Alternate Day Fasting Combined with Exercise for Weight Loss and
Cardio-Protection in Obese Adults

BY

SURABHI BHUTANI
B.Sc., University of Delhi, India, 2003
M.Sc., University of Delhi, India, 2005

THESIS
Submitted as partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Kinesiology, Nutrition, and Rehabilitation
in the Graduate College of the
University of Illinois at Chicago, 2013
Chicago, Illinois

Defense Committee:

Krista A. Varady, Chair and Advisor
Phyllis E. Bowen, Kinesiology and Nutrition
Shane A. Phillips, Physical Therapy
Giamila Fantuzzi, Kinesiology and Nutrition
David X. Marquez, Kinesiology and Nutrition
DEDICATION

I dedicate my dissertation work to my family who offered me unconditional love and support throughout the course of this thesis. Particularly to my understanding and patient husband, Nitin, who has put up with so many years of research. His unwavering encouragement and support has meant a lot to me and motivated me to set higher targets. I also want to thank my loving mother and brother for their words of encouragement over these years. I would also like to dedicate my thesis to the most wonderful in-laws who have supported me at each step of the process. And above all the almighty god for giving me healthy body, healthy mind and healthy spirit, that helped me finish my thesis.
ACKNOWLEDGEMENTS

I would like to thank all the people who have helped and inspired me during my doctoral thesis. I especially want to express my deepest appreciation to my committee chair, Dr. Krista Varady for her countless hours of reflecting, encouraging and most of all patience throughout this process. She has been a great inspiration and mentor who has worked tirelessly in giving me constructive criticism, ideas and corrections from proposal development to final write-up. Her intellectual skills, advice, commitment and close supervision have been a key to successful completion of this thesis. A special thanks to Dr. Phyllis Bowen, Dr. Giamila Fantuzzi and Dr. David Marquez for taking time to serve on my committee and for all their valuable suggestions throughout the research process. I am grateful as well to Dr. Shane Phillips for spending his valuable time and continuous support and for personally guiding me through FMD measurement and data analysis process.

This work would not have been possible without the all the undergrad and graduate interns who volunteered to help me through the data collection process. I am grateful to Melissa Goslawski for teaching FMD’s and Edita Norkeviciute for her painstaking analysis of that data. I would like to acknowledge American Heart Association for the pre doctoral fellow award and giving me an opportunity to complete my thesis and providing me the financial support to present my work internationally.

Special thanks to my friends and lab buddies Monica Klempel and Cynthia Kroeger for helping me with the data collection and keeping me sane. Thanks to John Trepenowski for those stimulating scientific discussions, moral support and encouragement in times when I was completely lost. I appreciate all your
effort for playing an important role in this journey. Lastly I would like to thank all the participants of the study. Without them this project would not have been possible.

SB
TABLE OF CONTENTS

CHAPTER  PAGE

I. INTRODUCTION .................................................................................................................. 1
A. Background and Rationale ................................................................................................. 1
B. Specific Aims ....................................................................................................................... 4
C. Significance ......................................................................................................................... 6
D. Innovation ............................................................................................................................ 7

II. LITERATURE REVIEW ......................................................................................................... 8
1. Obesity and coronary heart disease risk .............................................................................. 8
2. Alternate day fasting (ADF) for weight loss and cardio-protection .................................... 9
  2.1 ADF effect on body weight ............................................................................................. 9
  2.2 ADF effect on body composition ................................................................................. 9
  2.3 ADF effect on plasma lipids, LDL particle size, and blood pressure .......................... 10
  2.4 ADF effect on FMD ....................................................................................................... 12
  2.5 ADF effect on adipokines ............................................................................................ 12
  2.6 ADF effect on eating behaviors .................................................................................... 14
3. Endurance exercise for weight loss and cardio-protection ................................................ 15
  3.1 Endurance exercise effect on body weight ................................................................... 15
  3.2 Endurance exercise effect on body composition .......................................................... 16
  3.3 Endurance exercise effect on plasma lipids, LDL size, and blood pressure .................. 17
  3.4 Endurance exercise effect on FMD .............................................................................. 18
  3.5 Endurance exercise effect on adipokines .................................................................... 19
  3.6 Endurance exercise effect on eating behaviors ............................................................. 20

III. MANUSCRIPT 1 ................................................................................................................. 22
A. Abstract ............................................................................................................................... 23
B. Introduction ......................................................................................................................... 24
C. Materials and methods ....................................................................................................... 26
D. Results ................................................................................................................................. 32
E. Discussion ............................................................................................................................ 35
F. Acknowledgements ............................................................................................................. 40

IV. MANUSCRIPT 2 ............................................................................................................... 50
A. Abstract ............................................................................................................................... 51
B. Introduction ......................................................................................................................... 52
C. Materials and methods ....................................................................................................... 54
D. Results ................................................................................................................................. 58
E. Discussion ............................................................................................................................ 60
F. Acknowledgements ............................................................................................................. 62

V. MANUSCRIPT 3 ................................................................................................................ 66
A. Abstract ............................................................................................................................... 67
B. Background .......................................................................................................................... 68
C. Materials and methods ....................................................................................................... 69
TABLE OF CONTENTS (continued)

<table>
<thead>
<tr>
<th>CHAPTER</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. Results</td>
<td>74</td>
</tr>
<tr>
<td>E. Discussion</td>
<td>77</td>
</tr>
<tr>
<td>F. Acknowledgements</td>
<td>81</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VI. DISCUSSION</th>
<th>87</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1 Association between changes in body weight, body composition and plasma lipids</td>
<td>87</td>
</tr>
<tr>
<td>1.2 Association between changes in body weight, body composition, blood pressure, FMD and insulin resistance</td>
<td>88</td>
</tr>
<tr>
<td>1.3 Association between changes in leptin, blood pressure and FMD</td>
<td>89</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>VII. FUTURE DIRECTIONS</th>
<th>92</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>VIII. SUMMARY OF FINDINGS AND GENERAL CONCLUSION</th>
<th>93</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Summary of findings</td>
<td>93</td>
</tr>
<tr>
<td>B. Conclusion</td>
<td>94</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>APPENDICES</th>
<th>95</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appendix A</td>
<td>95</td>
</tr>
<tr>
<td>Appendix B</td>
<td>104</td>
</tr>
<tr>
<td>Appendix C</td>
<td>105</td>
</tr>
<tr>
<td>Appendix D</td>
<td>107</td>
</tr>
<tr>
<td>Appendix E</td>
<td>108</td>
</tr>
<tr>
<td>Appendix F</td>
<td>109</td>
</tr>
<tr>
<td>Appendix G</td>
<td>112</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CITED LITERATURE</th>
<th>117</th>
</tr>
</thead>
</table>

