This thesis is dedicated to my family, especially my very patient husband. Their encouragement through my current academic journey has been unwavering and they have always advocated for me to reach higher goals. I would also like to dedicate this thesis to one outstanding high school biology teacher who exemplifies the type of educator I would like to be. Bruce Buysee ignited my interest in exercise and nutrition with his passion for health sciences. I am truly grateful for all of these special people, and words cannot fully express my appreciation.
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KKH
CONTRIBUTION OF AUTHORS

Chapter 1 introduces the thesis questions and provides a framework for the novelty and significance of the following work. Chapter 2 is a literature review highlighting the relevant works in the area of alternate day fasting, meal timing, meal frequency, and appetite hormone alterations.

Chapter 3 represents the first published manuscript (Hoddy KK, Kroeger CM, Trepanowski JF, Barnosky A, Bhutani S, Varady KA. Meal timing during alternate day fasting: Impact on body weight and cardiovascular disease risk in obese adults. Obesity (Silver Spring). 2014;22:2524-31) from this study. As first author, I designed the experiment, ran the clinical trial, analyzed the data, and wrote the manuscript under the guidance of my doctoral advisor, Krista A. Varady. Cynthia A Kroeger, John F. Trepanowski, Adrienne Barnosky, and Surabhi Bhutani assisted with the conduction of the clinical trial and performed some of the lab analyses. Chapter 4 represents the second published manuscript (Hoddy KK, Kroeger CM, Trepanowski JF, Barnosky AR, Bhutani S, Varady KA. Safety of alternate day fasting and effect on disordered eating behaviors. Nutr J. 2015;14:44.) from this work. As first author, I designed the experiment, ran the clinical trial, analyzed the data, and wrote the manuscript under the guidance of my doctoral advisor, Krista A. Varady. Cynthia A Kroeger, John F. Trepanowski, Adrienne Barnosky, and Surabhi Bhutani assisted with the conduction of the clinical trial and performed some of the lab analyses. Chapter 5 represented a third published manuscript (Hoddy KK, Gibbons C, Kroeger CM, Trepanowski JF, Barnosky A, Bhutani S, Gabel K, Finlayson G, Varady KA. Changes in hunger and fullness in relation to gut peptides before and after 8 weeks of alternate day fasting. Clin Nutr. 2016 Mar 30. [Epub ahead of print]). As first author, I designed the experiment, ran the clinical trial, analyzed the data, and wrote the manuscript under the guidance of my doctoral advisor, Krista A. Varady. Catherine Gibbons and Graham Finlayson provided expert insight surrounding the analysis of
CONTRIBUTION OF AUTHORS (continued)

appetite hormone data and they assisted in writing the manuscript. Cynthia A Kroeger, John F. Trepanowski, Adrienne Barnosky, Surabhi Bhutani, and Kelsey Gabel assisted with the conduction of the clinical trial and performed some of the lab analyses. Chapter 6 summarizes this work in a discussion. Chapter 7 poses areas of interest in which future studies could expand upon. Chapter 8 ends with an all-encompassing conclusion highlighting clinical outcomes for reducing coronary heart disease risk factors.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1. Background and Rationale......</td>
<td>1</td>
</tr>
<tr>
<td>2. Specific Aims......</td>
<td>3</td>
</tr>
<tr>
<td>3. Significance......</td>
<td>5</td>
</tr>
<tr>
<td>4. Innovation......</td>
<td>5</td>
</tr>
<tr>
<td>II. LITERATURE REVIEW</td>
<td>6</td>
</tr>
<tr>
<td>1. Obesity and cardiometabolic disease risk</td>
<td>6</td>
</tr>
<tr>
<td>2. Effect of alternate day fasting on body weight and body composition</td>
<td>7</td>
</tr>
<tr>
<td>2.1 Effect of alternate day fasting on body weight</td>
<td>7</td>
</tr>
<tr>
<td>2.2 Effect of alternate day fasting on body composition</td>
<td>8</td>
</tr>
<tr>
<td>2.2.1 Fat mass</td>
<td>8</td>
</tr>
<tr>
<td>2.2.2 Fat free mass</td>
<td>8</td>
</tr>
<tr>
<td>2.2.3 Visceral fat mass</td>
<td>9</td>
</tr>
<tr>
<td>3. Effect of alternate day fasting on coronary heart disease risk factors</td>
<td>9</td>
</tr>
<tr>
<td>3.1 Total cholesterol</td>
<td>9</td>
</tr>
<tr>
<td>3.2 Low density lipoprotein cholesterol</td>
<td>9</td>
</tr>
<tr>
<td>3.3 High density lipoprotein cholesterol</td>
<td>10</td>
</tr>
<tr>
<td>3.4 Triglycerides</td>
<td>11</td>
</tr>
<tr>
<td>3.5 Blood pressure and heart rate</td>
<td>11</td>
</tr>
<tr>
<td>4. Effect of alternate day fasting on glucose, insulin, insulin resistance</td>
<td>12</td>
</tr>
<tr>
<td>5. Effect of meal-timing and meal frequency on body weight, coronary heart disease risk, and glucose regulation</td>
<td>12</td>
</tr>
<tr>
<td>5.1 Effect of meal frequency on body weight and body composition under energy balance</td>
<td>13</td>
</tr>
<tr>
<td>5.2 Effect of meal frequency on body weight and body composition under calorie restriction</td>
<td>15</td>
</tr>
<tr>
<td>5.3 Summary of meal frequency effects on body weight and body composition</td>
<td>16</td>
</tr>
<tr>
<td>5.4 Relationship between meal timing and body weight and composition</td>
<td>16</td>
</tr>
<tr>
<td>5.5 Effect of meal timing on body weight and body composition under energy balance</td>
<td>17</td>
</tr>
<tr>
<td>5.6 Effect of meal timing on body weight and body composition under calorie restriction</td>
<td>18</td>
</tr>
<tr>
<td>5.7 Summary of meal timing effect on body weight and body composition</td>
<td>19</td>
</tr>
<tr>
<td>6. Effects of meal-timing and meal frequency on plasma lipids and blood pressure</td>
<td>19</td>
</tr>
<tr>
<td>6.1 Acute effects of meal frequency and meal timing meal timing on plasma lipids</td>
<td>19</td>
</tr>
<tr>
<td>6.2 Short-term and moderate-term effects of meal frequency on plasma lipids</td>
<td>20</td>
</tr>
<tr>
<td>6.2.1 Short-term and moderate-term effects of meal frequency on plasma lipids under energy balance</td>
<td>20</td>
</tr>
</tbody>
</table>
TABLE OF CONTENTS (continued)

6.2.2 Short-term and moderate-term effects of meal frequency on plasma lipids under calorie restriction ............................................ 21
6.3 Short-term and moderate-term effects of meal timing on plasma lipids .......................................................... 22
6.3.1 Short-term and moderate-term effects of meal timing on plasma lipids under energy balance .................................................. 22
6.3.2 Short-term and moderate-term effects of meal timing on plasma lipids under calorie restriction .................................................. 22
6.4 Effects of meal frequency and meal timing on blood pressure ....................................................................................... 23
6.5 Summary of the effect of meal frequency and meal timing on coronary heart disease risk factors ................................................................. 24

7. **Meal frequency and meal timing’s effect on glucose regulation** ......................................................................................... 24
7.1 Acute effects of meal timing and meal frequency on glucose regulation .......................................................... 25
7.1.1 Acute effects of meal frequency on glucose regulation .................................................................................. 25
7.1.2 Acute effects of meal timing on glucose regulation .................................................................................. 25
7.2 Short-term and moderate-term effects of meal frequency on glucose regulation .......................................................... 26
7.2.1 Short-term and moderate-term effects of meal frequency on glucose regulation under energy balance .......................................................... 26
7.2.2 Short-term and moderate-term effects of meal frequency on glucose regulation under calorie restriction .................................................. 27
7.3 Short-term and moderate-term effects of meal timing on glucose regulation .......................................................... 28
7.3.1 Short-term and moderate-term effects of meal timing on glucose regulation under energy balance .......................................................... 28
7.3.2 Short-term and moderate-term effects of meal timing on glucose regulation under CR ........................................................................ 29
7.4 Summary of the effect of meal frequency and meal timing on glucose regulation .......................................................... 29

8. **Role of appetite hormones in regulating body weight** ................................................................................................. 30
8.1 Background of peptide tyrosine tyrosine, glucagon-like peptide 1, and ghrelin function .......................................................... 30
8.2 Relationship between appetite hormones and body weight status .............................................................................. 31
8.2.1 Relationship between ghrelin and body weight status .................................................................................. 31
8.2.2 Relationship between peptide tyrosine tyrosine and body weight status .......................................................... 33
8.2.3 Relationship between glucagon-like peptide 1 and body weight status .......................................................... 35
8.3 Effect of weight loss on appetite hormones .................................................................................................................. 36

9. **Safety concerns during alternate day fasting** .................................................................................................................. 38
TABLE OF CONTENTS (continued)

APPENDICES

<table>
<thead>
<tr>
<th>Appendix</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>103</td>
</tr>
<tr>
<td>B</td>
<td>103</td>
</tr>
<tr>
<td>C</td>
<td>110</td>
</tr>
<tr>
<td>D</td>
<td>111</td>
</tr>
<tr>
<td>E</td>
<td>112</td>
</tr>
<tr>
<td>F</td>
<td>113</td>
</tr>
<tr>
<td>G</td>
<td>114</td>
</tr>
<tr>
<td>H</td>
<td>115</td>
</tr>
<tr>
<td>I</td>
<td>117</td>
</tr>
<tr>
<td>J</td>
<td>122</td>
</tr>
<tr>
<td>K</td>
<td>123</td>
</tr>
</tbody>
</table>

CITED LITERATURE

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>125</td>
</tr>
</tbody>
</table>

VITA

<table>
<thead>
<tr>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>142</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. NUTRIENT COMPOSITION OF THE PROVIDED FAST DAY MEALS</td>
<td>48</td>
</tr>
<tr>
<td>II. SUBJECT CHARACTERISTICS AT BASELINE</td>
<td>53</td>
</tr>
<tr>
<td>III. CHANGES IN PLASMA LIPIDS AND LOW DENSITY LIPOPROTEIN PARTICLE SIZE DURING THE WEIGHT LOSS PERIOD</td>
<td>56</td>
</tr>
<tr>
<td>IV. CHANGES IN METABOLIC DISEASE RISK FACTORS DURING THE WEIGHT LOSS PERIOD</td>
<td>58</td>
</tr>
<tr>
<td>V. ADVERSE EVENTS REPORTED WITH 8 WEEKS OF ALTERNATE DAY FASTING</td>
<td>69</td>
</tr>
<tr>
<td>VI. CHANGES IN EATING DISORDER SYMPTOMS AND BODY IMAGE PERCEPTION AFTER 8 WEEKS</td>
<td>70</td>
</tr>
<tr>
<td>VII. CHANGES IN BODY COMPOSITION AND METABOLIC PARAMETERS OVER THE COURSE OF THE TRIAL</td>
<td>82</td>
</tr>
<tr>
<td>VIII. COMPARISON OF BASELINE CHARACTERISTICS OF COMPLETERS COMPARED TO NONCOMPLETERS</td>
<td>92</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>DESCRIPTION</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Study flow chart</td>
<td>45</td>
</tr>
<tr>
<td>2.</td>
<td>Changes in body weight and body composition during the weight loss period</td>
<td>55</td>
</tr>
<tr>
<td>3.</td>
<td>Changes in body weight over the course of the trial</td>
<td>81</td>
</tr>
<tr>
<td>4.</td>
<td>Fasting and postprandial levels of hunger and fullness before and after the intervention</td>
<td>84</td>
</tr>
<tr>
<td>5.</td>
<td>Fasting and postprandial levels of ghrelin, glucagon-like peptide-1, and peptide tyrosine</td>
<td>85</td>
</tr>
<tr>
<td>6.</td>
<td>Weight loss response after 8 weeks of alternate day fasting</td>
<td>94</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

ADF  Alternate day modified fasting
ADF-L Alternate day modified fasting with the fast day meal at lunch
ADF-D Alternate day modified fasting with the fast day meal at dinner
ADF-SM Alternate day modified fasting with the fast day meal throughout the day
ANOVA Analysis of variance
BMI Body mass index
BSQ Body Shape Questionnaire
CHD Coronary heart disease
CR Calorie restriction
CVD Cardiovascular disease
HDL High density lipoprotein
GLP-1 Glucagon-like peptide-1
LDL Low density lipoprotein
MF Meal Frequency
MT Meal Timing
MEADS Multidimensional Assessment of Eating-Disorder Symptoms
PYY Peptide tyrosine tyrosine
RMR Resting metabolic rate
SEM Standard error of mean
TFEQ Three factor eating questionnaire
VAS Visual analogue scale
SUMMARY

This randomized control trial in obese subjects investigates the effect of three different meal-time protocols during alternate day fasting (ADF) on measures of body composition and coronary heart disease risk factors. The study lasted 10 weeks and consisted of a two-week baseline period and followed by 8 weeks of ADF. Subjects were randomized to have their fast day meal either as lunch (ADF-L) dinner (ADF-D), or split into small meals throughout the day (ADF-SM). The fast day meal was provided to each subject on a weekly basis. Body weight, waist circumference, blood pressure, and heart rate were assessed weekly. Plasma lipids, glucose, insulin, leptin, body composition, and eating disorder symptoms were assessed at weeks 1, 3, and 10. A meal challenge was conducted at weeks 3 and 10. During this challenge, a liquid shake was provided and blood was drawn at intervals up to two hours to assess appetite hormones.

Participant adherence was high in all groups during the study. This is reflected by similar weight loss and body composition improvements across all three meal-timing protocols. There was no change observed total cholesterol, LDL cholesterol, HDL cholesterol, or triglycerides after 8 weeks of dieting. Improvements in LDL particle size occurred in all of the groups. This was marked by an increase in the proportion of large LDL particles in all intervention groups, and a decrease in the proportion of small LDL particles in only the ADF-D and ADF-SM groups. This shift in LDL particle size represents a reduction of atherosclerotic risk. Only the ADF-SM group experienced a decrease in systolic blood pressure, and only the ADF-L group reported a decrease in heart rate. Gluoregulatory factors, glucose, insulin, and insulin sensitivity, were unaffected. Surveying for adverse effects and eating disorder symptoms showed ADF to be a safe means for weight loss in the obese with only minor complaints.
SUMMARY (continued)

Lastly, findings from the meal challenge indicate a unique effect on appetite after 8 weeks of ADF.

Results show an increase in subjective fullness and increased plasma PYY along with unchanged subjective reports of hunger and a slight increase in ghrelin.
I. INTRODUCTION

1. Background and Rationale

Obesity increases an individual’s risk of coronary heart disease (CHD). (1) Excess fat is associated with dyslipidemia and hypertension. (1) A 5% weight loss has been shown to diminish deleterious consequences of obesity. (1) Alternate day fasting (ADF) is a novel diet regimen for weight loss. The diet consists of an alternating pattern of an ad libitum “feed day” followed by a “fast day”, the latter consisting of a day of eating 25% of energy needs (~450-600 kcal/d). (2) Recent human research reports that ADF decreases body weight by 5-8% in 8-12 weeks. (2) (3) These decreases in body weight were also accompanied by decreases in LDL cholesterol and blood pressure, and increases in LDL particle size. (2)

Adherence is essential to any weight loss protocol. At present, all studies of ADF have required that subjects consume the fast day meal at lunchtime (12.00-2.00pm). (2) However, some individuals may find it easier to adhere to the ADF regimen if the fast day meal was pushed back to dinnertime (6.00-8.00pm). This change would permit subjects to eat with their families at night on the fast day, which was not possible with the previous lunchtime regimen. Another option that may improve adherence with ADF would be to allow the fast day meal (~450-600 kcal) to be consumed as smaller meals at breakfast, lunch and dinner (150-200 kcal at each meal). This may help individuals to better cope with the hunger they may experience throughout the fast day. A comparison of the standard lunchtime meal (ADF-L) to a dinnertime meal (ADF-D) or three small meals throughout the day (ADF-SM) on dietary adherence, weight loss, and CHD risk reduction, has yet to be performed.
ADF is effective for weight loss. However, the safety of ADF has not yet been evaluated and apprehensions exist. In particular, concerns have been raised regarding gastrointestinal disturbances, sleep issues, and problems with energy levels (that may be related to lower blood sugar levels). ADF has also been criticized for potentially increasing disordered eating behaviours and negatively impacting body image perception. Whether or not ADF produces adverse events, changes in disordered eating symptoms, and alterations in body image perception, has yet to be tested.

The effect of ADF on appetite hormones also has yet to be elucidated. We have previously demonstrated that hunger decreases and fullness increases after two weeks of ADF (measured by hunger questionnaires). Whether this psychological effect is mimicked by a physiological effect (i.e. plasma levels of appetite hormones), remains unclear. For instance, ghrelin is a peptide released by the gastrointestinal tract that acts on hypothalamic cells to increase hunger. In contrast, peptide YY (PYY) and glucagon-like peptide-1 (GLP-1) are released by the small intestine and act to reduce appetite and increase fullness. Whether ADF is able to decrease ghrelin and increase PYY and GLP-1 in a way that promotes satiety, is an important question that has yet to be investigated. Thus, the specific aims of this study are:
2. **Specific Aims**

**Specific Aim 1:** To establish that the ADF-D and ADF-SM group will be more adherent to the diet, and experience greater weight loss and more favorable improvements in body composition, when compared to the ADF-L group.

**Hypothesis 1:** The ADF-D and ADF-SM group will have higher adherence, lower body weight, lower fat mass, lower visceral fat mass, and greater retention of lean mass, when compared to the ADF-L group after the 8-week intervention.

**Specific Aim 2:** To establish that the ADF-D and ADF-SM group will produce the most optimal shifts in CHD risk factors when compared to the ADF-L group, due to greater weight loss.

**Hypothesis 2:** The ADF-D and ADF-SM group will experience greater improvements in plasma lipid profile (i.e. decreases in total cholesterol, LDL cholesterol and triglycerides), and greater increases in LDL particle size, when compared to the ADF-L group after the 8-week intervention.

**Specific Aim 3:** To establish that the ADF-L, ADF-D and ADF-SM are safe diet therapies with no negative effects on eating disorder symptoms or body image.

**Hypothesis 3:** Subjects in the ADF-L, ADF-D and ADF-SM will experience minimal adverse events, and will have no negative impact on eating disorder symptoms or body image.
Specific Aim 4: To establish that the ADF-D and ADF-SM group will produce greater improvements in circulating appetite hormones when compared to the ADF-L group.

Hypothesis 4: The ADF-D and ADF-SM group will experience greater increases in circulating GLP-1 and PYY, and greater decreases in ghrelin levels, when compared to the ADF-L group after the 8-week intervention.
3. **Significance**

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

1. Improve clinical recommendations for ADF by showing that ADF-D and ADF-SM are more effective protocols than the original ADF-L regimen for reducing body weight and producing better CHD risk reduction in the obese population.
2. Further the field’s understanding of appetite hormones and appetite response after weight loss diets, such as, ADF.
3. Report the safety of ADF with regards to eating disorders, body image, and adverse events.

4. **Innovation**

This study will be the first study comparing three different fast day regimens during ADF to determine outcomes related to compliance, weight loss, and CHD risk reduction. Additionally, this study will be the first study to objectively evaluate appetite and investigate adverse outcomes related to ADF. In sum, the proposed study is innovative in that it will be the first to:

1. Directly compare the efficacy of alternative meal times and frequency during the fast day (ADF-L, ADF-D, ADF-SM) for weight loss and reduction of cardiometabolic risk.
2. Elucidate previous anecdotal reports of appetite alterations with objective evaluations of appetite hormones during a meal challenge.
3. Document changes in eating disorder symptoms, body shape perceptions, and side effects after the diet.
II. LITERATURE REVIEW

1. Obesity and cardiometabolic disease risk

The prevalence of obesity in the United States is approximately 35% according to a NHANES analysis. (4) This poses an urgent health concern considering excess adiposity is associated with chronic disease risk factors, such as, increased blood pressure, elevated glucose, dyslipidemia, and insulin resistance. (5) It has been shown that for every unit increase in BMI, the risk of eventual heart failure increases by 5% for males and 7% for females. (6) Interventions resulting in weight reductions as little as 2-5% can decrease chronic disease risk factors. (7) Additionally, active weight loss has been shown to improve blood lipids. (8) Therefore, lifestyle interventions to reduce adiposity and BMI are indicated to reduce and prevent the deleterious effects of obesity.

Calorie restriction (CR) of 15-40% is a common method for reducing body weight. (9) Alternate day fasting (ADF) is one particular form of CR that allows for greater dietary flexibility to the dieter. Typically, this diet consists of a “modified fast” day in which calories are restricted by 75% and a “feed” day in which at least 100% of calories needs are allowed. (2) ADF appears to improve chronic disease risk factors related to CHD and type 2 diabetes. (9, 10) Alterations to the standard ADF protocol have also been shown to be effective. For example, Klempel et al., 2013 (3) showed that a high fat ADF regimen, similar to a westernized diet, could reduce weight and CHD disease risk to a similar extent as a low fat diet. Eshghinia et al., 2013 (11) altered the ADF regimen to have only three modified fast days a week instead of 3-4 fast day due to an alternating fasting pattern, and noted significant weight loss and cardiometabolic risk reduction. The current ADF protocol calls for one fast day meal at lunchtime. No trials have examined the effectiveness of altered meal times and frequencies within an ADF
regimen. Understanding the potential for modifications to the standard ADF protocol is indicated to determine appropriate diet recommendations. The literature on ADF will be reviewed here.

2. **Effect of alternate day fasting on body weight and body composition**

2.1 **Effect of alternate day fasting on body weight**

Eight ADF studies have examined changes in body weight. Two early ADF trials conducted an ADF protocol with 100% energy restriction on the fast day alternated with compensatory calories on the feed day in normal weight subjects. Halberg et al., 2005 (12) observed no change in body weight in males after ADF for two weeks. Conversely, Heilbronn et al., 2005 (13) conducted a similar ADF regimen over three weeks in men and women and showed an unintentional 3% weight loss. Interestingly, weight loss was inversely associated with self-reports of being “big eaters”, with male subjects commonly reporting this response. (13) Early ADF participants also reported irritability on fast days. (13)

Subsequent ADF studies evaluated a modified fast ranging from 70-80% energy restriction on fast days. High adherence of about 90% has been reported in several studies (2, 14, 15) without a hyperphagic response. (16) Short-term studies ranging from 6-8 weeks report favorable weight loss findings. Johnson et al., observed the greatest weight loss over 8 weeks at 8%. (17) This could be due to an 80% restriction on the fast day. (17) Other studies with a 75% fast day restriction and similar duration have shown a 4-7% reduction in weight. (2, 11, 14) Interestingly, there appears to be no difference in weight loss between a high fat ADF diet versus the standard low fat ADF diet. (14) Studies lasting as long as 12 weeks further support ADF’s effectiveness for weight loss. Varady et al., 2011 (18) showed a moderate weight loss of 5% when overweight and obese subjects followed ADF. Bhutani et al., 2013 (19)
reported the lowest weight loss of 3% in obese participants. However, this could be due to the large attrition rate and a diminished effect due to intention to treat analysis. (19) ADF was also shown to be effective in a normal and overweight subject population when body weight decreasing by 7%. (15) Data from our lab reports the longest study (24 weeks) to date. (20) An 8% reduction in body weight was observed and did not differ significantly from the group assigned to CR. (20) As such, extended exposure to ADF does not seem to lead to greater weight loss. Overall, weight loss after ADF ranges from 3-8% over 6-24 weeks.

