percent energy restriction despite losing only ~15% of the weight that the CR group lost (95). Das and colleagues prescribed 30% CR to overweight (BMI between 25-30 kg/m²) men and women aged 24-42 y (96). One group assigned to consume a low-glycemic index diet achieved an actual 18% energy restriction, while another group assigned to consume a high-glycemic index diet achieved an actual 16% energy restriction (96). These values are slightly lower than the measured values in the present study despite similar weight loss between studies, and the reason for this discrepancy is unclear.

Whether ADF is a viable alternative to CR for weight loss remains an open question. Future work should examine whether ADF can specifically benefit individuals who did not respond to CR. We did not measure dietary adherence at multiple time points, and so it is unclear whether individuals become less adherent to ADF over time, similar to how adherence to CR gradually deteriorates (97). However, such deterioration in dietary adherence for the ADF group in this study seems likely when considering that weight loss following 24 weeks of intervention was similar to weight loss in other studies that examined ADF for 8 weeks (3, 83). It is also unclear whether participants in the ADF group adhered to the alternating feast day/fast day schedule, and this should therefore be measured in future research of ADF.

Older age and greater weight loss during the first two weeks of the intervention each independently albeit weakly predicted change in body weight at the end of the intervention. In contrast, neither baseline BMI nor baseline HOMA-IR predicted change in body weight at the end of the intervention. Older age has previously been shown to be associated with greater weight loss (98). This association has been speculated to be due to older participants having greater time to devote to a lifestyle intervention as a consequence of having fewer other responsibilities (e.g. work and social) (98). Early weight loss is a well-known predictor for overall weight loss (99). On the other hand, baseline BMI and baseline insulin sensitivity have each been reported to be unrelated to weight loss when expressed as a percentage (51, 100).

ADF and CR reduce fat mass similarly (~5 kg) despite highly dissimilar day-to-day energy intake prescriptions. Two trials (both lasting 8 weeks) have previously measured reduction in fat mass in response to ADF via DEXA (12, 82). While both studies reported lower weight loss (~4 kg) compared

to the present study, one study also reported lower loss of fat mass (~2 kg) (82), while another study reported similar loss of fat mass (~5 kg) (12). The reason for this discrepancy is unclear. ADF and CR had never been previously compared directly regarding changes to body composition, but two trials have compared CR to a 5:2 diet (i.e. 5 consecutive fast days repeatedly alternated with 2 consecutive fast days), which is another form of intermittent fasting (15, 16) . The shorter trial (lasting 3 months) found greater total fat mass loss for the 5:2 diet compared to CR despite comparable weight loss (16), while the longer trial (lasting 6 months) found comparable weight loss and total fat mass reduction for both diets (15).

Fat-free mass did not change for any of the intervention groups in the present study. This parameter also did not change in a previous study examining 6 mo of CR (94). Previous ADF trials measuring body composition via DEXA have reported either no change in fat-free mass or a slight decrease (~1.5 kg) following 8 weeks of intervention(12, 82). It has been suggested previously that ADF may better preserve fat-free mass compared to CR (12, 101) based on findings that this parameter did not change much during weight loss, but this hypothesis is not supported from the results of the current investigation.

Neither visceral fat mass nor subcutaneous fat mass changed in either intervention group relative to the control group. The reason for this is unclear but may be due to measurement error. Both visceral fat mass and subcutaneous fat mass were previously reported to decrease following 6 mo of CR (102).

HDL-cholesterol increased in the ADF group relative to the CR group. Previous studies of ADF have consistently reported that this plasma lipid does not change (3, 4, 12, 13). Also, previous comparisons between a 5:2 diet and a CR diet have noted no relative change for this parameter (15, 16). The other

blood lipids did not change in either intervention group relative to controls. Previous work also found that LDL-cholesterol were unchanged relative to controls following 6 month of CR (77). The same study reported reduced serum triglycerides in the CR group relative to controls, but this may have been due to a 22% increase in this parameter in the control group (77). The two trials that previously compared ADF to a control group also reported no relative changes in any blood lipid (4, 13).

Neither blood pressure nor heart rate changed in the intervention groups relative to controls. It has been reported previously that 6 mo of CR does not change blood pressure relative to controls (77). The two trials that previously compared ADF to a control group also reported no relative changes in blood pressure (4, 13). The only trial to previously compare changes in heart rate between ADF and a control group reported no relative change (13).

Fasting glucose, fasting insulin, and HOMA-IR did not change in the intervention groups relative to controls. A previous study also did not observe changes in the CR group for fasting glucose or fasting insulin relative to controls following 6 mo of treatment (103). The only trial to previously compare changes in fasting glucose, fasting insulin, and HOMA-IR between ADF and a control group reported no relative change for any parameter (13). Both trials that compared 5:2 to CR found greater reductions for the former for fasting insulin and HOMA-IR (15, 16), but this may have been due to the lack of within-group change in these parameters for the CR group in both studies.

In a sub-analysis, we found substantially greater weight loss in Caucasian intervention participants compared to African American intervention participants, although this finding was not statistically significant. The majority of participants in both ethnic groups were women, and it has been shown previously that African American women lost less weight than men and women of other ethnicities (104,

105). We did not collect data that might have explained why African American intervention participants lost less weight than Caucasian participants, but disparities in socioeconomic status, access to healthful foods, encouragement by family and peers, and perceived racial discrimination may have played a role (84, 106-111).

This study has several strengths. It was the first trial to directly compare ADF to CR, and the first to objectively measure energy intake under an ADF regimen. This is also one of few trials to compare an experimental diet group (ADF) to both a negative control group and a positive control group (CR). Additional strengths include the excellent retention rate, the confirmation of weight stability at baseline, the confirmation with accelerometry that physical activity was unchanged, and the use of MRI and DEXA for measurement of body composition. Another strength is the design of the dietary intervention, which involved provision of all food for the first 12 weeks of the trial, followed by dietary counseling for the remainder of the trial: This allowed comparisons to be made between ADF and CR under both laboratory and “real-world” conditions.

As for limitations, the provision of food for the first 12 weeks of study is also a weakness, because it limits the generalizability of our work. Another limitation is that the double-labeled water procedure was not performed at additional time points that would allow for metabolic adaptation to be factored in the calculation of energy intake (112). A third limitation is that respiratory quotient was not measured but rather assumed to equal 0.86. There is an estimated 1% error in the measurement of energy intake from doubly-labeled water for every 0.01 U difference between actual and estimated respiratory quotient (113). We estimated the respiratory quotient to be 0.86 in this study, which is higher than the measured respiratory quotient values in previous studies of ADF (0.83) (114) and CR (0.84) (22). However, because many of our participants were likely insulin resistant as indicated by their HOMA-IR values,

their respiratory quotient values many have been much lower, leading to a much larger magnitude of error in measuring energy intake. A fourth limitation is that physical activity was measured only at baseline (which occurred in the winter) and end of study (which occurred in the summer). Thus, it is possible that a seasonal effect on physical activity may have occurred, as has been reported in the literature previously (115), and may explain why the CR group experienced a (non-significant) 26% increase in the number of steps taken per day from pre-intervention to post-intervention. A fifth limitation is taking blood samples only on feast days, resulting in a longer duration of complete food abstinence prior to blood draw in the ADF group relative to the other groups. This may have affected the concentrations of some of our measured parameters (116). Finally, compliance to the alternating feast day and fast day schedule was not measured in the ADF group, and it remains possible that some of the participants in this group turned their dietary plan into *de facto* CR (i.e. they ate less than prescribed on the feast days, and more than prescribed on the fast days). Future research should measure compliance to the alternating feast day and fast day schedule using an objective marker. For example, plasma ketone bodies have been measured previously to assess compliance to an ADF regimen (83).

In conclusion, percent energy restriction, weight loss, and reduction of total fat mass are similar between ADF and CR, but these results do not translate into improvement in visceral fat mass or other measured cardiometabolic parameters for the first 6 month of dieting. It is possible that ADF is superior to CR for weight loss in some individuals and *vice versa*, resulting in similar group averages. On the opposite extreme, it may be the case that ADF does not benefit any individual who would not have benefitted similarly under CR. Future work needs to determine which possibility better reflects reality in order to determine whether ADF can provide additional benefit as a dietary alternative to standard approaches.

F. ACKNOWLEDGMENTS

JFT conducted the clinical trial, performed the laboratory analyses, performed the data analysis, and wrote the manuscript. CMK, AB, MK, SB, KKH assisted with the conduction of the clinical trial and the laboratory analyses. JR and ER conducted the doubly labeled water analysis. KAV designed the study, assisted with the data analysis, and assisted with manuscript preparation.

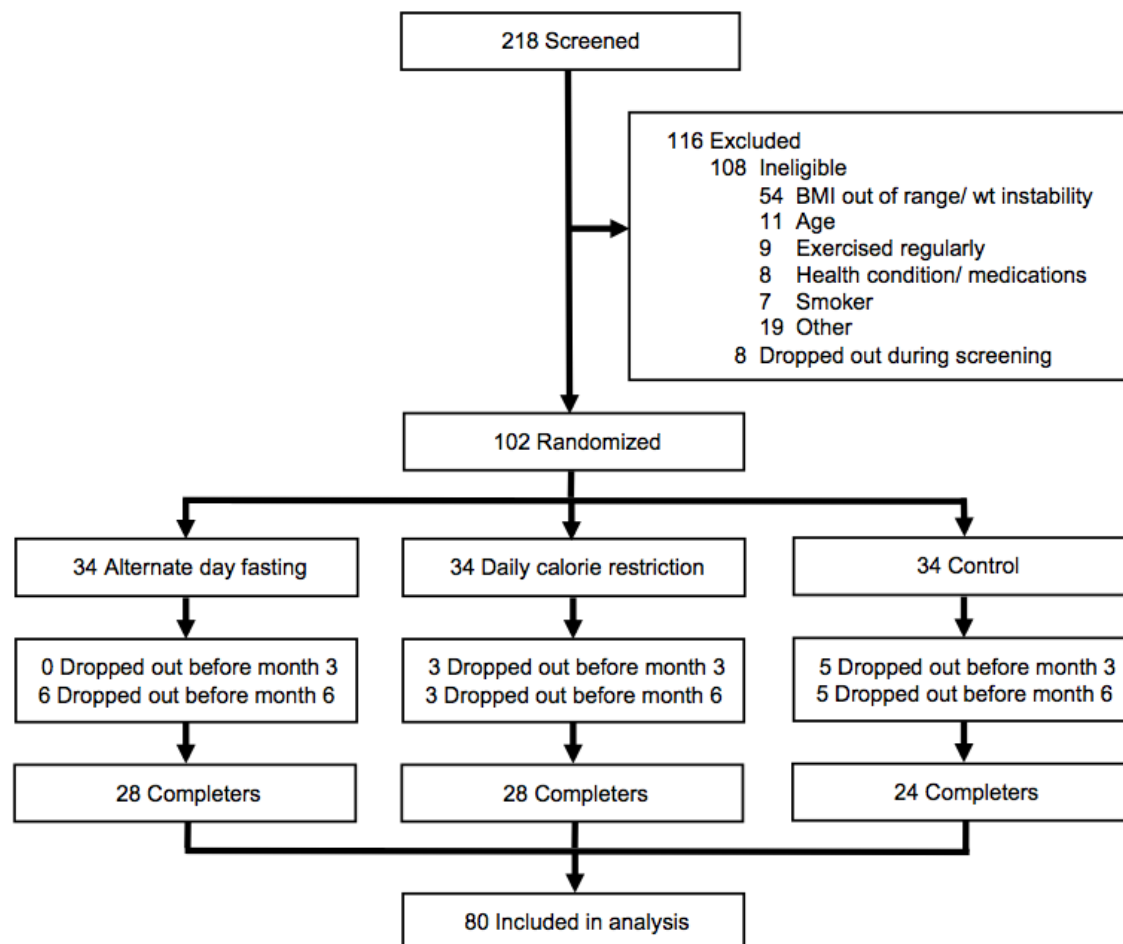


Figure 3.1. Participant flow in the trial

TABLE III.I. BASELINE CHARACTERISTICS OF THE STUDY COMPLETERS¹

	ADF	CR	Control	P-value ²
n	28	28	24	
Age (y)	44 ± 2	44 ± 2	43 ± 2	0.87
Sex (F/M)	25/3	23/5	20/4	
Ethnicity (n)				
African American	19	16	14	0.56
Caucasian	6	10	10	0.72
Hispanic	2	1	0	0.89
Other	1	1	0	0.95
Body weight (kg)	94 ± 3	100 ± 3	91 ± 3	0.12
Height (cm)	166 ± 2	168 ± 2	164 ± 2	0.34
Body mass index (kg/m ²)	34 ± 1	35 ± 1	34 ± 1	0.31
Visceral fat mass (kg)	1.9 ± 0.2	2.3 ± 0.2	2.0 ± 0.3	0.43
Subcutaneous fat mass (kg)	8.4 ± 0.4	8.7 ± 0.4	8.0 ± 0.7	0.56
Energy intake (kcal/d)	2663 ± 121	2931 ± 143	2698 ± 183	0.35
Physical activity (steps/d)	7422 ± 699	6895 ± 563	7363 ± 833	0.84
Lipids (mg/dL)				
Total Cholesterol	188 ± 7	184 ± 7	191 ± 6	0.72
LDL Cholesterol	110 ± 6	112 ± 6	114 ± 7	0.89
HDL Cholesterol	58 ± 3	52 ± 2	57 ± 3	0.17
Triglycerides	103 ± 12	97 ± 5	99 ± 9	0.91
Systolic blood pressure (mmHg)	125 ± 2	124 ± 3	120 ± 3	0.48
Diastolic blood pressure (mmHg)	83 ± 2	81 ± 2	80 ± 2	0.56
Heart rate (bpm)	76 ± 2	74 ± 2	73 ± 2	0.36
Glucose (mg/dL)	91 ± 2	92 ± 2	88 ± 2	0.34
Insulin (uIU/ml)	17 ± 3	18 ± 3	16 ± 2	0.89
HOMA-IR	4.0 ± 0.7	4.2 ± 0.7	3.5 ± 0.4	0.77

¹ Values reported as mean ± SEM. F: Female, M: Male.

² P-value between groups at screening (One-way ANOVA). No differences between groups for any parameter.

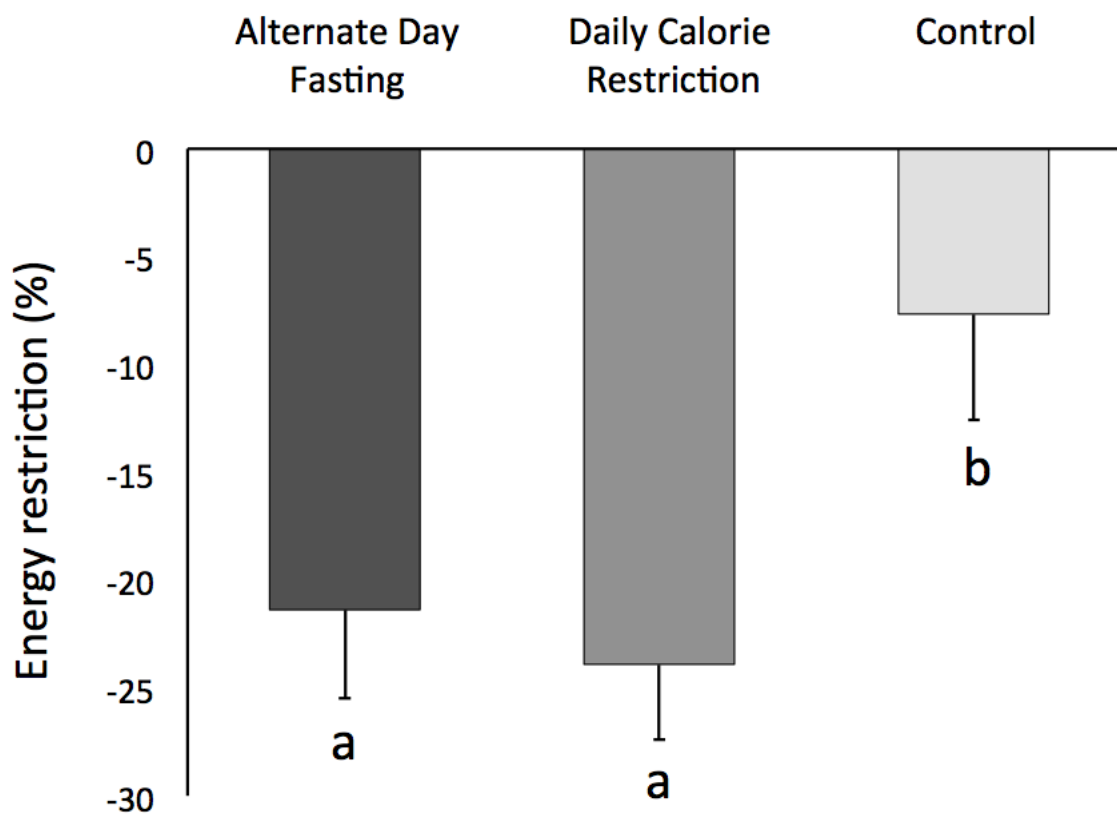


Figure 3.2. Mean energy restriction over 6 months assessed via doubly labeled water¹

¹ Values reported as mean $\pm$ SEM. Alternate day fasting group (n = 28), Daily calorie restriction group (n = 28), Control group (n = 24). Mean energy restriction was significantly different ($P < 0.05$) between the control group and the two intervention groups.