| VITA | 133 |
# LIST OF TABLES

<table>
<thead>
<tr>
<th>TABLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>III.I</td>
<td>NUTRIENT COMPOSITION OF THE FAST DAY DIET PROVIDED TO THE COMBINATION AND ADF GROUP</td>
</tr>
<tr>
<td>III.II</td>
<td>SUBJECT CHARACTERISTICS AT BASELINE</td>
</tr>
<tr>
<td>III.III</td>
<td>BODY WEIGHT AND BODY COMPOSITION DURING 12-WEEK TRAIL</td>
</tr>
<tr>
<td>III.IV</td>
<td>CORONARY HEART DISEASE RISKS DURING 12-WEEK TRIAL</td>
</tr>
<tr>
<td>III.V</td>
<td>LDL PARTICLE SIZE DURING 12-WEEK TRIAL</td>
</tr>
<tr>
<td>III.VI</td>
<td>HDL PARTICLE SIZE DURING 12-WEEK TRIAL</td>
</tr>
<tr>
<td>IV.I</td>
<td>BODY WEIGHT, BLOOD PRESSURE, AND ADIPOKINES DURING THE 12-WEEK TRIAL</td>
</tr>
<tr>
<td>V.I</td>
<td>SUBJECT CHARACTERISTICS AT BASELINE</td>
</tr>
<tr>
<td>V.II</td>
<td>CHANGES IN EATING BEHAVIORS DURING 12-WEEK STUDY</td>
</tr>
<tr>
<td>V.III</td>
<td>ENERGY AND MACRONUTRIENT INTAKE ON FEED DAYS DURING 12-WEEK STUDY</td>
</tr>
<tr>
<td>VI.I</td>
<td>SAMPLE SIZE NEEDED FOR COMBINATION GROUP TO ACHIEVE GREATER IMPROVEMENTS THAN ADF BY WEEK</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>FIGURE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Rationale for effect of the combination of ADF and exercise on body composition and plasma lipids</td>
</tr>
<tr>
<td>2.1</td>
<td>Rationale for effect of the combination of ADF and exercise on adipokines and endothelial function</td>
</tr>
<tr>
<td>3.1</td>
<td>Study flow chart</td>
</tr>
<tr>
<td>3.2</td>
<td>Experimental design</td>
</tr>
<tr>
<td>4.1</td>
<td>Flow mediated dilation (FMD) at baseline and post-treatment</td>
</tr>
<tr>
<td>5.1</td>
<td>Timing of the fast day exercise session and impact on food intake</td>
</tr>
<tr>
<td>6.1</td>
<td>Associations between weight loss and improved CHD risk by ADF</td>
</tr>
</tbody>
</table>
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADF</td>
<td>Alternate day fasting</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>Bpm</td>
<td>Beats per minute</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>CR</td>
<td>Calorie restriction</td>
</tr>
<tr>
<td>eNOS</td>
<td>Endothelial nitric oxide synthase</td>
</tr>
<tr>
<td>FMD</td>
<td>Flow mediated dilation</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
</tr>
<tr>
<td>HNRU</td>
<td>Human nutrition research unit</td>
</tr>
<tr>
<td>Hrmax</td>
<td>Heart rate maximum</td>
</tr>
<tr>
<td>LDL</td>
<td>Low density lipoprotein</td>
</tr>
<tr>
<td>METs</td>
<td>Metabolic equivalents</td>
</tr>
<tr>
<td>NO</td>
<td>Nitric oxide</td>
</tr>
<tr>
<td>RBC</td>
<td>Red blood cells</td>
</tr>
<tr>
<td>RMR</td>
<td>Resting metabolic rate</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of mean</td>
</tr>
<tr>
<td>TFEQ</td>
<td>Three factor eating questionnaire</td>
</tr>
<tr>
<td>VAS</td>
<td>Visual analogue scale</td>
</tr>
</tbody>
</table>
SUMMARY

A study examining whether the combination of alternate day fasting (ADF) plus exercise produces superior changes in body weight, body composition and coronary heart disease risk, when compared to each intervention alone, was conducted in obese adults. Sedentary, obese subjects were randomized to 1 of 4 groups for 12 weeks: 1) combination (ADF plus endurance exercise), 2) ADF, 3) exercise, or 4) control. The ADF intervention consisted of an ad libitum “feed day” alternated with a 75% energy restriction “fast day”. The exercise regimen consisted of moderate intensity aerobic exercise, 3 d/week, for 25-40 min duration. Body weight and body composition were measured weekly in all four groups. Blood samples were taken every four weeks to measure plasma lipids and adipokine levels. Endothelial function (measured by flow-mediated dilation; FMD) and eating behaviors were assessed before and after 12 weeks of intervention.

Body weight was reduced to a greater extent in the combination group, when compared to the ADF and exercise groups. The combination group demonstrated a decrease in fat mass, accompanied by retention in lean mass. LDL cholesterol decreased and HDL cholesterol increased in the combination group only. Both the combination and ADF groups demonstrated increased in LDL particle size. The proportion of small HDL particles decreased in the combination group only. Leptin decreased in the combination, ADF and exercise group, whereas adiponectin was not changed by any intervention. FMD increased only in the ADF group. Changes in FMD in the ADF group were not related to changes in leptin. As for behavioral effects, hunger decreased while satisfaction and fullness increased post-treatment in the ADF group only. Restrained eating increased and uncontrolled eating decreased in the combination and ADF groups. In conclusion, the combination of ADF plus exercise may yield greater weight loss and cardio-protection when compared to each intervention alone.
I. INTRODUCTION

A. Background and Rationale

Obesity is a key risk factor for the development of coronary heart disease (CHD) (1). Losing weight by means of dietary restriction greatly reduces an obese individual’s risk of CHD (1). Alternate day fasting (ADF) is a novel dietary restriction regimen that has been implemented to help obese individuals lose weight and lower their risk of CHD (2-4). ADF regimens include a “feed day” where food is consumed ad-libitum over a 24-h period, alternated with a “fast day”, where food intake is partially reduced for 24-h. Although this regimen is effective for weight loss and fat mass reduction, lean mass is not conserved, and visceral fat mass is only minimally reduced (2, 3). Endurance exercise has been shown to conserve lean mass and reduce visceral fat mass. Thus, adding an exercise regimen to this diet therapy may help obese individuals retain lean mass and experience additive reductions in visceral fat. The effect of this combination therapy on body weight and body composition has yet to be elucidated, however.

This combination intervention may also produce the most beneficial changes in lipid risk factors for CHD, when compared to each treatment alone. For instance, when ADF is applied by itself, only total and LDL cholesterol are lowered. In contrast, when exercise is applied alone, triglycerides are lowered, HDL cholesterol is augmented, while total and LDL cholesterol remain unaffected. Thus, when ADF and exercise are applied in combination all four-lipid parameters may be beneficially modulated. These improvements in CHD risk by this combination intervention may be mediated by changes in fat-cell derived hormones (i.e. adipokines) that occur with visceral fat mass reduction. More specifically, as visceral fat mass decreases, levels of the anti-atherogenic hormone, adiponectin, increase, while levels of the pro-atherogenic hormones, leptin and resistin, decrease (5). Whether greater reductions in visceral fat mass by this combination therapy can produce more pronounced improvements in adipokine profile and lipid profile, compared to each intervention alone, has yet to be investigated.
Figure 1.1. Rationale for effect of the combination of ADF and exercise on body composition and plasma lipids
Figure 2.1. Rationale for effect of the combination of ADF and exercise on adipokines and endothelial function
B. Specific Aims

Specific Aim 1: To establish that the combination (ADF and exercise) intervention will produce greater weight loss and more favorable improvements in body composition, when compared to the ADF intervention alone, or the exercise intervention alone.

**Hypothesis 1:** The combination group will have a lower body weight, lower fat mass, lower visceral fat mass, and greater retention of lean mass, when compared to the ADF group and the exercise group after the 12-week intervention.

Specific Aim 2: To establish that the combination intervention will produce the most optimal shifts in lipid risk factors for CHD when compared to the ADF intervention alone, or the exercise intervention alone.

**Hypothesis 2:** The combination group will experience greater improvements in plasma lipid profile (i.e. decreases in total cholesterol, LDL cholesterol and triglycerides, accompanied by increases in HDL cholesterol), greater increases in LDL and HDL particle size when compared to the ADF group and the exercise group after the 12-week intervention.

Specific Aim 3: To establish that the combination intervention will produce greater improvements in adipokine profile when compared to the ADF intervention alone, or the exercise intervention alone.

**Hypothesis 3:** The combination group will experience greater improvements in circulating adipokines (adiponectin, leptin, and resistin) when compared to the ADF group and the exercise group, due to greater reductions in visceral fat mass.
Specific Aim 4: To establish that the combination intervention will produce greater improvements in eating behaviors when compared to the ADF intervention alone, or the exercise intervention alone.

Hypothesis 4: The combination group will experience greater improvements in eating behaviors (hunger, satisfaction, fullness, restrained eating, uncontrolled eating, and emotional eating), when compared to the ADF group and the exercise group after 12-week intervention.
C. **Significance**

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

1. Advance clinical practice guidelines by showing that this combination therapy (ADF plus exercise) is more efficacious than individual therapies (ADF alone or exercise alone) to help obese individuals lose larger amounts of weight that will lead to more pronounced reductions in CHD risk in this population.

2. Show that this lifestyle therapy can be implemented as a cost-effective alternative to traditional drug therapy to improve plasma lipid profile (i.e. beneficially modulate all four key lipid parameters, which is not possible with current drug treatments).