### 2.2 Effect of alternate day fasting on body composition

#### 2.2.1 Fat mass

The preliminary 3-week study by Heilbronn et al., 2005 reported a small 1% reduction in fat mass without intentional energy restriction. (13) However, weight loss studies lasting 6-8 weeks have consistently shown larger reductions of fat mass from 10-12% after ADF. (2) (11) (14) Similarly, interventions extending 12-24 weeks show a 12-14% reduction in fat mass. (15) (20) Bhutani et al., 2013 (19) is the exception with an intention to treat analysis showing only a 4% decrease in fat mass.

#### 2.2.2 Fat free mass

There are mixed findings related to fat-free mass after ADF. For example, the shortest ADF intervention resulted in a 5% fat-free mass reduction when measured by DXA (13). Conversely, the longest study lasting 24 weeks showed no significant change in fat free mass measured by DXA. (20) Trepanowski et al., 2016(20) Klempel et al., 2013(14) and Varady et al., 2013(15) lasted 8 and 12 weeks, respectively, and also showed no change in fat free mass when measured by DXA. Additionally, one study using BIA showed a significant 2% reduction in fat free mass (19) and another showed no change. (2)
2.2.3 Visceral fat mass

The magnitude of visceral fat reduction may depend on the type of measurement technique. Studies indirectly measuring visceral fat via waist circumference (WC) have shown a 5-7% reduction (11) (14) (19). Interestingly the effect was augmented by endurance exercise and just as effective when following a high fat diet. (14)(19). Visceral fat measured by MRI showed a greater improvement with a 16% reduction after 24 weeks. (20). Therefore, it is possible that longer ADF exposure or adjunct interventions may lead to greater modulations of visceral fat.

3. Effect of alternate day fasting on coronary heart disease risk factors

3.1 Total cholesterol

Improvements in CHD risk factors are known to improve with weight loss. Four modified ADF studies show a decrease in total cholesterol of 10-21% over 8-12 weeks.(14) Varady et al., 2011 (21) reported the largest total cholesterol decrease of 21%. Why this study showed superior results is uncertain, as weight loss (6%) was comparable to other studies.(21) Additionally, modified ADF with a high fat diet elicited similar improvements in total cholesterol as modified ADF that was low in fat. (14) Despite these observations several studies showed no change in total cholesterol (18) (19) (11) (20).

3.2 Low density lipoprotein cholesterol

Similar to total cholesterol, LDL cholesterol has been shown to decrease 10-25% over 8-12 weeks of modified ADF.(21) (18) (15) Interestingly, Varady et al., 2011 (18) showed a 10% decrease in LDL cholesterol even without a decrease in total cholesterol. However, similar studies also show no effect on LDL cholesterol. (11) (17) (19) (20) All studies reporting LDL cholesterol particle size have shown a 1-2% improvement notwithstanding inconsistent changes in LDL cholesterol. (14, 15, 18) (19) (21) Additionally, the improvement in particle size seems to represent a shift in the proportion of
particle size. For example, Varady et al., 2011 (21) showed that 8 weeks of ADF resulted in an increase in the proportion of large particles with a decrease in both medium and small particles. In a 12-week study by Varady et al., 2011 (18) this pattern of particle redistribution was also observed in ADF but not in the comparable CR group despite similar improvements in overall LDL particle size. Klempel et al., 2013 (14) also showed that these beneficial improvements in LDL cholesterol parameters are conserved whether following a high fat of low fat ADF diet. Overall, findings related to LDL concentrations are inconsistent. However, the improvement seen with LDL particle size represents a beneficial alteration in blood lipids.

3.3 High density lipoprotein cholesterol

HDL cholesterol does not typically improve with diet alone. However, three ADF studies reported HDL improvements without exercise. Helibronn et al., 2005 (13), which utilized a complete fast, showed an increase in HDL in only women after 3 weeks of dieting without any weight loss. Conversely, Klempel et al., 2013 (14) showed no change in HDL in an entirely female study population. Johnson et al., 2007 (17) reported a 9% HDL increase in males and females after an 80% fast day regimen for 8 weeks. After 24 weeks, Trepanowski et al., (20) 2016 reported an 8% increase in HDL in males and females. The greatest improvement in HDL cholesterol (18%) was observed in Bhutani et al., 2013 (19) when a 75% ADF was combined a with endurance exercise. Of particular interest, the combined diet and exercise group out-performed the exercise only group. (19) While most studies indicate no improvement in HDL, some ADF studies show promise in decreasing cardiovascular risk factors by increasing HDL cholesterol.
3.4 **Triglycerides**

Triglyceride response to ADF varies widely from no significant effect to decreases of 14-40%. (11, 13, 14, 15, 17, 18, 19, 20, 21) Johnson et al., 2007(17) showed a 40% decrease in triglycerides. Participants in this study experienced a large weight loss (8%) across 8 weeks of ADF dieting and had high triglycerides at baseline (279 mg/dL). (17) Several studies enrolling overweight subjects showed no effect on triglyceride concentrations after 6-24 weeks of modified ADF. (11) (15) (20) It is possible that a certain threshold is necessary for an appreciable effect in triglyceride concentrations to be observed. Heilbronn et al., 2005 showed a decrease in triglyceride concentrations after three weeks of ADF with a complete fast without weight loss in only males with no change in females. (13) Most ADF studies have a high female enrollment. Therefore, an uneven distribution of sex could alter overall findings if triglyceride outcomes are sex specific.

3.5 **Blood pressure and heart rate**

A decrease of 3-8% in systolic blood pressure has been noted in four ADF studies. (2, 11, 19) Only three studies have shown a 2-10% decrease in diastolic blood pressure. (19) Interestingly, the inclusion of exercise by Bhutani et al., 2013 (19) did not enhance the effect of the diet on systolic blood pressure or diastolic blood pressure. Previously, the sample populations assessed have been relatively normotensive. To date, studies have not evaluated the effectiveness of ADF in a hypertensive only population or the have included individuals on anti-hypertensive pharmaceuticals in samples comingled with non-hypertensive subjects. Therefore, there could be a threshold of effectiveness that is not well represented by the available subjects. Heart rate does not typically change after ADF. Only one study reported a 5% decrease after 8 weeks of the diet and about 5% weight loss. (2) At this time, it is not known why this study resulted in the only decrease in heart rate. Overall, it would appear that
non-lipid CHD risk factors are less responsive to ADF intervention in mostly healthy overweight and obese subjects.

4. **Effect of alternate day fasting on glucose, insulin, insulin resistance**

Weight loss has been shown to decrease diabetes risk by reducing fasting glucose and insulin concentrations and improving insulin sensitivity. Short-term ADF studies do not show a change in glucose regulation after 6-12 weeks of ADF. (11, 17, 19) There may, however, be an acute effect on glucose regulation on the fast day. Both Halberg et al., 2005 (12) and Heilbronn et al, 2005 (13) reported a large decrease in insulin following a fast day by 50% and 57%, respectively. Halberg et al., 2005 (12) also reported a 9% decrease in glucose on the fast day. Insulin sensitivity assessed by HOMA has not shown any improvement. However, more robust measurements of insulin sensitivity, such as euglycemic clamp, have shown a 15% improvement in insulin sensitivity. (12) As such, fasting may have an acute effect that could culminate into metabolic improvements in interventions lasting greater than 12 weeks. This was demonstrated by Varady et al., 2016 (20) when ADF for 24 weeks resulted in a 40% decrease in both insulin and HOMA.

5. **Effect of meal-timing and meal frequency on body weight, coronary heart disease risk, and glucose regulation**

Data from NHANES reports approximately 80% of Americans eat an average of five times a day. (22). Popular health advice promotes the practice of eating smaller meals 2-3 hours apart resulting in 4-6 daily eating episodes. (22) Increasing meal frequency has been trending over the past 40 years, and snacking specifically represents the largest component to this increase. (23)
Observational studies have suggested that increased meal frequency could reduce body weight and improve body composition while also improving risk factors of chronic disease. (24, 25), (26) However, McCrory et al., 2011 (23) later showed the relationship between weight and meal frequency to be a byproduct of underreporting, specifically in obese or dieting subjects. It is well known that overall energy intake is the primary driver for obesity and chronic disease. Therefore, increased meal frequency potentially poses a concern when coupling both the availability of high energy density foods with increased eating occasions. The culmination of these factors could explain the approximate 700 kcal increase in energy intake since the 1950’s. (27, 28)

A potential consequence of increased meal frequency is the 23% decrease in duration between meals. (28) Reasonably, increased meal frequency has the potential to prolong feeding times and decrease intermittent fasts throughout the day. Meal timing influenced by skipping breakfast or consuming late meals has also been associated with increased obesity and chronic disease risk. (22, 25), (26), (29) As such, controversy has been raised in light of both meal frequency and timing in regards to obesity and health.

5.1 Effect of meal frequency on body weight and body composition under energy balance

Several research studies have examined the impact of meal frequency on body weight and composition under isocaloric conditions. These studies ranged from 2 to 8 weeks and involved 1 to 17 eating episodes. (30, 31, 32, 33, 34, 35, 36) Studies performed by Jenkins et al., 1989(33) and Jenkins et al., 1995 (36) represent the most extreme difference in meal frequencies (3 vs 17 meals). Neither study showed a difference between the meal conditions for overall body weight after 2 weeks in lean participants. (33, 36) Studies lasting 4 weeks show conflicting findings related to body weight. (31, 32,
King et al., 1999 (34) noted a significant decrease in body weight (2%) when participants were assigned to reduce their typically meal frequency from 5 meals per day to 3 meals per day over 4 weeks, and a time by treatment effect was reported for body weight. (34) Diets were self-selected, and weight loss was not intended. (34) It can be difficult to draw conclusions from this trial as there were 4 treatment groups with varying meal frequencies and fat content, and the authors did not note specific between group differences. Interventions with a reduction in body weight also reported an 11%-22% decrease in energy consumption compared to energy needs. (34) There was no body weight or energy intake change in either of the 5 meal conditions. (34) Therefore it is difficult to isolate the effect of meal frequency alone. Chapelot et al., 2006 (32) reported no difference between or within groups when evaluating 3 meals compared to 4 meals for body weight or body composition. There was no change in body weight and body fat percentage was increased significantly by 3% in the 3-meal condition with no change noted in the 4-meal condition. (32) The meal frequency groups only differed by one meal, this could explain why there was no significant difference between groups over the short time period. A studies by Arciero et al., 2013 (31) and Arnold et al., 1994., (35) investigated a wider range in meal frequencies over 4 weeks and also noted no significant difference in body weight or composition. Arciero et al., 2013 (31) reported beneficial alterations in body composition in both groups; however, this is most likely due to the high protein diet that both groups were placed on. A pilot study reported by Stote et al., 2007 (30) and Carlson et al., 2007 (37) investigated the effects of either one-meal per day or three-meals per day without calorie restriction over 8 weeks. Body weight and fat mass were reduced only in the 1-meal condition by 1% and 12%, respectively. (30) However, body composition was measured by BIA, and the time of day of post-intervention measurements differed between groups. Therefore a diurnal shift in body water could have affected the outcome.
5.2 **Effect of meal frequency on body weight and body composition under calorie restriction**

Studies evaluating meal frequency under caloric restriction are difficult to compare to each other due to differing frequencies, energy distributions, macronutrient distributions, and intervention durations. Several studies have compared three eating occasions to 6 eating occasions as either 3 meals versus 6 meals (31) or 3 meals versus 3 meals and 3 snacks. (38) (39) (40). Arciero et al., 2013 (31) evaluated a high protein diet under 25% CR over 4 weeks as either 3 meals or 6 meals. Overall, both the 3-meal and 6-meal conditions lost a similar amount of body weight (4% and 3%, respectively), fat mass (9% for both), and visceral fat (11% and 16%, respectively). (31) The 6-meal condition seemed to favor lean mass retention (-1%) compared to the 3-meal condition (-2%). (31) This could also be a consequence of the high protein diet dispersed more evenly throughout the day under CR. Cameron et al., compared three meals to three meals with three snacks over 8 weeks. Both groups were restricted by 700 kcal and similar decreased in bodyweight (5% and 4%, respectively), fat mass (8% and 7%, respectively), and lean mass (4% and 3%, respectively) mass similarly. (38) CR was not based on a percentage of individual energy needs, thus an arbitrary decrease by 700 kcal could create a discrepancy if relative calorie restriction was compared. CR studies with 3 meals compared to 3 meals and 3 snacks lasting 24 (39) and 52 weeks (40) also showed no significant difference between meal frequency groups. Schlundt et al., 1992 (41) compared 2 meals and 3 meals with the difference in meal frequency due to the presence or absence of breakfast. Both groups reduced body weight and improved body composition. (41) There was a trend for greater weight loss in the breakfast consuming (3 meals per day) group. (41) The authors commented that the most successful participants were assigned to a meal pattern that was different from their typical routine. (41) Poston et al., 2005 (42) assessed body weight response to 2 and 5 meals per day in males and females allowed 1500 kcal/day
and 1200 kcal/day, respectively, at 12 and 24 weeks. Weight loss ranged from 5-6%, and showed no meal frequency advantage. (42) Studies comparing a greater difference in meal frequencies (2 vs 6 meals/day) also do not support one meal frequency over another for weight loss. (43, 44) Except Iwao et al., 1996 (44), which reported a greater retention of absolute lean mass in the 6-meal condition. However, this could be due to a low calorie intake (1200 kcal/day) with simultaneously expending about 3000 kcal/day through exercise. It is possible that under extreme exercise conditions, a steady intake of protein and energy would have been advantageous for lean mass retention.

5.3 **Summary of meal frequency’s effect on body weight and body composition**

Based on the data presented, meal frequency alone or in the presence of CR does not seem to have a large bearing on bodyweight or body composition alterations overtime. There may be an effect under isocaloric conditions in favor of lower meal frequencies. However, this could also be a consequence of meal timing as the one meal was served in the evening. Longer interventions were performed in studies assigning CR. Therefore, it is also possible that no differences between groups under weight loss conditions were observed because the stimulus of energy deficit diminished a potential effect of meal frequency alone.

5.4 **Relationship between meal timing and body weight and body composition**

Humans display an increase in appetite in the evening and a decrease in the morning. (45) Skipping breakfast may be a strategy to reduce overall energy intake. (46) However, both skipping breakfast (47) and having larger evening meals (29) are associated with overweight and obesity. An increased waist circumference has also been associated with breakfast skipping and evening calorie intake. (48) (49) Findings from the National Weight Loss Registry show that 78% of successful dieters
regularly eat breakfast where as 4% regularly skip breakfast. (50) Interestingly, a large prospective study showed a negative association between BMI and energy consumed at breakfast despite an increase in overall energy intake. (51) Late calorie consumption (after 8:00 pm) has been shown to predict obesity even after controlling for sleep time and duration. (52) One weight loss study also showed that early eaters seemed to lose more weight over 20 weeks of dieting compared to late eaters. (53) Several studies on night shift workers and late night eaters also offer compelling evidence against late night eating. (54) (55) (56) However, none of these studies provide causal evidence. As such, it is important to consider the findings from interventional studies when evaluating the effectiveness of meal timing.

**5.5 Effect of meal timing on body weight and body composition under energy balance**

Four of the studies assessed meal timing under energy balance. (57, 58) (59, 60) Dhurandhar et al., 2014 (57) evaluated whether or not recommendations alone to either consume breakfast or not consume breakfast would affect body weight. The education material provided to the subjects was effective with over a 90% breakfast consumption compliance rate; however there was no difference in body weight after 16 weeks between the groups. (57) A crossover study lasting 2 weeks in lean women showed no difference in body weight, body fat percentage, or waist circumference when comparing a breakfast consuming condition compared to a breakfast skipping condition. (58) The authors noted that the participants consumed about 90 kcal more per day when they skipped breakfast during the self-selected eating period later in the day. (58) It is unknown whether or not this increase in energy intake would result in weight gain as the intervention period was of short duration. Hibi et al., 2013, also assessed lean women over 2 weeks and found no difference in body weight between participants asked to consume either a daytime or nighttime snack. (60) However, the difference in daily calorie
distribution may have been too small of a stimulus to elicit an effect over such a short time period. A study lasting 6 weeks found no difference in body weight, body composition, or waist circumference when 700 kcal were consumed as breakfast (before 11:00am, with 50% within 2 hours of waking) or as later in the day (after 12:00pm) (59). Interestingly, the breakfast group had an increased physical activity level and consumed about 500 kcal more than the group that skipped breakfast. (59) Overall, body weight and body composition are unaffected by meal timing alone. It is unclear whether or not humans compensate with additional calories under different meal timing conditions and how this affects long term weight maintenance.

5.6 Effect of meal timing on body weight and body composition under calorie restriction

Four studies assessing meal timing under calorie restriction employed a study design where the distribution of calories was shifted to be earlier or later in the day. (61, 62, 63, 64) Not surprisingly, all of the studies reported significant weight loss (4-16%) and, if assessed, significant losses in fat mass (5-17%), lean mass (1-4%) and waist circumference (3-8%) over 12 to 16 weeks of dieting. (61, 62, 63, 64) Jakubowicz et al., 2012 (63) was the only study to show similar weight loss between early and late feeding groups. However, the macronutrient distribution was also altered during this study to have more carbohydrates and greater calories earlier in the day over 16 weeks. (63) When this study was extended through a weight maintenance period, the early eaters continued to lose weight whereas the late eaters regained some of the lost weight. (63) However, it is difficult to determine whether this is an effect of energy timing, macronutrient timing, or just overall diet satisfaction. Similar to other studies (61) (62), Keim et al., 1997 (64) also showed greater weight loss in the early eating intervention. However, the late eaters showed better retention of lean mass compared to the early eaters (-1% vs -3%, respectively). (64) This study employs a more rigorous inpatient protocol. (64) However, the use of
a cross-over design is flawed under the presence of weight loss. For example, the first 6 weeks of the study showed greater fat mass loss than the second 6 weeks of the study. (64) These studies collectively indicate an advantage of early energy consumption in reducing body weight and body fat in those restricting calories. This body of research is limited, however, by a small number of studies that differ in macronutrient and energy distribution.

5.7 Summary of meal timing effect on body weight and body composition

As with meal frequency, meal timing has little bearing on body weight and body composition alone. There is support for the use of earlier meals for weight loss. However, it is unclear whether there is an ideal time, energy distribution, or macronutrient distribution to use for best results. There is also additional uncertainty regarding lean mass retention that requires further exploration.

6. Effects of meal timing and meal frequency on plasma lipids and blood pressure

Dyslipidemia and hypertension represent cardiovascular risk factors that are modifiable by lifestyle improvements, such as weight loss. However, these factors may also be influenced by the frequency and timing of meals.

6.1 Acute effects of meal timing and meal frequency on plasma lipids

Studies evaluating participants’ response to either 3 or 6 meals over 12 to 48 hours have shown no difference between groups for total cholesterol, LDL cholesterol, or HDL cholesterol. (65, 66) However, Jones et al., 1993 (65) showed that cholesterol synthesis was decreased in the 6-meal condition. Additionally, Heden et al., 2013 (66) reported lower triglyceride concentrations during the 3-meal condition compared to the 6-meal condition. One longer acute study lasting 7 days reported a
greater decrease in total cholesterol and LDL cholesterol when participants switched from consuming 3 meals per day to 6 meals per day compared to decreasing meal frequency from 6 to 3 meals per day. (67) Only one study assessed the effect of meal timing on blood lipids. (68) A protein supplement snack compared to a placebo in the evening had no effect on blood lipid values. (68) However, this does not explain how a mixed macronutrient snack or larger meal would affect lipids.

6.2 Short-term and moderate-term effects of meal frequency on plasma lipids

6.2.1 Short-term and moderate-term effects of meal frequency on plasma lipids

under energy balance

A few studies lasting 2 to 8 weeks support the use of increased meal frequency for the reduction of cholesterol. (30, 33, 36) Two of these studies, Jenkins et al., 1989(33) and Jenkins et al., 1995 (36) were performed as a crossover study evaluating 3 or 17 meals. In congruence with the study from McGrath et al., 1994, both studies showed the higher frequency meal pattern to produce lower total cholesterol and LDL cholesterol levels. (33, 36) Jenkins et al., 1989 reported a 16% and 18% reduction in total cholesterol and LDL cholesterol in the 17-meal condition with no change in the 3-meal condition. (33) No changes in fasting HDL cholesterol or triglycerides were observed, however postprandial triglycerides were lower with a higher meal frequency. (33) Stote et al., 2007(30) conducted an 8 week crossover study and observed no between group differences from baseline to post intervention for lipids. Oddly, the 1-meal condition increased their total cholesterol, LDL, and HDL by 18%, 25%, and 16%, respectively, even with weight loss. (30) However, these results should be interpreted with hesitation due to the study methodology. The post measurement data for the one-meal condition was collected in the afternoon while all other measurements were collected in the morning.(30) Additionally, the designed called for isocaloric conditions, but weight loss was
experienced. (30) An increase in HDL cholesterol was also seen in a 2-week study by Murphy et al., 1996 (69) in the lower meal frequency group (3 meals) compared to the higher meal frequency group (12 meals). However, no other lipid parameters were changed. (69) A study by King et al., 1999 (34) investigated low fat and meal frequency recommendations in only hypercholesterolaemic males noted significant reductions in total cholesterol by 7% and LDL cholesterol by 10% in the group following a low fat and low frequency (3 meals) diet for 4 weeks. (34) The group following a higher meal frequency pattern (5 meals) only experienced decreased total cholesterol by 9% when also following a low fat diet. (34) The authors noted a treatment by time effect for HDL cholesterol, however differences between groups were not specified and there was no significant within group difference observed. (34) However, as with Stote et al., 2007 (30), King et al., 1999 (34) did not intend for weight loss, but noted a 2% decrease in body weight. Therefore, energy restriction and consequential weight loss may be the primary driver behind these improvements in lipids. Another 4-week study in hypercholesterolaemic participants showed no difference between 3 meals and 2 snacks compared to 9 meals. (35) Additional influence from macronutrient distribution, energy deficits, meal timing, and a meal frequency threshold is possible. As such, comparison of these works is difficult because some interventions resulted in weight loss and selected studies are heterogeneous for subject groups and diet protocols.