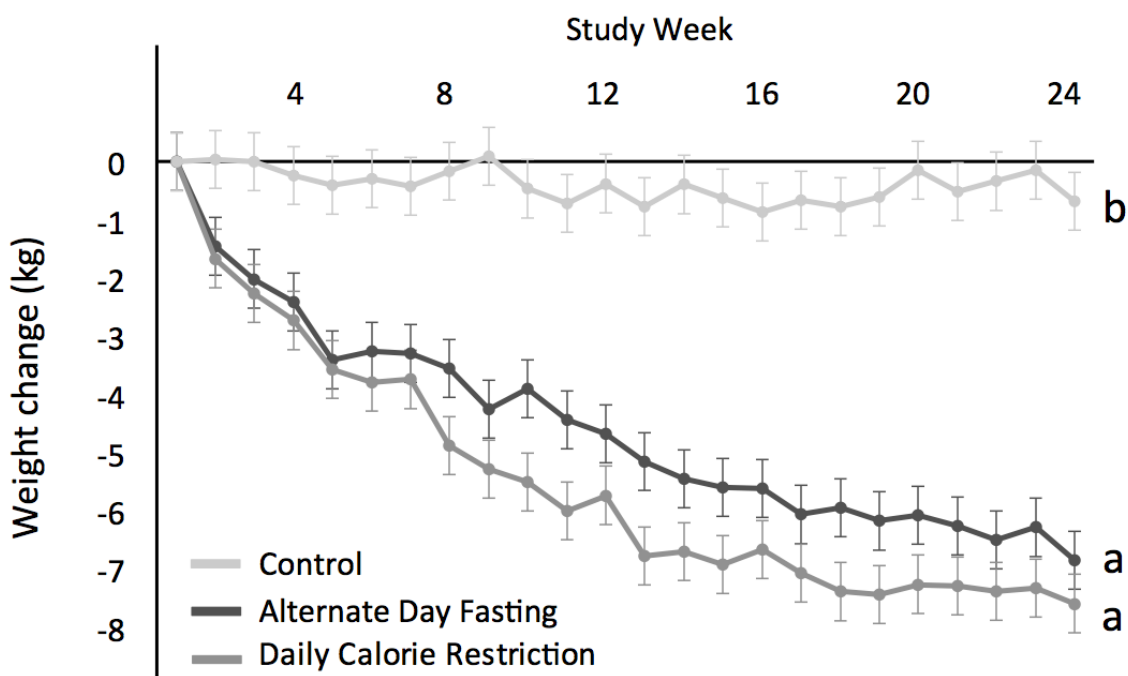


Figure 3.3. Weekly weight loss by group¹

¹ Values reported as mean $\pm$ SEM. Alternate day fasting group (n = 28), Daily calorie restriction group (n = 28), Control group (n = 24). Change in weight over time was significantly different between the control group and the two intervention groups ($P < 0.001$), with no difference between the Alternate Day Fasting and Daily Calorie Restriction group.

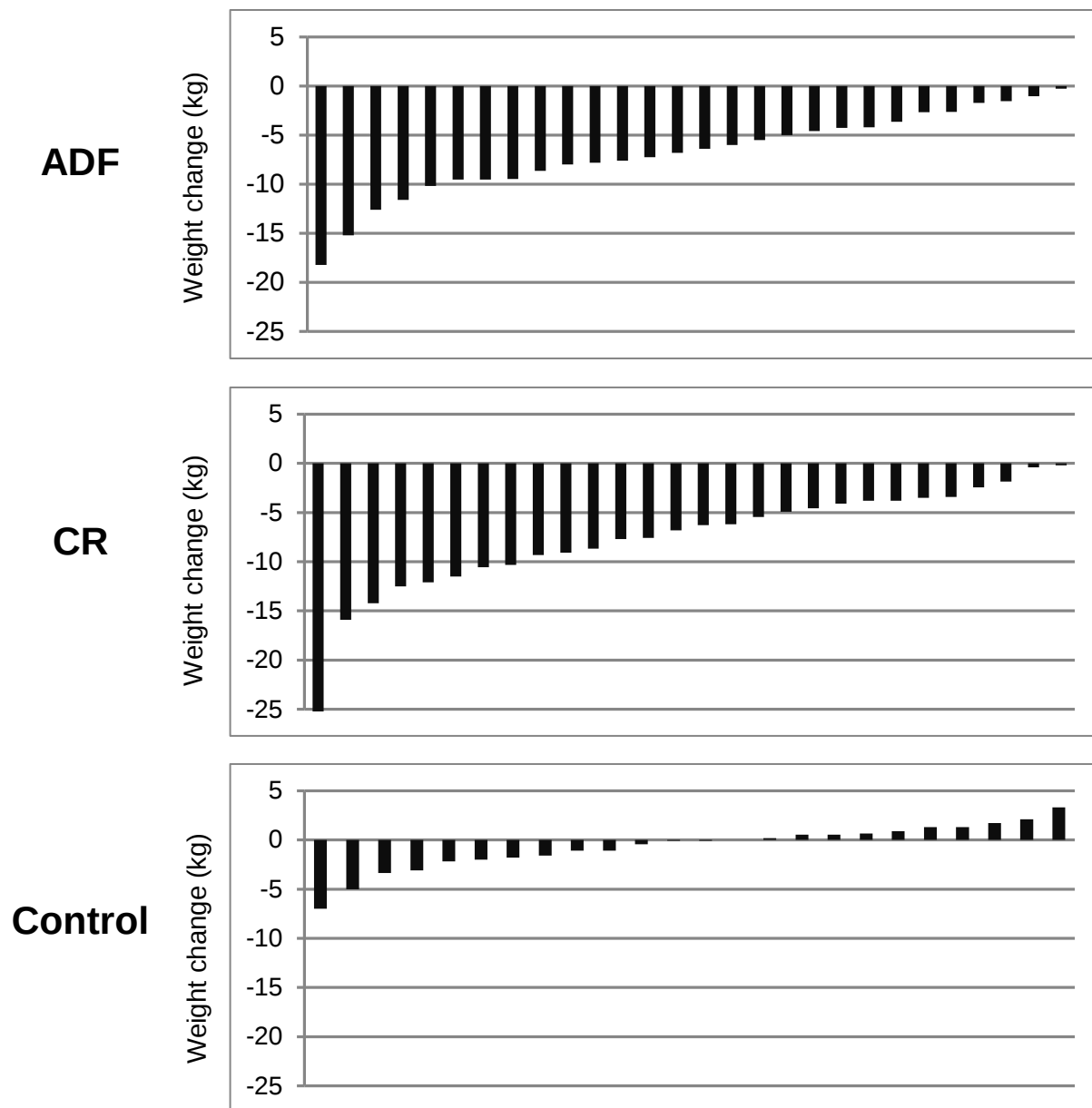


Figure 3.4. Individual body weight change¹

¹ Each bar represents an individual participant's body weight response.

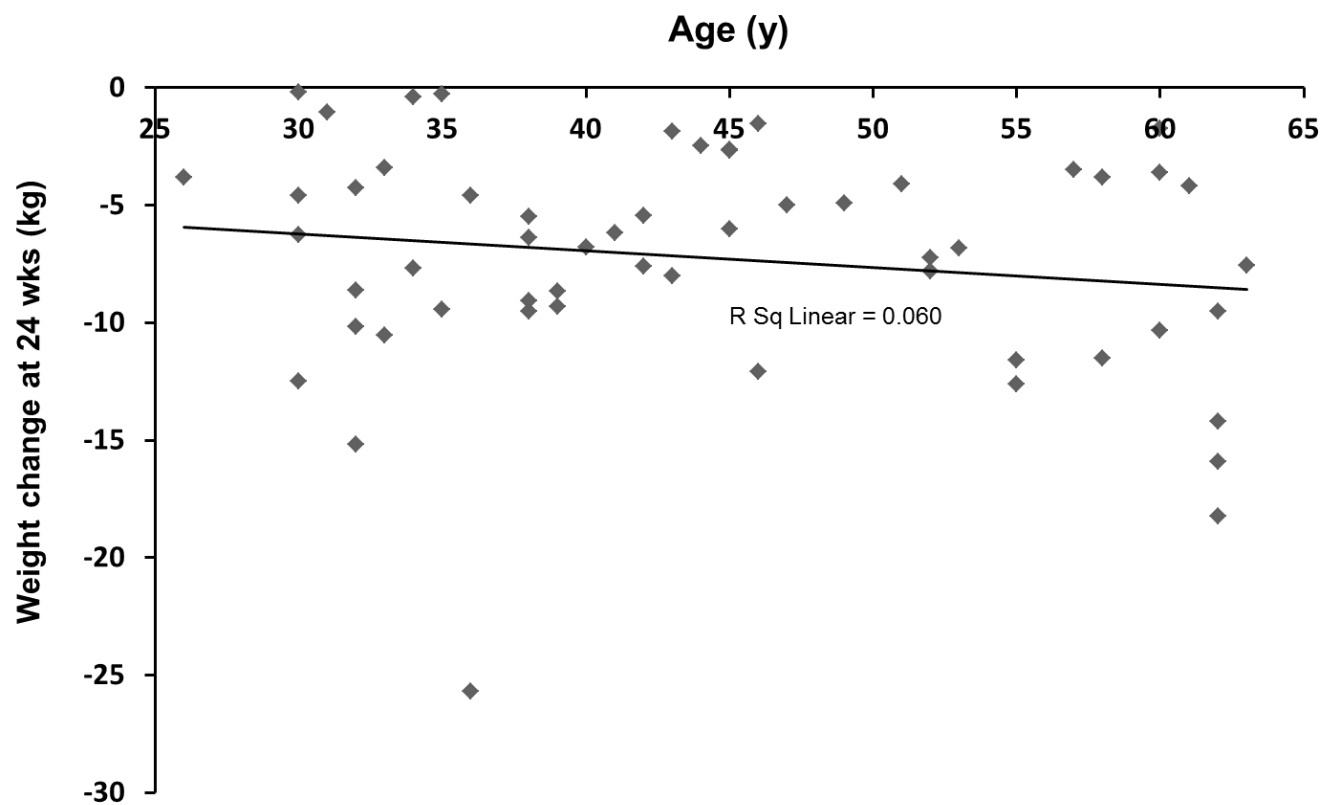


Figure 3.5. Relationship between age and weight loss¹

¹ Older age was associated with greater weight loss at the end of study ($P < 0.05$).

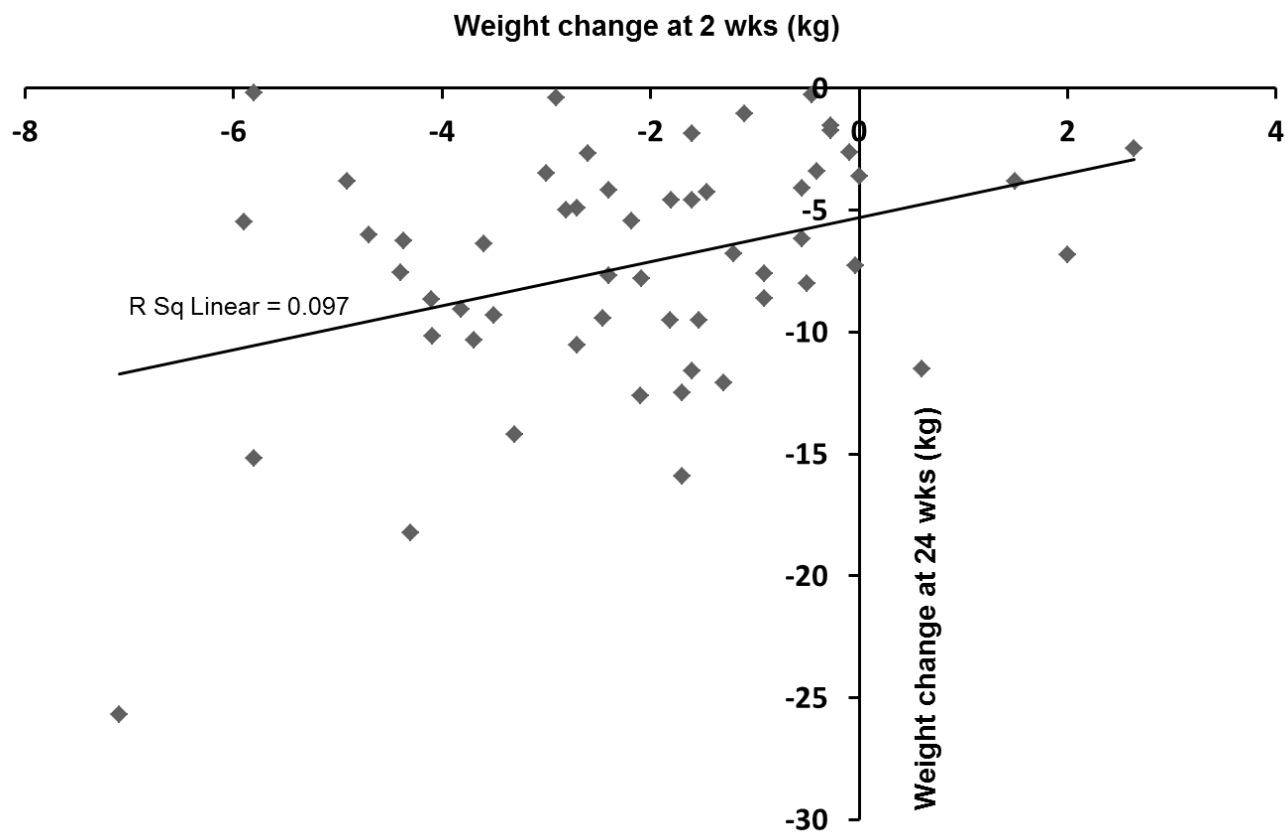


Figure 3.6. Relationship between initial and final weight loss¹

¹ Greater weight loss during the first 2 weeks of intervention was associated with greater weight loss at the end of study ($P < 0.05$).

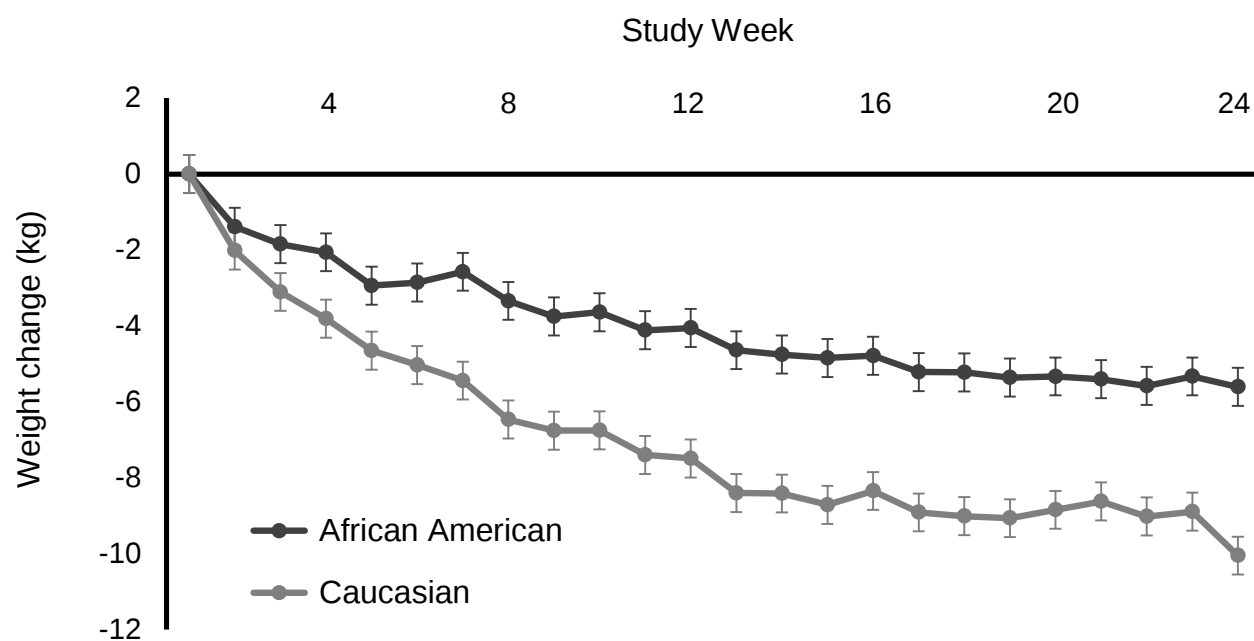


Figure 3.7. Weekly weight loss by ethnicity¹

¹ Values reported as mean $\pm$ SEM. African Americans (n = 35), Caucasians (n = 16). There was no statistically significant ethnicity by time interaction ($P > 0.05$).