3. Further uncover the intermediate role that adipose tissue plays in mediating CHD risk.
D. **Innovation**

This study will be the first to directly compare the time-course effects of this combination therapy to that of each intervention alone on physiological variables, and show that this combination therapy is more effective for weight loss and CHD risk reduction than individual therapies. This study is also unique in that it employs novel approaches, i.e. assessing adipose biology parameters, to evaluate changes in vascular disease risk. In sum, the proposed study is innovative in that it will be the first to:

1. Directly compare the efficacy of this combination therapy (ADF plus exercise) to that of each intervention alone (ADF alone or exercise alone) for weight loss and cardio-protection.

2. Employ novel markers, i.e. fat-cell derived hormones, to assess the impact of this lifestyle therapy on CHD risk.
II. LITERATURE REVIEW

1. Obesity and coronary heart disease risk

Obesity is a well-established risk factor for coronary heart disease (CHD) (1, 6, 7). According to the American Heart Association’s Heart Disease and Stroke 2013 report, 154.7 million (68%) US adults (≥ 20 years of age) are overweight or obese (8). Large prospective studies have shown a strong association between body mass index (BMI) and CHD mortality (9, 10). As obesity increases, median survival reduces by 2-4 years at a BMI of 30-35 kg/m² and by 8-10 years at a BMI of 40-45 kg/m² (9, 10). It has also been shown that with every 5 kg/m² increase in BMI above 25 kg/m², mortality rate due to CHD increases by 40% (10).

Weight loss can help reduce an individual’s risk of CHD (11). For every kilogram of weight loss the following favorable changes occur: fasting serum cholesterol, −1.0%; low-density lipoprotein cholesterol, −0.7%; triglycerides, −1.9%; high-density lipoprotein cholesterol, +0.2%; systolic blood pressure, −0.5%; diastolic blood pressure, −0.4%; and blood glucose, −0.2 mM (12). Calorie restriction (CR) is the most common weight loss strategy employed. CR involves reducing energy needs by 15-40% each day. Another form of dietary restriction that has gained popularity in recent years is alternate day fasting (ADF). The following section describes how this new diet therapy affects body weight and CHD risk.
2. **Alternate day fasting (ADF) for weight loss and cardio-protection**

2.1 **ADF effect on body weight**

ADF is a modified form of CR, consisting of a 24 h period of ad libitum feeding alternated with a 24 h period of 75% energy restriction. Only five trials have studied the effect of ADF on body weight. After 3 weeks of ADF in overweight subjects, Heilbronn et al. (13) reported a 4% decrease in body weight. Furthermore, with a slightly longer trial (8 weeks), Johnson et al. (14) reported a reduction in body weight of 8% in overweight adults. Findings from another 8 week ADF regimen in obese individuals by Varady et al. (3), reported a 6% decline in body weight. A weight loss of 5% from baseline was also noted in a study by Klempel et al. (15) after 8 weeks in obese subjects consuming a high-fat background diet. Contrary to these findings, Halberg et al. (16) showed no change in body weight with 2 weeks of ADF in normal weight individuals. A possible explanation for the lack of weight loss by Halberg et al. may be that the study duration was not long enough to produce weight changes. Taken together, these findings reveal that ADF can be an effective treatment for weight loss. Though more than 8 weeks of ADF may be required to reach a clinically significant body weight loss of 5%.

2.2 **ADF effect on body composition**

The primary goal of any weight reduction therapy is to decrease fat mass and preserve lean mass. Four trials studied the effect of ADF on body composition. Two weeks of ADF in the study by Halberg et al. (16) did not show any improvement in fat mass in overweight men. However, 3 weeks of ADF in a study by Heilbronn et al. (13) in overweight adults decreased fat mass by 4%. When the intervention was increased to 8 weeks, a reduction in body fat was observed (11%) by Varady et al. (3). In line with these data, Klempel et al. (15) reported a decrease in fat mass of 4.2 kg (9.6%) in obese individuals after
8 weeks of an ADF-high fat regimen. In sum, an overall decrease of 4-11% was observed in total body fat with short term ADF.

As for fat free mass, Heilbronn et al. (13) demonstrated a complete retention of lean mass after 3 weeks of ADF in overweight participants. Varady et al. (3) and Klempel et al. (15) also reported no change in fat free mass after 8 weeks of ADF in obese participants. These short-term studies (3-8 weeks) offer preliminary evidence indicating that fat free mass may be retained with ADF. Future trials are however necessary to confirm if this effect is maintained long-term (>24 weeks).

Visceral obesity is associated with increased CHD risk and metabolic disturbances (17). Magnetic resonance imaging (MRI) is the gold standard technique to accurately measure visceral fat. However, this method was not used in any of the ADF studies to date. Waist circumference (>88cm for women, and >102cm for men) is an alternative indicator of visceral fat mass and can predict visceral fat accumulation to some extent (18). Only two trials measured visceral fat changes with ADF. Bhutani et al. (2) demonstrated 4% reductions in visceral fat (measured by waist circumference) after 8 weeks of ADF in obese subjects. Klempel et al. (15) also reported a similar decline in visceral fat (5%) in obese participants with 8 weeks of ADF. Therefore, it appears that ADF can be an effective therapy to reduce visceral fat mass in short durations (8 weeks). However, longer-term trials implementing more robust methods (i.e. MRI) are required before more concrete conclusions can be reached.

2.3 ADF effect on plasma lipids, LDL particle size, and blood pressure

Obesity is a key risk factor for dyslipidemia (19). Weight loss of 5-10% has been shown to decrease LDL cholesterol concentrations by 10-20% in obese individuals. HDL cholesterol, however, does not seem to be affected by reductions in body weight (19). The majority of ADF studies measured plasma
lipids. Heilbronn et al. (13) reported a decrease in triglyceride levels in non-obese men with no change in total cholesterol levels after 3 weeks of ADF. In a slightly longer trial (8 weeks) by Johnson et al. (14), decreases in total cholesterol (9%) as well as triglyceride levels (42%) were observed in overweight subjects. Bhutani et al. (2) also reported a decline in triglycerides (32%) and LDL cholesterol (25%) after 8 weeks of ADF in obese subjects. Similar decreases in total cholesterol (16%), LDL cholesterol (18%) and triglyceride levels (14%) were observed in an 8-week study by Klempel et al. (15). HDL cholesterol levels increased only in normal weight and overweight subjects (9%) (13, 14), but not in obese individuals (2, 15) following ADF for 3-8 weeks. Overall, ADF may be an effective diet therapy to modulate total cholesterol, LDL cholesterol, triglycerides, but not HDL cholesterol, in human subjects.

Accumulating evidence suggests that small dense LDL particles are independent risk factors for CHD (20). Mechanisms that link small LDL particles to atherosclerosis include: longer residence time in plasma, high susceptibility to oxidation, and increased permeability through the endothelial barrier (21, 22). Furthermore, small LDL particle diameter has been correlated with increased fasting triglyceride levels in both men and women (23). To date, only one ADF study has measured LDL particle size. Klempel et al. (24) found that after 8 weeks of ADF, peak LDL particle size increased by 3 Å in obese men and women consuming a high-fat background diet. Additionally, the proportion of large LDL particles increased by 3% and the proportion of small particles decreased by 11 %, post intervention (24). These preliminary findings warrant further investigation into the impact of this novel diet therapy on this emerging indicator of CHD risk.

Blood pressure is another key indicator of CHD risk, and has been shown to decrease with weight loss. The mechanisms by which dietary restriction-induced weight loss may decrease blood pressure include:
reducing extracellular volume thus decreasing cardiac output, suppressing sympathetic nervous system, and normalizing renin-angiotensin-aldosterone system (25). Three ADF studies have examined blood pressure response. After 3 weeks of ADF in normal weight individuals, no change in systolic or diastolic blood pressure was observed by Heilbronn et al. (13). Longer-term (8 week) studies demonstrate conflicting findings. For instance, Varady et al. (3) reported a decline of 5% only in systolic blood pressure in obese participants, while Klempel et al. (15) showed no change. Based on this limited data, it is still unclear if ADF has favorable effects on blood pressure.