6.2.2 Short-term and moderate-term effects of meal frequency on plasma lipids under calorie restriction

Several meal frequency studies with caloric restriction collectively show an improvement in lipid profiles with weight loss. (40, 41, 42, 43, 70) However, Poston et al., 2005 (42) and Antoine et al., 1984 (42) imply that these improvements may be due to initial weight loss. Interestingly, two studies of CR showed a similar increase in HDL cholesterol in response to reduced meal frequency as the energy balance studies discussed earlier. (40, 43) However, the findings
reported by Alencar et al., 2015 (43) may not be clinically relevant as the 2-meal and 6-meal conditions showed small HDL increases of 1% and 0.1%, respectively.

6.3 Short-term and moderate-term effects of meal timing on plasma lipids

6.3.1 Short-term and moderate-term effects of meal timing on plasma lipids under energy balance

Three studies assessed the effect of meal timing on lipids under energy balance. (58, 59, 60) Farshchi et al., 2005 (58) demonstrated that skipping breakfast resulted in poor lipid outcomes with an increase in total and LDL cholesterol (6% and 13%, respectively) in typical habitual breakfast eaters over 2 weeks. Hibi et al., 2013 (60) also showed that later eating in the form of a late night snack had deleterious effects on plasma lipids. However, when breakfast skipping was compared to breakfast consuming over 6 weeks in a larger sample size, there was no change in lipids observed. As such, it is possible that fluctuations exist with initial meal timing alterations that do not persist at 6 weeks.

6.3.2 Short-term and moderate-term effects of meal timing on plasma lipids under calorie restriction

Meal timing alterations with calorie restriction findings are equivocal. Jakubowicz et al., 2012 (63) reported no changes to total cholesterol, LDL cholesterol, and HDL cholesterol after 16 weeks. Despite similar weight loss at 16 weeks, triglycerides were lowered by 23% in the groups with calories in the late feeding group with no changes in the early feeding group. (63) This could also be a consequence of the late feeding group also consuming a diet lower in carbohydrates. In contrast, Jakubowicz et al., 2013 (62) reported a 34% decrease in triglycerides in the early feeding group and a 15% increase in the late feeding group. Total cholesterol and HDL cholesterol also improved by about
5% in the breakfast group only. (62) Dieters in Lombardo et al., 2014 (61) experienced similar improvements in total cholesterol, LDL cholesterol, and HDL cholesterol. Similar to Jakubowicz et al., 2013 (62), HDL was significantly higher in the early feeders group. (61)

6.4 Effects of meal timing and meal frequency on blood pressure

Literature reporting the effect of meal frequency and meal timing on blood pressure and heart rate is lacking. Stote et al., 2007 (30) observed lower systolic and diastolic blood pressures after 8 weeks of consuming three-meals compared to one-meal and noted no difference in heart rate. Unfortunately, methodological errors within this study could explain this difference. Post-intervention measurements were not conducted at the same time as baseline and only in the one-meal intervention. Another study showed no difference in blood pressure due to meal frequency. (35) Studies employing a CR and meal frequency intervention noted similar reductions in blood pressure after the meal frequency intervention period. (40, 41, 42). This is most likely due to weight loss alone as the plateau in weight loss observed in Poston et al., 2005 (42) coincided with a plateau in blood pressure. Weight loss may also be a factor in meal timing studies. For example, Lombardo et al., 2014 (61) reported a 7% drop in diastolic blood pressure in only the early eating group. This group also had lower blood pressure values at baseline. The exception to greater weight loss resulting in greater changes in blood pressure was demonstrated by Jakubowicz et al., 2013 (62). Both early and late eating group experienced similar decreases in systolic blood pressure and diastolic blood pressure of about 3-7%, despite greater weight loss in the early eating group. (62)
6.5 Summary of the effect of meal frequency and meal timing on coronary heart disease risk factors

Extreme differences in meal frequency patterns (i.e. 1 vs 17) may influence total cholesterol and LDL concentrations in favor of higher meal frequencies. However, the practicality of very high meal frequencies is questionable and has not been assessed for longer than 2 weeks. Subtle differences in meal frequencies, differing by 1-3 meals per day, may not have a strong influence over total cholesterol and LDL cholesterol. The largest improvements were seen under CR conditions. Lower meal frequencies, conversely, may be better suited to increase HDL concentrations as improvements were observed during energy balance and CR. Additionally, meal frequency should be considered in the context of meal timing. Skipping breakfast may result in a shift of calories to later in the day, and it is possible that the body is better suited to accommodate greater calorie intakes in the early part of the day. The long-term effects of a late meal time under energy balance is not clear. Current research suggests that lipids respond favorably to early mealtimes during 12 weeks of CR. However, this may be due to greater weight loss sustained in the early feeding groups. Blood pressure does not seem to be affected by meal frequency in either energy balance or CR conditions. Collectively, cardiovascular risk factors seem to improve the most under weight loss conditions regardless of meal frequency or meal timing.

7. Meal timing and meal frequency's effect on glucose regulation

Support for increased meal frequency stems from the notion that small meals throughout the day will cause less fluctuation in insulin and glucose. It has also been shown that humans are less glucose tolerant at night.(71)
7.1 Acute effects of meal timing and meal frequency on glucose regulation

7.1.1 Acute effects of meal frequency on glucose regulation

Three studies with healthy volunteers show glycemic values within normal limits during acute testing (8-24 hours) of various meal frequencies (3-14 meals). (72) (73) (74) Solomon et al., 2008 (73) and Munsters et al., 2012 (74) observed more pronounced peaks and valleys in insulin and glucose consistent with the larger meals during the lower meal frequencies and a plateau effect when participants consumed more frequent meals (12 and 14, respectively). Ohkawara et al., 2013 (72) compared 6 meals to 3 meals over 24-hours. More frequent but smaller peaks and valleys with a lower overall insulin AUC in the 6 meal condition were noted. (72) This insulin response was consistent with Jones et al., 1993 (65) who reported a smaller total insulin concentration in a 6-meal condition. However, glucose responses have also been shown to be similar across meal frequencies. (65) (73) (72) In contrast, Munster et al., 2012 (74) showed AUC for insulin and glucose to be lower in the 3-meal condition compared to the 14-meal condition. One crossover study lasting 7 days showed no effect of 3 or 6 meals on fasting and insulin values. (67) As such, it would seem that increased meal frequency leads to more frequent spikes in insulin and in glucose. In some cases, this aggregate has the potential to culminate into a sustained insulin response during the feeding period. However, this response does not seem to affect fasting measurements after 7 days. Whether or not this is beneficial or hazardous to health remains unknown.

7.1.2 Acute effects of meal timing on glucose regulation

Four acute studies assessed glucoregulatory responses to meal timing. (68, 75, 76, 77) Sato et al., 2011(75) and Tsuchida et al., 2013 (77) both showed that a late meal could increase blood glucose responses to the next day’s morning meal. Additionally, Morgan et al., 2011(76) reported
findings that support the theory that a late meal could increase glucose and insulin resistance, and that the meal's glycemic index had an effect on response. Contrary to these findings, Kinsey et al., 2014 (68) demonstrated no difference for insulin, glucose, or HOMA-IR between groups given a protein supplement 2 hours after dinner and 30 minutes prior to bedtime compared to a placebo. Therefore, meals higher in protein or very low in glycemic index could, theoretically, mitigate the subsequent effects of a late night meal.

7.2 Short-term and moderate-term effects of meal frequency on glucose regulation

7.2.1 Short-term and moderate-term effects of meal frequency on glucose regulation under energy balance

Studies lasting 2 weeks or longer may offer better insight into metabolic adaptation. Two studies, lasting two weeks, by Jenkins et al., compared 3 meals to 17 meals throughout the day. (33, 36) Consistent with some acute studies, the higher meal frequency decreased insulin concentrations throughout testing. (33, 36) Total glucose concentrations and the response to an OGTT were similar between groups (33, 36); however, postprandial glucose concentrations were lower in the 17-meal condition. (33) Two studies lasting 4 weeks showed no difference across meal frequencies ranging from 2-9 meals. (31, 35) However, this could be due to a relatively high frequency of eating occasions in Arnold et al., 1994 (35) (5 v 9) and a high protein diet in Arciero et al., 2013 (2 v 6) (31). Chapelot et al., 2006 (32) designed a study in which participants were asked to switch from their typical meal pattern (3 v 4). When participants increased their meal pattern from 3 to 4 meals per day for 4 weeks, the AUC and mean concentrations for both insulin and glucose were significantly higher in the 4-meal condition compared to the 3-meal condition. (32) Carlson et al., 2007 (37) reports glucoregulatory data from Stote et al., (30) which consisted of an 8 week trail where 3 meals were
compared to 1 evening meal. Over the course of a two-hour OGTT there was no effect on insulin AUC. (37) However, the 3-meal condition showed better glucose AUC and fasting values compared to the 1 meal condition. (37) All glucose values, however, were within normal ranges. These findings should be interpreted with caution as the timing of the single meal was in the evening. As such, the effect of meal timing could confound meal frequency glucoregulatory outcomes.

7.2.2 Short-term and moderate-term effects of meal frequency on glucose regulation under calorie restriction

Alterations to meal frequency under CR show no advantage to either a high or low frequency meal pattern. (31, 40, 42, 43) However, these studies differ from the previously reviewed meal frequency studies in that they all compared more moderate meal frequency patterns. For example, all frequencies selected were under 6 meals or eating episodes per day. Meal frequencies showing an effect in the previous section were as high as 17 meals per day. These studies also represent interventions of longer duration lasting 12 to 52 weeks. (40, 42) Additionally, most of the studies reviewed under CR showed an improvement in glucose regulation. (40, 42, 43) Thus, discerning an effect based on meal frequency alone is difficult. The exception to this was Arciero et al., 2013(31) who compared 3 to 6 meals under a high protein 30% CR diet. It is surprising that this diet would not affect glucose regulation, although the lack of effect could be a consequence of a self-selected diet and weight loss <5%.
7.3 Short-term and moderate-term effects of meal timing on glucose regulation

7.3.1 Short-term and moderate-term effects of meal timing on glucose regulation under energy balance

Three studies ranging from 2-6 weeks assessed meal timing under energy balance. (58, 59, 60) Farchchi et al., 2005 (58) conducted a two-week study in normal weight women. In the early feeding group a breakfast of cereal and milk was provided between 7:00am and 8:00am and then a candy bar between 10:30am and 11:00am. (58) In the late feeding group this breakfast was given between 12:00 and 12:30 pm that fell after the candy bar between 10:30 and 11:00am. (58) Each group self-selected 2 meals and 2 snacks throughout the rest of the day for 2 weeks. (58) There were no within group or between group differences for fasting glucose, insulin, or HOMA-IR. (58) A 9:00 am meal challenge showed the early feeding group to have a decrease in insulin AUC that was significantly lower than that of late feeding group. (58) Changes in glucose AUC were unremarkable. (58) The change in insulin response seen in the early feeding group is unexpected as the study participants were all previously habitual breakfast eaters. There could have been a difference in metabolic response due to be given a candy bar as the first or second eating occurrence each day. Additionally, the meal test employed did not represent the eating conditions of each group. As such, the differences in response could be due to the body’s anticipation of energy intake at a certain time of day. Another study lasting 2 weeks found no difference in glucose regulation when comparing a daytime snack with a nighttime snack. (60) However, it is possible that the glycemic load of a snack was not enough to elicit a noticeable response. Betts et al., 2014 (59) also found no difference in fasting insulin, glucose, or HOMA-IR after 6 weeks of either consuming breakfast or skipping breakfast. Adipose tissue was assessed by biopsy and showed a 10% increase in insulin sensitivity in the breakfast group compared to
the breakfast skipping group. In a lean population, this occurrence could be negligible. However, higher amounts of adipose tissue in the obese could affect more general fasting measurements.

### 7.3.2 Short-term and moderate-term effects of meal timing on glucose regulation under calorie restriction

Weight loss could also be a major contributing factor when evaluating how the timing of a meal affects glucose regulation under CR. For example, the two studies showing superior glucose regulation outcomes also experienced greater weight loss. (61, 62) Likewise, Jakubowicz et al., 2012 (63) reported a similar decrease in glucose, insulin, and insulin resistance for both groups after 16 weeks of CR with similar weight loss. However, the macronutrient distribution was also altered in this study to shift a greater amount of carbohydrates to earlier in the day. The intervention groups being compared in each of these studies consumed the same amount of energy over a similar feeding interval. They all differed in how the calories were distributed. As such, the difference between early and late feeders may be augmented if the late eaters consumed their last meal later in the day.

### 7.4 Summary of the effect of meal frequency and meal timing on glucose regulation

Meal frequency and timing seem to have minimal influence on glucose regulation compared to weight loss. Interpretation of the available meal frequency research is muddled due to a wide range of frequencies tested (1-17 meals per day) and inconsistent meal times. Additionally, the majority of the studies indicating effects are 2 weeks or less in duration. Meal timing studies also have their own limitations. This would include short trial durations, not being able to discern the effect of weight loss separately, and employing study designs that only compare two meal frequencies.
8. Role of appetite hormones in regulating body weight

8.1 Background of peptide tyrosine tyrosine, glucagon-like peptide-1 and ghrelin function

Several hormones influence appetite in humans. These include peptide tyrosine-tyrosine (PYY), glucagon-like peptide one (GLP-1), and ghrelin. PYY and GLP-1 are stimulated by energy intake though the gastrointestinal tract, and ghrelin peaks just prior to energy intake. Collectively, these hormones are thought to play a role in the regulation of appetite, satisfaction, and hunger.

PYY is secreted from the L-cells of the lower gut in response to energy intake. A 50% increase in PYY has been demonstrated following a mixed nutrient meal. Increased plasma concentrations have been observed prior to nutrient passage through the lower gut. This anticipated rise is thought to be modulated by the luminal presence of gastric and bile acids, increased CCK concentrations, and vagus nerve stimulation. It acts to decrease hunger and increase satiety by delaying gastric emptying and inhibiting gastric and bile acid secretion. Increasing caloric intake has been shown to increase subsequent plasma PYY concentrations. Additionally, infusions of active PYY have been shown to decrease calorie intake in obese and lean subjects by about 30%.

PYY and GLP-1 appear to work synergistically with one another. GLP-1 is also secreted from the L-cells in proportion to calories consumed. It acts as an incretin hormone. Thus, it promotes glucose-stimulated insulin release and inhibits glucagon secretion. Additionally, it promotes insulin biosynthesis, β-cell proliferation, glucose uptake by muscles and adipocytes while decreasing β-cell apoptosis and inhibiting hepatic glucose production. In addition to glucose regulation, it has also been shown to slow gastric emptying, inhibit gastric acid secretion, and stimulate the CNS.
In opposition to other appetite hormones, ghrelin is the only known orexigenic hormone. It increases prior to meal initiation and rapidly decreases once nutrients are sensed. (84, 85) Secretion from the stomach and a minor contribution from the pituitary gland are primarily regulated by energy intake. (78) However, diurnal fluctuations have also been shown to occur and seem to be entrained by meals. (84) Infusions of ghrelin have been shown to increase perceived appetite and subsequent ad-lib energy intake. (86) In response to energy intake, ghrelin acts to increase gastrointestinal motility. (78) Additionally, there appears to be a dual relationship between insulin and ghrelin. (85) Ghrelin decreases in response to insulin in states of both hypoglycemia and hyperglycemia controlled by clamp procedures. (87) This suggests that insulin may suppress ghrelin independently of glucose. (87) Ghrelin may also increase insulin sensitivity. (88)

### 8.2 Relationship between appetite hormones and body weight status

#### 8.2.1 Relationship between ghrelin and body weight status

Several studies show an association between body weight and ghrelin concentrations. (84, 89, 90, 91, 92, 93) Tschöp et al., 2001(91) observed lower fasting ghrelin concentrations in obese Pima Indians compared to lean Caucasians. A negative correlation also existed between ghrelin and percent body fat. (91) Shiiya et al., 2002 (84) similarly observed a negative relationship between BMI and fasting ghrelin. This particular study assessed fasting ghrelin concentrations of 28 healthy controls, 17 anorexic participants, 11 obese participants, 23 lean type 2 diabetics, and 19 overweight and obese type two diabetics. (84) The obese and obese type two diabetics showed lower ghrelin concentrations that were only 68% and 66%, respectively, of the healthy controls’ concentrations. (84) Both the anorexic participants and the lean type two diabetics displayed an opposite comparison with higher ghrelin concentrations at 225% and 190% of the healthy controls. Altered fasting ghrelin
concentrations were also observed by Monteleone et al., 2005 (89). Both obese and non-obese women with binge eating disorder and obese women without binge eating disorder had decreased ghrelin levels compared to healthy controls. (89) It is possible that this decrease would serve to decrease appetite in populations demonstrating excess energy intake. Interestingly, weight cycling with intentional weight loss (>10lbs) has been associated with higher ghrelin levels in women. (94)

While there seems to be substantial evidence to suggest that ghrelin is reduced in the obese state, one study contradicts these findings by reporting no difference in fasting concentrations between lean and obese women. (95) It is unknown why this study did not find a relationship, as its design was similar to other studies showing an association.

Abnormal ghrelin responses in the obese also persist postprandially. (92, 96) For example, ghrelin is suppressed following a meal in normal weight individuals. (96) However, this suppression is blunted in obese, insulin-resistant individuals. (96) Additionally, Carlson et al., 2009(92) reported dissimilar ghrelin responses between lean and severely obese women over a 2-hour meal challenge. Overall, postprandial ghrelin concentrations were lower in the obese group compared to the lean group. (92) Another study utilizing a 2-hour meal challenge in lean and obese men and women also support these findings. (90)

In addition to body weight being a factor in ghrelin concentrations, insulin insensitivity seems to decrease ghrelin concentrations in the obese. (91, 96, 97) This was demonstrated by McLaughlin et al., 2004.(97) Obese participants with insulin resistance had fasting ghrelin concentrations that were only 61% of that of insulin sensitive obese participants. (97) Additionally, multiple linear regression
analysis revealed insulin resistance to be associated with a 145 pg/ml decrease in ghrelin. (97) As discussed previously, Roux et al., 2005 (96) reported a blunted ghrelin response in the insulin-insensitive obese participants compared to healthy controls. These findings would be strengthened by an evaluation of postprandial values in insulin-sensitive obese compared to insulin-insensitive obese to tease out the influence of body weight.

Collectively, these studies suggest that ghrelin is decreased in the obese state. Insulin resistance has the potential to further disturb ghrelin concentrations in the obese. Additional studies are needed to determine ghrelin’s role in the pathophysiology of obesity and the associated co-morbidity of insulin resistance.

8.2.2 Relationship between peptide tyrosine tyrosine and body weight status

Batterham et al., 2003 (82) is the seminal study comparing PYY concentrations based on weight status. They were the first to show fasting PYY to be lower in obese subjects compared to lean subjects. (82) A negative correlation was also discovered between BMI and fasting PYY. (82) Another study from this research group supports these initial findings.(81)Roux et al., 2006 (81) compared 20 obese subjects to 20 lean subjects over the course of 6 test meals with varying calorie content and volume. Fasting PYY was lower in the obese subjects compared to the lean subjects. (81) Both weight groups displayed an incremental increase in PYY with increasing calorie intake; however, the obese group responded with significantly lower plasma PYY concentrations. Almost twice the calorie content was required to elicit a similar PYY response in the obese group compared to the lean group. The obese group also reported a lower level of fullness when assessed by visual analogue scale. One study contradicts these initial findings. Ukkola et al., 2011(98) reported a positive relationship between BMI
and the active form of PYY, PYY\textsubscript{3-36}, in 428 coronary artery disease patients with diabetes and 440 coronary artery disease patients. There was also an independent relationship established between high PYY\textsubscript{3-36} and type two diabetes. (98) For example, the highest tertile of PYY\textsubscript{3-36} was also associated with high BMI, waist circumference, hemoglobin A1C, fasting glucose, and insulin. (98) It is possible that the disease status of this cohort affected PYY differently than obesity alone would.

There are several studies that do not support any relationship between obesity and altered PYY concentrations. (93, 99, 100), (101) Pfluger et al., 2007(99) compared total fasting PYY in anorexic, lean, obese, and morbidly obese subjects. PYY was highest in the anorexic subjects compared to all other groups; however, PYY was similar in the both obese categories compared to the lean subjects. (99) There was also no difference between lean and obese subjects when PYY\textsubscript{3-36} was assessed. (99) Kim et al., 2005, performed a 2-hour glucose tolerance test. (93) Total PYY peaked at 20 minutes and returned toward baseline for the remaining of the 2-hour test. There was no relationship observed between PYY and BMI and no difference in PYY concentrations between lean and obese subjects. (93)

The largest cross-sectional study (n=2095) to date by Cahill et al., 2014(100) also reported no difference in fasting PYY concentrations across normal weight, overweight, and obese subject groups when categorized by either percent body fat or BMI. Interestingly, women in the highest 33% for waist circumference, percent body fat, and trunk fat had significantly higher PYY than women in the lowest 33% for these parameters. (100)

The studies reporting on the relationship between PYY and obesity are equivocal. This could be due to the reliance of fasting measurements and the inconsistency in PYY species assessed. Most of the studies reviewed evaluated only fasting measurements. This limits interpretation of PYY between
weight classes, as PYY is known to respond to nutrient intake. Furthermore, peak concentrations have been shown at 20, 90, and 120 minutes. (81, 93), (99) The lack of consensus of peak response over time can add additional complication to study design and interpretation.

8.2.3 Relationship between glucagon-like peptide-1 and body weight status

Two initial studies by Ranganath et al., (102, 103) report lower GLP-1 concentrations in obese subjects compared to lean in a 180-minute meal challenge with carbohydrates. The earliest study assessed 6 lean and 6 obese subjects over the course of either a carbohydrate meal challenge or a fat meal challenge (340 kcal each). (102) GLP-1 concentrations were tested 30 minutes prior to the meal and at intervals for 180 minutes following the meal. (102) Fasting GLP-1 was lower prior to the carbohydrate meal in the obese with no difference between weight categories prior to the fat meal. (102) This is odd as no meal was provided to elicit a response. Following the carbohydrate meal, the obese group experienced a GLP-1 peak at 15 minutes whereas the lean group’s GLP-1 peaked at 90 minutes. (102) Incremental AUC for GLP-1 was also lower in the obese compared to the lean group during the carbohydrate meal. (102) There were no differences noted between groups during the meal challenge with fat. (102) A subsequent study by the same group reported lower total and incremental AUC in the obese women compared to lean women. (103) However, GLP-1 peaked at 30 minutes in both groups. (103) The subsequent return to baseline differed between groups. (103) For example, in the lean GLP-1 concentrations remained elevated above baseline at 90 minutes whereas the obese group experienced a GLP-1 return to baseline at 90 minutes. (103) The blunted response of GLP-1 to the carbohydrate meal could provide a link to insulin resistance as seen in Vilsbøll et al., 2003. Obese type two diabetics had a significantly decreased GLP-1 response compared to weight matched healthy obese when provided a mixed meal. (104) However, no difference was noted between the obese and
lean subject groups. (104) Kim et al., 2005 (93) performed a 2-hour glucose tolerance test and noted no relationship between GLP-1 and BMI and no difference in GLP-1 concentrations between lean and obese subjects. Unlike Ranganath et al., 1996 (102), GLP-1 peaked at 20 minutes and returned toward baseline for the remaining of the 2-hour test. (93) Given the differences in late response seen in Ranganath et al., 1996 (102), it is possible that a longer meal challenge is necessary. A study conducting a 3-hour meal challenge noticed no difference in fasting GLP-1 concentrations between weight categories, but they did observe a decreased GLP-1 AUC in the obese compared to the lean group. (105)

These studies suggest there may be a relationship between obesity and attenuated GLP-1 postprandial responses. This relationship may be mediated by a state of insulin resistance or glucose intolerance in the obese. Future studies must aim to control for insulin resistance in their study populations to determine whether weight status alone is a factor in GLP-1 concentrations.