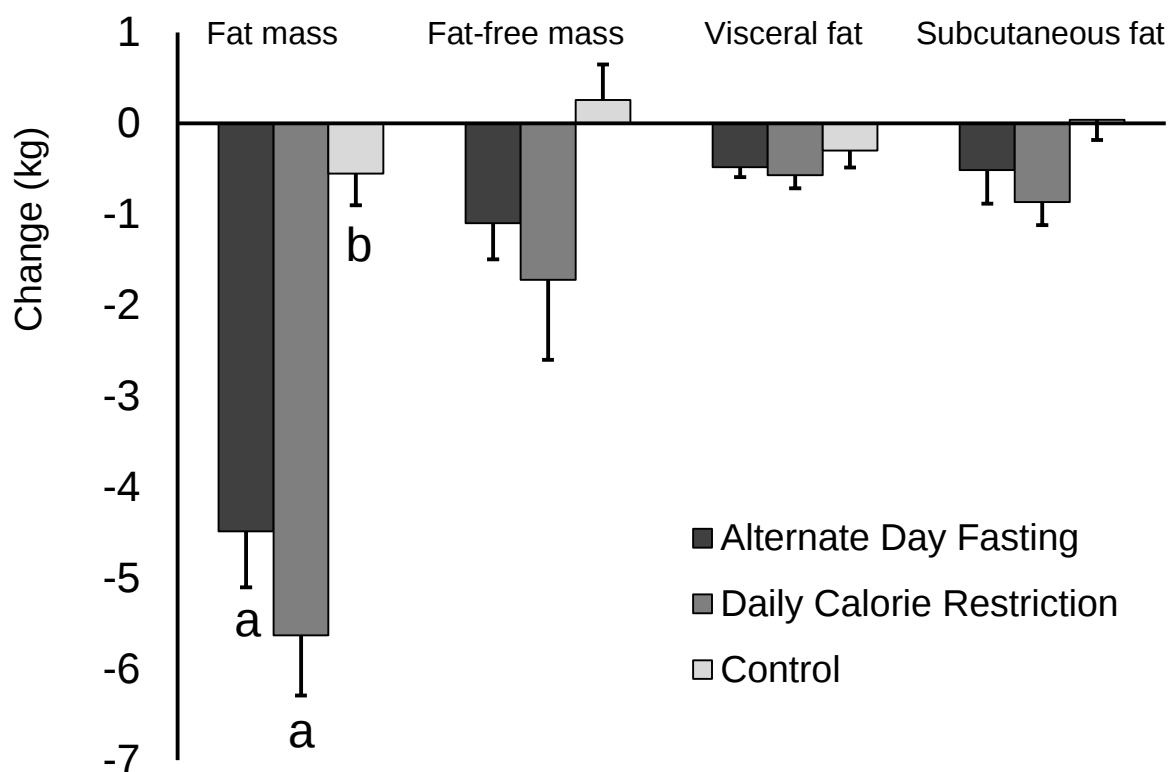


Figure 3.8. Change in fat mass, lean mass, visceral fat mass and subcutaneous fat mass by group at month 6¹

¹ Values reported as mean $\pm$ SEM. Alternate day fasting group (n = 28), Daily calorie restriction group (n = 28), Control group (n = 24). Change in fat mass was significantly different ($P < 0.05$) between the control group and the two intervention groups.

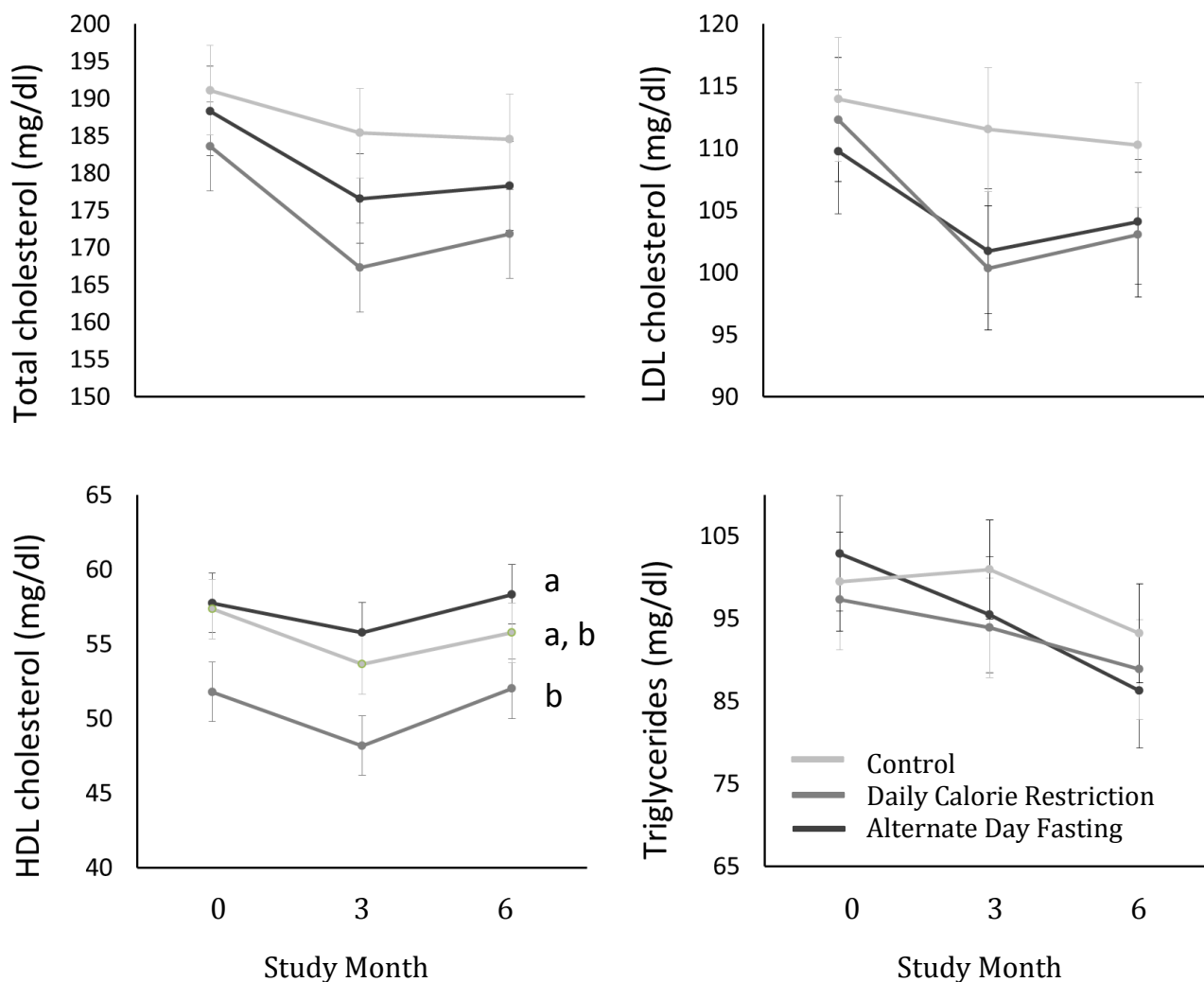


Figure 3.9. Fasting plasma lipids at baseline, month 3, and month 6¹

¹ Values reported as mean $\pm$ SEM. Alternate day fasting group (n = 28), Daily calorie restriction group (n = 28), Control group (n = 24). Change in HDL-cholesterol over time was significantly different between the Alternate Day Fasting group and the Daily Calorie Restriction group ($P < 0.05$), with no difference between the Alternate Day Fasting group and control group.

Table III.II. CHANGE IN CORONARY HEART DISEASE RISK FACTORS AT BASELINE, MONTH 3, AND MONTH 6¹

	Alternate day fasting				Daily calorie restriction				Control			
	Baseline	Month 3	Month 6	Change ²	Baseline	Month 3	Month 6	Change ²	Baseline	Month 3	Month 6	Change ²
Systolic BP (mm Hg)	125 ± 2	120 ± 3	118 ± 3	-7 ± 3	124 ± 3	120 ± 3	115 ± 2	-9 ± 3	120 ± 3	120 ± 2	118 ± 2	-2 ± 3
Diastolic BP (mm Hg)	83 ± 2	78 ± 2	78 ± 2	-5 ± 3	81 ± 2	78 ± 2	76 ± 2	-5 ± 2	80 ± 2	79 ± 2	78 ± 2	-2 ± 2
Heart rate (bpm)	76 ± 2	73 ± 1	69 ± 2	-7 ± 2	74 ± 2	73 ± 2	72 ± 2	-2 ± 2	73 ± 2	71 ± 2	72 ± 2	-1 ± 2
Glucose (mg/dL)	91 ± 2	91 ± 2	92 ± 1	1 ± 2	92 ± 2	90 ± 2	96 ± 2	4 ± 2	88 ± 2	87 ± 2	93 ± 2	5 ± 2
Insulin (uIU/ml)	17 ± 3	12 ± 1	10 ± 1	-7 ± 2	18 ± 3	11 ± 1	14 ± 2	-4 ± 2	16 ± 2	17 ± 2	17 ± 2	1 ± 2
HOMA-IR	4.0 ± 0.7	2.7 ± 0.3	2.2 ± 0.3	-1.8 ± 0.6	4.2 ± 0.7	2.5 ± 0.2	3.4 ± 0.5	-0.8 ± 0.5	3.5 ± 0.4	3.6 ± 0.4	4.1 ± 0.7	0.6 ± 0.6

¹ Values expressed as mean ± SEM. Alternate day fasting group (n = 28), Daily calorie restriction group (n = 28), Control group (n = 24).

² Absolute change between baseline and month 6 values.

No differences between groups for any parameter at any time point.

IV. MANUSCRIPT 2

Impact of alternate day fasting versus daily calorie restriction on visceral fat-mediated inflammation

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Keywords: Calorie restriction, alternate day fasting, body weight, inflammation, coronary heart disease, obese adults

A. ABSTRACT

Background: Previous work indicates that energy intake affects changes in visceral fat mass and bioactive molecules that are secreted from visceral adipose tissue independently of weight loss.

Alternate-day modified fasting (ADF) and daily calorie restriction (CR) are two dietary weight-loss strategies that have very different patterns of energy intake.

Objective: Accordingly, this study examined whether changes in visceral fat mass and/or secreted molecules differ in response to ADF and CR.

Design: In a controlled feeding trial, overweight and obese participants ($n = 102$) were randomized to 1 of 3 groups: 1) ADF (alternating every 24-h between energy intake = 25% or 125% of needs); 2) CR (energy intake = 75% of needs every day); or 3) control (energy intake = 100% of needs every day). The intervention period was 6 months, and consisted of a 3-month controlled feeding period followed by a 3-month self-selected feeding period. Visceral fat mass was measured at baseline and end of study, while total fat mass and circulating parameters were measured every 3 mo.

Results: Compared to the control group ($P < 0.05$ for all comparisons), the intervention groups experienced greater reductions in body weight (ADF: $-7 \pm 1\%$; CR: $-8 \pm 1\%$), total fat mass (ADF: $-12 \pm 2\%$; CR: $-14 \pm 2\%$), and leptin (ADF: $-17 \pm 7\%$; CR: $-30 \pm 9\%$). Within-group reductions were also observed for visceral fat mass in both intervention groups (ADF: $-22 \pm 4\%$; CR: $-25 \pm 5\%$; $P < 0.05$ for both), while adiponectin ($18 \pm 15\%$) and resistin ($-15 \pm 6\%$) changed within the CR group only ($P < 0.05$ for both). Correlational analyses between changes in fat mass (visceral or total) and changes in circulating parameters found a significant association only between leptin and total fat mass in the ADF group ($r = 0.43$; $P < 0.03$).

Conclusion: Despite highly dissimilar patterns of energy intake, ADF and CR affect changes in visceral fat mass and secreted molecules similarly.

B. INTRODUCTION

Excess adiposity is a pathological condition, and evidence suggests that visceral adipose tissue (VAT) is more pathogenic than subcutaneous adipose tissue (SAT) (117, 118). Visceral obesity is associated with cardiometabolic derangements that include insulin resistance (119), low HDL-C level (120), high serum triacylglycerol level (120) and a small-dense phenotype of LDL particles (121). It is therefore unsurprising that visceral obesity is associated with elevated prevalence of type 2 diabetes (122), hypertension (123) and other forms of cardiovascular disease (124).

Emerging evidence suggests that the above-mentioned cardiometabolic derangements observed in visceral obesity are largely mediated by VAT-secreted cytokines and other circulating proteins and amino acids that are disturbed in the obese state. Examples of these include tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), leptin, resistin, insulin-like growth factor-1 (IGF-1), C-reactive protein (CRP) and homocysteine (125-127). Additionally, levels of adiponectin, an anti-inflammatory cytokine, are reduced in visceral obesity (128).

Diet-induced weight loss is known to reduce VAT (129) and modulate circulating levels of the above-mentioned bioactive molecules (49, 130-133). These results are not simply due to weight loss *per se*; rather, the degree of energy restriction also appears to have an independent effect. Indeed, the degree of energy restriction is independently associated with the preferential loss of VAT in studies of very-low calorie diets (76). Mechanistically this could be explained by the greater responsiveness of visceral adipocytes to lipolytic stimuli (134). Furthermore, the responsiveness of visceral adipocytes to lipolytic stimuli *itself* might be increased during negative energy balance (but not weight loss *per se*), as this phenomenon has been previously observed in abdominal subcutaneous adipocytes (135). The adipokine profile is also modulated by energy restriction in a manner that is independent of weight loss (136).

These findings suggest the possibility that alternate-day modified fasting (ADF) and daily calorie restriction (CR) may differentially affect VAT and the above-mentioned bioactive molecules even in the presence of similar weight loss, because the two diets restrict energy differently. While CR involves moderate (e.g. 25%) energy restriction every single day, ADF alternates between 24-h periods of either intense (e.g. 75%) energy restriction or no restriction (or even slight energy surplus). The two diets' different patterns of energy restriction could be perceived by visceral adipocytes as unique stimuli with unique effects on visceral fat mass and circulating levels of adipokines and related bioactive molecules. Therefore, a key secondary aim of our randomized controlled trial designed to compare ADF and CR (137) was to determine whether one diet preferentially modulated VAT and/or the above-mentioned bioactive molecules.

Research has shown that baseline insulin sensitivity may predict weight-loss response to diet therapy. For example, insulin-resistant individuals lose more weight on a low-carbohydrate diet than on a low-fat diet (138-142). Therefore, we were also interested to see if insulin sensitivity at baseline could predict whether individuals would respond more favorably to ADF or CR in the present study.

C. SUBJECTS AND METHODS

Subjects and screening

Overweight and obese (body mass index, BMI: 25.0-39.9 kg/m²) men and women aged 18-65 y were recruited from the local community by advertisement. Participants were excluded if they smoked, self-reported to exercising more than 3 h/wk, were diabetic, had a history of cardiovascular disease, were pregnant or had an irregular menstrual cycle, had excessive body weight fluctuation (> 4 kg) immediately prior to study enrollment, or were contraindicated to magnetic resonance imaging (for measurement of VAT). Individuals were also excluded if they took weight-loss or lipid-lowering medications. However, following medications were taken by enrolled participants: angiotensin 2 receptor blocker, angiotensin converting-enzyme inhibitor (x2), antihistamine, beta blocker (x2), calcium channel blocker (x2), corticosteroid, levothyroxine (x2), nonsteroidal anti-inflammatory drug, proton-pump inhibitor (x2), selective serotonin reuptake inhibitor, and tricyclic antidepressant. Details of the screening process have been described previously (137). The study protocol was approved by the Office for the Protection of Research Subjects at the University of Illinois, Chicago. Written consent was provided by all participants in the study.