2.4 ADF effect on FMD

Obesity is also linked to abnormal endothelial function, marked by reduced vasodilation to an increased blood flow (endothelium-dependent flow-mediated dilation; FMD). Endothelial dysfunction is an early predictor of future vascular events, and most CHD risk factors are correlated with reduced FMD (26). A recent meta-analysis estimated that with every 1% increase in FMD, future risk of cardiovascular events decrease by 13% (27). To date, only one study examined the effect of ADF on FMD (28). After 8 weeks of diet, obese participants increased FMD by 2% with a low-fat background diet, and decreased FMD by 2% with a high-fat background diet (28). These very limited findings indicate that endothelial health may be improved by ADF, but only when a low-fat background is implemented.

2.5 ADF effect on adipokines

Adiponectin is an anti-atherogenic hormone (adipokine) released by adipocytes. Adiponectin may protect against vascular disease by inhibiting the action of scavenger receptor-A and preventing the conversion of macrophages into atherosclerotic foam cells (29). Additionally, this adipokine may inhibit the release of inflammatory factors (i.e. TNF-α, IL-6, and IL-8), while promoting the release of cardio-
protective factors (i.e. IL-10 and tissue inhibitor of metalloproteases-1 (TIMP-1)). Adiponectin also improves endothelial function and stimulates smooth muscle cell proliferation, thus inhibiting aortic lesion formation (29). Moreover, adiponectin has been shown to be inversely related to body weight (30). To date, only three trials of ADF have measured plasma adiponectin levels. Halberg et al. (16) conducted an ADF study on normal weight subjects for 2 weeks. Results reveal increases in adiponectin by 37% in the absence of body weight or fat mass loss. (16). Furthermore, Bhutani et al. (2) reported that after 8 weeks of ADF in obese subjects, adiponectin levels increased by 30%. This increase in adiponectin was accompanied by a decrease of 6% in body weight along with 3% decrease in total body fat (2). In line with these findings, adiponectin increased by 51% after 8 weeks of an ADF high-fat diet in the study by Klempel et al. (corresponding to 5% weight loss) (28). These preliminary findings suggest that ADF-induced weight loss may increase plasma adiponectin concentrations.

Evidence suggests that plasma leptin levels increase with obesity in both men and women. High leptin levels are positively associated with central adiposity, total body fat, triglycerides, and CRP, and are negatively correlated with HDL cholesterol concentrations. Four ADF trials measured leptin concentrations. In the study by Halberg et al. (16), 2 weeks of ADF decreased leptin levels in normal weight individuals with no change in body weight. Leptin levels also decreased in overweight subjects after 8 weeks by ~22% (corresponding to 8% weight loss) in the study by Johnson et al.(14). In the Bhutani et al.(2) study, leptin concentrations decreased (21%) after weeks 8 of ADF in obese men and women. Lower leptin levels were significantly associated with decreased in body weight (6%) and fat mass (11%) (2). Complementary to these findings, leptin levels decreased by 24% in obese participants after 8 weeks of a high-fat ADF diet, with 5% weight loss (28). Taken together, weight loss resulting from ADF may decrease circulating concentrations of leptin.
2.6 ADF effect on eating behaviors

Subjective feelings of hunger and satiety play a role in development of obesity. Overconsumption of high fat food suppresses appetite related feelings, leading to positive energy balance and excess fat storage (31). Additionally, adiposity is also associated with low dietary restraint and high disinhibition (32). Therefore several diet restriction studies have tried to understand the changes in eating behavior in obese individuals. Changes in eating behaviors have been assessed in two ADF studies to date. Heilbronn et al. (13) studied the effect of 3 weeks of ADF on hunger and fullness levels using a visual analogue scale (VAS). On the first fast day of the study, hunger ratings were high and fullness ratings were low. After 3 weeks of diet, hunger remained elevated, but feelings of fullness increased slightly over time (13). In the study by Johnson et al. (14), hunger levels were moderate at the beginning of the study and decreased slightly by week 8 of ADF. The impact of ADF on dietary restraint (the intent and ability to restrict caloric intake) and dietary disinhibition (the tendency to overeat) has also been investigated (13). After 3 weeks, dietary restraint increased, while disinhibition decreased (13). Moreover, men considered themselves “big eaters” while women frequently reported that they “watched what they ate”. Weight loss was also shown to correlate negatively with considering oneself a big eater (13). These preliminary findings suggest that ADF may be effective at improving eating behaviors. However more studies are required before solid conclusions can be reached.
3. **Endurance exercise for weight loss and cardio-protection**

3.1 **Endurance exercise effect on body weight**

The American College of Sports Medicine (ACSM) suggests that 150-250 min/week of moderate intensity exercise (60-80% Heart rate max (HRmax)) be employed for modest weight loss (<5%). To achieve greater weight loss (>5%), at least 250 min/week of moderate intensity exercise must be implemented (33). A negative energy balance created by physical activity results in a reduction of body weight. The larger the negative energy balance is, the greater the weight loss. Extreme physical activity like military training (34) and high intensity sports (35, 36) can result in substantial weight loss. However, this extreme energy expenditure is difficult to achieve by the majority of Americans. Therefore, most studies test the effect of moderate intensity exercise 3-5 times per week.

Thorogood et al. (37) performed a meta-analysis examining the effect of endurance exercise on weight loss in overweight/obese individuals. Studies were classified according to the length of the program, with weekly exercise sessions ranging from 120-240 minutes at intensities 40-85% HRmax (37). After 12 weeks of training, mean weight loss was 1.6 kg. After 24 and 52 weeks of training, body weight was further reduced by 1.7 kg and 2.1 kg, respectively, in obese individuals (37). Another review by Shaw et al. (38) examined the effect of 12-24 weeks of moderate intensity training (3-5 d/week, 15-60 min sessions,) in overweight/obese subjects. Body weight decreased by 0.5-4 kg, with more weight lost occurring with higher intensities of exercise (38). These effects occurred in the absence of any dietary modifications (38). In sum, endurance exercise alone at a moderate intensity results in a modest body weight loss (0.5-4 kg from baseline) in obese subjects.
3.2 **Endurance exercise effect on body composition**

Endurance exercise has been shown to be effective for reducing fat mass in most studies (39), but not all (40). In a meta-analysis by Cornelisson et al. (39), 4-52 weeks of endurance exercise (intensity of 30-88% heart rate reserve) decreased fat mass by 1.4% in healthy overweight and obese participants. However, in another meta-analysis, Kelley et al. (40) showed no effect on fat mass by moderate intensity exercise. Thus, the effect of endurance exercise on fat mass is still unclear.

Fat free mass is an important predictor of basal metabolic rate (41). Therefore, weight loss interventions that can effectively increase or preserve fat free mass are highly recommended for obese individuals. Garrow et al. (42) performed a meta-analysis to determine if physical training can prevent the loss of fat free mass. Pooled data from 28 exercise studies revealed conservation of fat free mass in sedentary, overweight individuals with a significant body weight loss (42). In another meta-analysis, it was determined that 75% of exercise trials report a retention in fat free mass, while the other 25% of studies, show an increase in fat free mass (43). Recent work suggests that the intensity of exercise may determine whether or not a person *increases* fat free mass. Trapp et al. (44) compared the effect of high intensity exercise (80-90% HRmax) to moderate intensity exercise (60-80% HRmax) in young women for 15 weeks. Results reveal that the women participating in the high intensity exercise increased fat free mass (0.6 kg), while the women participating in the moderate intensity exercise saw no increase in fat free mass (44). Taken together, endurance exercise appears to preserve fat free mass at moderate intensities, and increase fat free mass at higher intensities.

Evidence suggests that endurance exercise may also reduce visceral fat mass in obese individuals independent of weight loss (45, 46). Ismail et al. (47) pooled data from 27 endurance exercise studies (3-5d/week, 60-75% HRmax). Endurance exercise decreased visceral fat mass by 0.33 kg versus controls
(47). Vissers et al. (48) also reported a decrease of 30 cm² and 40 cm² in overweight women and men, respectively, with endurance exercise. This study also demonstrated that moderate to vigorous intensity exercise produced greater decreases in visceral fat than low intensity exercise. (48). Thus it can be concluded that endurance exercise is an effective therapy to reduce visceral fat mass.