8.3 Effect of weight loss on appetite hormones

Studies with 5% weight loss or less show GLP-1 to decrease and conflicting PYY and ghrelin findings. (13, 106, 107) Heilbronn et al., 2005(13) was the shortest intervention at 3 weeks. A 3% weight loss by ADF had no effect on fasting ghrelin.(13) Hill et al., (106) also reported 3% weight loss but over a longer period of time (12 weeks) with 70% energy intake. (106) Fasting and postprandial ghrelin increased 21% and14%, respectively. Interestingly, ghrelin primarily increased later in the day. (106) No changes were noted in PYY after weight loss in this study. (106) In opposition to Hill et al., 2013 (106), Beck et al., 2010 (107) found fasting GLP-1 and PYY to increase by 14% with no change in ghrelin after an approximate 5% weight loss in overweight and obese women.
This study was originally a three arm parallel study assessing diets with varied amounts of fiber. There was no difference between groups, so the data was combined. However, the authors noted large standard deviations that could have reduced the chance of significant findings between groups despite 56 subjects evaluated. (107)

It is possible that changes in certain appetite hormones require greater weight loss. Sumithran et al., 2011 (108) conducted a 10-week very low calorie diet with the goal of achieving 10% weight loss. It is the only study reviewed to assess fasting and postprandial concentrations of PYY, GLP-1, and Ghrelin. Fasting PYY decreased 25% and the postprandial 4-hour AUC decreased 12%, and preceded to decrease over a year of weight maintenance. (108) Fasting GLP-1 decreased by 14% with no change in postprandial AUC. (108) Fasting and postprandial ghrelin decreased considerably (57% and 47%, respectively) and maintained this reduction following weight maintenance. (108) Moran et al., 2005 (109) partially contradicts these findings. Nine percent weight loss over twelve weeks with an additional 4 weeks of weight maintenance showed increased fasting ghrelin (39%) and a trend for ghrelin’s AUC to decrease postprandially. (109) Additionally, the decrease in ghrelin was earlier post-diet at 60 minutes compared to baseline at 120 minutes. (109) This earlier decline could have resulted in a trend for lower AUC ghrelin. The alterations to GLP-1 in Adam et al., 2005 (110) was unlike Sumithran et al., 2011 in that fasting was unchanged and postprandial measurements were decreased. Interestingly, despite these alterations to GLP-1 the subjects reported an increase in satiety and a decrease in hunger. (110) It is surprising that the GLP-1 concentrations between the two studies were dissimilar as the caloric intake in each was similar. It is possible that the longer duration of 8 to 10 weeks in Sumithran et al., 2011 (108) is capable of representing a return to baseline for GLP-1 as
studies utilizing a weight maintenance period show no alteration to GLP-1 from baseline. (105, 108)

The only other study reviewed to assess PYY with similar weight loss to Sumithran et al., 2011 reported about a 10% reduction in both fasting and 2.5-hour postprandial PYY concentrations. (111)

The majority of these studies indicate a consequential imbalance that occurs with weight loss. The decreases in PYY and GLP-1 with concurrent increases in ghrelin could promote eventual weight regain. However, it is unclear how these hormonal changes translate to perceived appetite, as subject response was not typically reported in these studies. There also appears to be discordant changes in appetite hormones throughout the studies. This could diminish reciprocal or synergistic associations seen between hormonal regulators of appetite. Unfortunately, all but one study failed to report a comprehensive array of appetite hormones. It also remains unknown whether some of the alterations are due to an increase in hormone sensitivity. Trials comparing weight-matched participants to compare could help to clarify this issue.

9. **Safety concerns during alternate day fasting**

There is concern among weight loss practitioners that a diet recommending a feed and fast regimen, as done with ADF, has the potential to elicit deleterious moods and behaviors such as depression and uncontrolled eating, respectively. Binge eating is prevalent in the obese population (112), and has been associated with eating behaviors that have higher disinhibition and lower restraint scores.(113)

It is a common practice for weight reducing diet regimens to promote restraint and disinhibition simultaneously in order to induce energy restriction. Increases in restraint have been associated with
decreases in both dishibition and hunger. (113) CR literature indicates an improvement in mood and eating behaviors after dieting. For example, the CALERIE (114) study observed improved depression, binge eating, and body shape scores with increased restrictiveness after three months following 25% CR. Foster et al., 1998 (113) reported an interesting relationship between participant restraint scores and weight loss. This studied showed a 5 kg weight loss advantage between those in the highest tertile for restraint change and the lowest tertile.(113) Additionally, a meta-analysis by Frabicatore et al., (115) reported overall improvements in depression with weight loss in the obese.

The affect of ADF on mood and binge eating has never been assessed. However, previous studies and anecdotal reports suggest that ADF does not cause uncontrolled eating. For example, Klempel et al., 2010(16) showed that ADF dieters do not overcompensate energy intake on the feed days. Furthermore, Bhutani et al., 2013 (116) reported beneficial alterations to restrained and disinhhibited eating during ADF and ADF combined with endurance exercise. ADF combined with exercise was the only group to experience an improvement in emotional eating and this could be a consequence of exercise.(116)

Side effects may also occur with dieting due to decreased energy consumption. (117) Examples of frequently cited adverse events would include: dry skin and mouth, hair loss, gastrointestinal disturbances, headache, dizziness or lightheadedness, fatigue and tiredness, cold intolerance, and irregular menstruation in females. (117) Unfortunately, studies reporting adverse events are limited and inconsistent in the outcomes measured. One early ADF study by Heilbronn et al., 2005 (13) calling
for 100% energy restriction on the fast day, noted that participants felt irritable on the fast day and reported constipation and lightheadedness as minor adverse events throughout the 3 week trial. It will be of interest for future studies to examine what adverse events occur when subjects participate in a modified ADF regimen, where 25% of energy needs are permitted on the fast day.
III. MANUSCRIPT 1


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Running head: Meal timing during alternate day fasting

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Copyright Agreement available in Appendix I
1. **Abstract**

**Objective:** Alternate day fasting (ADF; 24-h feeding/24-h 25% energy intake at lunchtime), is effective for weight loss, but diet tolerability is questionable. Moving the fast day meal to dinnertime, or dividing it into smaller meals, may improve tolerability. Accordingly, this study compared the effects of ADF with three meal times on body weight and heart disease risk. **Methods:** Obese subjects (n = 74) were randomized to 1 of 3 groups for 8 weeks: 1) ADF-L: lunch, 2) ADF-D: dinner, or 3) ADF-SM: small meals. **Results:** Body weight decreased similarly (P < 0.001) in all groups (ADF-L: 3.5 ± 0.4 kg, ADF-D 4.1 ± 0.5 kg, ADF-SM 4.0 ± 0.5 kg). Reductions (P < 0.001) in fat mass and visceral fat were also comparable. Plasma lipids remained unchanged, and LDL particle size increased (P < 0.05) in all groups (1.3 ± 0.5 Å). Systolic blood pressure decreased (P < 0.05) by ADF-SM only. Fasting glucose, insulin, and HOMA-IR remained unchanged. **Conclusion:** Thus, allowing individuals to consume the fast day meal at dinner or small meals produces similar weight loss and cardio-protection as consuming the meal at lunch. This flexibility in meal timing may increase tolerability and long-term adherence to ADF protocols.

**Keywords:** Alternate day fasting, calorie restriction, meal timing, coronary heart disease, insulin resistance, obese adults
2. **Introduction**

Obesity increases an individual’s risk of coronary heart disease (CHD) (5). A collection of evidence suggests that weight loss as low as 3-5%, can improve plasma lipid profile, decrease blood pressure, and reduce insulin resistance (7). Alternate day fasting (ADF) is a novel diet regimen that has gained considerable popularity over the past decade. In animal models, ADF consists of an ad libitum “feed day” alternated with a 100% restriction “fast day”. In humans, this protocol is often modified to allow for a small amount of food consumption on the fast day (i.e. approximately 25% of the individual’s energy needs). Findings from recent modified ADF trials demonstrate 4-8% reductions in body weight after 8-12 weeks (119, 120, 121, 122). These decreases in body weight are usually accompanied by reductions in LDL cholesterol (120, 121, 122), triglycerides (120, 121, 122), systolic blood pressure (122), insulin resistance (19), and increases in LDL particle size (119, 123, 124).

Adherence and diet tolerability are essential to any weight loss protocol. Previous studies of ADF have generally required that subjects consume the fast day meal as a lunch (i.e. between 12.00-2.00 pm) (119, 120, 121, 122). Unfortunately, many obese individuals are hesitant to try ADF, as they fear that they will not be able to comply with the rigid lunchtime fast day protocol. In view of these issues, it has been speculated that allowing subjects to consume the meal as either a dinner (i.e. between 6.00-8.00pm) or as small meals throughout the day, may increase diet tolerability. To elaborate, the dinnertime protocol is beneficial in that it would permit subjects to eat with their families at night on the fast day, which was not possible with the previous lunchtime regimen. On the other hand, breaking up the meal into smaller meals at breakfast, lunch and dinner could help individuals better cope with the hunger they may experience throughout the day. Whether or not these novel fast day meal
schedules would produce the same degree of weight loss and cardio-protection as the traditional lunchtime protocol is an important question that has yet to be tested.

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

3. Methods

Subjects

Subjects were recruited from the Chicago area by means of advertisements placed around the University of Illinois, Chicago campus. A total of 159 individuals expressed interest, 97 subjects were deemed eligible to participate after screening via a preliminary questionnaire and BMI assessment, 23 subjects declined participation after qualifying, and 74 subjects were randomized to the interventions (Figure 1). Inclusion criteria were as follows: BMI between 30 and 39.9 kg/m²; age between 25 and 65 years; pre-menopausal or post-menopausal (absence of menses for more than 2 years); lightly active (< 3 h/week of light intensity exercise at 2.5 to 4.0 metabolic equivalents (METs) for 3 months prior to the study); weight stable for 3 months prior to the beginning of the study (< 4 kg weight loss or weight gain); non-diabetic; no history of cardiovascular disease (myocardial infarction or stroke); non-smoker; and not taking weight loss, lipid- or glucose-lowering medications. The experimental protocol was approved by the University of Illinois, Chicago, Office for the Protection of Research Subjects, and all research participants gave their written informed consent to participate in the trial.
Figure 1. Study flow chart

- n = 159 Screened
  - n = 62 Did not meet 1+ inclusion criteria
  - n = 23 Declined participation after qualifying
  - n = 74 Randomized
    - n = 24 ADF-L group
      - n = 4 Dropouts
        - n = 2 Issues with diet
        - n = 2 Personal reasons
      - n = 20 Completers
    - n = 25 ADF-D group
      - n = 6 Dropouts
        - n = 3 Issues with diet
        - n = 2 Personal reasons
        - n = 1 Not specified
      - n = 19 Completers
    - n = 25 ADF-SM group
      - n = 5 Dropouts
        - n = 1 Issues with diet
        - n = 2 Scheduling issues
        - n = 2 Not specified
      - n = 20 Completers
**Study design**

Experimental design

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

Baseline control period (weeks 1-2):

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

Weight loss ADF period (weeks 3-10):

Subjects were then randomized by a stratified random sample (based on sex, age, and BMI) into 1 of 3 interventions: 1) ADF-Lunch (ADF-L), 2) ADF-Dinner (ADF-D), or 3) ADF-Small meals (ADF-SM). Total energy expenditure (TEE) was calculated using the Mifflin equation (125). All subjects consumed 25% of their baseline energy needs on the fast day (24 h), and ate ad libitum on each alternating feed day (24 h). The feed and fast days began at midnight each day. Subjects were asked to refrain from staying up until midnight on feed days, or getting up just after midnight following a fast day, to eat. Subjects were provided with meals on each fast day, and ate ad libitum at home on the feed day. All ADF fast day meals were prepared in the metabolic kitchen of the Human Nutrition Research Center (HNRU) at the University of Illinois, Chicago. Fast day meals were provided as a 3-day rotating menu, and were formulated based on the American Heart Association (AHA) guidelines (126) (TABLE I.). Subjects were permitted to consume energy-free beverages, tea, coffee, and sugar-free gum, and were encouraged to drink plenty of water on the fast day. Each intervention group consumed the fast day meal at
different times throughout the day. The ADF-L group consumed their entire meal between 12.00 pm and 2.00 pm on each fast day. In contrast, the ADF-D group consumed their meal between 6.00 pm and 8.00 pm. The ADF-SM group divided their fast day meal up into 3 mini meals, and consumed ~100 kcal between 6.00 am and 8.00 am, ~300 kcal between 12.00 pm and 2.00 pm, and ~100 kcal between 6.00 pm and 8.00 pm.
## TABLE I. NUTRIENT COMPOSITION OF THE PROVIDED FAST DAY MEALS

<table>
<thead>
<tr>
<th>Foods</th>
<th>Fast day 1</th>
<th>Fast day 2</th>
<th>Fast day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entrée</td>
<td>Chicken enchilada</td>
<td>Lasagna w/meat</td>
<td>Roasted turkey</td>
</tr>
<tr>
<td>Fruit/Vegetable</td>
<td>Grapes</td>
<td>Carrot sticks</td>
<td>Grapes</td>
</tr>
<tr>
<td>Dessert/ Snack</td>
<td>Peanuts</td>
<td>Cookie</td>
<td>Crackers</td>
</tr>
<tr>
<td>Dairy</td>
<td>Yogurt</td>
<td>Yogurt</td>
<td>Yogurt</td>
</tr>
</tbody>
</table>

### Nutrients

<table>
<thead>
<tr>
<th>Nutrients</th>
<th>Fast day 1</th>
<th>Fast day 2</th>
<th>Fast day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal)</td>
<td>500</td>
<td>500</td>
<td>500</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>15 (27%)</td>
<td>13 (24%)</td>
<td>12 (22%)</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>5</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Monounsaturated fat (g)</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Polyunsaturated fat (g)</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Trans fat (g)</td>
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<td>0</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>32</td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>20 (16%)</td>
<td>23 (18%)</td>
<td>22 (17%)</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>72 (57%)</td>
<td>73 (58%)</td>
<td>78 (61%)</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>11</td>
<td>11</td>
<td>11</td>
</tr>
</tbody>
</table>

¹ No differences between meals for any nutrient when meals matched for total kcal. ADF-Lunch (ADF-L) group consumed entire fast day meal between 12.00 pm and 2.00 pm. ADF-Dinner (ADF-D) group consumed entire fast day meal between 6.00 pm and 8.00 pm. ADF-Small meals (ADF-SM) group divided the fast day meal during the day, and consumed 100 kcal between 6.00 am and 8.00 am, 300 kcal between 12.00 pm and 2.00 pm, and 100 kcal between 6.00 pm and 8.00 pm.

² Percent of energy (kcal).
Blood collection protocol

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

Analyses

Adherence with the ADF diet

Subjects were instructed to eat only the foods provided on each fast day, and to report any extra food items consumed using an “Extra food log”. If the log indicated that the subject ate extra food items (totalling >75 kcal) on a fast day, that day was labelled as “not adherent”. If the log revealed that the subject did not eat an extra food item, that day was labelled as “adherent”. Adherence data was assessed each week as: \[
\text{% Adherence to kcal goal} = \left( \frac{\# \text{ fast days adherent}}{\# \text{ of fast days in the week}} \right) \times 100.
\]

Compliance to the timing of the ADF meal was assessed using a “Fast day food checklist” (specific for each intervention group). The checklist contained a list of food items for that particular fast day, and clearly indicated the time range in which each food item was to be consumed. The checklist also contained a column where the subject was to report the specific time of day that each food item was eaten. If the checklist indicated that the subject ate one or more food items outside of the specified time range, that day was labelled as “not adherent”. If the checklist revealed that the subject ate all the food items during the correct time range, that day was labelled as “adherent”. Adherence data was
assessed each week as: \( \text{% Adherence to Meal Timing} = \left( \frac{\# \text{ Fast Days Adherent}}{\# \text{ of Fast Days in the Week}} \right) \times 100. \)

**Physical activity maintenance assessment**

All subjects were asked to maintain their physical activity habits during the trial. Physical activity was quantified by the use of a validated (127) pattern recognition monitor (Sense Wear Mini, BodyMedia, Pittsburgh, PA). Subjects wore the lightweight monitor on their upper arm for 7 d (approx. 23 h/d) at week 3 and 10. The data were processed using Bodymedia Software V.7.0 (127).

**Weight loss and body composition**

Body weight was assessed to the nearest 0.25 kg at the beginning of every week without shoes and in light clothing using a balance beam scale at the research center (HealthOMeter, Boca Raton, FL). BMI was assessed as kg/m\(^2\). Body composition (fat mass, lean mass, visceral fat mass) was measured using dual x-ray absorptiometry (128) (DXA; iDXA, General Electric Inc).

**Plasma lipids and LDL particle size**

Plasma total cholesterol, direct LDL cholesterol, HDL-cholesterol, and triacylglycerol concentrations were measured in duplicate using enzymatic kits (Biovision Inc., Mountainview, CA) at week 1, 3 and 10. LDL particle size was measured by linear polyacrylamide gel electrophoresis (Quantimetrix Lipoprint System, Redondo Beach, CA, USA) (118). Lipoware computer software (Quantimetrix, Redondo Beach, CA, USA) was then used to divide LDL into small (<255 Å), medium (255-260 Å), and large (>260 Å) particles, and to assess mean LDL particle size (21).

**Metabolic disease risk factors**
All measurements were taken at week 1, 3 and 10 (after a feed day). Blood pressure and heart rate were measured in triplicate using a digital automatic blood pressure/heart rate monitor (Omron HEM 705 LP, Kyoto, Japan) with the subject in a seated position after a 10-min rest. Resting metabolic rate (RMR) was measured by a handheld open circuit indirect calorimeter (129) (MedGem Indirect Calorimeter, Microlife, USA). RMR measurements were taken between 6.00 am and 9.00 am. Subjects were instructed to abstain from food, drink, and exercise for 12 h prior to the visit, and the timing since the last meal (12 h) was standardized for each subject prior the RMR measurement. Subjects first rested in a dark room in the supine position for 25 min, then a mouthpiece/nose clip were placed on the subject, and oxygen consumption was measured until it reached a stable flow (approx. 10 min). Fasting glucose concentrations were measured with a hexokinase reagent kit (Abbott, South Pasadena, CA). Fasting insulin was measured as total immunoreactive insulin (Coat-A-Count Insulin, Los Angeles, CA). Insulin resistance (IR) was calculated using the HOMA (Homeostasis Model Assessment) method, by applying the following formula: \[ \text{HOMA-IR} = \frac{\text{Fasting insulin} \, (\mu \text{U/ml}) \times \text{Fasting glucose} \, (\text{mg/dL})}{405}. \]

**Statistics**

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

Subject baseline characteristics and dropouts

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

<table>
<thead>
<tr>
<th></th>
<th>ADF-L</th>
<th>ADF-D</th>
<th>ADF-SM</th>
<th>P-value $^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>19</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>45 ± 3</td>
<td>45 ± 3</td>
<td>46 ± 2</td>
<td>0.88</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>17/ 3</td>
<td>15 / 4</td>
<td>18 / 2</td>
<td>0.58</td>
</tr>
<tr>
<td>Ethnicity (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>10</td>
<td>3</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>6</td>
<td>9</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>3</td>
<td>7</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>94 ± 2</td>
<td>97 ± 3</td>
<td>90 ± 2</td>
<td>0.16</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>165 ± 2</td>
<td>168 ± 2</td>
<td>163 ± 2</td>
<td>0.08</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
<td>35 ± 1</td>
<td>34 ± 1</td>
<td>34 ± 1</td>
<td>0.90</td>
</tr>
<tr>
<td>Lipids (mg/dL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Cholesterol</td>
<td>190 ± 11</td>
<td>199 ± 8</td>
<td>185 ± 8</td>
<td>0.57</td>
</tr>
<tr>
<td>LDL Cholesterol</td>
<td>114 ± 11</td>
<td>120 ± 6</td>
<td>110 ± 7</td>
<td>0.66</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>55 ± 3</td>
<td>57 ± 4</td>
<td>57 ± 3</td>
<td>0.95</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>104 ± 15</td>
<td>108 ± 14</td>
<td>94 ± 9</td>
<td>0.69</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>115 ± 3</td>
<td>117 ± 3</td>
<td>119 ± 2</td>
<td>0.70</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>78 ± 2</td>
<td>79 ± 2</td>
<td>83 ± 1</td>
<td>0.10</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>69 ± 3</td>
<td>71 ± 2</td>
<td>72 ± 3</td>
<td>0.63</td>
</tr>
</tbody>
</table>

Values reported as mean ± SEM.
F: Female, M: Male.
$^1$ P-value between groups at baseline (week 1): One-way ANOVA.
**Adherence to the ADF diet and physical activity maintenance**

Adherence to the fast day energy goal was similar ($P = 0.475$) for each group throughout the course of the trial (ADF-L: 91 ± 1%, ADF-D: 92 ± 2%, ADF-SM: 89 ± 2%). Compliance to the timing of the ADF meal was high in each intervention group (ADF-L: 98 ± 1%, ADF-D: 99 ± 1%, ADF-SM: 98 ± 1%). Maintenance of physical activity habits was measured using an activity monitor. There were no differences in activity from baseline to post-treatment in any of the groups (ADF-L week 3: 5663 ± 650 steps/d, week 10: 5995 ± 633 steps/d; ADF-D week 3: 5733 ± 1304 steps/d, week 10: 5847 ± 627 steps/d; ADF-SM week 3: 7113 ± 938 steps/d, week 10: 6457 ± 812 steps/d). Moreover, there were no differences between groups for activity level at week 3 ($P = 0.361$) or week 10 ($P = 0.326$).