Study design

Following a 4-week baseline period, participants were randomized (stratified by age, BMI, and sex) into one of three groups for 24 weeks: an ADF group ($n = 34$), a CR group ($n = 34$) or a non-intervention control group ($n = 34$) (**Figure 4.1**). Participants in the intervention groups received a controlled diet for the first 12 weeks of intervention. They then self-selected their diets based on their individual daily calorie goals for the final 12 weeks of intervention. Participants in the control group consumed their

usual diet throughout the entire trial and visited the research center at the same frequency as the intervention groups.

Baseline (Week B1-B4): The baseline period lasted 4 weeks in order to stabilize participants' body weights, determine individual energy requirements, and conduct baseline testing. The energy deficit required during the intervention was calculated from total energy expenditure assessed during a 14-d period by doubly-labeled water as previously described (137). This procedure was repeated at end of study as part of a calculation of dietary adherence (see below).

Controlled feeding period (Week 1-12): Participants in the intervention groups received a diet based on the guidelines from the American Heart Association (85), with approximately 25% of energy deriving from fat, 60% of energy deriving from carbohydrate, and 15% of energy deriving from protein (**Table IV.I**). All meals were provided as a 3-day rotating menu for the first 12 weeks of intervention. The energy content of the meals was formulated to produce an average 25% energy restriction (ER) over a 48-h period: Participants in the CR group consumed 75% of energy needs every day, while participants in the ADF group repeatedly alternated between a 24-h period involving consumption of 25% of energy needs ('fast day') and a 24-h period involving consumption of 125% of energy needs ('feast day'). Fast day meals were consumed between 12:00 pm and 2:00 pm in order to standardize the duration of complete food abstinence among participants in the ADF group. The intervention groups collected their meals at the beginning of each week in rolling insulated coolers, and all meals were consumed outside of the research center. Subjects were permitted to consume calorie free beverages such as black coffee, black tea, and diet sodas during the controlled feeding period. Additionally, subjects were encouraged to consume plenty of water throughout the trial.

Self-selected feeding period (Week 13-24): For the final 12 weeks of intervention, participants in the intervention groups met with a dietician weekly and self-selected a diet based on their individual calorie targets. No food was provided to the subjects during this period. Briefly, subjects took part in one-on-one counselling sessions on a weekly basis to learn how to maintain the ADF or CR regimen on their own at home. During each counselling session, the dietician worked with the subject to develop individualized meal plans. These plans included menus, food lists, and portion sizes that were consistent with the subject's food preferences and target calorie levels. During these sessions, subjects were also instructed how to make general healthy food choices by choosing low fat meat/dairy options and increasing fruit and vegetable intake.

Physical activity maintenance

Physical activity was assessed for 7 consecutive days at baseline and end of study with a validated (86) multi-sensor activity monitor, as described previously (137).

Energy intake and energy restriction

Energy restriction during the intervention was reported previously (137) and is reported again in the present paper to provide context to the changes in body composition and circulating bioactive molecules reported here. Briefly, actual energy intake during the course of the intervention period was compared to the prescribed 25% ER. Energy intake at baseline was assumed to be equal to total energy expenditure due to the stable body weight of the participants. Energy intake during the course of the intervention was calculated based on total energy expenditure at end of study after correction for changes in body energy stores (for weight loss, 1 g of fat mass = 9.3 kcal and 1 g of fat free mass = 1.1 kcal) (22).

Body weight and body composition

Body weight was measured weekly after an overnight fast while the participants were wearing only underwear and a hospital gown. Total fat mass and fat-free mass were assessed at baseline and end of study by dual energy X-ray absorptiometry (DEXA) (QDR 4500W, Hologic Inc. Arlington, MA). VAT was assessed at baseline and end of study by MRI, as described previously (137).

Circulating inflammatory parameters

Blood samples were taken at baseline and end of study following a 12-h fast. Participants refrained from strenuous exercise for 48 h prior to sample collection. Blood was centrifuged for 10 min at 1000 *g* and 4°C to separate plasma from RBC, and was stored at -80°C until analyzed. Adiponectin (intra-assay coefficient of variation: 3.4%), leptin (3.0%), resistin (5.3%), IGF-1 (4.3%), IL-6 (1.6%), and TNF- α (4.2%) were quantified using high-sensitivity ELISA kits (R&D Systems, Minneapolis, MN). High-sensitivity CRP (intra-assay coefficient of variation: 7.7%) and homocysteine (9.3%) were analyzed off-site (Alverno Clinical Laboratories, LLC; Hammond, IN) using immunonephelometric and enzymatic assaying methods, respectively.

Statistical analysis

Data are presented as mean $\pm$ SEM. Significance for all statistical tests was accepted at $P < 0.05$. SPSS (v. 20; SPSS Inc, Chicago, IL) was used for analysis. Normality was assessed by the Kolmogorov-Smirnov test, and no variables were found to be not normal. Baseline differences between the ADF, CR, and control groups were assessed by a one-way ANOVA. Post hoc comparisons between groups were performed by Tukey's tests as appropriate. Changes in variables over the course of the trial were assessed by repeated-measures ANOVA. To examine whether baseline insulin sensitivity predicted success with ADF or CR, we used a general linear model to test for a diet-by-HOMA-IR interaction.

D. RESULTS

Subject dropouts and baseline characteristics

One hundred and two subjects commenced the study ($n = 34$ per group). After 24 weeks of treatment, $n = 6$ dropped out of the ADF group, $n = 6$ dropped out of the CR group, and $n = 10$ dropped out of the control group. Reason for subject dropout was similar in each group, and included: dissatisfaction with intervention assignment, dissatisfaction with study diets, scheduling conflicts, pregnancy, or medical complications not related to the study. The number of completers in each group was as follows: ADF ($n = 28$), CR ($n = 28$), and control ($n = 24$). Subject baseline characteristics did not differ between groups (Table 2).

Energy restriction and physical activity

The three groups had similar energy intake (ADF: 2663 ± 121 kcal/d; CR: 2931 ± 143 kcal/d; control: 2698 ± 183 kcal/d) and physical activity (ADF: 7422 ± 699 steps/d; CR: 6895 ± 563 steps/d; control: 7363 ± 833 steps/d) at baseline (137). Percent ER during the course of the intervention was similar between the intervention groups (ADF: $21 \pm 4\%$; CR: $24 \pm 4\%$, $P = 0.89$), and lower in the control group ($P \leq 0.01$ for both comparisons) (137). Change in physical activity from baseline to end of study was not significantly different within or between any groups (ADF: 489 ± 642 steps/d; CR: 1696 ± 972 steps/d; control: -415 ± 555 steps/d).

Body weight and body composition

There was a diet-by-time interaction ($P < 0.01$) for weight loss, with post-hoc testing revealing that the ADF group and CR group lost more weight (ADF: $-7.3 \pm 0.9\%$; CR: $-7.7 \pm 1.0\%$) than the control group but a similar amount of weight as each other (Table IV.III). The intervention groups also lost more total fat mass (ADF: $-11.8 \pm 1.6\%$; CR: $-15.0 \pm 2.0\%$) compared to the control group ($P < 0.001$ for both

comparisons), with no differences between the intervention groups. Fat free mass remained unchanged after 24 weeks in all groups. Visceral fat mass decreased in the ADF ($-22 \pm 4\%$) and CR groups ($-25 \pm 5\%$), with no differences between groups. Moreover, these changes in visceral fat mass were not different from controls at any time point.

Insulin sensitivity and body weight

The diet-by-HOMA-IR interaction was not significant. We then combined the data from the diet groups and ran a mixed-design ANOVA to determine if HOMA-IR was related to weight loss over time. The time-by-HOMA-IR interaction was not significant.

Circulating inflammatory parameters

Changes in adiponectin, leptin, and resistin from baseline to week 24 are displayed in **Figure 4.2**.

Adiponectin increased from baseline to end of study within the CR group only, but this change was not significant when compared to the controls. The intervention groups experienced greater reductions in leptin compared to the control group, although there were no differences between the intervention groups. Resistin decreased from baseline to end of study within the CR group only, but this change was not significant when compared to the controls. Circulating levels of CRP, homocysteine, IGF-1, IL-6, and TNF- α over the course of the trial are shown in **Table IV.IV**. None of these parameters changed within or between groups at either the midpoint (week 12) or end of the study (week 24).

Relationship between energy restriction, visceral fat, and inflammatory parameters

An analysis was performed to determine whether the statistically significant within-group changes to the adipokines correlated with within-group changes to either total fat mass or visceral fat mass. Only one

correlation was statistically significant: For the ADF group, the reduction in circulating leptin was associated with the reduction in total fat mass ($r = 0.43$; $P < 0.03$).

E. DISCUSSION

Based on previous findings that VAT (76) and circulating bioactive molecules (136) that derive from (or are modulated by) VAT respond to energy restriction independently of weight loss, we hypothesized that these parameters would respond differently to ADF versus CR. However, we found that visceral fat mass decreased similarly between the intervention groups, and only circulating leptin level changed in the intervention groups relative to controls. These findings suggest that there is no effective difference in our measured parameters between moderate daily energy restriction versus alternating between intense energy restriction and small energy surplus, so long as average energy restriction is similar.

Despite sizeable numerical reductions in visceral fat mass for both intervention groups (22-25%), this result did not reach significance when compared to controls. This may have been due to the 15% reduction in this parameter that was observed in the control group. As body weight only decreased by 1% in the controls, this reduction in visceral fat mass likely reflects measurement error. A previous report found that CR (25%) reduced visceral fat mass by 37% despite weight loss that was comparable (8 kg) to the weight loss by the intervention groups in the present study (102). Visceral fat mass was actually much lower at baseline for the report that observed the greater change; therefore the reason for the discrepant finding is unclear.

Of the measured adipokines, only leptin decreased in the intervention groups relative to the control group. A reduction in leptin is a common finding in research of diet-induced weight loss (143). In contrast, whether adiponectin changes during dietary intervention appears to have little relation to the amount of body weight that is lost (143). Interestingly, circulating adiponectin level appears to be more responsive to diet-induced weight loss in men (143), which may explain why this parameter did not change in our mostly female cohort. Resistin has been shown to either decrease (49) or not respond to

ADF (4). Similarly inconsistent findings have been observed in response to CR (144, 145). TNF- α has been previously reported to decrease in response to ADF (83) but not CR (95) despite similar weight loss between the studies. The reason for these discrepant results is unclear, especially in light of the fact that TNF- α does not appear to decrease in response to energy restriction *per se* (136). Weight loss > 8% appears to be required for changes in circulating IL-6 to be observed (143), which may explain why this parameter did not change in the present investigation.

CRP, Homocysteine, and IGF-1 also did not change in either intervention group. Recent studies have reported no change for CRP in response to either ADF (83) or CR (2, 77). Homocysteine level often increases in response to weight loss, but this may be due to inadequate intake of folate or vitamin B12 (125). Energy restriction without protein restriction does not appear to modulate circulating IGF-1 (146).

The only significant association found in this study was the association between reduction in circulating leptin and reduction in total fat mass, but not visceral fat mass. This is consistent with other investigations that have reported sizeable reductions in leptin with minimal change in visceral fat mass (143).

In our study insulin-sensitive and insulin-resistant individuals lost similar amounts of weight, and diet group assignment had no effect on this. Previous work suggests that insulin-sensitive and insulin resistant individuals experience similar weight loss when given the same treatment (51, 53). Also, insulin-resistant individuals lose more weight on a low-carbohydrate diet than on a low-fat diet (138-142).

A key strength of this study is that circulating bioactive molecules were measured after 12 weeks of controlled feeding. By providing all meals to participants during this phase, dietary noncompliance was likely minimized. Our sample size is another strength of this study, especially when compared to other dietary weight-loss studies that have previously examined our chosen parameters.

The main weakness of this study was not measuring the coefficient of variation for the measurement of visceral fat mass, either within or between observers. It is possible that high intra- or inter-observer variability explains why difference in visceral fat mass reduction between the intervention groups and the control group did not reach statistical significance. A second limitation is drawing blood samples after fast days, thus increasing the duration of complete food abstention prior to blood draw in the ADF group relative to the other groups. The concentrations of some of our measured parameters may have been affected by the longer duration of complete food abstention (89). A third limitation of this paper is not measuring other fat depots such as superficial and deep subcutaneous adipose tissue. Measuring physical activity only before and after the intervention is a fourth limitation, because seasonal variability (115) may have biased our results. Finally, parameters related to the metabolic health of subcutaneous adipocytes (e.g. secretion of adipokines, responsiveness to lipolytic stimuli) were not measured.

In conclusion, energy restriction *per se*, whether undergone every day at a moderate level or every other day at an intense level, does not appear to appreciably alter visceral fat mass or related bioactive molecules. Rather, weight loss appears to be the main driver of these changes, and the minimal amount needed to elicit these changes appears to be >8%, but the exact amount remains to be discovered.

F. ACKNOWLEDGMENTS

JFT conducted the clinical trial, performed the laboratory analyses, performed the data analysis, and wrote the manuscript. CMK, AB, MK, SB, KKH assisted with the conduction of the clinical trial and the laboratory analyses. JR and ER conducted the doubly labeled water analysis. KAV designed the study, assisted with the data analysis, and assisted with manuscript preparation.

TABLE IV.I. NUTRIENT COMPOSITION OF THE PROVIDED DIETS DURING THE CONTROLLED FEEDING PHASE

	ADF		CR
Nutrients ^{1, 2}	Fast day	Feed day	Every day
Energy (kcal)	500	2500	1500
Fat (g)	14 (25%) ³	72 (26%) ³	42 (25%) ³
Saturated fat (g)	5	39	22
Monounsaturated fat (g)	5	18	11
Polyunsaturated fat (g)	4	15	9
Trans fat (g)	0	0	0
Cholesterol (mg)	32	163	96
Protein (g)	20 (16%) ³	88 (14%) ³	53 (14%) ³
Carbohydrate (g)	74 (59%) ³	375 (60%) ³	229 (61%) ³
Fiber (g)	11	31	22

¹ Values are for an example individual with a daily total energy expenditure of 2000 kcal. Each participant received an individualized nutrient prescription based on his or her total energy expenditure.

² No differences between groups for any nutrient when diets matched for total kcal over 48-h.

³ Percent of energy (kcal).