3.3 **Endurance exercise effect on plasma lipids, LDL size, and blood pressure**

Exercise-induced weight loss may have beneficial effects on cholesterol levels. Recent reports suggest that 1 kg weight loss can decrease plasma cholesterol levels by 1% and triglycerides by 1.9% (49). Pattyn et al. (50) conducted a meta-analysis of the effect of exercise on plasma lipids. The duration of the studies was 8-52 weeks, the intensity was moderate to high, and the frequency was 2-5 d/wk (45 min each). In the majority of studies, HDL cholesterol levels increased (6%), with no change in triglyceride levels (50). Results from another meta-analysis reveal consistent increases in HDL cholesterol levels (900%), in conjunction with consistent reductions in triglycerides (100%) (38). In all meta-analyses, total and LDL cholesterol did not change no matter what intensity or duration of exercise was applied. Overall, endurance exercise increases HDL cholesterol while decreasing triglyceride levels, with no effect on total or LDL cholesterol.

Endurance exercise may have beneficial effects on LDL and HDL particle size. Altena et al (51) and Varady et al (52) observed an increase in mean LDL particle size after 4 weeks (51) and 8 weeks (52), respectively, of moderate intensity endurance exercise. In a 24-week study, Halverstadt et al. (53) reported increases in LDL particle size with moderate intensity endurance exercise (3d/week, 50-70% VO2 max) in overweight and obese adults. A decrease in the proportion of small HDL particles was also observed in this study (53). In the STRRIDE study (54), no change in LDL and HDL particle size was reported with low intensity exercise, while high intensity exercise decreased the proportion of small
LDL particles (54). In sum, exercise may be effective in increasing LDL particle size with moderate and high intensity protocols, while the effect on HDL particle size is not certain.

The National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure states that a 2 mm Hg reduction in systolic blood pressure results in a 6% decrease in stroke mortality and a 4% decrease in CHD mortality. As systolic blood pressure values decrease further to 5 mm Hg, stroke mortality is further reduced by 14%, and CHD mortality is further reduced by 9% (55). A pooled analysis of blood pressure data from 72 endurance exercise trials (4-52 weeks, 30-88% heart rate reserve) report a mean decrease of 3 mmHg in systolic blood pressure, with a decline of 2.4 mmHg in diastolic blood pressure (39). The Cochrane review (38), however, reported significant decrease in diastolic blood pressure, with no changes in systolic blood pressure with endurance exercise. In another meta-analysis, it was determined that hypertensive individuals experience greater decreases in blood pressure with endurance exercise (systolic: 4%, diastolic: 5%) when compared to normotensive individuals (systolic: 2%, diastolic: 1%) (40). Therefore, it can be concluded that endurance exercise reduces resting diastolic blood pressure, but the effects on systolic blood pressure are not clear.

3.4 Endurance exercise effect on FMD

The effect of endurance exercise on FMD has been examined in a few recent reports. A 3d/week moderate intensity (70% HRmax) exercise program, did not show any improvements in FMD in hypertensive patients after 12 weeks (56). In contrast, 5-7d/week of moderate intensity exercise for 12 weeks resulted in improved FMD in conjunction with increased nitric oxide (NO) release (57). In a longer-term trial (24 weeks), Spence et al. (58) reported a 33% increase in brachial artery FMD in healthy obese subjects. Similar results were observed in diabetics (59) and subjects with coronary heart disease (60). The influence of exercise intensity on FMD has also been examined. Tjonna et al. (61)
compared vascular health between participants following high intensity (90-95% HRmax) versus moderate intensity (75% HRmax) endurance exercise program. FMD improved to a greater extent in the high intensity group (9%) as compared to the moderate intensity group (5%)(61). The investigators speculated that the high intensity exercise program produced greater sheer stress on the blood vessel walls, thus generating positive molecular changes (61). Therefore, endurance exercise is generally shown to increase FMD with higher intensities (>90% HRmax) thus improving endothelial function.

3.5 Endurance exercise effect on adipokines

Several studies have examined the effect of endurance exercise on circulating adiponectin levels in obese individuals. After 12 weeks of training (2d/week, 70 min sessions, 60-80 % HRmax), no increase in adiponectin levels was observed by Ando et al. (62). Similarly, Christiansen et al. (63) did not observe any change in adiponectin after 12 weeks of training (3d/week, 60-75 min sessions, 500 kcal energy expenditure per session). However, in a slightly longer-term trial (16 weeks, 3 d/week, 60 min session, 60-70% VO2max), adiponectin levels increased by 51% (64). In a meta-analysis by Simpson et al. (65), it was shown that consistent increases in adiponectin (15-97%) could be seen with 19 weeks of moderate intensity training (3 h/week). However, some long-term studies (24-52 weeks) demonstrate no change in adiponectin with moderate intensity (66) (67). The impact of high versus moderate intensity exercise on adiponectin has also been investigated. Marcell et al. (68) reported greater increases in adiponectin (7%) with high intensity (80-90% HRmax, 5 d/week, 30 min sessions), compared to moderate intensity exercise (5%). Taken together, it is still not clear whether endurance exercise can produce consistent increases in plasma adiponectin levels.

The effect of endurance exercise on leptin levels has also been well studied in obese populations. A few studies have reported decreases in leptin levels with exercise (9-45%), while others have shown no
significant effect (6-23%) (69-72). For instance, 4 weeks of moderate intensity training (5 d/week, 45 minute sessions, 60-80% HRmax) significantly decreased leptin levels by 13% in obese subjects (73). In contrast, after 9 weeks of a similar training protocol, leptin levels did not change (74). However, it is possible that leptin did not decrease in this study since there was no change in fat mass post-treatment (74). In long-term studies (52 weeks), a moderate intensity exercise regimen decreased leptin by 16% in subjects with ≥2kg fat mass loss and 2% with ≤2kg body fat loss (75). Overall, endurance exercise may decrease circulating leptin levels, but a concomitant decrease in fat mass must occur to observe this effect.

3.6 Endurance exercise effect on eating behaviors

Acute and chronic exercise have been shown to influence eating behaviors (76). There is a substantial amount of data available on the effect of acute endurance exercise on eating behaviors. Acute vigorous exercise has been associated with a temporary state of “exercise induced anorexia” (76). However, after the exercise session is over, hunger tends to increase 30-40 min post-training (76). As for chronic exercise, the findings are less clear. For instance, after 12 weeks of training (5 d/wk, 40-60 min sessions, 70-80% HRmax) hunger levels did not change, while perceived fullness increased over the course of the trial (77). These findings are supported by Martins et al. (78). After 6 weeks of moderate intensity exercise (4 d/week, 65–75% HRmax) appetite regulation increased substantially post-intervention (78). The effect of exercise on dietary restraint is not as well studied. In a study by Keim et al. (79), obese women were classified as “over-eaters” and “under-eaters” and partook in a moderate intensity exercise regimen for 4 weeks. Results reveal that dietary restraint decreased in all women post-exercise, regardless of whether they were previously diagnosed as over-eaters or under-eaters (79). In contrast, Bryant et al. (80) demonstrated increased dietary restraint after 12 weeks of moderate intensity training (5d/week, 70% VO2max). This trial also demonstrated that individuals who showed high dietary
restraint had lower body weights post-intervention when compared to individuals with low dietary restraint (80). Thus, acute exercise blunts hunger during the period of training, and increases hunger post-training. As for chronic exercise, the effects on hunger, fullness, and dietary restraint are inconclusive. More research in this area is well warranted.
III. MANUSCRIPT 1

Alternate day fasting and endurance exercise combine to reduce body weight and favorably alter plasma lipids in obese humans

Surabhi Bhutani, Monica C. Klempel, Cynthia M. Kroeger, John F. Trepanowski, Krista A. Varady

Author affiliation:
Department of Kinesiology and Nutrition, University of Illinois at Chicago, Chicago, IL, USA.