**Weight loss and body composition**

There were no changes in body weight or body composition during the baseline period (Figure 2). Body weight was reduced ($P < 0.001$) by 3.5 ± 0.4 kg (3.8 ± 0.5%) in the ADF-L group, 4.1 ± 0.5 kg (4.2 ± 0.5%) in the ADF-D group, and 4.0 ± 0.5 kg (4.6 ± 0.6%) in the ADF-SM group, with no differences between groups post-treatment. Fat mass and lean mass decreased ($P < 0.001$) similarly by all interventions. Visceral fat mass also declined ($P < 0.001$) in all groups (ADF-L: 0.075 ± 0.027 kg, ADF-D: 0.135 ± 0.042 kg, ADF-SM: 0.135 ± 0.032 kg), with no differences between groups at week 10. BMI was reduced ($P < 0.05$) by ADF-L (1.3 ± 0.2 kg/m$^2$), ADF-D (1.4 ± 0.2 kg/m$^2$), and ADF-SM (1.5 ± 0.2 kg/m$^2$) after 8 weeks of treatment.
Figure 2. Changes in body weight and body composition during the weight loss period

A. Absolute change in body weight, fat mass, and lean mass from week 3 to 10 in the Alternate day fasting-Lunch (ADF-L), Alternate day fasting-Dinner (ADF-D), and Alternate day fasting-Small Meals group (ADF-SM). B. Absolute change in visceral fat mass from week 3 to 10 of the trial for each intervention group. Values reported as mean ± SEM. *Week 3 values significantly (P < 0.001) different from week 10 values within group (Paired t-test). No differences between groups for absolute change in body weight, fat mass, lean mass, or visceral fat mass (One-way ANOVA).

Plasma lipids and LDL particle

Total cholesterol, LDL cholesterol, HDL cholesterol, and triglyceride concentrations remained unchanged during the control phase, and during the weight loss phase in all groups (Table III). Mean LDL particle size increased (P < 0.05) after 8-weeks of treatment in the ADF-L (1.3 ± 0.5 Å), ADF-D (1.3 ± 0.5 Å), and ADF-SM (1.3 ± 0.6 Å) group, with no differences between group
TABLE III. CHANGES IN PLASMA LIPIDS AND LOW DENSITY LIPOPROTEIN PARTICLE SIZE DURING THE WEIGHT LOSS PERIOD

<table>
<thead>
<tr>
<th></th>
<th>Intervention</th>
<th>Week 3</th>
<th>Week 10</th>
<th>P-value ₁</th>
<th>P-value ₂</th>
<th>Change   ³</th>
<th>P-value ⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>ADF-L</td>
<td>185 ± 12</td>
<td>184 ± 9</td>
<td>0.92</td>
<td>0.75</td>
<td>-1 ± 7</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>ADF-D</td>
<td>195 ± 8</td>
<td>190 ± 8</td>
<td>0.19</td>
<td></td>
<td>-5 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADF-SM</td>
<td>182 ± 8</td>
<td>181 ± 7</td>
<td>0.82</td>
<td></td>
<td>-1 ± 4</td>
<td></td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>ADF-L</td>
<td>115 ± 9</td>
<td>113 ± 9</td>
<td>0.54</td>
<td>0.75</td>
<td>-2 ± 3</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>ADF-D</td>
<td>119 ± 6</td>
<td>119 ± 8</td>
<td>0.98</td>
<td></td>
<td>0 ± 6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADF-SM</td>
<td>109 ± 7</td>
<td>110 ± 7</td>
<td>0.79</td>
<td></td>
<td>1 ± 3</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>ADF-L</td>
<td>54 ± 3</td>
<td>52 ± 3</td>
<td>0.22</td>
<td>0.80</td>
<td>-2 ± 1</td>
<td>0.91</td>
</tr>
<tr>
<td></td>
<td>ADF-D</td>
<td>54 ± 4</td>
<td>54 ± 3</td>
<td>0.70</td>
<td></td>
<td>0 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADF-SM</td>
<td>56 ± 3</td>
<td>55 ± 3</td>
<td>0.69</td>
<td></td>
<td>-1 ± 2</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>ADF-L</td>
<td>102 ± 12</td>
<td>96 ± 14</td>
<td>0.38</td>
<td>0.54</td>
<td>-6 ± 7</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td>ADF-D</td>
<td>111 ± 14</td>
<td>102 ± 12</td>
<td>0.10</td>
<td></td>
<td>-9 ± 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADF-SM</td>
<td>85 ± 8</td>
<td>84 ± 9</td>
<td>0.91</td>
<td></td>
<td>-1 ± 8</td>
<td></td>
</tr>
<tr>
<td>LDL particle size (Å)</td>
<td>ADF-L</td>
<td>257.6 ± 0.8</td>
<td>258.9 ± 0.8</td>
<td>0.02</td>
<td>0.95</td>
<td>1.3 ± 0.5</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>ADF-D</td>
<td>257.3 ± 0.9</td>
<td>258.6 ± 1.0</td>
<td>0.01</td>
<td></td>
<td>1.3 ± 0.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADF-SM</td>
<td>257.7 ± 0.7</td>
<td>259.0 ± 0.7</td>
<td>0.02</td>
<td></td>
<td>1.3 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>% Large LDL particles</td>
<td>ADF-L</td>
<td>37 ± 2</td>
<td>42 ± 2</td>
<td>0.02</td>
<td>0.59</td>
<td>5 ± 2</td>
<td>0.90</td>
</tr>
<tr>
<td></td>
<td>ADF-D</td>
<td>34 ± 2</td>
<td>40 ± 2</td>
<td>0.01</td>
<td></td>
<td>6 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADF-SM</td>
<td>35 ± 2</td>
<td>40 ± 2</td>
<td>0.01</td>
<td></td>
<td>5 ± 2</td>
<td></td>
</tr>
<tr>
<td>% Medium LDL particles</td>
<td>ADF-L</td>
<td>37 ± 2</td>
<td>36 ± 2</td>
<td>0.61</td>
<td>0.86</td>
<td>-1 ± 1</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>ADF-D</td>
<td>37 ± 2</td>
<td>37 ± 2</td>
<td>0.93</td>
<td></td>
<td>0 ± 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADF-SM</td>
<td>35 ± 1</td>
<td>36 ± 2</td>
<td>0.65</td>
<td></td>
<td>1 ± 1</td>
<td></td>
</tr>
<tr>
<td>% Small LDL particles</td>
<td>ADF-L</td>
<td>26 ± 1</td>
<td>22 ± 2</td>
<td>0.09</td>
<td>0.75</td>
<td>-4 ± 2</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>ADF-D</td>
<td>29 ± 3</td>
<td>23 ± 2</td>
<td>0.02</td>
<td></td>
<td>-6 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADF-SM</td>
<td>30 ± 3</td>
<td>24 ± 2</td>
<td>&lt;0.001</td>
<td></td>
<td>-6 ± 2</td>
<td></td>
</tr>
</tbody>
</table>

Values reported as mean ± SEM. ADF-L: Alternate day fasting-Lunch, ADF-D: Alternate day fasting-Dinner, ADF-SM: Alternate day fasting-Small meals.

₁ P-value between week 3 and week 10: Paired t-test.
₂ P-value between groups at week 10: One-way ANOVA.
₃ Absolute change between week 3 and week 10 values.
₄ P-value between groups for absolute change: One-way ANOVA.
Metabolic disease risk factors

There were no changes in any metabolic disease risk factors during baseline. Systolic blood pressure was reduced (P < 0.05) in the ADF-SM group only (6 ± 3 mmHg) (TABLE IV). Diastolic blood pressure remained unchanged in all groups. Heart rate decreased (P < 0.001) in the ADF-L group only (7 ± 2 bpm). RMR was reduced (P < 0.001) from baseline to post-treatment in the ADF-D group only (198 ± 48 kcals/d). Fasting glucose, insulin, and HOMA-IR remained unchanged in all groups after 8 weeks of diet.
<table>
<thead>
<tr>
<th></th>
<th>Intervention</th>
<th>Week 3</th>
<th>Week 10</th>
<th>P-value&lt;sup&gt;1&lt;/sup&gt;</th>
<th>P-value&lt;sup&gt;2&lt;/sup&gt;</th>
<th>Change&lt;sup&gt;3&lt;/sup&gt;</th>
<th>P-value&lt;sup&gt;4&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Systolic blood pressure (mm Hg)</strong></td>
<td>ADF-L</td>
<td>114 ± 2</td>
<td>112 ± 3</td>
<td>0.57</td>
<td>0.99</td>
<td>-2 ± 2</td>
<td>0.52</td>
</tr>
<tr>
<td></td>
<td>ADF-D</td>
<td>117 ± 5</td>
<td>112 ± 2</td>
<td>0.07</td>
<td></td>
<td>-5 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADF-SM</td>
<td>118 ± 3</td>
<td>112 ± 3</td>
<td>0.04</td>
<td></td>
<td>-6 ± 3</td>
<td></td>
</tr>
<tr>
<td><strong>Diastolic blood pressure (mm Hg)</strong></td>
<td>ADF-L</td>
<td>77 ± 2</td>
<td>76 ± 2</td>
<td>0.56</td>
<td>0.25</td>
<td>-1 ± 2</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>ADF-D</td>
<td>79 ± 3</td>
<td>76 ± 1</td>
<td>0.15</td>
<td></td>
<td>-3 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADF-SM</td>
<td>80 ± 2</td>
<td>79 ± 1</td>
<td>0.60</td>
<td></td>
<td>-1 ± 2</td>
<td></td>
</tr>
<tr>
<td><strong>Heart rate (bpm)</strong></td>
<td>ADF-L</td>
<td>71 ± 2</td>
<td>64 ± 2</td>
<td>&lt;0.001</td>
<td>0.63</td>
<td>-7 ± 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>ADF-D</td>
<td>71 ± 2</td>
<td>68 ± 2</td>
<td>0.05</td>
<td></td>
<td>-3 ± 2&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADF-SM</td>
<td>72 ± 3</td>
<td>72 ± 3</td>
<td>0.80</td>
<td></td>
<td>0 ± 2&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td><strong>Resting metabolic rate (kcal/d)</strong></td>
<td>ADF-L</td>
<td>1423 ± 48</td>
<td>1336 ± 51</td>
<td>0.06</td>
<td>0.22</td>
<td>-87 ± 42</td>
<td>0.22</td>
</tr>
<tr>
<td></td>
<td>ADF-D</td>
<td>1557 ± 62</td>
<td>1359 ± 74</td>
<td>&lt;0.001</td>
<td></td>
<td>-198 ± 48</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADF-SM</td>
<td>1356 ± 52</td>
<td>1324 ± 48</td>
<td>0.52</td>
<td></td>
<td>-32 ± 47</td>
<td></td>
</tr>
<tr>
<td><strong>Fasting glucose (mg/dL)</strong></td>
<td>ADF-L</td>
<td>96 ± 2</td>
<td>94 ± 2</td>
<td>0.28</td>
<td>0.23</td>
<td>-2 ± 2</td>
<td>0.87</td>
</tr>
<tr>
<td></td>
<td>ADF-D</td>
<td>100 ± 3</td>
<td>99 ± 2</td>
<td>0.77</td>
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<td>-1 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADF-SM</td>
<td>101 ± 3</td>
<td>99 ± 2</td>
<td>0.20</td>
<td></td>
<td>-2 ± 2</td>
<td></td>
</tr>
<tr>
<td><strong>Fasting insulin (µU/ml)</strong></td>
<td>ADF-L</td>
<td>12 ± 1</td>
<td>12 ± 1</td>
<td>0.49</td>
<td>0.14</td>
<td>0 ± 1</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td>ADF-D</td>
<td>11 ± 1</td>
<td>9 ± 2</td>
<td>0.09</td>
<td></td>
<td>-2 ± 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADF-SM</td>
<td>16 ± 2</td>
<td>14 ± 2</td>
<td>0.07</td>
<td></td>
<td>-2 ± 7</td>
<td></td>
</tr>
<tr>
<td><strong>HOMA-IR</strong></td>
<td>ADF-L</td>
<td>3.0 ± 0.2</td>
<td>2.7 ± 0.3</td>
<td>0.44</td>
<td>0.10</td>
<td>-0.3 ± 0.4</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>ADF-D</td>
<td>3.0 ± 0.4</td>
<td>2.2 ± 0.4</td>
<td>0.09</td>
<td></td>
<td>-0.8 ± 0.3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ADF-SM</td>
<td>4.2 ± 0.6</td>
<td>3.4 ± 0.5</td>
<td>0.09</td>
<td></td>
<td>-0.8 ± 0.4</td>
<td></td>
</tr>
</tbody>
</table>

Values reported as mean ± SEM. ADF-L: Alternate day fasting-Lunch, ADF-D: Alternate day fasting-Dinner, ADF-SM: Alternate day fasting-Small meals, HOMA-IR: Homeostatic model assessment-Insulin resistance.

<sup>1</sup>P-value between week 3 and week 10: Paired t-test.
<sup>2</sup>P-value between groups at week 10: One-way ANOVA.
<sup>3</sup>Absolute change between week 3 and week 10 values.
<sup>4</sup>P-value between groups for absolute change: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test).
5. **Discussion**

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

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

Body weight and body composition improved to the same extent in each group, due to similar levels of adherence. Specifically, body weight, fat mass, and lean mass decreased by approximately 4 kg, 3 kg, and 1 kg, respectively, by each intervention. Previous studies have reported similar modulations in body weight and fat mass after 8 weeks of ADF (120, 121, 122). However, the present trial differs from previous studies (121, 122) in terms of lean mass findings. For instance, in a study by Klempel et al., (121), obese subjects were provided with either a high-fat or low-fat diet during 8 weeks of ADF. This study demonstrated impressive reductions in body weight (4 kg) and fat mass (4 kg), with a complete retention in lean mass (121). Similarly, Varady et al., (122) reported a 5 kg reduction in body weight/fat
mass, with no change in lean mass. Johnson et al., (120) demonstrated the greatest decreases in body weight (8 kg) after 8 weeks of ADF, but failed to report changes in body composition. The percentage of body weight lost as fat mass versus lean mass, also differed considerably between present and previous trials (121, 122). While previous ADF studies suggests that 90% of weight is lost as fat and 10% is lost as lean (121, 122), the present study demonstrates a shift in this ratio to 75% lost as fat and 25% lost as lean. The reason for these contradictory findings is not clear, but may be related to the instruments used to measure body composition. For example, previous work used bioelectrical impedance analysis (BIA) (121, 122), while the present study used DXA. Results from validation studies demonstrate that BIA routinely overestimates lean mass by 5-10% (130, 131) when compared to DXA. These systematic errors in accuracy may therefore partly explain why lean mass findings differed between studies. Visceral fat mass was also assessed. Results from DXA analysis reveal comparable modest reductions (0.1 kg) by all three mealtime protocols. Previous short-term ADF studies also report reductions in visceral fat (121, 122). However, it is difficult to compare findings, as past studies (121, 122) used an indirect measure (i.e. waist circumference reported in cm), while the present study used DXA (i.e. reported as kg).

Plasma lipids remained unchanged in all intervention groups after 8 weeks of treatment. These findings are inconsistent with previous trials (120, 121, 122). For instance, other 8-week ADF studies have demonstrated fairly reliable reductions in total cholesterol (9-21%), LDL cholesterol (12-31%), and triglyceride concentrations (14-42%) (120, 121, 122). Only one other ADF study reported no effect on these lipid parameters (119). The reason for this lack of effect is not clear, as weight loss and baseline lipid values were similar between present and previous studies (120, 121, 122). No ADF trial to date has demonstrated increases in HDL cholesterol levels. This is not surprising as HDL cholesterol
concentrations are generally only augmented with exercise (132, 133). The effect of these interventions on LDL particle size was also investigated. Small dense LDL particles are more atherogenic than their larger counterparts, due to increased oxidizability and permeability through the endothelial barrier (134). We show here that LDL particle size increased by 1 Å by all interventions, despite no change in HDL cholesterol or triglyceride concentrations (135). Thus, each of these mealtime protocols produces similar cardiovascular benefits by increasing mean LDL particle size.

The impact of these interventions on metabolic disease risk factors was also assessed. Systolic blood pressure was reduced in the ADF-SM group only (6 mmHg) after 8 weeks of diet. The reason for this selective effect is not clear, as all groups lost comparable amounts of body weight, fat mass and visceral fat mass. However, it should be noted that the ADF-SM group had the highest systolic values at baseline (though not significantly). Since these subjects were closer to being hypertensive than the other groups, this may explain why their systolic blood pressure was more responsive to the treatment. None of the interventions had any effect on diastolic blood pressure. The minimal effect on blood pressure is most likely due to the normotensive status of the subjects at baseline (<120 mm Hg systolic, <80 mm Hg diastolic). Previous ADF studies that demonstrated reductions in systolic (4-6 mm Hg) and diastolic blood pressure (2 mm Hg) recruited individuals with borderline hypertension (119, 122). Thus, the hypertensive status of the sample may be a key determinant of blood pressure change by ADF. Heart rate was also evaluated. Heart rate was reduced by ADF-L only. The reason why the lunchtime protocol produced this specific effect is uncertain, as all groups had similar heart rates at baseline (71-72 bpm) and the activity levels of the ADF-L subjects did not increase to a greater extent than the other groups (136). This finding is also somewhat surprising, as ADF does not generally improve heart rate (119, 121). As for glucose, insulin, and insulin resistance, no changes were observed
for any intervention. Seeing as baseline levels of glucose and insulin were within the normal range for each group, it is not surprising that these diets had little effect on these parameters. The effect of ADF on RMR was also evaluated. Interestingly, RMR was maintained in the ADF-L and ADF-SM group, but declined considerably by ADF-D (200 kcal/d from baseline). The reason for this is unclear as all groups lost the same amount of lean mass, and lean mass is a key predictor of RMR(137). Whether or not these RMR findings can be reproduced using a more robust technique for measuring energy expenditure, such as doubly labeled water (138), is well warranted.

This study has several limitations. First and foremost, this trial did not include a “breakfast” arm to test the effects of consuming the fast day meal between 6.00 am and 8.00 am. Thus, it is still uncertain if consuming the meal as breakfast produces similar adherence and weight loss as the other mealtimes. Second, activity data was only collected for 80% of the subjects in each intervention group due to malfunctioning activity monitors. As such, the study may not be powered adequately to identify changes in physical activity from baseline to post-treatment. Third, adherence to the fast day protocol was determined using self-reports. It is well known that obese subjects underestimate energy intake by 10-20%(139, 140), thus the reliability of our adherence data is questionable. Fourth, due to the nature of the “small meals” intervention, this group was not technically taking part in an ADF protocol. Instead, this group was following a very low calorie diet, which should be considered when interpreting the present findings. Fifth, our study lacked a control group, so whether or not the present findings can truly be attributed to these treatments, remains unknown. Sixth, since the ADF-SM group consumed the majority of their calories (~300 kcal) between 12.00 pm and 2.00 pm on the fast day, this intervention may not be that different from the traditional lunchtime approach. In retrospect, the ~500
kcal meal for the ADF-SM group should have been divided evenly over the three meals, to more effectively test our hypotheses.

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

6. **Acknowledgements**

KKH designed the experiment, ran the clinical trial, analyzed the data, and wrote the manuscript. CMK, JFT, AB, and SB assisted with the conduction of the clinical trial and performed the laboratory analyses. KAV assisted with the design of the experiment, data analyses and the preparation of the manuscript. The authors have no competing interests.
V. MANUSCRIPT 2

Safety of alternate day fasting and effect on disordered eating behaviors

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Running head: Safety of alternate day fasting

Funding source: Departmental funding, Kinesiology and Nutrition, University of Illinois at Chicago

Proof of Open Access available in Appendix J
1. Abstract

Background: Alternate day fasting (ADF; ad libitum intake “feed day” alternated with 75% restriction “fast day”), is effective for weight loss, but the safety of the diet has been questioned. Accordingly, this study examined occurrences of adverse events and eating disorder symptoms during ADF. Findings: Obese subjects (n = 59) participated in an 8-week ADF protocol where food was provided on the fast day. Body weight decreased (P < 0.0001) by 4.2 ± 0.3%. Some subjects reported constipation (17%), water retention (2%), dizziness (<20%), and general weakness (<15%). Bad breath doubled from baseline (14%) to post-treatment (29%), though not significantly. Depression and binge eating decreased (P < 0.01) with ADF. Purgative behavior and fear of fatness remained unchanged. ADF helped subjects increase (P < 0.01) restrictive eating and improve (P < 0.01) body image perception.

Conclusions: Therefore, ADF produces minimal adverse outcomes, and has either benign or beneficial effects on eating disorder symptoms.

Keywords: Calorie restriction, alternate day fasting, safety, adverse events, eating disorder behaviors, obese adults
2. **Introduction**

Alternate day fasting (ADF) is an innovative form of dietary restriction capable of decreasing body weight by 4-8% after 8-12 weeks. (119, 120, 121, 122) ADF allows dieters to alternate between a “feed-day” involving ad libitum food intake, and a “fast-day” consisting of 75% energy restriction. Although ADF is effective for weight loss, the safety of the diet has been questioned. In particular, concerns have been raised regarding gastrointestinal disturbances, sleep issues, and problems with energy levels (that may be related to lower blood sugar levels). ADF has also been criticized for potentially increasing disordered eating behaviors and negatively impacting body image perception. Accordingly, this study examined the occurrence of adverse events, changes in disordered eating symptoms, and alterations in body image perception after 8 weeks of ADF in obese subjects.

3. **Methods**

**Subject selection**

Obese subjects were recruited from the Chicago area by advertisements. Key inclusion criteria were as follows: age 25–65 y, BMI between 30 and 39.9 kg/m$^2$, weight stable, previously sedentary, non-diabetic, non-smoker, and no history of cardiovascular disease. The experimental protocol was approved by the Office for the Protection of Research Subjects at the University of Illinois, Chicago, and all volunteers gave written informed consent.

**Study design and diet protocol**

An 8-week trial was implemented to test the study objectives. The primary outcome measures were body weight, adverse events, disordered eating symptoms, and body image perception. Total energy expenditure was quantified by the Mifflin equation (125) and appropriate activity factors. All subjects
consumed 25% of their baseline energy needs on the fast day (24 h), and ate ad libitum on each
alternating feed day (24 h). Subjects were provided with meals on each fast day, and ate ad libitum on
the feed day. All meals were consumed outside of the research center. Fast day meals were provided
as a 3-day rotating menu, based on American Heart Association (AHA) guidelines. (141) Body weight
was measured using a balance beam scale (HealthOMeter, Boca Raton, FL), and body composition was
assessed by dual energy X-ray absorptiometry (DXA; iDXA, GE Inc.).

**Adverse event, eating disorder, and body image questionnaires**

An adverse event questionnaire was administered at baseline (i.e. before the subjects began the
intervention) and post-treatment. Eating disorder symptoms were measured using the
Multidimensional Assessment of Eating-Disorder Symptoms (MAEDS). The MAEDS is a validated (142)
self-report inventory that measures six symptom domains related to eating disorders, including: binge
eating, restrictive eating, purgative behavior, fear of fatness, avoidance of forbidden foods, and
depression. Body image was assessed by the Body Shape Questionnaire (BSQ). The BSQ is a self-report
questionnaire that measures excessive concern about one’s body size and shape. (143) Higher scores
on the BSQ indicate greater concerns with body size and shape.