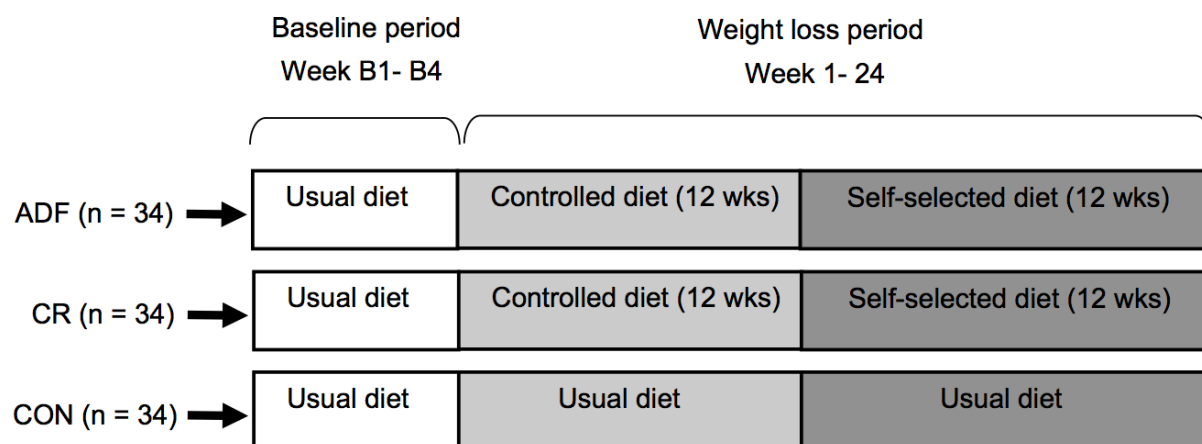


Figure 4.1. Experimental design

TABLE IV.II. BASELINE CHARACTERISTICS OF STUDY COMPLETERS¹

	ADF	CR	Control	P-value ²
n	28	28	24	
Age (y)	44 ± 2	44 ± 2	43 ± 2	0.87
Sex (F/M)	25/3	23/5	20/4	
Body weight (kg)	94 ± 3	100 ± 3	91 ± 3	0.12
Height (cm)	166 ± 2	168 ± 2	164 ± 2	0.34
Body mass index (kg/m ²)	34 ± 1	35 ± 1	34 ± 1	0.31
Visceral fat mass (kg)	1.9 ± 0.2	2.3 ± 0.2	2.0 ± 0.3	0.43
Subcutaneous fat mass (kg)	8.4 ± 0.4	8.7 ± 0.4	8.0 ± 0.7	0.56
HOMA-IR	4.0 ± 0.7	4.2 ± 0.7	3.5 ± 0.4	0.77
Adiponectin (ng/mL)	4389 ± 564	3964 ± 373	4689 ± 374	0.53
Leptin (ng/mL)	64 ± 6	71 ± 10	50 ± 7	0.17
Resistin (ng/mL)	13 ± 2	14 ± 1	14 ± 2	0.88
TNF-alpha (pg/mL)	1.5 ± 0.1	1.9 ± 0.2	1.7 ± 0.2	0.18
IL-6 (pg/mL)	2.1 ± 0.3	2.2 ± 0.2	2.8 ± 0.8	0.41
CRP (µg/mL)	0.3 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.17
Hcy (µmol/L)	10 ± 1	10 ± 1	10 ± 1	0.87
IGF-1 (ng/mL)	84 ± 4	103 ± 7	97 ± 10	0.16

¹ Values reported as mean ± SEM. F: Female, M: Male.

² P-value between groups at screening (One-way ANOVA). No differences between groups for any parameter.

TABLE IV.III. CHANGE IN BODY WEIGHT AND BODY COMPOSITION AT BASELINE, WEEK 12, AND WEEK 24¹

	Alternate day fasting				Daily calorie restriction				Control			
	Baseline	Week 12	Week 24	% Change ²	Baseline	Week 12	Week 24	% Change ²	Baseline	Week 12	Week 24	% Change ²
Body weight (kg)	94 ± 3	89 ± 3	87 ± 3	-7 ± 1*	100 ± 3	94 ± 3*	92 ± 3*	-8 ± 1*	91 ± 3	91 ± 3	90 ± 3	-1 ± 1
Fat mass (kg)	38 ± 1	34 ± 1	33 ± 1*	-12 ± 2*	40 ± 2	36 ± 3	35 ± 2	-14 ± 2*	35 ± 2	35 ± 2	34 ± 2	-2 ± 1
Fat free mass (kg)	54 ± 2	53 ± 2	53 ± 2	-2 ± 1	58 ± 2	56 ± 2	56 ± 2	-3 ± 2	54 ± 2	54 ± 2	54 ± 2	0 ± 1
Visceral fat (kg)	1.9 ± 0.2	-	1.5 ± 0.2	-22 ± 4	2.3 ± 0.2	-	1.7 ± 0.2	-25 ± 5	2.0 ± 0.3	-	1.7 ± 0.3	-15 ± 6
Subcutaneous fat (kg)	8.4 ± 0.4	-	7.9 ± 0.5	-6 ± 5	8.7 ± 0.4	-	7.8 ± 0.5	-11 ± 3	8.0 ± 0.7	-	8.0 ± 0.7	1 ± 2

¹ Values expressed as mean ± SEM. Alternate day fasting group (n = 28), Daily calorie restriction group (n = 28), Control group (n = 24).

² Percent change between baseline and week 24 values.

* Significantly different from control group (P < 0.0001). No differences between ADF and CR group for any parameter at any time point.

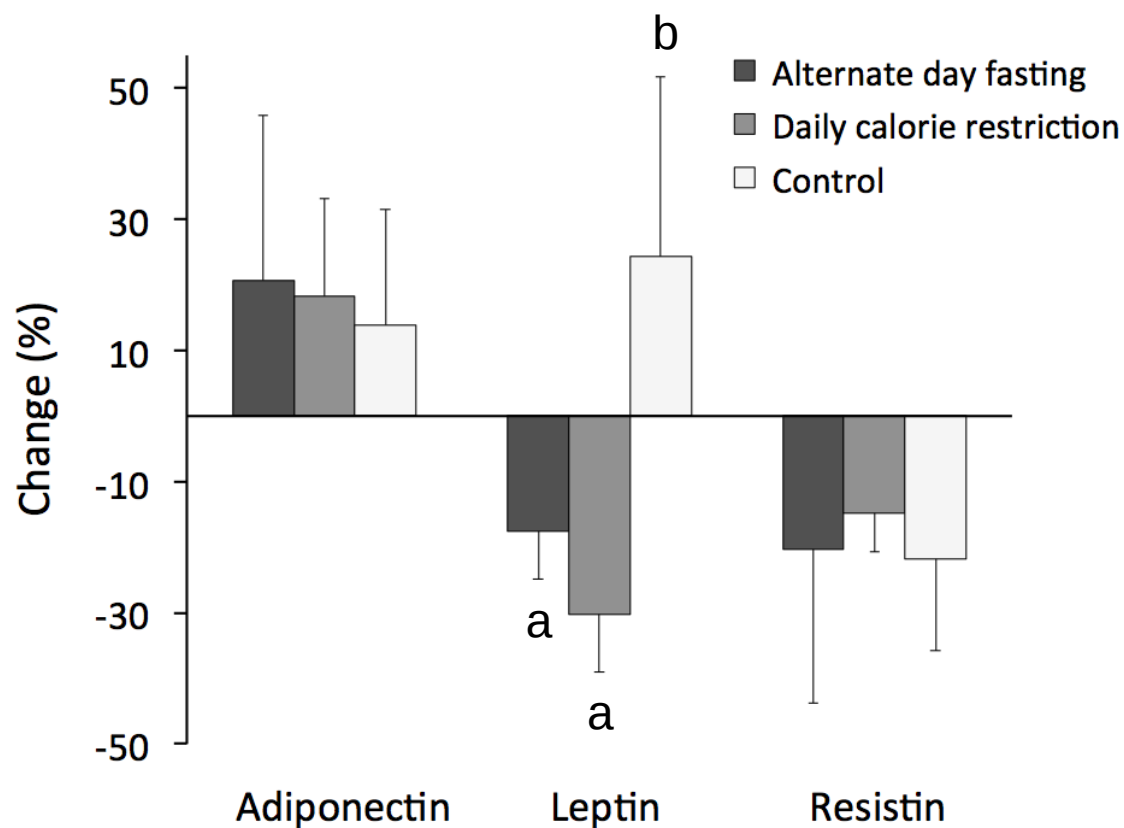


Figure 4.2. Change in adipokines after 24 weeks of treatment¹

¹ Values reported as mean $\pm$ SEM. Alternate day fasting group (n = 28), Daily calorie restriction group (n = 28), Control group (n = 24).

Leptin was significantly different ($P < 0.05$) between the control group and the two intervention groups.

TABLE IV.IV. CHANGE IN INFLAMMATORY PARAMTERS AT BASELINE, WEEK 12, AND WEEK 24¹

	Alternate day fasting				Daily calorie restriction				Control			
	Baseline	Week 12	Week 24	% Change ²	Baseline	Week 12	Week 24	% Change ²	Baseline	Week 12	Week 24	% Change ²
TNF-alpha (pg/ml)	1.5 ± 0.1	1.6 ± 0.2	1.6 ± 0.1	0.1 ± 0.1	1.9 ± 0.2	2.0 ± 0.2	2.0 ± 0.2	0.1 ± 0.1	1.7 ± 0.2	1.6 ± 0.2	1.4 ± 0.2	-0.3 ± 0.2
IL-6 (pg/ml)	2.1 ± 0.3	2.6 ± 0.3	2.5 ± 0.4	0.4 ± 0.4	2.2 ± 0.2	2.5 ± 0.3	2.5 ± 0.4	0.3 ± 0.4	2.8 ± 0.8	2.5 ± 0.3	3.0 ± 0.4	0.2 ± 0.7
CRP (ug/ml)	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0 ± 0.1
Hcy (umol/L)	10 ± 1	10 ± 1	10 ± 1	0 ± 1	10 ± 1	10 ± 1	9 ± 1	-1 ± 1	10 ± 1	9 ± 1	9 ± 1	-1 ± 1
IGF-1 (ng/ml)	84 ± 4	86 ± 5	87 ± 5	3 ± 4	103 ± 7	108 ± 6	113 ± 10	10 ± 8	97 ± 10	93 ± 9	88 ± 8	-9 ± 7

¹ Values expressed as mean ± SEM. Alternate day fasting group (n = 28), Daily calorie restriction group (n = 28), Control group (n = 24).

TNF-alpha: Tumor necrosis factor-alpha, IL-6: Interleukin-6, CRP: C-reactive protein, Hcy: Homocysteine, IGF-1: Insulin-like growth factor-1.

² Absolute change between baseline and week 24 values. No differences between baseline, week 12, and week 24 values for any parameter. No differences between groups for any parameter at any time point.

V. DISCUSSION

Findings from previous work suggested that, compared to CR, ADF might be able to promote greater dietary adherence (3), elicit greater weight loss (3), preserve more fat-free mass (12, 101), better improve insulin sensitivity, and better improve other traditional markers of coronary heart disease risk (blood lipids and blood pressure) as well as emerging markers of coronary heart disease risk (visceral fat mass and circulating inflammatory cytokines). This project was undertaken to examine these hypotheses, and found that ADF is not superior to CR regarding any of these parameters.

A well-known deficiency of CR regimens is diminished adherence over time. Indeed, adherence to CR regimens begins to decline as early as 6 weeks and progressively worsens thereafter (97). ADF was hypothesized to foster better adherence than CR on the basis that the former dietary regimen allows the patient to eat an unrestricted amount every other day (3). Patients on ADF therefore always have a day of unrestricted eating “to look forward to”, and this mindset was believed to promote greater adherence to the diet (3). Greater adherence would in turn be expected to yield greater weight loss and improvement in parameters affected by weight loss, including blood lipids and blood pressure. Early work in our laboratory appeared to provide evidence suggesting that dietary adherence would be superior for ADF: specifically it was reported that participants in ADF groups adhere to the fast day energy prescription and do not compensate by binging on the feast day for 8 weeks of dieting (147). However, it should be noted that these findings derived from self-reported energy intake. Self-reported energy intake has been consistently shown to be underreported – this is especially so for overweight and obese individuals (20) – and therefore should not be used as the primary measurement of energy intake in obesity research (148). The present study objectively measured energy intake using the doubly-labeled water method and found that dietary adherence was not superior for ADF compared to CR. It also found that ADF was not superior when compared to CR at promoting weight loss or improving

blood lipids or blood pressure. These findings, along with the observation that self-report yields invalid results for energy intake (148), should call into question the idea that patients may be better able to adhere to ADF when compared to CR.

It has also been suggested that ADF may be better able to preserve fat-free mass during weight loss when compared to CR. This suggestion first appeared in a literature review of ADF and CR on the basis that the ADF diets appeared to elicit a lower ratio of fat-free mass reduction to total mass reduction (101). However, this review contained several limitations. First, it featured no studies that directly compared ADF to CR (as none had been performed at the time of publication). Second, it only examined three published ADF studies. Third, although two of the three published ADF studies found via bioelectrical impedance that the ratio of fat-free mass reduction to total mass reduction was smaller than the average ratio in the reviewed CR studies (3, 13), the other ADF study found via DEXA that this ratio was *larger* than the average ratio in the reviewed CR studies (101). Following publication of this review, another study in our lab determined via DEXA that ADF produced sizable reductions in fat mass (4-5 kg) without affecting fat-free mass following 8 weeks of treatment (12). However, a subsequent study in our lab of equal duration found that ADF reduced fat-free mass by approximately 1-1.5 kg despite similar weight loss to the previous study (82). In the present study, we found with DEXA that ADF and CR produce comparable numerical reductions in fat-free mass. Thus, given the limitations of the aforementioned review (101), the findings from Hoddy and colleagues (82), and most importantly the results from the only direct comparison between ADF and CR to date (the present study), it should be concluded that there is insufficient evidence to suggest that ADF can better preserve fat-free mass compared to CR.

Previous findings have suggested the possibility that ADF may be able to improve insulin sensitivity better than CR even if weight loss were similar between the two diets. There were two lines of evidence to suggest this possibility. First, it has been shown that, in the absence of change in body weight, ADF improves the glucose infusion rate (largely reflecting skeletal muscle insulin sensitivity) and the ability of insulin to reduce interstitial glycerol concentrations (reflecting adipose tissue insulin sensitivity) during a euglycemic-hyperinsulinemic clamp (149). Second, two studies comparing CR to a 5:2 diet (which is similar to ADF but with two consecutive fast days alternated with 5 consecutive feast days) found that the latter diet produced greater improvement in HOMA-IR (15, 16). However, we found in the present study that, although the magnitude of improvement in HOMA-IR was over twice as large for the ADF group compared to the CR group (-1.8 au vs. 0.8 au), this difference did not reach statistical significance. It may be the case that the present study was underpowered to detect a difference of this magnitude. Alternatively it is possible that HOMA-IR is not sensitive enough compared to the euglycemic-hyperinsulinemic clamp (150) to detect a putative difference in insulin sensitivity between ADF and CR. Therefore, future work will need to compare improvement in insulin sensitivity between ADF and CR using the euglycemic-hyperinsulinemic clamp.

We also hypothesized that ADF would be better able to reduce visceral fat mass and improve circulating factors that are related to visceral fat mass when compared to CR even if weight loss were similar between the two diets. This hypothesis was based on previous findings that these parameters are affected by energy restriction *per se* in addition to weight loss. For example, a meta-analysis found that the degree of energy restriction is independently associated with the preferential loss of VAT (76), and another study found that the adipokine profile is modulated by energy restriction in a manner that is independent of weight loss (136). We therefore hypothesized that ADF would preferentially improve these parameters when compared to CR, because the greater degree of energy restriction during the fast

days of ADF would be perceived by visceral adipocytes as a stronger stimulus for these favorable changes. However, we found that ADF and CR affect visceral fat mass and our measured circulating factors similarly. This suggests that during dietary weight loss, average energy restriction is more important than day-to-day energy balance for affecting these parameters.

VI. LIMITATIONS

The results of this thesis project should be considered in the context of its limitations. Providing food to the intervention participants for the first 12 weeks of the intervention was perhaps the biggest limitation, because this interfered with our ability to test the primary hypothesis that adherence to ADF would be greater than adherence to CR. Future studies should compare the rate of adherence between ADF and CR under more “real-world” conditions.

Another limitation is that our doubly-labeled water measurements did not account for metabolic adaptation, which would lead to an underestimation of energy intake by roughly 3 absolute percentage points (112). Another limitation of our doubly-labeled water procedure was estimating respiratory quotient rather than measuring it. It has been estimated that there is a 1% error in the estimation of energy intake from doubly-labeled water for every 0.01 U difference between actual and estimated respiratory quotient (113). We estimated that the respiratory quotient was 0.86 in this study, which is higher than the measured respiratory quotient values in previous studies of ADF (0.83) (114) and CR (0.84) (22). However, because many of our participants were likely insulin resistant as indicated by their HOMA-IR values, their respiratory quotient values many have been much lower, leading to a much larger magnitude of error in measuring energy intake. Therefore, future weight-loss research should incorporate the doubly-labeled water procedure following 4 weeks of weight loss (so as to factor in metabolic adaptation) and measure the respiratory quotient in order to provide the most accurate measurement of energy intake.

An additional limitation is not measuring compliance to the alternating feast and fast day schedule of ADF. Without this measurement, it cannot be ruled out that ADF participants crossed over to the CR

regimen over time. Future research should measure compliance to ADF with ketone bodies (83) or the breath carbon isotope ratio method (151).

Another limitation is that blood samples were always taken following a fast day. Thus, the ADF participants experienced a longer duration of complete food abstinence prior to blood draw than the participants in the other two groups, and this may have affected the levels of some of our measured parameters (116). Future work should draw blood on consecutive feast and fast days in order to account for a potential effect of duration of food abstinence.