Correspondence and reprint requests:
Surabhi Bhutani, M.S.
Department of Kinesiology and Nutrition
University of Illinois at Chicago
1919 West Taylor Street, Room 103, Chicago, IL, 60612
Tel: 312-996-7897 Fax: 312-413-0319
Email: sbhuta2@uic.edu

Running head: Alternate day fasting and exercise for weight loss

Funding source: American Heart Association 12PRE8350000; University of Illinois, Chicago, Departmental funding
A. Abstract

Objective: This study examined whether the combination of Alternate day fasting (ADF) plus exercise produces superior changes in body composition and plasma lipid levels when compared to each intervention alone. Designs and Methods: Obese subjects (n = 64) were randomized to 1 of 4 groups for 12 weeks: 1) combination (ADF plus endurance exercise), 2) ADF, 3) exercise, or 4) control.

Results: Body weight was reduced ($P < 0.05$) by $6 \pm 4$ kg, $3 \pm 1$ kg, and $1 \pm 0$ kg in the combination, ADF, and exercise group, respectively. Fat mass and waist circumference decreased ($P < 0.001$) while lean mass was retained in the combination group. LDL cholesterol decreased ($12 \pm 5\%, P < 0.05$) and HDL cholesterol increased ($18 \pm 9\%, P < 0.05$) in the combination group only. LDL particle size increased ($P < 0.001$) by $4 \pm 1$ Å and $5 \pm 1$ Å in the combination and ADF group, respectively. The proportion of small HDL particles decreased ($P < 0.01$) in the combination group only. Conclusions: These findings suggest that the combination produces superior changes in body weight, body composition, and lipid indicators of heart disease risk, when compared to individual treatments.
B. Introduction

Overweight and obesity are key risk factors for the development of coronary heart disease (CHD) (81). A moderate weight loss of 5-10% significantly lowers CHD risk (82). Different forms of dietary restriction, including alternate day fasting (ADF), have been shown to be effective for weight loss and improving vascular health (83, 84). ADF involves consuming 25% of energy needs on the “fast” day and eating ad libitum on alternating “feed” days. Three trials of ADF that ran for 2-3 weeks have demonstrated a decrease of 3% in body weight, in conjunction with decreased triglyceride levels (13, 16). Longer-term trials of ADF have shown greater weight loss (i.e. 8% from baseline) and decreased visceral fat mass (3, 14). LDL cholesterol, LDL particle size, and triglyceride levels also improved significantly in this 8-week trial (3, 85). Thus, ADF is effective in lowering body weight, visceral fat mass, total cholesterol, triglyceride and LDL cholesterol levels. Despite these beneficial effects, ADF has no impact on HDL cholesterol, and does not help in the retention of lean mass (3). Endurance exercise, on the other hand, has been shown to prevent the loss of lean mass, increase HDL cholesterol, decrease the proportion of small HDL particles, and augment visceral fat loss (86). What is not yet known, however, is whether ADF combined with exercise can enhance this weight loss without the loss of lean mass. Also, it is not clear if this combination therapy can improve all four lipid parameters (i.e. decrease total, LDL cholesterol, and triglyceride levels, in conjunction with increasing HDL cholesterol).

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

Subjects

Independently living subjects were recruited from the University of Illinois at Chicago campus by means of flyers. Of the 146 individuals that expressed interest in the study, 83 were deemed eligible to participate according to a preliminary questionnaire and body mass index assessment (Figure 3.1). Key inclusion criteria were as follows: age 25 to 65 years; body mass index between 30 and 39.9 kg/m²; weight stable for 3 months prior to the beginning of the study (i.e. less than 5 kg weight loss or weight gain); non-diabetic; no history of cardiovascular disease; no polycystic ovarian syndrome (PCOS); not vegan or vegetarian; no food allergies; lightly active (i.e. <3 h/week of light intensity exercise at 2.5 to 4.0 metabolic equivalents (METs) for 3 months prior to the study); non-smoker; no history of bariatric surgery; and not taking weight loss, lipid or glucose lowering medications. Peri-menopausal women were excluded from the study, and post-menopausal women (absence of menses for more than 2 years) were required to maintain their current hormone replacement therapy regimen for the duration of the study. The experimental protocol was approved by the Office for the Protection of Research Subjects at the University of Illinois, Chicago, and all volunteers gave their written informed consent to participate in the trial.

Experimental design and randomization

A 12-week, randomized, controlled, parallel-arm feeding trial was implemented to test the effects of ADF, exercise, and ADF combined with exercise (combination group) on body weight, body composition, and CHD risk reduction in obese adults. Subjects were recruited and randomized by the clinical coordinator (SB). Eligible subjects were stratified on the basis of BMI, age, and sex, and then randomized into 1 of 4 groups: 1) combination group; 2) ADF group; 3) exercise group; 4) control group.
(Figure 3.2). Randomization was performed for each stratum by selecting an intervention at random from an opaque envelope. The 12-week clinical trial was run 3 times. Recruitment took place during a 4-week period before the beginning of each trial. During the second and third run of the trial, additional subjects were randomized to groups that had high dropout rates (i.e. the ADF and exercise group). This ensured that the total number of subjects would be the same in each group at the end of the study.

**Diet protocol**

Only the combination and ADF group participated in the dietary intervention, which consisted of two periods: 1) a 4-week controlled feeding period, and 2) an 8-week self-selected feeding period. During the controlled feeding period (week 1-4) participants consumed 25% of their baseline energy needs on the "fast day" (24 h) and consumed food ad libitum on each "feed day" (24 h). The baseline energy requirements for the subjects were assessed by the Mifflin equation (87). The diet consisted of a 3-day rotating menu plan, and all fast day meals were prepared in the metabolic kitchen of the Human Nutrition Research Unit (HNRU). Fast day meals were consumed between 12.00 pm and 2.00 pm to ensure that each subject was undergoing the same duration of fasting. The nutrient composition of the provided fast day meals is described in **Table III.1.** Subjects picked up their food from the HNRU at the beginning of each week, and consumed the food outside of the research center. Subjects were encouraged to drink plenty of water, and were permitted to consume calorie-free foods such as black coffee, tea, water and sugar-free gum on the fast days. During the self-selected feeding period (week 8-12) subjects continued with the ADF regimen but no fast day food was provided to them. Instead, each subject met with a dietician at the beginning of each week to learn how to maintain the ADF regimen on his or her own at home. During each counseling session, the dietitian worked with the subject to develop individualized fast day meal plans. Subjects were also instructed how to make healthy food choices on the ad libitum feed days, by choosing low fat meat and dairy options and increasing fruit and vegetable
intake. Throughout the 12-week study, combination and ADF group subjects consumed 1 meal on the fast day, and an ad libitum number of meals on the feed day. Control and exercise group subjects were asked to maintain their regular food habits, and were not provided with any food or dietary counseling. Control and exercise group subjects consumed an ad libitum number of meals everyday.

**Exercise protocol**

Only the combination and exercise groups participated the exercise intervention. These subjects participated in a moderate intensity exercise program 3 times per week under supervised conditions, for 12 weeks. Exercise was performed using stationary bikes and elliptical machines at the HNRU. Training intensity was estimated for each individual using an age-predicted heart rate maximum (HRmax) equation \[209 - (0.7 \times \text{age})\] (88) and a Polar Heart Rate Monitor (Polar USA, Inc., NY). Each training session began with a 5 min warm-up period, and ended with a 5 min cool-down. At the beginning of the study (weeks 1 to 4), each exercise session ran for a 25 min duration and corresponded to 60% of the subject’s HRmax. Training duration and intensity increased incrementally at week 4, 7 and 10 by 5 min and 5% HRmax. As such, by week 10, each subject was exercising for a 40 min duration at an intensity of 75% HRmax. ADF and control subjects were asked to maintain their regular activity habits, and to refrain from joining an exercise class during the study.