**Statistics**

Results are presented as mean ± SEM. A paired t-test was used to assess changes from baseline to
post-treatment for continuous variables, while a McNemar’s test was used for categorical variables.
Differences were considered significant at $P < 0.05$. All data was analyzed using SPSS software (version 21.0, SPSS Inc, Chicago, IL).

4. Results

Subject dropouts and weight loss

Seventy-four subjects began the study, and 59 subjects (age $46 \pm 1$ y) completed the 8-week trial.

Reasons for dropouts were as follows: inability to comply with the diet ($n = 6$), scheduling conflicts ($n = 2$), personal reasons ($n = 4$), and not specified ($n = 3$). Body weight decreased ($P < 0.0001$) from $93.5 \pm 1.4$ kg to $89.7 \pm 1.5$ kg ($-4.2 \pm 0.3\%$) after 8 weeks of diet. Fat mass was reduced ($P < 0.0001$) from $41.3 \pm 1.0$ kg to $39.1 \pm 1.0$ kg, and visceral fat decreased ($P < 0.001$) from $1.2 \pm 0.1$ kg to $1.1 \pm 0.1$ kg. Lean mass was also reduced ($P < 0.001$) from $48.7 \pm 1.1$ kg to $47.3 \pm 1.0$ kg.

Adverse events

Constipation was experienced by 17% of subjects with no change throughout the intervention period (Table V). There were no reports of diarrhea, and a small proportion of subjects (2%) experienced water retention. Some subjects reported bad breath at baseline (14%), and this value doubled (29%) after 8 weeks, though not significantly ($P = 0.15$). Inability to fall asleep was not reported, though a small proportion of subjects (<10%) were unable to stay asleep. Dizziness and general weakness were reported by some subjects (<15%), with no change after 8 weeks of diet.
### Table V. Adverse events reported with 8 weeks of alternate day fasting

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-treatment</th>
<th>P-value ¹</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gastrointestinal issues</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Constipation (%)</td>
<td>17</td>
<td>17</td>
<td>1.00</td>
</tr>
<tr>
<td>Diarrhea (%)</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>Water retention (%)</td>
<td>2</td>
<td>2</td>
<td>1.00</td>
</tr>
<tr>
<td>Bad breath (%)</td>
<td>14</td>
<td>29</td>
<td>0.15</td>
</tr>
<tr>
<td><strong>Sleep disturbances</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inability to fall asleep (%)</td>
<td>0</td>
<td>0</td>
<td>1.00</td>
</tr>
<tr>
<td>Inability to stay asleep (%)</td>
<td>7</td>
<td>2</td>
<td>0.50</td>
</tr>
<tr>
<td><strong>Other adverse effects</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dizziness (%)</td>
<td>10</td>
<td>17</td>
<td>0.38</td>
</tr>
<tr>
<td>General weakness (%)</td>
<td>14</td>
<td>10</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Values reported as mean % occurrences at each time point (n = 59).

¹ P-value between baseline and post-treatment: McNemar test.
Eating disorder symptoms and body image perception

Depression and binge eating decreased (P < 0.01) after 8 weeks of ADF (Table VI). Purgative behavior was low at baseline and remained unchanged. Fear of fatness was moderate, and did not change from baseline to post-treatment. The intervention helped subjects increase (P < 0.01) restrictive eating, but had no impact on avoidance of forbidden foods. Body image perception improved (P < 0.01) from baseline to post-treatment.

**Table VI.** CHANGES IN EATING DISORDER SYMPTOMS AND BODY IMAGE PERCEPTION AFTER 8 WEEKS

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Post-treatment</th>
<th>P-value $^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eating disorder symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>27 ± 1</td>
<td>25 ± 1</td>
<td>0.01</td>
</tr>
<tr>
<td>Binge eating</td>
<td>33 ± 1</td>
<td>30 ± 1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Purgative behavior</td>
<td>9 ± 1</td>
<td>9 ± 1</td>
<td>0.38</td>
</tr>
<tr>
<td>Fear of fatness</td>
<td>43 ± 2</td>
<td>43 ± 1</td>
<td>0.71</td>
</tr>
<tr>
<td>Restrictive eating</td>
<td>26 ± 1</td>
<td>31 ± 1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Avoidance of forbidden foods</td>
<td>37 ± 2</td>
<td>39 ± 2</td>
<td>0.14</td>
</tr>
<tr>
<td>Body image</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Concerns about body size/shape</td>
<td>53 ± 2</td>
<td>48 ± 2</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Values reported as mean ± SEM (n = 59).

$^1$ P-value between baseline and post-treatment: Paired t-test.
5. **Discussion**

Subjects undergoing ADF experienced mild gastrointestinal issues, occasional problems with staying asleep, and minor dizziness/weakness. The rate of adverse events reported with ADF appears to be similar to that of daily calorie restriction. For instance, Cuevas et al., (144) and Lin et al., (145) observed minimal adverse outcomes with 30-50% restriction after 4-12 weeks (<15% of participants reported constipation, diarrhea, or dizziness). In the present study, the most commonly reported adverse event was bad breath. However, this side effect may be lessened by consuming more water throughout the day and chewing sugar-free gum between meals.

As for eating disorder symptoms, ADF does not increase the rate depression, binge eating, purgative behavior, fear of fatness, or avoidance of forbidden foods, and may have small beneficial effects on body image perception. Interestingly, restrictive eating was moderately increased with ADF, suggesting that the diet may help control unrestrained eating behaviors. (142) These findings for ADF are comparable to those of calorie restriction. In a recent study by Williamson et al., (146) it was demonstrated that 25% daily restriction did not increase eating disorder symptoms and had no other harmful psychological effects. Taken together, it is possible that neither ADF nor calorie restriction increase eating disorder symptoms in obese adults, but these findings require confirmation in a longer-term randomized control trial comparing the two diets.

This study has several limitations. Firstly, the adverse event questionnaire was not very comprehensive, and was developed based on adverse events reported by subjects in previous ADF trials conducted by our lab. Assessing the impact of ADF on cold intolerance, hair loss, headaches, muscle cramps, and difficulty concentrating, would provide a more comprehensive portrayal of the
diet’s safety profile. Second, the intensity of these adverse events (mild, moderate or severe) were not examined and recorded. Third, much of the adverse event data reported in this study was qualitative, and therefore may underestimate all potential adverse events associated with ADF. However, it should be noted that there is mounting quantitative data from previous studies (119, 120, 121, 122) indicating that ADF may protect against the development of cardiovascular and metabolic diseases. Thus, the present data should be evaluated in the context of these previous reports. (119, 120, 121, 122) Fourth, this study is limited in that it was short term (8 weeks), had a small sample size (n = 59), and did not directly compare the effects of ADF versus calorie restriction on these endpoints.

In summary, ADF appears to be a safe and effective way to lose weight, and produces little or no gastrointestinal, sleep, or energy level disturbances. We also show here that this diet does not increase eating disorder symptoms, and has benign or beneficial effects on body image perception. These preliminary findings are an encouraging first step in this field, but obviously warrant confirmation in a larger-scale, longer-term clinical trial.

6. Acknowledgements
KKH designed the experiment, ran the clinical trial, analyzed the data, and wrote the manuscript. CMK, JFT, AB, and SB assisted with the conduction of the clinical trial and performed the laboratory analyses. KAV assisted with the data analyses and the preparation of the manuscript. Sources of funding for all authors: Departmental funding, Kinesiology and Nutrition, University of Illinois at Chicago.
V. MANUSCRIPT 3

Changes in hunger and fullness in relation to gut peptides before and after 8 weeks of alternate day fasting (Previously published in Clinical Nutrition, In Press [Epub ahead of print])

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Running head: Alternate day fasting effect on gut peptides

Funding source: Kinesiology and Nutrition, University of Illinois, Chicago

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1. **Abstract**

**Background and aims:** Alternate day fasting (ADF; 25% energy intake “fast day”, alternated with an ad libitum intake “feed day”) is effective for weight loss. Whether or not ADF modulates hunger, fullness and gut peptides in a way that enhances dietary compliance and weight loss, remains unknown. Accordingly, this study examined the effect of ADF on postprandial appetite ratings and gut peptides.

**Methods:** Obese subjects (n = 59) participated in an 8-week ADF protocol where food was provided on the fast day. **Results:** Body weight decreased (P < 0.0001) by 3.9 ± 0.6 kg after 8 weeks of diet. Reductions (P < 0.05) in fat mass (-2.2 ± 0.2 kg), fat free mass (-1.4 ± 0.2 kg), visceral fat mass (-0.1 ± 0.1 kg), and resting metabolic rate (RMR; -104 ± 28 kcal/day) were also observed. Fasting leptin and insulin decreased (P < 0.05), while AUC ghrelin levels increased (P < 0.05). Despite these metabolic changes, there was no increase in subjective hunger by the end of the study. Furthermore, fullness and PYY increased (P < 0.05). Fat free mass and RMR were not related to hunger or ghrelin at any time point.

**Conclusion:** These findings suggest that the absence of a compensatory increase in hunger in conjunction with an increase in sensations of fullness may contribute to the weight loss efficacy of an 8-week ADF regimen.

**Keywords:** Alternate day fasting, calorie restriction, hunger, fullness, gut peptides, obese adults
2. **Introduction**

Alternate day fasting (ADF) is a novel weight loss approach that has gained recent popularity (147). ADF consists of a 25% energy intake “fast day” alternated with an ad libitum intake “feed day”. Recent studies show that obese adults lose 4-8% of body weight after 8-12 weeks (119, 120, 121, 122, 148, 149), and that adherence to ADF remains high (90-95%) throughout the duration of the trial (150).

Difficulty in managing sensations of hunger is one of the main reasons given for aborted or unsuccessful attempts to diet (151, 152). The precise reason why compliance remains elevated with ADF is not clear, but may be related to favorable changes in hunger and fullness (153). For instance, in a trial by Klempel et al., (150) hunger decreased on the fast day after 8 weeks of ADF. Complementary to these findings, Bhutani et al., (154) observed reductions in hunger and increases in fullness on the fast day after 12 weeks of ADF. It should be noted, however, that these findings are limited in that subjective ratings of hunger and fullness were only measured at one time point on the evening of a fast day (pre and post treatment), and that post-prandial levels before and after ADF were not assessed (150, 154). Moreover, none of these studies (150, 154) employed objective hormonal measures of appetite, such as ghrelin (indicator of hunger) or PYY and glucagon like peptide-1 (indicators of fullness). Thus, whether or not these findings for hunger and fullness can be reproduced when more robust measures are employed, warrants investigation.

Also of interest, is the role of fat free mass and resting metabolic rate (RMR) in mediating these decreases in hunger. Previous findings suggest that fat free mass and RMR generally decrease in response to weight loss (155, 156), and that reductions in these parameters are related to lower hunger levels (157). Although the data for ADF are limited, evidence suggests that fat free mass and RMR typically decrease with 4-8% weight loss (120, 148, 149, 158), while some studies show no change
(119, 121, 122). Whether or not reductions in fat free mass and RMR are associated with decreases in hunger during ADF is an important question that has yet to be addressed.

Accordingly, this study examined the effect of 8-weeks of ADF on fasting and postprandial appetite ratings and gut peptide concentrations in obese adults. The degree to which changes in fat free mass and RMR correlate with changes in hunger and gut hormone concentrations, was also assessed.

3. Methods

Subjects

Participants were recruited from the Chicago area by flyers. Subjects were obese (BMI between 30 and 39.9 kg/m²), 25 to 65 years of age, pre-menopausal or post-menopausal, physical activity level at < 3 h/week at 2.5 to 4.0 metabolic equivalents (METs) for 3 months prior to the study, weight stable for 3 months prior to the study (< 4 kg weight loss or gain), non-diabetic, no history of cardiovascular disease, non-smokers, and not taking any medications that would affect study outcomes. A total of 159 subjects were screened, and 74 began the study. The experimental protocol was approved by the Office for the Protection of Research Subjects at the University of Illinois at Chicago (Protocol #2013-0220). All participants gave their written informed consent to participate in the trial.

Diet intervention

A 10-week trial consisting of a 2-week baseline period followed by an 8-week weight loss ADF period, was employed to test the study objectives (158). During the baseline control period, subjects maintained a stable body weight by continuing their usual diet. During the weight loss period, all subjects participated in an ADF protocol where 25% of baseline energy needs were consumed on the
fast day (24 h), and ad libitum intake was permitted on each alternating feed day (24 h). Subjects were provided with meals on each fast day, and ate ad libitum at home on the feed day. The Mifflin equation (125) was used to calculate total energy expenditure (TEE) for each individual, and these values were used to estimate fast day energy intake. For the fast day meals, a 3-day rotating menu was employed. The macronutrient distribution of the provided fast day meals was as follows: ~24% kcal as fat, ~16% kcal as protein, and ~60% kcal as carbohydrates (158). Participants visited the research center on a weekly basis to pick up their meals for the week. Subjects were allowed to consume zero-calorie beverages, such as black coffee, tea and diet soda, on the fast day.

**Diet adherence and physical activity maintenance**

As described previously (158) compliance with the fast day was assessed weekly using an “Extra food log”. Any alteration in physical activity habit was measured by a validated accelerometer (159) (Sense Wear Mini, Bodymedia, Pittsburg, PA). Each participant wore the accelerometer on their upper arm for 7 d (23 h/d) at baseline and post-treatment.

**Dietary intake**

Each subject completed a 7-d food record at baseline and week 10 of the trial. Subjects were provided with food diaries, and were instructed to record food items in as much detail as possible. Each subjects met with the Research Dietitian at baseline to learn how to properly document their food intake in the diaries. All dietary information was analyzed using the food analysis program, Nutritionist Pro (Axxya Systems). The program was used to calculate total daily intake of energy, fat, protein, and carbohydrates.
Body weight and body composition

Body weight was measured weekly at the research center using a balance beam scale (HealthOMeter, Boca Raton, FL). Measurements were taken to the nearest 0.25 kg in a hospital gown and without shoes. BMI was assessed as kg/m$^2$. Fat mass, fat free mass, visceral fat mass were quantified by dual x-ray absorptiometry (DXA; iDXA, General Electric Inc) (128).

Blood sampling and test meal protocol

Baseline blood draw: At the beginning of the control period (week 1), a baseline 12-h fasting blood sample was collected between 6.00 am and 9.00 am. Meal challenge blood draws: The meal challenge protocol was performed before (week 3) and after the weight loss period (week 10). Subjects stopped the ADF regimen two to three days prior to the meal challenge, and were instructed to eat their usual diet. Subjects were fasted for 12-h. The initial baseline blood draw was performed at −15 min. After the baseline draw, subjects waited 10 minutes, and then consumed two bottles of a nutritionally complete liquid meal served at 4 °C (237 ml Ensure Original Nutrition Shake, Abbott, South Pasadena, CA). The macronutrient profile of the test meal was as follows: 24% kcal from fat, 16% kcal from protein, 60% kcal from carbohydrate, with a total energy value of 440 kcal. The test meal was designed to have a similar energy content and macronutrient profile as a typical breakfast meal. Blood was then drawn immediately after consumption of the liquid meal (0 min). Subsequent blood samples were drawn at 30, 90, and 120 min. Following the methods of Gibbons et al., (160), hunger and fullness ratings were assessed using a visual analog scale (0–100 mm) immediately before each blood draw.

Blood samples were collected in tubes containing EDTA. After collection, blood was centrifuged for 15
min at 2800 RPM at 4°C to separate plasma from red blood cells. The plasma was then immediately pipetted and stored at -80°C therefore limiting the time for degradation.

**Biochemical assays**

Authors of the current paper have experience in measuring both total and acylated ghrelin, with both forms of ghrelin giving the same meal-induced responses (160). Therefore, for the current study, we decided to measure total ghrelin levels, due to the lower CV values. Total ghrelin was measured in duplicate by ELISA from EMD Millipore, Billerica, MA (intra-assay coefficient of variation (CV) = 5.4, inter-assay CV = 6.6). Total glucagon like peptide-1 (GLP-1) was assayed in duplicate with ELISA from EMD Millipore (intra-assay CV = 27.2, inter-assay CV = 15.3). The majority of circulating PYY is known to be PYY_{3-36}, therefore the measurement of total PYY (which was the only feasible option in the current study) effectively measured PYY_{3-36}. Total peptide YY (PYY) was quantified in duplicate by ELISA from EMD Millipore (intra-assay CV = 4.3, inter-assay CV = 7.0). Leptin was measured in duplicate using an ELISA kit from R&D Systems, Minneapolis, MN (intra-assay CV = 5.5, inter-assay CV = 6.2). All samples were within the measurable range of the kit. Fasting glucose was assessed in duplicate with a hexokinase reagent kit (Abbott, South Pasadena, CA). Fasting insulin was quantified in duplicate as total immunoreactive insulin (Coat-A-Count Insulin, Los Angeles, CA).

**Resting Metabolic Rate**

A handheld indirect calorimeter was used to measure resting metabolic rate (RMR) (129) (MedGem Indirect Calorimeter, Microlife, USA). Participants were instructed not to consume food, water, or perform any exercise for 12-h prior to the RMR measurement. Each patient rested in the supine position for 25 min in a dark room before the measurement. After this resting period, a mouthpiece
was placed on the participant, and oxygen consumption was measured until stable flow was reached (10-15 min).

**Statistics**

Results are presented as means ± standard error of the mean (SEM). One-way ANOVA was performed to determine differences for variables measured at week 1, 3 and 10. The Bonferroni correction was used to assess significance. Differences in fasting/postprandial levels of hormones and appetite ratings between baseline (week 3) and post-treatment (week 10) were assessed by repeated measures ANOVA. Area under the curve (AUC) was calculated for gut hormone levels and appetite ratings using trapezoidal approximation. Differences between baseline and post-treatment AUC were assessed by a paired t-test. Correlation analyses were performed to evaluate the relationship between appetite ratings, gut hormones, fat free mass and RMR. A test for normality was included in the model. P-values were considered significant at < 0.05. Data were analyzed by using SPSS software (Mac v.21, SPSS Inc., Chicago, IL).

**4. Results**

**Baseline characteristics and dropouts**

Of the 74 subjects who commenced the study, 59 completed the entire protocol. Dropouts were primarily due to scheduling conflicts (n = 2), personal reasons (n = 4), issues with the ADF diet (n = 6, i.e. dislike of foods provided on the fast day), and unspecified reasons (n = 3). Subjects (50 females/9 males) who completed the study were middle age (46 ± 1 y), obese (93.6 ± 2.2 kg; BMI 34 ± 1 kg/m²), and not physically active.
Dietary adherence, body weight, body composition, and RMR

As reported previously (158), subjects met their energy goal on 91 ± 2% of fast days. Subjects did not change their activity patterns from baseline (6042 ± 530 steps/d) to post-treatment (5851 ± 411 steps/d). At screening, subjects were classified as “low active” (161) via a study-specific screening questionnaire. This “low active” level of activity was reconfirmed via the activity monitor (“low active” classified as 5000-7499 steps/d (161)). Changes in body weight over the course of the trial are displayed in Figure 3. Body weight remained stable during the control period, and decreased (P < 0.0001) by 3.9 ± 0.6 kg during the weight loss period. Fat mass, fat free mass, and visceral fat mass also decreased (P < 0.001) during the weight loss period (TABLE VII). RMR decreased (P < 0.05) by 104 ± 28 kcal/d after 8 weeks of intervention. There were no differences between males and females for dietary adherence, percent weight loss, and RMR.

Figure 3. Changes in body weight over the course of the trial

Values presented as mean ± SEM (n = 59). * Significantly different (P < 0.0001) from week 3 values.
TABLE VII. CHANGES IN BODY COMPOSITION AND METABOLIC PARAMETERS OVER THE COURSE OF THE TRIAL

<table>
<thead>
<tr>
<th></th>
<th>Week 1 Start of control period</th>
<th>Week 3 Start of intervention</th>
<th>Week 10 End of intervention</th>
<th>Change Week 3 to 10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat mass (kg)</td>
<td>41.3 ± 1.0</td>
<td>41.3 ± 1.0</td>
<td>39.1 ± 1.0 *</td>
<td>-2.2 ± 0.2</td>
</tr>
<tr>
<td>Fat free mass (kg)</td>
<td>48.7 ± 1.1</td>
<td>48.7 ± 1.1</td>
<td>47.3 ± 1.0 *</td>
<td>-1.4 ± 0.2</td>
</tr>
<tr>
<td>Visceral fat mass (kg)</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.1 ± 0.1 *</td>
<td>-0.1 ± 0.1</td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>53.4 ± 4.9</td>
<td>52.7 ± 4.4</td>
<td>43.8 ± 4.5 **</td>
<td>-8.9 ± 2.4</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>98.9 ± 2.2</td>
<td>100.0 ± 2.3</td>
<td>96.2 ± 1.4 **</td>
<td>-3.8 ± 1.3</td>
</tr>
<tr>
<td>Insulin (µlU/ml)</td>
<td>13.2 ± 0.9</td>
<td>13.5 ± 1.0</td>
<td>10.6 ± 0.7 **</td>
<td>-2.9 ± 0.7</td>
</tr>
</tbody>
</table>

Values presented as mean ± SEM (n = 59). No differences from week 1 to 3 for any parameter.
* Significantly different from week 3 values (P < 0.001).
** Significantly different from week 3 values (P < 0.05).

Dietary intake

A 7-day food record was collected at baseline to assess usual intake, and another 7-day food record was collected at week 10 to assess dietary intake on feed days. At baseline, subjects consumed 1775 ± 64 kcal/d, 73 ± 3 g of protein (16% of total energy), 208 ± 11 g of carbohydrates (48% of total energy), and 72 ± 3 g of fat (36% of total energy). At week 10 on feed days, subjects consumed 1620 ± 68 kcal/d, 64 ± 3 g of protein (15% of total energy), 180 ± 11 g of carbohydrates (49% of total energy), and 65 ± 3 g of fat (36% of total energy). Baseline values did not differ from post-treatment values for energy or macronutrient distribution.
Subjective appetite ratings

Fasting and postprandial levels of hunger and fullness are shown in Figure 4. Hunger ratings declined after consumption of the test meal (P < 0.05), though the AUC for hunger did not differ between week 3 and 10. Fullness ratings increased (P < 0.01) after ingestion of the meal, and the AUC for fullness was higher at week 10 (P < 0.05) when compared to week 3. There were no differences between males and females for subjective appetite ratings.