The decision to measure physical activity only at baseline and end of study is an additional limitation of our work. Physical activity increases in the summer and decreases in the winter (115). Therefore, by only measuring physical activity at baseline (winter) and end of study (summer), we may have overestimated the change in physical activity that occurred throughout the trial. This is especially relevant for the CR group, which was observed to experience a 26% numerical increase in the number of steps taken per day.

Finally, the decision to not measure intra- or inter-observer variability in the measurement of visceral fat mass is another limitation of our work. The control group experienced roughly 50% of the reduction of visceral fat mass that was experienced by the intervention groups, a finding that appears dubious. Examining intra- or inter-observer variability could have potentially pinpointed a source of error in our measurements if such error exists.

VII. FUTURE DIRECTIONS

There are two main directions to take in future comparisons between ADF and CR. One is to test the comparison under more controlled conditions than the present study. The other is to test the comparison under more ‘real-world’ conditions than the present study.

The present investigation involved providing all food to participants in the intervention groups for the first 12 weeks, followed by providing no food for the final 12 weeks. This design is interesting in that it allows for the same study to compare ADF and CR under both laboratory conditions and real-world conditions. Unfortunately, this design also entails that the two experimental conditions (laboratory and real-world) confound the interpretation of each other. For example, it is difficult to assess whether ADF truly promotes greater dietary adherence than CR when all food is provided to the participants for the first 12 weeks, which will assuredly artificially boost adherence in both groups. Another example is that it is difficult to assess whether ADF improves insulin sensitivity better than CR independently of weight loss when participants are provided no food for the final 12 weeks of study: In the absence of food provision, many participants in the ADF group may cross over into the CR intervention, which is to say that they may restrict energy by a moderate amount each day rather than keeping with the prescribed alternating feast day/fast day schedule. The solution to this problem is to utilize two separate studies to examine the comparison between ADF and CR under laboratory controlled and real-world conditions. For the laboratory controlled study, the paramount concern is that the ADF and CR groups do not cross over. The best way to prevent this is to examine the participants in an inpatient setting where all food intake (and physical activity) is experimentally controlled. Robust measurements of metabolic health should be examined in this study. For example the euglycemic-hyperinsulinemic clamp should be employed to assess insulin sensitivity. It should also be examined whether a putative greater improvement in insulin sensitivity for ADF compared to CR leads to more favorable metabolic handling

of mixed meals, and whether over time this results in less intra-organ lipid storage (e.g. pancreatic fat) and better metabolic function in these organ (e.g. greater beta cell function as estimated by the disposition index). A respiratory chamber should be used to compare resting energy expenditure between the diets, because a previous study found that ADF lowers this parameter without changing body weight (152). ADF might therefore lead to a larger effect on adaptive thermogenesis than CR, thereby predisposing individuals to regain the body weight that they lost while on this dietary plan. The real-world study will assess whether participants can actually adhere to the alternating feast day/fast day schedule of ADF, and whether overall adherence to ADF is greater than adherence to CR. Prior to the present investigation, our laboratory only had utilized self-report to assess adherence to the ADF schedule. This approach is invalid in obesity research (148). A previous study measured ketone bodies in plasma to assess adherence to the ADF schedule, but this study only lasted 8 weeks (83). The proposed real-world study would measure breath carbon stable isotope ratios (151) as well as plasma ketone bodies to determine day-to-day compliance to the ADF regimen. Doubly-labeled water would be used to compare average energy restriction between ADF and CR. Dietary counseling but not food would be provided as part of the intervention to maximize the generalizability of the results and to increase the likelihood that one diet would emerge as superior to the other. This study would also last 52 weeks so that the trajectories of adherence to the two diets could be compared over time.

VIII. SUMMARY OF FINDINGS AND GENERAL CONCLUSION

A. Summary of findings

This is the first study to directly compare ADF and CR in humans. It was undertaken to determine whether following an ADF regimen could yield greater improvement in dietary adherence, weight loss, fat-free mass preservation, insulin sensitivity, blood lipids, blood pressure, visceral fat mass reduction, and the inflammatory profile when compared to CR. ADF was shown to not be superior to CR regarding any of these variables. Based on comparisons with a control group, the use of robust measurement techniques (e.g. the doubly-labeled water method), and our excellent retention rate and completeness of data collection, we believe that these null findings demonstrate that ADF is not superior to CR regarding these parameters.

B. Conclusion

As this is the lone direct comparison between ADF and CR, the results from this study should supersede speculation derived from indirect comparisons between the two dietary regimens. The results from the present investigation contradict previously published speculation (3, 12, 101), and suggest that ADF is not superior to CR regarding its effect on any of our measured parameters.

ADF and CR produce similar weight loss. From a glass half full perspective, this means that overweight and obese individuals have an additional choice for dietary weight loss. From a glass half empty perspective, the modest weight loss after 24 weeks (average of ~7 kg) is well below the amount needed for many obese individuals to reach the upper bounds of normal weight (e.g. ~45 kg for a 5'9" person with a BMI of 40 to slim down to a BMI just below 25). This would suggest that ADF and CR are equally ineffective.

Which perspective is correct? It is tempting to choose the glass half full perspective for the sake of being positive, but this choice would be motivated by an unscientific reason. Rather, the perspective that one takes should be motivated by the answer to the following question: does introducing ADF as an additional choice for dietary weight loss lead to clinical benefit? The answer may be 'yes', but it is not *necessarily* so. Sometimes additional choices do not translate into additional benefit.

Interestingly, a similar debate of glass half full versus glass half empty perspective is happening in the study of low-fat versus low-carbohydrate diets (141). Prior to the year 2003, the low-fat diet had been regarded as the standard method of dietary weight loss, and the low-carbohydrate diet was viewed as the new competitor that could potentially result in greater weight loss and greater clinical benefit. Then starting in year 2003, a number of major trials compared the two diets head-to-head. In support of the

glass half empty perspective, the results to date have been modest, with trials lasting 6 months or longer yielding average weight losses typically ranging from 2-5 kg. However, the range of weight change within a dietary group has been consistently much larger than the difference in average weight loss between groups. Consider a trial that compared the Atkins, Zone, Weight Watchers, and Ornish diets for 12 months (153). Average weight loss was modest in all four diet groups (~4-7 kg), but the range of response in each diet group was between ~23 kg and ~40 kg (with some individuals gaining ≥ 5 kg and other individuals losing ≥ 15 kg). Or consider the A to Z study, which compared the Atkins, Zone, LEARN, and Ornish diets for 12 months (154). Average weight loss was modest in all four diet groups (~2-5 kg), but the range of response in each diet group was between ~30 kg and ~35 kg (with some individuals gaining ≥ 5 kg and other individuals losing ≥ 15 kg).

The wide range of weight-change response within a low-fat diet group or a low-carbohydrate diet group suggests the possibility that some individuals may respond better to one diet over the other. If this were confirmed, and if a method for predicting which individuals would respond more favorably to one diet or another were identified, then the glass half full perspective would be correct. Work on identifying such a predictor has already begun. Study results suggest that insulin-resistant individuals lose more weight on low-carbohydrate diets, while insulin-sensitive individuals either lose the same amount of weight on either diet, or slightly more weight on a low-fat diet (138-142). The investigation of whether one's genotype can predict weight change response to a low-fat versus a low-carbohydrate diet has already begun, and so far it has been discovered that individuals with the IRS1 rs2943641 CC genotype lose more weight on a low-fat diet (155).

The results from studies examining insulin sensitivity or genotype as predictors of success indicate that we should take a glass half full perspective regarding the common finding that low-fat and low-

carbohydrate diets yield similar average weight loss in head-to-head comparisons: there is reason to believe that *individuals* will respond differently to the two diets, even if their group averages will be similar. Is this also the case regarding the comparison between ADF and CR? There currently is no evidence that demonstrates that some individuals lose more weight on an ADF or CR diet. But absence of evidence is not evidence of absence, and a variable that predicts individual success on an ADF or CR diet may emerge in the future. It is worthwhile for future research to attempt to identify such a variable.

APPENDICES

Appendix A – Consent form

Leave box empty - For office use only STARTS APPROVAL EXPIRES NOV 05 2014 TO APR 15 2015 UNIVERSITY OF ILLINOIS AT CHICAGO INSTITUTIONAL REVIEW BOARD
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University of Illinois at Chicago
Research Information and Consent for Participation in Biomedical Research
Alternate day fasting: Effects on lipid metabolism and cardiovascular health

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 will sign the consent document first then you will be given 2 weeks to decide whether or not you would like to participate. You should feel free to ask the researchers any questions you may have.

Human Nutrition Research Unit: 312-355-0542
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 comparing alternate day fasting to calorie restriction for weight loss and weight maintenance. 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 150 subjects may be involved in this research at UIC.

What is the purpose of this research?

This research is being done to better understand how alternate day reductions in calorie intake or daily reductions in calorie intake can help a person lose weight, maintain weight loss, and reduce their risk of heart disease.

What procedures are involved?

This research will be performed at the Human Nutrition Research Unit (HNRU), 1919 W Taylor St., First floor, Room 121C, in the Applied Health Sciences Building at UIC. You will need to come to the study site 45 times over the next 52 weeks. Each of those visits will take about 30 minutes. However the first and last visit during the weight loss and the weight maintenance period may take up to 6 hours.

Alternate day fasting, lipid metabolism, and cardiovascular health
Version 8, October 27 2014

1 of 11

The study procedures are:

Before you begin the main part of the study you will need to have the following “screening” tests or procedures done 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 overweight or 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.
- **Blood pressure:** Your blood pressure will be measured during the screening visit. If your blood pressure does not fall between 120-139 mm Hg (systolic blood pressure) and 80-89 mm Hg (diastolic blood pressure), you will not be eligible to participate.
- **Blood draw:** Your blood will be drawn during the screening visit. If your LDL cholesterol level is not higher than 130 mg/dl, you will not be eligible to participate.
- **Complete a 7-day food record:** You will be given a 7-day food record to complete. You will return the food record 10 days after your first screening visit. If the food record is not adequately completed, you will not be eligible to participate.

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 of these groups:

1. **Alternate day fasting group:** If you fall in this category you will be asked to limit your food intake on the “fast day” to 75% less food than a person of your weight, and eat whatever you wish to eat on the “feed day”.
2. **Calorie restriction group:** If you fall in this category you will consume 25% less food every day than a person of your weight throughout the study.
3. **Control group/ No treatment group:** If you fall in this category you will be asked not to change your diet in anyway. You will be asked to do this to reduce variability to better see if calorie reduction has an effect in the two treatment groups.

If you are in the alternate day fasting group or the calorie restriction group, you will be provided with food only during study participation and not after completion. You will be encouraged to continue with the diet after the study, however, food will no longer be provided.

You will take part in a study that is 52 weeks long, and at each visit you will be asked to do the following:

Baseline period (Weeks B1-B4)

Week B1 visit: During your first visit, you will go to the Human Nutrition Research Unit to have a blood draw (about 4 tablespoons of blood) to measure heart disease risk factors. You will also have your blood pressure taken and your body weight / percent fat measured. At this visit, you will also drink a small amount of “heavy water” (70-90 ml) to measure the amount of energy you burn on a daily basis. You will be asked to sit for 6 hours following the “heavy water” administration to make sure that you don’t feel dizzy and to collect urine samples. During this 6-hour period, we will collect 3 urine samples from you (at hour 3, 4.5 and 6). After you have sat for 6 hours, it will be safe for you to leave the center.

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 B2 visit: You will go to the Human Nutrition Research Unit to have your urine collected.

Week B3 visit: You will go to the Human Nutrition Research Unit to have your urine collected.

Weight loss/ Controlled diet period (Weeks 1-12)

Week 1 visit: You will go to the 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. If you are in the alternate day fasting group or the calorie restriction group, you will be given your meals for the rest of the week. A small sample of fat (2 ml, or about a quarter of a teaspoon) will be taken from just under the skin in the lower back or upper buttocks region to measure the size of your fat cells. You will have 2 fat samples taken throughout the study. The samples will be frozen immediately after they are collected, and stored for up to 6 months (until they are analyzed). First, you will have a local anesthetic (lidocaine) applied to the area. This will sting briefly, and then it will feel numb. Once the anesthetic has taken effect, a small cut (1 cm) will be made to expose fat tissue underneath the skin. Approximately 2 ml of fat tissue (about the size of a finger nail) will be removed from the area. The incision will be closed with steristrips and covered with a waterproof bandage. At this visit, you will also drink a small amount of "heavy water" (70-90 ml) to measure your fat metabolism. You will be asked to sit for an hour following the "heavy water" administration to make sure that you don't feel dizzy. After you have sat for an hour, it will be safe for you to leave the research center. No urine samples will be collected at this time, so you will not have to stay for 6 hours after your heavy water dose. You will also have your body fat distribution measured by magnetic resonance imaging (MRI). Additionally, you will be given a small activity monitor to wear on your arm for the following 7 days.

Week 2 visit: During this visit you will have your blood pressure taken, and your body weight/percent fat measured. If you are in the alternate day fasting group or the calorie restriction group, you will be given your meals for the rest of the week.

Week 3 visit: During this visit you will have your blood pressure taken, and your body weight/percent fat measured. If you are in the alternate day fasting group or the calorie restriction group, you will be given your meals 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. If you are in the alternate day fasting group or the calorie restriction group, you will be given your meals for the rest of the week.

Week 5 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 have an ultrasound done on your arm. If you are in the alternate day fasting group or the calorie restriction group, you will be given your meals 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. If you are in the alternate day fasting group or the calorie restriction group, you will be given your meals for the rest of the week.

Week 7 visit: During this visit you will have your blood pressure taken, and your body weight/percent fat measured. If you are in the alternate day fasting group or the calorie restriction group, you will be given your meals 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. If you are in the alternate day fasting group or the calorie restriction group, you will be given your meals for the rest of the week. At this visit, you will also drink a small amount of "heavy water" (70-90 ml) to measure the amount of energy you burn on a daily basis. You will be asked to sit for 6 hours following the "heavy water" administration to make sure that you don't feel dizzy. During this time, we will collect 3 urine samples from you. After you have sat for 6 hours, it will be safe for you to leave the research center.

Week 9 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, urine collected, and your body weight/percent fat measured. You will also have an ultrasound done on your arm. If you are in the alternate day fasting group or the calorie restriction group, you will be given your meals for the rest of the week.

Week 10 visit: During this visit you will have your blood pressure taken, urine collected, and your body weight/percent fat measured. If you are in the alternate day fasting group or the calorie restriction group, you will

be given your meals for the rest of the week.

Week 11 visit: During this visit you will have your blood pressure taken, and your body weight/percent fat measured. If you are in the alternate day fasting group or the calorie restriction group, you will be given your meals for the rest of the week.

Week 12 visit: During this visit you will have your blood pressure taken, and your body weight/percent fat measured. If you are in the alternate day fasting group or the calorie restriction group, you will be given your meals for the rest of the week.

Weight loss/ Behavioral treatment period (Weeks 13-24)

Week 13 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 have an ultrasound done on your arm. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to keep losing weight on your own at home. During this period, food will no longer be provided to you.

Week 14 visit: During this visit you will have your blood pressure taken, and your body weight/percent fat measured. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to keep losing weight on your own at home. During this period, food will no longer be provided to you.

Week 15 visit: During this visit you will have your blood pressure taken, and your body weight/percent fat measured. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to keep losing weight on your own at home. During this period, food will no longer be provided to you.

Week 16 visit: During this visit you will have your blood pressure taken, and your body weight/percent fat measured. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to keep losing weight on your own at home. During this period, food will no longer be provided to you.

Week 17 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 have an ultrasound done on your arm. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to keep losing weight on your own at home. During this period, food will no longer be provided to you.

Week 18 visit: During this visit you will have your blood pressure taken, and your body weight/percent fat measured. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to keep losing weight on your own at home. During this period, food will no longer be provided to you.

Week 19 visit: During this visit you will have your blood pressure taken, and your body weight/percent fat measured. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to keep losing weight on your own at home. During this period, food will no longer be provided to you.

Week 20 visit: During this visit you will have your blood pressure taken, and your body weight/percent fat measured. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to keep losing weight on your own at home. During this period, food will no longer be provided to you.

Week 21 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 have an ultrasound done on your arm. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to keep losing weight on your own at home. During this period, food will no longer be provided to you.

Week 22 visit: During this visit you will have your blood pressure taken, and your body weight/percent fat measured. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to keep losing weight on your own at home. During this period, food will no longer be provided to you. At this visit, you will also drink a small amount of “heavy water” (70-90 ml) to measure the amount of energy you burn on a daily basis. You will be asked to sit for 6 hours following the “heavy water” administration to make sure that you don’t feel dizzy. During this time, we will collect 3 urine samples from you. After you have sat for 6 hours, it will be safe for you to leave the research center.