**Diet and exercise compliance**

During the controlled feeding phase (week 1-4), subjects were instructed to eat only the fast day food provided, and to report any extra food item consumed using an “Extra food log”. During the self-selected feeding phase, subjects were provided with individualized meal plans that were consistent with their food preferences and prescribed calorie levels for the fast day. Each subject was asked to report any extra food item consumed on the fast day that did not comply with their prescribed plan using the “Extra
food log”. The log was collected and reviewed by study personnel each week. If the log indicated that the subject ate an extra food item on a fast day, that day was labeled as “not adherent”. If the log revealed that the subject did not eat any extra food item, that day was labeled as “adherent”. Percent adherence was calculated by applying the following formula: \[
\% \text{ADHERENCE} = \frac{\# \text{FAST DAYS ADHERENT}}{\# \text{OF FAST DAYS IN THE WEEK}} \times 100.
\]

Exercise compliance was assessed by recording attendance at each supervised exercise session. Exercise was performed in the morning or evening. If an exercise session was missed, the subject was required to make up for the missed session that same week. Subjects were allowed to miss a maximum of 4 out of 36 total sessions. Percent adherence was calculated by applying the following formula: \[
\% \text{ADHERENCE} = \frac{\# \text{EXERCISE SESSION ATTENDED}}{\# \text{TOTAL NUMBER OF EXERCISE SESSIONS}} \times 100
\]

**Blood collection protocol**

Twelve-hour fasting blood samples were collected between 6.00 am and 10.00 am at baseline and week 12 (Figure 3.2). Subjects were instructed to avoid exercise, alcohol and coffee for 24 h before each visit. Blood was centrifuged for 10 minutes at 520x g and 4°C to separate plasma from RBC and was stored at -80°C until analyzed.

**Body weight and body composition assessment**

Body weight measurements were taken to the nearest 0.5 kg at the beginning of each week with subjects wearing light clothing and without shoes using a balance beam scale (HealthOMeter; Sunbeam Products, Boca Raton, FL, USA). BMI was assessed as kg/m². Fat mass and fat free mass were assessed each week in triplicate using a tetra-polar bioelectrical impedance analyzer (BIA; Omron HBF-500; Omron Health Care, Bannockburn, IL, USA). Waist circumference was measured by a flexible tape to the
Coronary heart disease risk indicator determination

Plasma total cholesterol, HDL-cholesterol, and triglyceride concentrations were measured in duplicate using enzymatic kits (Biovision Inc, Mountainview, CA) and analyzed using a microplate reader (iMark Microplate Reader; Bio-Rad Laboratories Inc, Richmond, CA). The concentration of LDL cholesterol was calculated using the Friedewald equation (89).

Blood pressure and heart rate were measured in triplicate using a digital automatic blood pressure/heart rate monitor (Omron HEM 705 LP, Kyoto, Japan) with the subject in a seated position after a 10-min rest. Fasting plasma glucose concentrations were measured with a hexokinase reagent kit in duplicate (A-gent glucose test, Abbott, South Pasadena, CA). Fasting insulin was measured as total immunoreactive insulin (Coat-A-Count Insulin, LA, California). Insulin resistance (IR) was calculated using the HOMA (Homeostasis Model Assessment) method, by applying the following formula:

\[
\text{HOMA-IR} = \frac{\text{Fasting insulin (µU/mL)} \times \text{Fasting glucose (mg/dL)}}{405}. 
\]

C-reactive protein (CRP) was measured by enzyme-linked immunosorbent assay (Linco Inc., St Charles, MO).

LDL and HDL particle size assessment

LDL and HDL particle size were measured by linear polyacrylamide gel electrophoresis (Quantimetrix Lipoprint System, Redondo Beach, CA, USA) (90). High-resolution 3% polyacrylamide gel tubes were used for electrophoresis. Briefly, 25 µL of sample was mixed with 200 µL of liquid loading gel containing Sudan black, and added to the gel tubes. After photopolymerization at room temperature for 30 min, samples were electrophoresed for 1 h (3 mA/gel tube). Lipoware computer software
(Quantimetrix, Redondo Beach, CA, USA) was then used to divide LDL into small (<255 Å), medium (255-260 Å), and large (>260 Å) particles, and HDL into small (<73 Å), medium (73-88 Å), and large (>88 Å) particles (90).

**Statistical analysis**

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

Subject baseline characteristics and dropouts

There were sixteen completers in each group at the end of the study (Figure 3.1). There were a greater number of dropouts in the ADF (n = 9) and exercise group (n = 8) when compared to the combination (n = 2) and control group (n = 0). The main reason for subject dropout was scheduling conflicts. There were no between-group differences for age, sex, ethnicity, body weight, height, BMI, waist circumference, plasma lipids or heart rate at baseline (Table III.II). Systolic and diastolic blood pressure values, however, differed between groups at baseline (Table III.II). Characteristics of the dropouts (n = 19, age: 36 ± 3 y, weight: 95 ± 3 kg, or BMI 35 ± 1 kg/m^2), were not significantly different from those of the completers (age: 42 ± 3 y, weight: 93 ± 2 kg, or BMI 35 ± 1 kg/m^2).

Diet and exercise adherence

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

Body weight and body composition

Changes in body weight and body composition are reported in Table III.III. Body weight was reduced (P < 0.05) in all three intervention groups after 12 weeks. However, weight loss in the combination group (6 ± 4 kg, 7 ± 2% change from baseline) was greater than the weight loss observed in ADF group (3 ± 1 kg, 3 ± 1% change from baseline) and exercise group (1 ± 0 kg, 1 ± 0% change from baseline).
BMI also decreased ($P < 0.05$) in all three intervention groups, with greater reductions occurring in the combination group ($2 \pm 0 \text{ kg/m}^2$), versus the ADF group ($1 \pm 0 \text{ kg/m}^2$) and exercise group ($1 \pm 0 \text{ kg/m}^2$). Fat mass decreased ($P < 0.01$) in the combination ($5 \pm 1 \text{ kg}$) and ADF ($2 \pm 1 \text{ kg}$) groups only, with greater decreases noted in the combination group. Fat free mass was retained in all the intervention groups. Waist circumference decreased ($P < 0.001$) by $8 \pm 1 \text{ cm}$ and $5 \pm 1 \text{ cm}$ in both the combination and ADF group. Less pronounced decreases ($P < 0.001$) in waist circumference were also noted in the exercise group ($3 \pm 1 \text{ cm}$).

**Coronary heart disease risk indicators**

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

**LDL and HDL particle size**

Changes in LDL particle size are reported in Table III.V. An increased proportion of small, dense LDL and HDL particles is associated with increased risk of CHD (91, 92). In the present trial, LDL particle size increased ($P < 0.001$) after 12-weeks of treatment in both the combination ($4 \pm 1 \text{ Å}$) and ADF group ($5 \pm 1 \text{ Å}$). The proportion of small atherogenic LDL particles was reduced ($P < 0.01$) in the combination
and ADF groups, while the proportion of large anti-atherogenic LDL particles was increased ($P < 0.001$) in the ADF group only. The proportion of medium LDL particles remained unchanged in all groups. The exercise intervention had no effect on any parameter of LDL particle size. Changes in HDL particle size are presented in Table III.VI. A decrease ($P < 0.01$) in the proportion of small HDL particles was noted in the combination group only (week 1: 15 ± 2%, week 12: 11 ± 1%). No other parameters of HDL particle size were affected by the combination, ADF or exercise interventions.
E. Discussion

This study is the first to show that the combination of ADF and exercise produces superior changes in body weight, body composition, and lipid indicators of CHD risk, when compared to ADF or exercise alone. More specifically, we report here that the combination group lost more weight (6 kg), and experienced greater decreases in fat mass (5 kg), when compared to individual interventions after 12 weeks. The combination therapy also elicited retention in lean mass, however, this preservation of lean mass was also noted in the ADF and exercise groups. As for CHD risk indicators, the combination of ADF plus exercise decreased LDL cholesterol (12% from baseline) while increasing HDL cholesterol (18% from baseline); a change that was not noted for any other intervention. The combination group also experienced an increase in LDL particle size, and a reduction in the proportion of small LDL and HDL particles.