Gut hormone concentrations

Fasting and postprandial levels of ghrelin, GLP-1 and PYY are displayed in Figure 5. Ghrelin demonstrated significant meal-related decreases (P < 0.05), and the AUC for ghrelin was higher at week 10 (P < 0.05) when compared to week 3. GLP-1 increased after consumption of the test meal (P < 0.001), but the AUCs did not differ between baseline and post-treatment. PYY demonstrated meal-related increases (P < 0.01), and the AUC at week 10 was higher (P < 0.05) than week 3. There were no differences between males and females for gut peptides at baseline or post-treatment.
Figure 4. Fasting and postprandial levels of hunger and fullness before (week 3) and after the intervention (week 10)

Values presented as mean ± SEM (n = 59). A. Hunger ratings decreased (P < 0.05) after the meal (ingested at 0 min) and remained low at 120 min post ingestion. The AUC for hunger did not differ between week 3 and week 10. B. Fullness ratings increased (P < 0.01) after the meal (ingested at 0 min) and remained higher at 120 min post ingestion. The AUC for fullness was higher at week 10 (P < 0.05) when compared to week 3.
Figure 5. Fasting and postprandial levels of ghrelin, glucagon-like peptide-1, and peptide tyrosine tyrosine before (week 3) and after the intervention (week 10)

Values presented as mean ± SEM (n = 59). A. Ghrelin levels decreased (P < 0.05) after the meal (ingested at 0 min), and the AUC for ghrelin was higher at week 10 (P < 0.05) when compared to week 3. B. GLP-1 levels increased (P < 0.001) after the meal (ingested at 0 min), with no differences in AUC between week 3 and week 10. C. PYY levels increased (P < 0.01) after the meal (ingested at 0 min), and the AUC was higher at week 10 (P < 0.05) when compared to week 3.
**Fasting leptin, glucose and insulin**

Leptin concentrations remained stable during the control period, and were reduced (P < 0.05) during the weight loss period by 8.9 ± 2.4 ng/ml (TABLE VII). Fasting glucose and insulin levels remained unchanged during the control period, and decreased (P < 0.05) by 3.8 ± 1.3 mg/dL and 2.9 ± 0.7 µIU/ml, respectively, by week 10.

**Relationship between appetite ratings, gut hormones, fat free mass and RMR**

Hunger was not related to ghrelin concentrations, fat free mass, or RMR at any time point. Fullness AUC was correlated with PYY AUC post-treatment (r = 0.33; P = 0.03). Fullness was not related to fat free mass or RMR at any time point.

5. **Discussion**

This is the first study to examine the impact of ADF on appetite ratings and gut peptide concentrations in response to a test meal. We show here that despite significant reductions in body weight (fat and fat free mass), leptin and insulin, and an increase in AUC ghrelin, post-prandial hunger levels did not change when baseline was compared to post-treatment after 8 weeks of ADF. Furthermore, AUC fullness and PYY levels increased from baseline to post-treatment, while GLP-1 levels remained unchanged. Changes in fat free mass and RMR were not related to hunger or ghrelin concentrations.

Compensatory increases in hunger following weight loss from continuous energy restriction are well documented for diet (162, 163, 164) and exercise induced weight loss (165). However, the effect of ADF-induced weight loss on hunger sensations has only been assessed in a handful of studies (120, 148, 150, 154). Contrary to continuous regimens, results from these trials indicate that hunger
decreases or remains unchanged after 3-12 weeks of intervention (120, 148, 150, 154). However, these previous studies are flawed in that hunger ratings were measured only at one time point, in a non-fasted state. The primary goal of the present trial was to see if these reductions in hunger by ADF could be replicated if a meal challenge test was imposed before and after 8 weeks of diet. We show here that AUC for hunger did not increase when baseline was compared to post-treatment. We also measured post-prandial changes in ghrelin. Ghrelin is a peptide produced by the gastrointestinal tract (166, 167). When the stomach is empty, ghrelin is secreted, and the hormone acts on the hypothalamus to increase hunger (166, 167). In the present trial, ghrelin AUC increased after 8 weeks of ADF. An increase in ghrelin could signal an increase in hunger (168), but as mentioned above, under ADF conditions, hunger remained unchanged over the course of the trial. Our findings for hunger and ghrelin are therefore inconsistent with published findings from calorie restriction (CR) studies (169, 170, 171), which tend to demonstrate increases in both hunger and ghrelin with 3-8% weight loss (169, 170, 171). However, ADF could affect ghrelin differently from CR due to cycling between feed and fast day, and this should be considered when comparing the two approaches. It is possible that the increase in ghrelin (with accompanying decreases in leptin and insulin) is reflecting the “normalization” of adiposity-related hormones in the obese subjects. Obese individuals tend to have lower ghrelin levels without any impact on hunger. Thus, this increase in ghrelin could indicate that appetite control is returning to that of a lean/normal weight system.

Also of interest was the effect of ADF on fullness ratings. In the present trial, fullness AUC increased after 8 weeks of treatment. We also observed an increase in PYY AUC from baseline to post-treatment, with no change in GLP-1. PYY and GLP-1 are peptides released from the gastrointestinal tract in response to feeding (172). These two hormones have been shown to reduce appetite/increase fullness
by inhibiting gastric motility (amongst other mechanisms) (172). The present findings are similar to what has been reported previously for ADF. For instance, Bhutani et al., (154) reported significant increases in fullness after 12 weeks of ADF in obese subjects consuming 25% of their energy on the fast day. Heilbronn et al., 2005 (148) also noted higher fullness ratings after only 3 weeks of ADF when normal weight subjects consumed no energy on the fast day. Although the data is limited, previous (148, 154) and present findings suggest that ADF may lead to higher fullness ratings over the course of the trial, which may augment dietary adherence. This increase in fullness may begin as early as 3 weeks, and may be sustained for 8-12 weeks or beyond. The reason why ADF promotes fullness is not clear, but may involve increases in the satiety-promoting gut peptide, PYY (168).

Overall, in the present study, ghrelin levels increased with no subsequent change in hunger levels. Simultaneously, both fullness and PYY levels increased. It is important to remember that there are several gastrointestinal peptides that have been linked to roles in appetite control; hunger and fullness are not the result of the changes in one single peptide (173). Therefore, the present study should emphasize considerations for a multi-peptide response influencing both hunger and fullness. For example, in the present study, the increase in PYY (and possibly other peptides not measured) had a stronger effect on subjective appetite (increasing fullness but no change in hunger) than the change in ghrelin levels.

As a secondary objective, we wanted to see if changes in fat free mass and RMR by ADF would be related to hunger and ghrelin concentrations. We hypothesized that reductions in fat free mass and RMR by ADF may suppress hunger (157, 174), and that these beneficial adaptions would encourage compliance with ADF. Contrary to what we hypothesized, reductions in fat free mass and RMR were
not related to hunger or ghrelin at any point. However, the clinical significance of these findings is
difficult to discern, as this study was not designed to directly test this hypothesis. It is also likely that
our study was not adequately powered to test these relationships, as our sample size was quite small
(n = 59). Thus, it remains unclear if ADF can modulate free mass and RMR in a way that would decrease
hunger.

Decreases in fasting leptin and insulin were also observed after 8 weeks of ADF. Leptin mediates long-
term energy balance by suppressing food intake (175). Obese individuals have high levels of leptin but
are leptin resistant (176). Insulin acts similarly to leptin in that it suppresses appetite and reduces food
consumption (177). During weight loss, leptin and insulin generally decrease (178). When the
circulating concentrations of these hormones are reduced, the brain becomes less sensitive to meal-
generated satiety signals (179). As a result, more calories than normal need to be consumed before a
sufficient signal is generated to stop food intake. Thus, decreases in leptin and insulin with weight loss
can result in increased appetite, food intake, and weight regain (179). What remains to be established
is how these appetite-stimulating changes in leptin and insulin, can be counteracted by the appetite-
suppressing changes in PYY observed with ADF. Evaluating the long-term impact of ADF on these
hormones during weight loss and weight maintenance could help answer these important questions.

This study has several limitations. First, meals were not standardized the day preceding the meal
challenge. Although all subjects were fasted for 12-h before the test, the varying energy and
macronutrient compositions of the previous night’s meal may have impacted our findings. Second, we
did not perform post-prandial assessments of leptin or insulin at baseline and post-treatment due to
budgetary constraints. Third, due to malfunctioning activity monitors, we were only able to collect
activity data for 80% of the subjects. As such, it remains unknown whether the other 20% of subjects maintained their level of physical activity throughout the trial. Fourth, ketone bodies were not measured during the study. Ketones are related to changes in hunger and fullness (180), thus measuring ketones could be helpful in interpreting the gut hormone data. Fifth, protease inhibitors were not used during the blood collection. However, it should be noted that all GLP-1 samples were within detectable limits even during fasting; therefore, we are confident that limited degradation occurred. It can also be presumed that any degradation that did occur would be the same for all samples and for all participants; thus, the findings of the study are not in question.

In summary, we show here that ADF results in no compensatory increase in sensations of hunger, and increases postprandial fullness and PYY levels in a way that may encourage ongoing compliance with the diet. Future trials should examine whether these changes in appetite and gut peptides are transient or persist over time with added weight loss and sustained weight maintenance.

6. **Acknowledgements**

KKH designed the experiment, ran the clinical trial, analyzed the data, and wrote the manuscript. CMK, JFT, AB, SB and KG assisted with the conduction of the clinical trial and performed the laboratory analyses. CG and GF helped with the data analysis and the preparation of the manuscript. KAV assisted with the design of the experiment, data analyses and the preparation of the manuscript.
VI. DISCUSSION

1. Adherence to ADF during varying mealtimes and frequencies

There was no statistical difference between completers and non-completers (Table VIII). Varady et al., 2015 (181) has recently shown that successful ADF dieters are typically older (50-59 y) and Caucasian with no differences based on gender or initial weight status. Participants in the current study reported similar fast day adherence (~90%) across all interventions. Thus, the timing of the meal does not seem to alter adherence. Previous ADF studies, which required individuals to consume the fast day meal as a lunch, observed adherence between 80%-90%. (2) (16) (19) Thus, altering the timing of the fast day meal appears to have no impact on level of adherence, when compared to previous studies. (2, 16) (19)
Table VIII.

COMPARISON OF BASELINE CHARACTERISTICS OF COMPLETERS COMPARED TO NONCOMPLETERS

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Completers (all groups pooled)</th>
<th>Non-completers (all groups pooled)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)¹</td>
<td>46 ± 1</td>
<td>40 ± 3</td>
</tr>
<tr>
<td>Sex (F/M)²</td>
<td>49/9</td>
<td>9/4</td>
</tr>
<tr>
<td>Ethnicity (n)²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>African</td>
<td>28</td>
<td>3</td>
</tr>
<tr>
<td>American</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>Hispanic</td>
<td>17</td>
<td>3</td>
</tr>
<tr>
<td>Caucasian</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)¹</td>
<td>94 ± 1</td>
<td>100 ± 3</td>
</tr>
<tr>
<td>Height (cm)¹</td>
<td>165 ± 1</td>
<td>167 ± 2</td>
</tr>
<tr>
<td>BMI (kg/m²)¹</td>
<td>34 ± 0.4</td>
<td>36 ± 1</td>
</tr>
</tbody>
</table>

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

¹P-Value assessed by way of independent samples t-test.
²P-Value for categorical variables assessed by Chi-Squared.

No difference between completers and non-completers for any parameter.
2. The effect of meal timing and frequency during alternate day fasting on body weight and body composition

Overall body weight changes are depicted in figure 6. While only weight gain was experienced in female subjects there was no statistical significance noted for percent body weight change between males and females. Body weight and composition improved similarly in each group. The approximate 4% (4 kg) body weight reduction in all three groups is reflective of the high diet adherence discussed previously. Fat mass was reduced by about 4-7% (2 kg) regardless of ADF intervention. These reductions are similar to previous ADF findings. Previous studies with a 25% energy intake on the fast day lasting 8-12 weeks have resulted in weight loss ranging from 3-6%. (2, 3, 18, 19) Two of which report higher fat loss at 10-12%. (2, 3)
This is the only ADF study to measure changes in visceral fat from DXA. In the present study, the intervention groups experienced a reduction in visceral body fat from 6-12%. There were no differences between groups. Previous studies reported a 5-7% decrease in waist circumference as an indirect proxy for visceral fat. (2, 3, 19) Therefore, it is difficult to compare across studies. Considered together, ADF appears to be capable of decreasing visceral fat, and this has the potential to reduce chronic disease risk.
Lean mass was also reduced to a similar extent in all groups by about 1 kg. This reduction is not consistent with earlier ADF reports indicating lean mass retention. (2, 3) One other study lasting 12 weeks showed a similar lean mass loss (19). However, findings from the 12-week study should be interpreted with caution due to the high dropout rate and subsequent intention to treat analysis. (19)

The present study used DXA to assess body composition. Bioelectrical impedance analyzer (BIA) has been used in previous studies (2, 19). However, results from BIA are less reliable at high levels of body fat due to wider hydration fluctuations. (182) Discrepancies could result due to high adiposity in the study population and lack of researcher control over hydration status.

3. **The effect of meal timing and frequency during alternate day fasting on lipid parameters**

The present ADF study showed no change in total cholesterol, LDL cholesterol, HDL cholesterol, or triglycerides after 8 weeks of dieting. Studies of similar duration and weight loss have reported decreases in total cholesterol by 13-21% and LDL cholesterol by 15-31%. (3, 21) Most ADF interventions have not shown HDL cholesterol to change (3, 11, 18, 19, 21). However, one study with 8% weight loss over 8 weeks resulted in a 9% increase in HDL cholesterol. (17) Two eight-week studies from our lab have shown triglycerides to decrease by 14-32%. (3, 21) However, findings related to triglycerides are equivocal as studies with both longer and shorter intervention durations have reported varying results. (11, 17, 19) The reason for varying lipid outcomes is unclear. Participants in the current study did not have hyperlipidemia. For this reason the outcomes may have not resulted in significant results.

LDL particle size increased by 1.3 Å in all of the groups. Prior research has shown LDL particle size to increase 2-5 Å. (3, 18, 19, 21) These collective improvements in LDL particle size represent a 1-2%
increase. Neither the concentration of LDL particle size or the change in LDL particle size were correlated to visceral fat. In addition, the atherosclerotic profile of the particles improved. There was an increase in the proportion of large LDL particles in the ADF-L (5%), ADF-D (6%), and ADF-SM (5%) groups. The proportion of small LDL particles decreased by 6% in the ADF-D and ADF-SM groups only. The decrease in the proportion of small LDL particles with concomitant increases in the proportion of large LDL particles is consistent with previous ADF research. (3, 18, 19, 21) These findings suggest that a dieter’s coronary heart disease risk can improve despite no change in total or LDL cholesterol.

4. **The effect of meal timing and frequency during alternate day fasting on metabolic disease outcomes**

The impact of the three feeding protocols on other metabolic disease risk factors was also assessed. Systolic blood pressure was reduced by 5% in the ADF-SM group alone after 8 weeks of the diet. A trend for a 4% decrease in systolic blood pressure occurred in the ADF-D group. None of the groups noted a significant decrease in diastolic blood pressure. Baseline blood pressure values were within normal ranges for all groups. However, mean systolic blood pressure in the ADF-SM group was insignificantly higher at baseline than the other groups. This could explain why only this group experienced a significant within group change after the diet. The reduction in systolic blood pressure in the ADF-SM group is similar to the 3-8% reductions observed by several other ADF studies ranging from 6-12 weeks. (2, 11, 19) (15) Only three previous ADF studies have reported a decrease in diastolic blood pressure. (11, 15, 19) However, two of these studies lasted 12 weeks.(15, 19) Therefore, weight loss over a longer period of time may be necessary to see systolic blood pressure decreases.
Heart rate decreased by 7 bpm (10%) in the ADF-L group with no changes in the ADF-D and ADF-SM groups. It is not clear why heart rate was reduced by ADF-L only, as all groups had similar baseline values (71-72 bpm). This finding is also somewhat surprising, as ADF does not generally improve heart rate. (3, 19)

None of the interventions in the present study changed glucose, insulin, or insulin sensitivity. This could be a consequence of normal values at baseline. The lack of change in these glucoregulatory factors may also be related to the intervention duration. For example, 12 weeks of ADF resulted in a modest 3 mg/dosecrease in glucose. (19) Two studies short-term studies with a complete fast on the fast day showed improvements in glucose regulation on the fast day only. (12, 13) Therefore, it is possible that the effect of ADF on glucose regulation requires either a longer intervention duration or a more extreme calorie depletion.

5. **Safety of alternate day fasting and effect on eating disorder symptoms**

This study is the first to show that ADF is a safe way to lose weight, and produces little or no gastrointestinal, sleep, or energy level disturbances. Side effects based on anecdotal reports from previous dieters were used for assessment. Constipation was initially the most frequent complaint at 17%, and remained consistent at the end of the study. Bad breath and dizziness were issues at the beginning (14% and 10%, respectively) of the diet which increased as time when on (29% and 17%, respectively). An inability to stay asleep and general weakness were reported initially (7% and 14%, respectively), but decreased once participants became acclimated to the diet (2% and 10%, respectively) Collectively, these represent modest occurrences of adverse events that affected less than 15% of the participants in the study. The present findings are similar to very low calorie diets
reporting the occurrence of constipation and gastrointestinal distress. (183) (184) (185) Adverse events seem to occur more often with increasing calorie restriction. (185) Very low calorie diets ranging from 450-800 kcal/day have shown adverse events occur 45-29% of time, respectively. (183) However, additional parameters, such as headaches, mood, and hair loss were not assessed in the current study. As such, the overall total of adverse events with ADF could be higher.

This study also shows that that ADF does not increase eating disorder symptoms, and has benign or beneficial effects on body image perception. Participants’ self-reports indicate an improvement in binge eating (9%), depression (7%), body image scores (9%), and restrictive eating (19%). These findings are similar to the CALERIE study where depression, binge eating, body shape, restrictive eating improved after three months of a 25% calorie restriction. (114) Additionally, results from a meta-analysis show improvements in depression with weight loss. (186) Hence, weight loss by way of ADF can actually be psychosocially beneficial. However, it cannot be recommended populations at risk for psychological disturbances.

6. Change in appetite hormones after weight loss from pooled ADF data

This study is unique in that it shows advantageous changes to subjective appetite and gut hormones. This is represented by an increase in subjective fullness and concurrent increase in circulating PYY (fullness hormone). Additionally, participant reported hunger remained unchanged notwithstanding a modest increase in ghrelin (hunger hormone).

Ghrelin increased 5% and subjective hunger remained unchanged after 8 weeks of ADF. Typically, ghrelin increases prior to a meal to signal hunger and is suppressed upon energy intake. Ghrelin
response is thought to be dysfunctional in the obese. This is denoted by a lower fasting concentration and impaired postprandial suppression. (187) As such, the increase in ghrelin without a change in hunger could represent a return to normalcy in this weight reduced obese population. One other ADF study utilizing a complete fast showed no change in morning fasting ghrelin concentrations. (13) Calorie restriction studies with weight loss of 3-10% show that fasting and postprandial ghrelin concentrations commonly increase with weight loss. (106) (38, 108). Moderate calorie restriction at 30% has resulted in a 14% increase in ghrelin AUC (106). More extreme depletion in a very low calorie diet showed ghrelin to increase as high as 47%. (108). These increases are much higher than those reported in the present study. Additionally, an increase in hunger has been shown after diets resulting in higher ghrelin profiles. (108) The cycling feed and fast protocol employed by ADF diets has the potential to affect ghrelin and hunger differently from traditional CR. Reports from previous ADF studies show a consistent decrease in hunger. (14, 15, 16, 116) Ghrelin concentrations have been show to correspond to subjective hunger. (188) However, there was no relationship between ghrelin and hunger in the present study. As such, there appears to be a decoupling of ghrelin and hunger after ADF. This may have beneficial outcomes related to diet success and weight maintenance.

PYY AUC increased 16% while there was no change in GLP-1. Both hormones are anorexigenic hormones that are secreted after a meal and may be reduced in obese states. (82, 102, 189) Previous CR studies report equivocal PYY findings, (38, 106, 108, 111) and macronutrient composition and adiposity may muddy observations. (190) Fasting GLP-1 decreased and showed nonsignificant postprandial outcomes after a very low calorie diet. (108) No previous ADF studies assessed PYY or GLP-1 concentrations. However, many have reported subjective increases in fullness from 47-66%. (14, 15, 116) In agreement with this, postprandial fullness increased after weight loss and was correlated
with PYY in the present study. These findings suggest that ADF may be advantageous in its ability to promote fullness while dieting.

Leptin decreased by 17% in the current study. The obese tend to have high leptin concentrations and leptin resistance. Leptin is an adipokine that acts to increase metabolic rate and suppress appetite. (191, 192) Leptin has been shown to decrease relative to caloric deficit (193), and can occur in a little as 12 hours of a fast. (194). Previous work from our lab showed a 24-40% decrease with similar weight loss. (14, 195) (15) Moderate CR diets have resulted in a similar decrease of about 30% over 8-12 weeks (106) (193) In light of these comparisons, ADF and CR seem to have similar leptin outcomes. Prior to this study, the relationship of leptin with body weight and appetite had not been evaluated. As expected, leptin correlated with body fat throughout the study duration. Both leptin and body fat were inversely related to hunger at week 10. Decreases in leptin have been shown to correlate with higher reports of hunger following weight loss. (196) It remains unseen why this ADF study resulted in a blunted decrease in leptin compared to other ADF work. It is possible that this smaller change in leptin was not great enough to elicit undesirable alterations to appetite.

These findings suggest that ADF’s effectiveness at mitigating subjective hunger and increasing sensations of fullness may be attributed to distinctive alterations in appetite hormone profiles. Collectively, this characteristic stands to improve adherence and success during an 8-week ADF regimen.
VII. FUTURE DIRECTIONS

It is now understood that the fast day meal can be consumed at any time throughout the day during an ADF diet. This offers dieters the potential option to consume this meal at a time that best suits their lifestyle. However, a self-selected mealtime has not been directly studied during ADF. As such, a flexible meal may be more accommodating to dieters trying to lose weight and maintain normalcy within social situations. However, the lack of ridged structure on the fast day could also be detrimental to someone requiring a restricted mealtime to stay on track.

It would be interesting to further explore appetite modulations that occur with ADF. This was the first study to approach the topic by assessing both objective hormone data and subjective reports during a meal challenge. The data suggests interesting appetite alterations after ADF that may support diet adherence and weight maintenance. However, this study was not powered or funded appropriately to adequately evaluate the full scope of appetite.