Week 23 visit: During this visit you will have your blood pressure taken, urine collected, and your body weight/percent fat measured. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to keep losing weight on your own at home. During this period, food will no longer be provided to you.

Week 24 visit (2 visits at the beginning of the week): You will go to the Human Nutrition Research Unit to have a blood draw (about 4 tablespoons of blood). You will also have your blood pressure taken, urine collected, 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. A small sample of fat (2 ml, or about a quarter of a teaspoon) will be taken from just under the skin in the lower back or upper buttocks region to measure the size of your fat cells. You will have 2 fat samples taken throughout the study. The samples will be frozen immediately after they are collected, and stored for up to 6 months (until they are analyzed). First, you will have a local anesthetic (lidocaine) applied to the area. This will sting briefly, and then it will feel numb. Once the anesthetic has taken effect, a small cut (1 cm) will be made to expose fat tissue underneath the skin. Approximately 2 ml of fat tissue (about the size of a finger nail) will be removed from the area. The incision will be closed with steristrips and covered with a waterproof bandage. At this visit, you will also drink a small amount of “heavy water” (70-90 ml) to measure your fat metabolism. You will be asked to sit for an hour following the “heavy water” administration to make sure that you don’t feel dizzy. After you have sat for an hour, it will be safe for you to leave the research center. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session. You will also have your body fat distribution measured by magnetic resonance imaging (MRI). You will be given a small activity monitor to wear on your arm for 7 days.

Weight maintenance/ Behavioral treatment period (Weeks 25–48)

Week 28 visit (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. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to maintain your weight loss. You will also be given eating questionnaires to complete before you leave the research center.

Week 32 visit (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. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to maintain your weight loss. You will also be given eating questionnaires to complete before you leave the research center.

Week 36 visit (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. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to maintain your weight loss. You will also be given eating questionnaires to complete before you leave the research center.

Week 40 visit (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. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to maintain your weight loss. You will also be given eating questionnaires to complete before you leave the research center.

Week 44 visit (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. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to maintain your weight loss. You will also be given eating questionnaires to complete before you leave the research center.

Week 48 visit (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. If you are in the alternate day fasting group or the calorie restriction group, you will attend a dietary counseling session to learn how to maintain your weight loss. You will also be given eating questionnaires to complete before you leave the research center. You will also have your body fat distribution measured by magnetic resonance imaging (MRI). Additionally, you will be given a small activity monitor to wear on your arm for the following 7 days.

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 520 ml of blood will be drawn during the study.
2. **Calorie restriction/alternate day fasting:** 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 DEXA x-ray scanning:** You will have 2 x-ray DEXA scans performed throughout the study to measure your body fat. The amount of radiation you will be exposed to during DEXA 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.
5. **Fat tissue sampling:** There is risk of adverse reactions to lidocaine. Should you experience any adverse reactions to lidocaine, the fat biopsy will not be performed. Lidocaine can also result in an allergic reaction, which is a risk with all medications. An allergic reaction can range from minor itching and a rash to severe respiratory arrest and death. The dosage of lidocaine used for this study is below the toxic threshold, however, if an allergic reaction is evident, medical treatment will be provided. If you have a history of allergic reactions, you will be excluded from study participation. Fat biopsies are well tolerated and associated with minimal discomfort. Risks are rare and include: excessive bleeding and discomfort at the site, fainting, wound rupture, and infection. Temporary bleeding or bruising can also occur at the incision site. To minimize these risks, fat biopsy procedures will be performed under sterile conditions and sustained pressure will be applied to incision sites. Wounds should heal within 3 to 5 days. After the first biopsy, you will be monitored closely to assess impairment of wound healing. If there is evidence of wound rupture or infection, the second biopsy will not be performed. We will give you follow-up phone calls to assess healing. You should report any bleeding or opening of your wounds immediately to Dr. Varady or Karen Vuckovic.
6. **Heavy water administration:** Heavy water (also called deuterated water or doubly labeled water) is a labeled form of water, which can be measured in trace amounts within your blood. It tastes and feels identical to water, contains no radioactivity, and has no known harmful effects at the doses given here. During the initial dosing it may cause some temporary dizziness in some people (about one out of every 30 people), although this is rare. In the rare event that dizziness occurs, this should go away within 1 hour, and will be treated symptomatically in the interim. Usually, lying down and resting for a brief period (an hour at most) is enough to allow the dizziness to go away. If dizziness happens more than once or persists, you will be removed from the study. Heavy water does not pose any risk to reproductive organs, including sperm or male fertility.
7. **Magnetic resonance imaging (MRI):** There are no known hazards to MRI examinations other than the discomfort associated with confinement and remaining stationary for the duration of the study. We will carefully screen out individuals who have an implanted electrical device (i.e. cardiac pacemakers), since these devices can malfunction when a person is undergoing MRI scanning. We will also screen out claustrophobic individuals.

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 calorie restriction 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. If you are assigned to the no treatment group, you are not expected to directly benefit from participating in this research.

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 and 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?

In return for your time and travel expenses, you will be paid \$1000 for completing the entire study. The stipend payment will be by check. The reimbursement will be pro-rated as follows:

- \$11 for each visit attended at the research center (45 in total)
- \$25 for each heavy water administration to assess energy burned (3 in total)
- \$10 for each blood draw (26 in total)
- \$20 for each magnetic resonance imaging scan (MRI) (3 in total)
- \$55 for each fat tissue sample (2 in total)

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 research coordinator (312-355-0542) or Dr. Krista Varady (312-996-7897)

- 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.

Who might benefit financially from this research?

Dr. Varady, the person leading this study, is an author of the book "The Every Other Day Diet." Dr. Varady has received financial compensation and will receive further compensation from sales of this book, if research shows the diet method is safe and effective.

The possible benefit to Dr. Varady is not likely to affect your safety. If you would like more information, please ask the researchers or the study coordinator.

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

Summary of study activities during the 52-week trial

Study week	Alternate day fasting group	Calorie restriction group	Control group
Baseline period (Weeks B1-B4)			
B1	• Blood draw • Body weight • Heavy water (energy)	• Blood draw • Body weight • Heavy water (energy)	• Blood draw • Body weight • Heavy water (energy)
B2	• Urine sample	• Urine sample	• Urine sample
B3	• Urine sample	• Urine sample	• Urine sample
Weight loss/ controlled diet period (Weeks 1-12)			
1	• Blood draw • Body weight/DEXA • Ultrasound • Fat sample • Heavy water (fat metab) • Food pickup • MRI scan	• Blood draw • Body weight/DEXA • Ultrasound • Fat sample • Heavy water (fat metab) • Food pickup • MRI scan	• Blood draw • Body weight/DEXA • Ultrasound • Fat sample • Heavy water (fat metab) • MRI scan
2	• Bodyweight • Food pickup	• Body weight • Food pickup	• Body weight
3	• Bodyweight • Food pickup	• Body weight • Food pickup	• Body weight
4	• Bodyweight • Food pickup	• Body weight • Food pickup	• Body weight
5 (2 visits)	• Blood draw • Body weight • Ultrasound • Food pickup	• Blood draw • Body weight • Ultrasound • Food pickup	• Blood draw • Body weight • Ultrasound
6	• Bodyweight • Food pickup	• Body weight • Food pickup	• Body weight
7	• Bodyweight • Food pickup	• Body weight • Food pickup	• Body weight
8	• Bodyweight • Food pickup • Heavy water (energy)	• Body weight • Food pickup • Heavy water (energy)	• Body weight • Heavy water (energy)
9 (2 visits)	• Blood draw • Body weight • Ultrasound • Food pickup • Urine sample	• Blood draw • Body weight • Ultrasound • Food pickup • Urine sample	• Blood draw • Body weight • Ultrasound • Urine sample
10	• Bodyweight • Food pickup • Urine sample	• Body weight • Food pickup • Urine sample	• Body weight • Urine sample
11	• Bodyweight • Food pickup	• Body weight • Food pickup	• Body weight
12	• Bodyweight • Food pickup	• Body weight • Food pickup	• Body weight
Weight loss/ Behavioral treatment period (Weeks 13-24)			
13 (2 visits)	• Blood draw • Body weight • Ultrasound • Diet counseling	• Blood draw • Body weight • Ultrasound • Diet counseling	• Blood draw • Body weight • Ultrasound

14	• Bodyweight • Diet counseling	• Body weight • Diet counseling	• Body weight
15	• Bodyweight • Diet counseling	• Body weight • Diet counseling	• Body weight
16	• Bodyweight • Diet counseling	• Body weight • Diet counseling	• Body weight
17 (2 visits)	• Blood draw • Body weight • Ultrasound • Diet counseling	• Blood draw • Body weight • Ultrasound • Diet counseling	• Blood draw • Body weight • Ultrasound
18	• Bodyweight • Diet counseling	• Body weight • Diet counseling	• Body weight
19	• Bodyweight • Diet counseling	• Body weight • Diet counseling	• Body weight
20	• Bodyweight • Diet counseling	• Body weight • Diet counseling	• Body weight
21 (2 visits)	• Blood draw • Body weight • Ultrasound • Diet counseling	• Blood draw • Body weight • Ultrasound • Diet counseling	• Blood draw • Body weight • Ultrasound
22	• Bodyweight • Diet counseling • Heavy water (energy)	• Body weight • Diet counseling • Heavy water (energy)	• Body weight • Heavy water (energy)
23	• Bodyweight • Diet counseling • Urine sample	• Body weight • Diet counseling • Urine sample	• Body weight • Urine sample
24 (2 visits)	• Blood draw • Body weight/DEXA • Ultrasound • Fat sample • Heavy water (fat metab) • Urine sample • MRI scan	• Blood draw • Body weight/DEXA • Ultrasound • Fat sample • Heavy water (fat metab) • Urine sample • MRI scan	• Blood draw • Body weight/DEXA • Ultrasound • Fat sample • Heavy water (fat metab) • Urine sample • MRI scan
Weight maintenance/ Behavioral treatment period (Weeks 25-48)			
28 (2 visits)	• Blood draw • Body weight • Diet counseling	• Blood draw • Body weight • Diet counseling	• Blood draw • Body weight
32 (2 visits)	• Blood draw • Body weight • Diet counseling	• Blood draw • Body weight • Diet counseling	• Blood draw • Body weight
36 (2 visits)	• Blood draw • Body weight • Diet counseling	• Blood draw • Body weight • Diet counseling	• Blood draw • Body weight
40 (2 visits)	• Blood draw • Body weight • Diet counseling	• Blood draw • Body weight • Diet counseling	• Blood draw • Body weight
44 (2 visits)	• Blood draw • Body weight • Diet counseling	• Blood draw • Body weight • Diet counseling	• Blood draw • Body weight
48 (2 visits)	• Blood draw • Body weight • Diet counseling • MRI scan	• Blood draw • Body weight • Diet counseling • MRI scan	• Blood draw • Body weight • MRI scan

Appendix B – Recruitment poster

Volunteers needed for a Weight Loss Study

Volunteers are needed for a 52-week research study of the effects of alternate-day food restriction or daily food restriction for weight loss, weight maintenance, and heart disease prevention.

The study is open to men and women who are:

- Between the ages of 18 and 65 •
- Obese, not diabetic, sedentary or moderately active •

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

Weight loss study
312-355-0542
Monica Klemm

Weight loss study
312-355-0542
Monica Klemm

Weight loss study
312-355-0542
Monica Klemm

Weight loss study
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Weight loss study
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University of Illinois at Chicago
Department of Kinesiology and Nutrition
1919 West Taylor Street, Chicago, IL
Krista Varady, Ph.D., Principal Investigator

UIC

*Alternate day fasting, lipid metabolism, and cardiovascular health
Version 4, February 1 2012*

Appendix C – Screening questionnaire

Screening questionnaire		Subject ID: 012 –
Date of screening: _____		
First name: _____ Middle initial: _____ Last: _____		
Phone: _____ Email: _____		
Age: _____ (Must be 18-65 yrs) DOB: _____ Sex: ☐ Female ☐ Male		
Weight: _____ Height: _____ BMI: _____ (must be 25-39.9kg/m ²)		
Ethnicity ☐ Hispanic/Latino ☐ Not Hispanic/Latino		
Race ☐ American Indian ☐ Asian ☐ African American ☐ Native Hawaiian/Pacific Islander ☐ White		
Do you smoke?	☐ yes ☐ no (If yes, disqualify)	
Claustrophobic/ have implanted electrical device?	☐ 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)	
Undergone weight loss surgery in the past?	☐ yes ☐ no (If yes, disqualify)	
Vegan or vegetarian?	☐ yes ☐ no (If yes, disqualify)	
Can you commit to these diets for 52 wks?	☐ yes ☐ no (If yes, 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)		
Are you on any medications?	☐ yes ☐ no	
Medication 1: _____		
Medication 2: _____		
Medication 3: _____		
Medication 4: _____		
(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)	
Post-menopausal (absence of menses for > 2 y)?	☐ yes ☐ no	
Pregnant or trying to become pregnant?	☐ yes ☐ no (If yes, disqualify)	
Alternate day fasting, lipid metabolism, and cardiovascular health		
Version 3, November 1 2011		

Appendix D – Alternate day fasting diet instructions

|Alternate day fasting – Take home meal instructions

General instructions:

- Use the checklist provided to keep track of what you eat each day.
- RECORD ANY EXTRA FOOD ITEMS that you eat in the “Extra food log”.
- For food/diet problems please speak to the study coordinator.

When eating the “feed day” meal:

- Eat ONLY the food we provide.
- Eat ALL of the food (even the little pieces).

When eating the “fast day” meal:

- Eat ONLY the lunchtime meal we provide.
- Eat ALL of the food (even the little pieces).
- **Eat all the food between 12 pm and 2 pm on the fast day.**

Zero calorie items that you can eat at any time:

- We recommend you DRINK 6-8 glasses of water each day.
- You may not drink other beverages except those listed here:
 - Water as desired
 - Black coffee (without sugar and/or cream) 2 cups per day max
 - Black tea (without sugar or milk) 2 cups per day max
 - Diet soda as desired
- You may also chew sugar-free chewing gum throughout the day.

Appendix E – Alternate day fasting diet checklist during controlled feeding period

Alternate day fasting diet checklist

Subject ID: _____

Study week: 1 7

SATURDAY	Meal	Food Item	Quantity given	Eaten?	Amount leftover if not eaten
Feed-3	Breakfast	Bagel	2 small		
		Butter	2 packets		
		Jam	1 packet		
		String Cheese	1 package		
		Apple	1 medium		
	Lunch	Macaroni and Cheese	1 package		
		Yogurt	1 container		
		Orange	1 medium		
		Peanuts			
	Dinner	Roasted Turkey	1 package		
		Milk	1 carton		
		Grapes			
		Brownie			
SUNDAY	Meal	Food Item	Quantity given	Eaten?	Amount leftover if not eaten
Fast-3	Lunch	Lasagna with Meat Sauce	1 package		
		Carrots Baby			
		Cheezits			

Appendix F – Calorie restriction diet instructions

Calorie restriction – Take home meal instructions

General instructions:

- Use the checklist provided to keep track of what you eat each day.
- RECORD ANY EXTRA FOOD ITEMS that you eat in the “Extra food log”.
- For food/diet problems please speak to the study coordinator.

When eating the meals each day:

- Eat ONLY the food we provide.
- Eat ALL of the food (even the little pieces).

Zero calorie items that you can eat at any time:

- We recommend you DRINK 6-8 glasses of water each day.
- You may not drink other beverages except those listed here:
 - Water as desired
 - Black coffee (without sugar and/or cream) 2 cups per day max
 - Black tea (without sugar or milk) 2 cups per day max
 - Diet soda as desired
- You may also chew sugar-free chewing gum throughout the day.