One possible study would be a similar in duration and subject characteristics; however this study would ask dieters to select their mealtime throughout the intervention. The study would also recruit a larger number of subjects. This would allow for more robust measurements related to appetite. For example, a meal-challenge could be conducted over a longer duration and also assess leptin, insulin, CCK, and glucose over the course of the challenge.
In conclusion, ADF with a fast day meal served throughout the day, as one lunch, or as one dinner are all effective diet therapies for weight loss in the obese. Consistent with previous ADF research, adherence to the diet regimes in this study was high. The high adherence could be a down-stream effect of ADF increasing fullness and not resulting in increased hunger. Additionally, coronary artery disease risk may be reduced due to beneficial alterations to LDL particle size proportions. Findings in the present study do not support the use of ADF to reduce insulin and glucose. However, a glucoregulatory effect many only be seen in a diabetic or pre-diabetic population. Blood pressure also showed varying results based on mealtime. Collectively, ADF with varying fast day meal times may be implemented to facilitate weight loss and potentially reduce risk of CHD in obese populations.
APPENDICES

APPENDIX A – CONSENT FORM

University of Illinois at Chicago
Research Information and Consent for Participation in Biomedical Research

Meal timing during alternate day fasting: Effect on heart disease risk and hunger in obese humans
You are being asked to participate in a research study. Researchers are required to provide a consent form such as this one to tell you about the research, to explain that taking part is voluntary, to describe the risks and benefits of participation, and to help you to make an informed decision. You should feel free to ask the researchers any questions you may have.

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

Why am I being asked?
You are being asked to be a subject in a research study about alternate day fasting for weight loss and heart disease risk reduction. You have been asked to participate in the research because you responded to our ad and may be eligible to participate. 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 90 subjects may be involved in this research at UIC.

What is the purpose of this research?
This research is being done to test the effects of meal timing during alternate day reductions in calorie intake on weight loss and heart disease risk factors.

What procedures are involved?
This research will be performed at the Human Nutrition Research Unit (HNRU), 1919 W Taylor St., Room 121C, Applied Health Sciences Building at UIC. You will need to come to the study site 11 times over the next 10 weeks. Each of those visits will take about 20 minutes. However the third and last visit may take 3 hours.

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

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

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

1. **Alternate day fasting-lunchtime meal group**: If you fall in this category you will be asked to consume one meal provided to you on the “fast day” between 12.00 – 2.00 pm. On the “feed day”, you will be allowed to eat whatever and whenever you want.

2. **Alternate day fasting-dinnertime meal group**: If you fall in this category you will be asked to consume one meal provided to you on the “fast day” between 6.00 – 8.00 pm. On the “feed day”, you will be allowed to eat whatever and whenever you want.

3. **Alternate day fasting-small meals group**: If you fall in this category you will be asked to consume three small meals provided to you on the “fast day”, one meal at each of these times: 6.00 – 8.00 am, 12.00 – 2.00 pm, and 6.00 – 8.00 pm. On the “feed day”, you will be allowed to eat whatever and whenever you want.

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

<table>
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<tr>
<th>Study week</th>
<th>Lunchtime meal group</th>
<th>Dinnertime meal group</th>
<th>Small meals group</th>
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<tr>
<td><strong>Baseline period, no weight loss (Weeks 1 and 2)</strong></td>
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| 1 | • Blood draw  
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• X-ray for body composition  
• Ultrasound  
• Metabolism mask  
• Eating questionnaires  
• Photo taken | • Blood draw  
• Blood pressure  
• Body weight  
• X-ray for body composition  
• Ultrasound  
• Metabolism mask  
• Eating questionnaires  
• Photo taken | • Blood draw  
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• X-ray for body composition  
• Ultrasound  
• Metabolism mask  
• Eating questionnaires  
• Photo taken |
| 2 | • Blood pressure  
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• Eating questionnaires | • Blood pressure  
• Body weight  
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• Body weight  
• Eating questionnaires |
| **Weight loss/diet period (Weeks 3-10)** | | | |
| 3 | • Blood draw  
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• Blood pressure  
• Body weight  
• X-ray for body composition  
• Ultrasound  
• Metabolism mask | • Blood draw  
• Serial blood draw*  
• Blood pressure  
• Body weight  
• X-ray for body composition  
• Ultrasound  
• Metabolism mask | • Blood draw  
• Serial blood draw*  
• Blood pressure  
• Body weight  
• X-ray for body composition  
• Ultrasound  
• Metabolism mask |
**APPENDIX A – CONSENT FORM (continued)**

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*Serial blood draw description: After the initial blood draw, you will consume a meal replacement shake and a snack bar. Once you have consumed these food items, you will have your blood drawn at 1 min, 30 min, 60 min, and 120 min post-meal (about 2 tablespoons at each time point)*

*Meal timing during alternate day fasting*

*Main Consent, Version 6, November 18, 2013*
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 140 ml of blood (16 tablespoons) will be drawn during the study.

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

3. **Radiation exposure with X-ray scanning:** The amount of radiation you will be exposed to during X-ray scanning is relatively small (equivalent to 10 days of normal daily radiation exposure). Radiation exposure can cause cell mutations that may lead to cancer. But the amount of radiation you’re exposed to during an X-ray is so small that the risk of any damage to cells in your body is extremely low. 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 rare.

5. **Loss of confidentiality:** Loss of confidentiality is another potential risk. We will make every attempt to keep your personal information as confidential as possible. To maintain confidentiality, code numbers only will identify lab specimens and forms, and the 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.

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 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. You may not directly benefit from the research.

What other options are there?

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

**What about privacy and confidentiality?**

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

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

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

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

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

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

If you do seek medical treatment, please take a copy of this document with you because it may help the doctors where you seek treatment. It will also provide the doctors where you seek treatment with information they may need if they want to contact the research doctors. You may contact the researcher: Dr. Krista Varady at 312-996-7897 to talk to her about your illness or injury. You or your insurance company will be billed for this medical care. Your insurance company may not pay for some or all of this medical care because you are participating in a research study. There are no plans for the University to provide free medical care or to pay for research-related illnesses or injuries, or for the University to provide other forms of compensation (such as lost wages or pain and suffering) to you for research related illnesses or injuries. By signing this form you will not give up any legal rights.

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

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

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

You will not be offered payment for being in this study.
Can I withdraw or be removed from the study?
If you decide to participate, you are free to withdraw your consent and discontinue participation at any time without affecting your future care at UIC. You have the right to leave a study at any time without penalty. For your safety, however, you should consider the investigator’s advice about how to leave the study. If you leave the study before the final planned study visit, the investigator may ask you to complete the final steps.

The researchers and sponsor also have the right to stop your participation in this study without your consent if:
- They believe it is in your best interest.
- You were to object to any future changes that may be made in the study plan.
- If you become ill during the research or you develop certain conditions during the study.
- If you don’t follow the prescribed procedure.

Requests to share your data with another study

Dr. Melissa Lamar and her research team in the Department of Psychiatry at the University of Illinois at Chicago (UIC) are conducting a related study titled “White matter microstructure, vascular risk & cognition in aging”. Information collected from you in this study may also be very valuable to her study.

Who is doing the research, and why is it being done?
The research is being conducted by Melissa Lamar, PhD in the Department of Psychiatry at the University of Illinois at Chicago (UIC). The study is funded by the National Institute on Aging.

The purpose of this study is to better understand the relationship between brain structure, vascular risk factors like high blood pressure and diabetes and their genetic associates, and cognitive abilities in older adults. This is because vascular risk factors may impact the aging process.

Participation in both studies is the only requirement to share data with the “White matter microstructure, vascular risk & cognition in aging” study. May Dr. Varady share your information with Dr. Lamar’s “White matter microstructure, vascular risk & cognition in aging” study? If yes, then please enter your initials below:

_____ “Yes, my information may be shared with Dr. Lamar’s study should I take part in it”
_____ “No, I decline to have my information shared with Dr. Lamar’s study should I take part in it”
_____ “I do not wish to be considered for Dr. Lamar’s study”

Who should I contact if I have questions?

Contact the researchers Dr. Krista Varady (312-996-7897) or Kristin Hoddy (312-355-0542)
- If you have any questions about this study or your part in it,
- If you feel you have had a research-related injury (or a bad reaction to the study treatment), and/or
- If you have questions, concerns or complaints about the research.

What are my rights as a research subject?
APPENDIX A – CONSENT FORM (continued)

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

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

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

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

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

_____________________________  ____________________________
Signature                                      Date

_____________________________
Printed Name

_____________________________  ____________________________
Signature of Person Obtaining Consent          Date (must be same as subject’s)

_____________________________
Printed Name of Person Obtaining Consent

Meal timing during alternate day fasting
Main Consent, Version 6, November 18, 2013
Volunteers needed for a Weight Loss Study

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

The study is open to men and women who are:
- Between the ages of 25 and 70 •
- Obese, not diabetic, sedentary or moderately active •

For more information, please call: 312-355-0542
## APPENDIX C – SCREENING QUESTIONNAIRE

| Date of screening: ___________________ | Phone: __________________________ |
| First name: _________________________ | Email: ____________________________________ |
| Middle initial: ____ | Age: _______________ (Must be 25-65 yrs) |
| Last: _____________________________ | DOB: _______________ |
| Phone: ____________________________ | Sex: □ Female □ Male |
| Email: ____________________________________ | Weight: ___________   Height: _________ |
| Age: _______________ (Must be 25-65 yrs) | BMI: ___________  (must be 30-39.9kg/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) | Do you exercise? □ yes □ no (If exercise >5 d/wk, disqualify) |
| Are you a night-shift worker? □ yes □ no (If yes, disqualify) | Kind of exercise: __________________________ | Total hours/week: __________________________ |
| Currently Dieting? □ yes □ no (If yes, disqualify, not weight 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) | Can you commit to ADF for 8 weeks? □ yes □ no (If no, disqualify) |
| Vegan or vegetarian? □ yes □ no (If yes, disqualify) | Food allergies? (Nuts, soy, etc) __________________________________________________________ |
| Can you commit to ADF for 8 weeks? □ yes □ no (If no, disqualify) | Do you have any health problems: □ yes □ no |
| If yes, explain: ______________________________________________________________________ | Are you on any medications? □ yes □ no |
| (Disqualify if diabetic, history of heart disease or stroke, history of eating disorders/meal skipping) | Medication 1: _____________________ |
| Are you on any medications? □ yes □ no | Medication 2: _____________________ |
| Medication 3: _____________________ | Medication 4: _____________________ |
| (Must not be taking lipid lowering, glucose lowering, weight loss medications) | Peri-menopausal (3-6 missed periods in 12 mo)? □ yes □ no (If yes, disqualify) |
| 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) | Pregnant or trying to become pregnant? □ yes □ no (If yes, disqualify) |
APPENDIX D – ALTERNATE DAY FASTING DIET INSTRUCTIONS

Alternate day fasting – Take-home meal instructions

After you pick up the meal:

• REFRIGERATE AND FREEZE FOOD IMMEDIATELY.
• Meal preparation requires you to have a refrigerator, freezer, and microwave oven.
• Meals must be frozen until they are needed, and then microwaved until hot.

When eating the “fast day” meal at home/work:

• Eat ONLY the food we provide.
• Eat ALL of the food (even the little pieces).
• RETURN any foods you do not eat to the Nutrition Research Center for weighing.
• For food/diet problems please speak to the study coordinator.
• We recommend you DRINK 6-8 glasses of water each day
• You may not drink other beverages except as listed here:
  o Water as desired
  o Black coffee (without sugar and/or cream) 2 cups per day max
  o Black tea (without sugar or milk) 2 cups per day max
  o Diet soda as desired
• You may also chew sugar-free chewing gum throughout the day

FAST DAY MEAL TIMING:

• ADF-lunch time group: Eat the fast day meal between 12pm and 2pm
• ADF-dinner time group: Eat the fast day meal between 6pm and 8pm
• ADF-small meals group: Eat the small meals at 6.00 – 8.00 am, 12.00 – 2.00 pm, and 6.00 – 8.00 pm

On the “feed day”, you can eat whatever and whenever you want.
### Food Record – Day 1

☐ Feed day  ☐ Fast day

<table>
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<tr>
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<th>Food Description</th>
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### APPENDIX F – HUNGER VISUAL ANALOG SCALE

Hunger Assessment

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<th>Subject: ___________________</th>
<th>Date: ___________________</th>
<th>Time: Before bedtime</th>
<th>Week: _____</th>
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</thead>
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Please mark this line with a dash "|" to indicate how hungry you are at this moment.

1) How hungry do you feel?

- Not Hungry at all
- Extremely hungry

2) How satisfied do you feel?

- Feel completely empty
- Can't eat another bite

3) How full do you feel?

- Not at all full
- Totally full
**APPENDIX G – BODY SHAPE QUESTIONNAIRE (BSQ)**

Body Shape Questionnaire (BSQ)

We would like to know how you have been feeling about your appearance over the past two weeks. Please read each question and circle the appropriate number. Please answer all the questions.

<table>
<thead>
<tr>
<th>Number</th>
<th>Question</th>
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<th>Rarely</th>
<th>Sometimes</th>
<th>Often</th>
<th>Very often</th>
<th>Always</th>
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<td>1</td>
<td>Have you been so worried about your shape that you have been feeling that you ought to diet?</td>
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<td>2</td>
<td>3</td>
<td>4</td>
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<td>2</td>
<td>Has being with thin people made you feel self-conscious about your shape?</td>
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<tr>
<td>3</td>
<td>Have you ever noticed the shape of other people and felt that your own shape compared unfavourably?</td>
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<tr>
<td>4</td>
<td>Has being undressed, such as when taking a bath, made you feel fat?</td>
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<td>5</td>
<td>Has eating sweets, cakes or other high calorie food made you feel fat?</td>
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<td>6</td>
<td>Have you felt excessively large and rounded?</td>
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<tr>
<td>7</td>
<td>Have you felt ashamed of your body?</td>
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<tr>
<td>8</td>
<td>Has worry about your shape made you diet?</td>
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APPENDIX G – BODY SHAPE QUESTIONNAIRE (BSQ) (continued)

Body Shape Questionnaire (BSQ)

We would like to know how you have been feeling about your appearance over the past two weeks. Please read each question and circle the appropriate number. Please answer all the questions.

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<tr>
<th>Question</th>
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MAEDS

Name: _________________________________ Date: ____________

Instructions: Using the scale below, please rate the following items on a scale from 1 to 7. Please answer as truthfully as possible.

1 = Never
2 = Very Rarely
3 = Rarely
4 = Sometimes
5 = Often
6 = Very Often
7 = Always
APPENDIX H – MULTIFACTORIAL ASSESSMENT OF EATING DISORDER SYMPTOMS (MAEDS) (continued)

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1. Fasting is a good way to lose weight.
2. My sleep isn't as good as it used to be.
3. I avoid eating for as long as I can.
4. Certain foods are "forbidden" for me to eat.
5. I can't keep certain foods in my house because I will binge on them.
6. I can easily make myself vomit.
7. I feel that being fat is terrible.
8. I avoid greasy foods.
9. It's okay to binge and purge once in a while.
10. I don't eat certain foods.
11. I think I am a good person.
12. My eating is normal.
13. I can't seem to concentrate lately.
APPENDIX H – MULTIFACTORIAL ASSESSMENT OF EATING DISORDER SYMPTOMS (MAEDS) (continued)

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<tr>
<th>1</th>
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<td>SOMETIMES</td>
<td>OFTEN</td>
<td>VERY</td>
<td>ALWAYS</td>
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</table>

15. I vomit to control my weight.
   1  2  3  4  5  6  7

16. Lately nothing seems enjoyable anymore.
   1  2  3  4  5  6  7

17. Laxatives help keep you slim.
   1  2  3  4  5  6  7

18. I don't eat red meat.
   1  2  3  4  5  6  7

19. I eat so rapidly I can't even taste my food.
   1  2  3  4  5  6  7

20. I do everything I can to avoid being overweight.
   1  2  3  4  5  6  7

21. When I feel bloated, I must do something to rid myself of that feeling.
   1  2  3  4  5  6  7

22. I overeat too frequently.
   1  2  3  4  5  6  7

23. It's okay to be overweight.
   1  2  3  4  5  6  7

24. Recently I have felt that I am a worthless person.
   1  2  3  4  5  6  7

25. I would be very upset if I gained 2 pounds.
   1  2  3  4  5  6  7

26. I crave sweets and carbohydrates.
   1  2  3  4  5  6  7

27. I lose control when I eat.
   1  2  3  4  5  6  7

28. Being fat would be terrible.
   1  2  3  4  5  6  7
APPENDIX H – MULTIFACTORIAL ASSESSMENT OF EATING DISORDER SYMPTOMS (MAEDS) (continued)

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<th>Question</th>
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<tr>
<td>29. I have thought seriously about suicide lately.</td>
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<tr>
<td>30. I don't have any energy anymore.</td>
<td>1 2 3 4 5 6 7</td>
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<tr>
<td>31. I eat small portions to control my weight.</td>
<td>1 2 3 4 5 6 7</td>
</tr>
<tr>
<td>32. I eat 3 meals a day.</td>
<td>1 2 3 4 5 6 7</td>
</tr>
<tr>
<td>33. Lately I have been easily irritated.</td>
<td>1 2 3 4 5 6 7</td>
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<tr>
<td>34. Some foods should be totally avoided.</td>
<td>1 2 3 4 5 6 7</td>
</tr>
<tr>
<td>35. I use laxatives to control my weight.</td>
<td>1 2 3 4 5 6 7</td>
</tr>
<tr>
<td>36. I am terrified by the thought of being overweight.</td>
<td>1 2 3 4 5 6 7</td>
</tr>
<tr>
<td>37. Purging is a good way to lose weight.</td>
<td>1 2 3 4 5 6 7</td>
</tr>
<tr>
<td>38. I avoid fatty foods.</td>
<td>1 2 3 4 5 6 7</td>
</tr>
<tr>
<td>39. Recently I have felt pretty blue.</td>
<td>1 2 3 4 5 6 7</td>
</tr>
<tr>
<td>40. I am obsessed with becoming overweight.</td>
<td>1 2 3 4 5 6 7</td>
</tr>
<tr>
<td>41. I don't eat fried foods.</td>
<td>1 2 3 4 5 6 7</td>
</tr>
<tr>
<td>42. I skip meals.</td>
<td>1 2 3 4 5 6 7</td>
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<tr>
<td>43. Fat people are unhappy.</td>
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<tr>
<td>44. People are too concerned with the way I eat.</td>
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<tr>
<td>45. I feel good when I skip meals.</td>
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<tr>
<td>46. I avoid foods with sugar.</td>
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<tr>
<td>47. I hate it when I feel fat.</td>
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<tr>
<td>48. I am too fat.</td>
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<tr>
<td>49. I eat until I am completely stuffed.</td>
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<tr>
<td>50. I hate to eat.</td>
<td></td>
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<tr>
<td>51. I feel guilty about a lot of things these days.</td>
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<tr>
<td>52. I'm very careful of what I eat.</td>
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<tr>
<td>53. I can &quot;hold off&quot; and not eat even if I am hungry.</td>
<td></td>
</tr>
<tr>
<td>54. I eat even when I am not hungry.</td>
<td></td>
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<tr>
<td>55. Fat people are disgusting.</td>
<td></td>
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<tr>
<td>56. I wouldn't mind gaining a few pounds.</td>
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APPENDIX I – COPYRIGHT AGREEMENT FOR MANUSCRIPT 1

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Hello,

I recently published a piece in Nutrition Journal. How do I receive permission to include this manuscript in my dissertation write up?

Thank you,

Kristin Hoddy  
ref_00020001_400200x0sNAAQref
## APPENDIX K – COPYRIGHT AGREEMENT FOR MANUSCRIPT 3

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CHICAGO, IL 60607  
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38. Cameron JD, Cyr M-J, Doucet É. Increased meal frequency does not promote greater weight loss in subjects who were prescribed an 8-week equi-energetic energy-restricted diet. *British Journal of Nutrition* 2010;103: 1098-1101.


52. Baron KG, Reid KJ, Kern AS, Zee PC. Role of sleep timing in caloric intake and BMI. *Obesity* 2011;19: 1374-1381.


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VITA – Kristin K. Hoddy

EDUCATION

<table>
<thead>
<tr>
<th>Degree</th>
<th>Institution</th>
<th>Program</th>
<th>Dates</th>
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<tbody>
<tr>
<td>Ph.D. RD</td>
<td>University of Illinois at Chicago – College of Applied Health Sciences</td>
<td>Human Nutrition</td>
<td>Expected May 2016</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kinesiology</td>
<td>Supervisor: Dr. Krista A. Varady, Ph.D.</td>
</tr>
<tr>
<td>Thesis title:</td>
<td>Meal Timing during Alternate Day Fasting: Effect on Body Weight and Cardiovascular Disease Risk</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B.S.</td>
<td>University of Illinois at Chicago – College of Applied Health Sciences</td>
<td>Exercise Science</td>
<td>August 2006 – May 2010</td>
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ACADEMIC AND RESEARCH APPOINTMENTS

<table>
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<tr>
<th>Position</th>
<th>Institution</th>
<th>Program</th>
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<tbody>
<tr>
<td>Course Instructor</td>
<td>University of Illinois at Chicago – Department of Kinesiology and Nutrition</td>
<td>Nutrition Science II (Undergraduate)</td>
<td>January 2016 – May 2016</td>
</tr>
<tr>
<td>Research Assistant</td>
<td>University of Illinois at Chicago – Department of Kinesiology and Nutrition</td>
<td>Human Nutrition Research Center</td>
<td>September 2013 – Present</td>
</tr>
<tr>
<td>Clinical Coordinator</td>
<td>University of Illinois at Chicago – Department of Kinesiology and Nutrition</td>
<td>Human Nutrition Research Center</td>
<td>May 2013 – April 2014</td>
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<tr>
<td>Teaching Assistant</td>
<td>University of Illinois at Chicago – Department of Kinesiology and Nutrition</td>
<td>Advanced Exercise Assessment (Undergraduate)</td>
<td>June 2013 – August 2013</td>
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<tr>
<td>Course Co-Instructor</td>
<td>University of Illinois at Chicago – Department of Kinesiology and Nutrition</td>
<td>Advanced Exercise Assessment and Programming (Undergraduate)</td>
<td>September 2012 – May 2014</td>
</tr>
<tr>
<td>Teaching Assistant</td>
<td>University of Illinois at Chicago – Department of Kinesiology and Nutrition</td>
<td>Advanced Exercise Assessment (Undergraduate)</td>
<td>September 2011 – May 2012</td>
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<tr>
<td>Teaching Assistant</td>
<td>University of Illinois at Chicago – Department of Kinesiology and Nutrition</td>
<td>Anatomy and Physiology (Undergraduate)</td>
<td>September 2009 – May 2010</td>
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HONORS AND AWARDS

Nov 2014  University of Illinois at Chicago – Graduate College Travel Award  $1000
Nov 2015  University of Illinois at Chicago – Graduate College Travel Award  $1000

PROFESSIONAL AFFILIATIONS

Member of the Academy of Nutrition and Dietetics since 2012
Member of the Obesity Society since 2013
Member of the American Society for Nutritional Sciences since 2014
Member of the International Society of Sports Nutrition since 2015

PUBLICATIONS


ABSTRACTS


11. 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 In: Ravussin E (ed). *The Obesity Society Annual Scientific Meeting*: Atlanta, Georgia, USA 2013. [Poster presentation]