Appendix G – Calorie restriction diet checklist during controlled feeding period

Calorie restriction diet checklist

Subject ID: _____

Study week: 1 4 7 10

WEDNESDAY	Meal	Food Item	Quantity given	Eaten?	Amount leftover if not eaten
Feed-1	Breakfast	Bread	1 slice		
		Butter	1 packet		
		Jam	1 packet		
		Yogurt	1 container		
		Grapes			
	Lunch	Chicken Enchilada	1 package		
		Carrots Baby			
		Cheezits			
	Dinner	Pepperoni Pizza	1 package		
		String Cheese	1 package		
		Celery	4 sticks		
		Chocolate chip cookies			
THURSDAY	Meal	Food Item	Quantity given	Eaten?	Amount leftover if not eaten
Feed-2	Breakfast	Oatmeal	1 packet		
		Milk	1 carton		
		Orange	1 medium		
	Lunch	Lasagna with Meat Sauce	1 package		
		Grapes			
		Brownie			
	Dinner	Sante Fe Style Rice and Beans	1 package		
		Yogurt	1 container		
		Carrots			
		Cheezits			

Appendix H – Extra food log for intervention subjects during controlled feeding period

Appendix I - Doubly labeled water administration protocol

Doubly labeled water administration protocol

Baseline

1. Weigh the subject, and calculate the DLW dose (DLW Dose = 1 g of DLW * body weight in kg, e.g. 90 kg person will require 90 g of DLW).
2. Weigh out the DLW for the subject in grams.
3. Collect two urine samples prior to DLW dosing (this can be one complete void separated into two conical tubes). Label the two conical tubes Baseline A and Baseline B. Record the time of collection on the logsheet.
4. Administer the DLW dose to the subject. Instruct the subject to consume all of the DLW in the cup and be careful not to spill the DLW. Add 50 mL of tap water to the container, swirl, and consume. Add another 50 mL of tap water to the container, swirl, and consume. Record the time of the DLW dose on the logsheet.
5. Have the participant void at 3 hours post dose. Discard the urine sample.
6. Collect a urine sample at 4.5 hours after the dose. Label the conical tube Day 0 – 4.5 hours. Record the time of collection on the logsheet.
7. Collect a urine sample at 6 hours after the dose. Label the conical tube Day 0 – 6 hours. Record the time of collection on the logsheet.

Day 7

8. On day 7, the subject will return to the Center. Upon arriving at the Center, the subject will collect a urine sample. This specimen is labeled Day 7A. A metabolic weight will be obtained and documented on the logsheet.
9. Approximately 1 hour after collection of the 7A sample, the subject will collect another urine sample. This specimen is labeled Day 7B.

Day 14

10. On day 14, the subject will return to the Center. Upon arriving at the Center, the subject will collect a urine sample. This specimen is labeled Day 14A. A metabolic weight will be obtained and documented on the logsheet.
11. Approximately 1 hour after collection of the 14A sample, the subject will collect another urine sample. This specimen is labeled Day 14B.

Notes: All urine samples must be at least 5 mL, if not then the participant must try to collect another sample. When the sample is adequate this will become the collection time that is to be recorded on the logsheet. **DO NOT FILL THE CONICAL TUBE PAST 10 ML (OR THE TUBE WILL EXPLODE WHEN FROZEN).**

DLW Logsheet**BASELINE**

Date: _____

Subject ID: _____

Subject Weight (kg) _____

DLW dose (g): _____

Baseline A Time: _____**Baseline B** Time: _____

DLW Dose Time: _____

Day 0 – 4.5 hour Time: _____**Day 0 – 6 hour** Time: _____**DAY 7**

Date: _____

Day 7 weight (kg) _____

Day 7A Time: _____**Day 7B** Time: _____**DAY 14**

Date: _____

Day 14 weight (kg) _____

Day 14A Time: _____**Day 14B** Time: _____

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VITA

EDUCATION

- PH.D. **University of IL., Chicago – Department of Kinesiology and Nutrition**
Human Nutrition August 2011 – August 2015
Supervisor: Dr. Krista A. Varady, Ph.D.
- Thesis title: Alternate-day fasting versus daily calorie restriction for weight loss and cardio-protection
- M.S. **University of Memphis – Department of Health and Sport Sciences**
Kinesiology August 2008 – May 2011
Supervisor: Dr. Richard J. Bloomer, Ph.D.
- Thesis title: Effects of a 21-day Daniel Fast with and without krill oil supplementation on blood lipids and lipid peroxidation
- B.A. **Rhodes College – Department of Philosophy**
Philosophy August 2003 – May 2007

ACADEMIC AND RESEARCH APPOINTMENTS

- Clinical Coordinator **University of IL., Chicago – Department of Kinesiology and Nutrition**
Human Nutrition Research Center
August 2011 – August 2015
- Teaching Assistant **University of IL., Chicago – Department of Kinesiology and Nutrition**
Exercise Physiology (Undergraduate)
August 2012 – May 2014
- Course Instructor **University of Memphis – Department of Health and Sport Sciences**
Exercise Physiology (Undergraduate)
June 2011 – August 2011
- Research Assistant **University of Memphis – Department of Health and Sport Sciences**
Cardiorespiratory-Metabolic Laboratory
August 2009 – August 2011

FELLOWSHIP

Pre-doctoral Fellowship **University Fellowship, University of IL., Chicago**
 August 2011 – August 2015, \$25,000/year for 2 years
 Awarded to top 1% of applicants

HONORS AND AWARDS

October 2014	The Obesity Society – Travel Grant	\$500
January 2014	University of IL., Chicago – Graduate Student Council Travel Award	\$275
December 2013	University of IL., Chicago – Travel Grant	\$1000
April 2013	University of IL., Chicago – Outstanding Research Award	\$1500
April 2011	University of Memphis – Premier Publication Award	\$500

PROFESSIONAL AFFILIATIONS

Member of the Obesity Society since 2011
 Member of the American Society for Nutritional Sciences since 2011

REVIEWER FOR JOURNALS

British Journal of Nutrition, Cell Metabolism, European Journal of Endocrinology, Journal of Hepatology, Lipids in Health and Disease, Nutrition Journal

PUBLICATIONS

1. **Trepanowski JF***, Kroeger CM*, Barnosky AR, Hoddy KK, Ravussin E, Varady KA. Effect of 12 months of alternate day fasting versus daily calorie restriction on body weight, body composition and metabolic disease risk in overweight and obese adults. * co-first authors. In preparation.
2. **Trepanowski JF**, Kroeger CM, Barnosky AR, Varady KA. Impact of alternate day fasting versus daily calorie restriction on biomarkers of inflammation. In preparation.
3. Kroeger CM, **Trepanowski JF**, Barnosky AR, Varady KA. Improvements in eating behaviors during alternate day fasting contribute to successful weight maintenance. In preparation.
4. Barnosky AR, **Trepanowski JF**, Kroeger CM, Varady KA. Impact of alternate day fasting versus daily calorie restriction on bone health in obese adults. In preparation.
5. Varady KA, **Trepanowski JF**, Kroeger CM. Comparison of adherence metrics in dietary weight-loss intervention: doubly-labeled water versus self-report. In preparation.
6. Varady KA, **Trepanowski JF**, Kroeger CM. LDL particle size during weight loss and maintenance. In preparation.
7. Hoddy KK, Kroeger CM, **Trepanowski JF**, Barnosky A, Bhutani S, Varady KA. Safety of alternate day fasting and effect on disordered eating behaviors. *Nutr J*. In review.
8. **Trepanowski JF**, Mey J, Varady KA. Fetuin-A: a novel link between obesity and related complications. *Int J Obes*. Epub ahead of print.
9. Hoddy KK, Kroeger CM, **Trepanowski JF**, Bhutani S, Barnosky A, Varady KA. Meal timing during alternate day fasting: effects on body weight and coronary heart disease risk in obese adults. *Obesity (Silver Spring)*. 2014. Epub ahead of print.
10. **Trepanowski JF**, Varady KA. Veganism is a viable alternative to conventional diet therapy for improving blood lipids and glycemic control. *Crit Rev Food Sci Nutr*. 2014. Epub ahead of print.
11. Varady KA, Bhutani S, Klempel MC, Kroeger CM, **Trepanowski JF**, Haus JM, Hoddy KK, Calvo Y. Alternate day fasting for weight loss in normal weight and overweight subjects: a randomized controlled trial. *Nutr J*. 2013; 12:146.
12. Bhutani S, Klempel MC, Kroeger CM, Aggour E, Calvo Y, **Trepanowski JF**, Hoddy KK, Varady KA. Effect of exercising while fasting on eating behaviors and food intake. *J Int Soc Sports Nutr*. 2013; 10:50.
13. Bhutani S, Klempel MC, Kroeger CM, **Trepanowski JF**, Phillips SA, Norkeviciute E, Varady KA. Alternate day fasting with or without exercise: Effects on endothelial function and adipokines in obese humans. *e-SPEN J*. 2013; 8:e205-9.
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15. Bhutani S, Klempel MC, Kroeger CM, **Trepanowski JF**, Varady KA. Alternate day fasting and endurance exercise combine to reduce body weight and favorably alter plasma lipids in obese humans. *Obesity (Silver Spring)*. 2013; 21:1370-9.
16. Klempel MC, Kroeger CM, Bhutani S, **Trepanowski JF**, Varady KA. Intermittent fasting combined with calorie restriction is effective for weight loss and cardio-protection in obese women. *Nutr J*. 2012; 11:98.

PUBLICATIONS - CONTINUED

17. Kroeger CM, Klempel MC, Bhutani S, **Trepanowski JF**, Tangney CC, Varady KA. Improvement in coronary heart disease risk factors during an intermittent fasting/calorie restriction regimen: Relationship to adipokine modulations. *Nutr Metab (Lond)*. 2012; 9:98.
18. **Trepanowski JF**, Kabir MM, Alleman RJ Jr, Bloomer RJ. A 21-day Daniel fast with or without krill oil supplementation improves anthropometric parameters and the cardiometabolic profile in men and women. *Nutr Metab (Lond)*. 2012; 9:82.
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20. **Trepanowski JF**, Farney TM, McCarthy CG, Schilling BK, Craig SA, Bloomer RJ. The effects of chronic betaine supplementation on exercise performance, skeletal muscle oxygen saturation and associated biochemical parameters in resistance trained men. *J Strength Cond Res*. 2011; 25:3461-71.
21. **Trepanowski JF**, Canale RE, Marshall KE, Kabir MM, Bloomer RJ. Impact of caloric and dietary restriction regimens on markers of health and longevity in humans and animals: a summary of available findings. *Nutr J*. 2011; 10:107
22. Trabelsi K, El Abed K, **Trepanowski JF**, Stannard SR, Ghilisi Z, Ghazzi H, Masmoudi L, Jammoussi K, Hakim A. Effects of ramadan fasting on biochemical and anthropometric parameters in physically active men. *Asian J Sports Med*. 2011; 2:134-44.
23. Bloomer RJ, Farney TM, **Trepanowski JF**, McCarthy CG, Canale RE. Effect of betaine supplementation on plasma nitrate/nitrite in exercise-trained men. *J Int Soc Sports Nutr*. 2011; 8:5.
24. Bloomer RJ, Kabir MM, **Trepanowski JF**, Canale RE, Farney TM. A 21 day Daniel Fast improves selected biomarkers of antioxidant status and oxidative stress in men and women. *Nutr Metab (Lond)*. 2011; 8:17.
25. **Trepanowski JF**, Bloomer RJ. The impact of religious fasting on human health. *Nutr J*. 2010; 9:57.
26. Bloomer RJ, Kabir MM, Canale RE, **Trepanowski JF**, Marshall KE, Farney TM, Hammond KG. Effect of a 21 day Daniel Fast on metabolic and cardiovascular disease risk factors in men and women. *Lipids Health Dis*. 2010; 9:94.
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BOOK CHAPTERS

1. **Trepanowski JF**, Varady KA. Intermittent fasting, body weight and plasma lipids. *Nutrition and cardiometabolic health*, 1st edition. CRC Press, Taylor & Francis Group. In preparation.
2. **Trepanowski JF**, Varady KA. Intermittent versus daily calorie restriction in visceral fat loss. *Nutrition in the prevention and treatment of abdominal obesity*. London, UK: Academic Press, 2014, 181-8.

ABSTRACTS

1. **Trepanowski JF**, Kroeger CM, Barnosky AR, Hoddy KK, Rood JC, Ravussin E, Varady KA. Dietary adherence and weight loss are similar between alternate-day fasting and daily calorie restriction during a 24-week trial. The Obesity Society Annual Meeting. Boston, Massachusetts, USA, 2014. [Oral Presentation].
2. Hoddy KK, Kroeger CM, Barnosky AR, **Trepanowski JF**, Varady KA. How does meal timing during alternate day fasting affect weight loss and coronary heart disease risk in obese adults? The Obesity Society Annual Meeting. Boston, Massachusetts, USA, 2014. [Oral Presentation].
3. **Trepanowski JF**, Kroeger CM, Klempel MC, Calvo Y, Varady KA. Alternate-day fasting versus daily calorie restriction for weight loss and cardio-protection. Federation of American Societies for Experimental Biology (FASEB) Annual Meeting. San Diego, CA, USA, 2014. [Oral Presentation].
4. Kroeger CM, **Trepanowski JF**, Klempel MC, Bhutani S, Hoddy KK, Varady KA. Alternate day fasting is effective for weight loss and weight maintenance in obese adults. Federation of American Societies for Experimental Biology (FASEB) Annual Meeting. San Diego, CA, USA, 2014. [Poster Presentation].
5. Hoddy KK, Kroeger CM, **Trepanowski JF**, Bhutani S, Barnosky A, Varady KA. Meal timing during alternate day fasting: effects on body weight and coronary heart disease risk in obese adults. Federation of American Societies for Experimental Biology (FASEB) Annual Meeting. San Diego, CA, USA, 2014. [Oral Presentation].
6. **Trepanowski JF**, Kroeger CM, Klempel MC, Calvo Y, Varady KA. Alternate-day fasting versus daily calorie restriction for weight loss and cardio-protection. The Obesity Society Annual Meeting. Atlanta, Georgia, USA, 2013. [Poster presentation].
7. **Trepanowski JF**, Kroeger CM, Klempel MC, Calvo Y, Varady KA. Alternate-day fasting versus daily calorie restriction for weight loss and cardio-protection. University of IL., Chicago: College of Medicine Research Forum. Chicago, Illinois, USA, 2013. [Poster presentation].
8. Kroeger CM, **Trepanowski JF**, Klempel MC, Bhutani S, Hoddy K, Varady KA. Alternate day fasting is effective for weight loss and weight loss maintenance in obese adults. University of IL., Chicago: College of Medicine Research Forum. Chicago, Illinois, USA, 2013. [Poster presentation].
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10. Kroeger CM, Klempel MC, Bhutani S, **Trepanowski JF**, Tangney CC, Varady KA. Improvement in coronary heart disease risk factors during an intermittent fasting/calorie restriction regimen: Relationship to adipokine modulations. University of IL., Chicago: Diabetes and Research Day. Chicago, IL, USA, 2013. [Poster presentation].
11. Bhutani S, Klempel MC, Kroeger CM, **Trepanowski JF**, Varady KA. Alternate day fasting combined with exercise: An effective treatment for weight loss and cardio-protection in obese humans. Federation of American Societies for Experimental Biology (FASEB) Annual Meeting. San Diego, CA, USA, 2012. [Poster presentation].
12. Farney TM, Kabir MM, **Trepanowski JF**, Canale RE, Bloomer RJ. A 21 day Daniel Fast favorably impacts biomarkers of oxidative stress in healthy men and women. American College of Sports Medicine Annual Meeting. Denver, Colorado, USA, 2011. [Poster presentation].
13. **Trepanowski JF**, Fisher-Wellman KH, Bloomer RJ. Impact of exercise training status on postprandial oxidative stress. American College of Sports Medicine Annual Meeting. Baltimore, Maryland, USA, 2010. [Poster presentation].

ABSTRACTS - CONTINUED

14. Canale RE, Kabir MM, Marshall KE, **Trepanowski JF**, Williams SA, Farney TM, Hammond KG, Bloomer RJ. Impact of the Daniel Fast on risk factors for metabolic and cardiovascular disease. American College of Sports Medicine Annual Meeting. Baltimore, Maryland, USA, 2010. [Poster presentation].
15. Weiss LW, Ferreira LC, Schilling BK, Hammond KG, **Trepanowski JF**, Landrum D, Grindle M. Comparative force outputs from non-centered accelerometers during loaded barbell jump squats. National Strength and Conditioning Association 2009 Annual Meeting. Las Vegas, Nevada, USA, 2009. [Poster presentation].
16. Weiss LW, Ferreira LC, Schilling BK, Hammond KG, **Trepanowski JF**, Landrum D, Grindle M. Validity of accelerometer-derived peak force output during loaded jump squats. National Strength and Conditioning Association 2009 Annual Meeting. Las Vegas, Nevada, USA, 2009. [Poster presentation].