Although this is the first trial to test the effects of ADF combined with exercise, several other investigations have been performed to evaluate the effects of daily calorie restriction (CR) combined with endurance exercise on body weight (93-95). In a recent study by Redman et al. (94), obese men and women participated in an intervention that combined daily CR (25% reduction in energy intake) with a supervised endurance exercise program (5 d/week, 60 minute duration, moderate intensity). After 12 weeks of treatment, body weight was reduced by 6% from baseline (94). Interestingly, subjects in the Redman et al. (94) study lost a similar amount of weight (6%) after 12 weeks when compared to the subjects in the present study (7%), despite exercising more frequently per week and for longer durations. One possible explanation for the similar weight loss in both trials, despite the different exercise prescriptions, may be the degree to which the subjects were supervised. Although the Redman et al. (94) intervention required that the subjects exercise 5 d/week, only 3 of these sessions were performed under supervised conditions. In contrast, our trial only required that the subjects exercise 3 d/week, but all of
these sessions were supervised. Thus, it is possible that the subjects in the Redman et al. (94) study did not perform the exercise as stringently on their own, as they would under supervision. This would result in less total energy expended during exercise per week, which would result in a total weight loss similar to what was observed in the present trial. As for the effect of ADF alone on body weight, the present findings differ slightly with what has been reported previously (3, 14). For instance, in two recent trials of ADF that ran for 8 weeks, body weight was reduced by 8% (14) and 6% (3) from baseline, which is greater than the weight loss experienced in the present trial (3%). The less pronounced weight loss in the present trial may partly be explained by the use of an intention-to-treat analysis, which may have diluted the effect. As for the impact of exercise alone on body weight, the majority of previous studies report very minimal reductions (1-2 kg) after 12 weeks of training (96, 97). In a 12-week study by Christiansen et al., a supervised exercise program (3 d/week, 60-75 minute duration, 70% HRmax, with an estimated energy expenditure 500-600 kcal/session) reduced body weight by 3.5 kg (98). This minimal effect of exercise on body weight may be explained by the compensatory eating that generally occurs post-exercise training (99). Although exercise blunts hunger acutely for 30 minutes post-training, most individuals experience a spike in hunger 45-60 min post-exercise (100). This surge of hunger generally results in compensatory energy consumption that negates the extra energy expended with exercise (99). As such, energy intake balances out with energy expenditure, and little weight is lost as a result.

Body composition was also preferentially altered in the combination group when compared to the individual interventions. We report here that the combination group lost a greater degree of fat mass (5 kg), when compared to the ADF group (2 kg). The combination therapy also resulted in retention of lean mass. These results suggest that combining ADF plus exercise may preserve lean mass at the expense of fat mass during period of energy restriction. Retention of lean mass is highly beneficial as it maintains resting metabolic rate (RMR) (41). More specifically, maintaining lean mass while dieting ensures that
RMR will be kept high, which can allow for a higher hourly energy burning capacity and greater weight loss. This preservation of lean mass was also noted in the ADF and exercise groups. This finding is in accordance with previous 12-week endurance exercise trials (97, 101). On the other hand, the effects of ADF on lean mass are equivocal. While one study indicates a possible retention in lean mass with 8 weeks of ADF (2), another trial indicates a reduction in lean mass (14). More work in this area is evidently required before solid conclusions can be reached regarding the efficacy of ADF for lean mass retention. Visceral fat mass (measured indirectly by waist circumference) was decreased in the combination (8 cm) and ADF group (5 cm). Reductions in waist circumference were also noted in the exercise group (3 cm). These findings for waist circumference are in line with what has been reported previously for ADF (2) and short-term exercise interventions (98, 102).

Previous studies of ADF consistently demonstrate reductions in LDL cholesterol and triglyceride concentrations (2, 13, 14). Short-term endurance exercise interventions (≤12 weeks), on the other hand, have been shown to increase HDL cholesterol levels (103, 104), although results are not consistent (105, 106). In the present study, only the combination intervention observed decreases in LDL cholesterol and increases in HDL cholesterol concentrations. No interventions lowered triglyceride levels. The reason why LDL cholesterol and triglycerides were not reduced in the ADF-only group, and why HDL cholesterol did not increase in the exercise-only group, is not clear. LDL-cholesterol has been estimated to decrease by 2.0 mg/dl per kg of weight loss (49). As such, it possible that the decrease in body weight by the ADF group was not substantial enough to produce significant reductions in LDL cholesterol. In addition, it should be noted that the varying effects of these interventions on LDL cholesterol may be attributable to baseline LDL cholesterol levels. For instance, LDL cholesterol of ADF group at baseline (113 mg/dl) was lower (though not significantly) than that of the combination group (125 mg/dl). Since
the LDL cholesterol concentration of the ADF group was already near optimal (19), this could possibly explain why less of a LDL cholesterol-lowering effect was noted in this group.

Remarkably, both the combination and ADF groups experienced increases in LDL particle size and reductions in the proportion of small LDL particles post-treatment. An increased proportion of small, dense LDL particles is strongly associated with the development of CHD (107). Potential mechanisms that link small LDL particles to increased risk of vascular events include: augmented oxidizability (108) and increased permeability through the endothelial barrier (109). In view of this, our findings suggest that both the combination and ADF groups may confer cardio-protection by increasing LDL particle size and lowering the proportion of small particles. This increase in particle size in the absence of reduced LDL cholesterol concentrations (as noted in the ADF group) is not common, but has been reported previously (110). The combination group also experienced decreases in the proportion of small HDL particles. The mechanisms that link small HDL particles to increased CHD risk have yet to be firmly established, but may involve the altered activity of lipases involved with the maturation and transformation of lipoproteins (111). This beneficial effect of the combination therapy on HDL particle size further strengthens our key finding that this lifestyle therapy is more cardio-protective than ADF or exercise alone.

There are some limitations that should be considered when interpreting these results. First of all, our trial duration (12 weeks) may have not been long enough to observe changes in plasma lipids (86). Evidence suggests that HDL cholesterol may require >16 weeks to be altered with endurance training (86). Second, the exercise intensity (60-75% HRmax) and frequency (3 d/week) may have not been sufficient to alter CHD risk indicators (112). HDL cholesterol concentrations generally only show consistent improvements with an exercise intensity exceeding 75% HRmax at a frequency of 5 d/week (112).
Third, we used BIA to measure fat mass and fat free mass instead of a more robust method, such as dual-energy X-ray absorptiometry (DXA). BIA is limited in that it may underestimate fat mass and overestimate fat free mass in obese individuals. Fourth, we recruited subjects solely from the University of Illinois, Chicago campus, which undoubtedly impacts the generalizability of our findings. More specifically, the subjects that partook in the study may differ from the general population in terms of race, education, and income. Fifth, the large number of dropouts in the ADF and exercise groups is also a limitation. Nevertheless, since the majority of these dropouts were due to scheduling conflicts and not adherence issues, these lifestyle therapies can still be considered viable options for weight loss and CHD risk reduction. Sixth, our randomization procedure may be flawed in that we chose to randomize additional subjects into groups that had high dropout rates. This uneven allocation of subjects to the intervention groups may have negatively impacted the internal validity of the study. These factors should be considered when interpreting the study findings.

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

Surabhi Bhutani designed the experiment, conducted the clinical trial, analyzed the data, and wrote the manuscript. Monica C Klempel and Cynthia M Kroeger assisted with the conduction of the clinical trial. John F Trepanowski assisted with the preparation of the manuscript and the analysis of the data. Krista A Varady assisted with the design of the experiment, and wrote the manuscript.

Disclosure

The authors have no conflicts of interest to report.
Figure 3.1. Study flow chart
Figure 3.2. Experimental design
**TABLE III. NUTRIENT COMPOSITION OF THE FAST DAY DIET PROVIDED TO THE COMBINATION AND ADF GROUP**

<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>Vegetarian pizza</td>
<td>Chicken enchilada</td>
<td>Chicken fettuccini</td>
</tr>
<tr>
<td>Fruit/Vegetable</td>
<td>Apple</td>
<td>Orange</td>
<td>Carrot sticks</td>
</tr>
<tr>
<td>Snack</td>
<td>Peanuts</td>
<td>Crackers</td>
<td>Cookie</td>
</tr>
<tr>
<td><strong>Nutrients</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Energy (kcal)</td>
<td>450</td>
<td>450</td>
<td>450</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>11 (26%) (^2)</td>
<td>12 (22%) (^2)</td>
<td>13 (24%) (^2)</td>
</tr>
<tr>
<td>Saturated fat (g)</td>
<td>4</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Monounsaturated fat (g)</td>
<td>4</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Polyunsaturated fat (g)</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Trans fat (g)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>30</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>29 (22%) (^2)</td>
<td>27 (26%) (^2)</td>
<td>25 (24%) (^2)</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>60 (52%) (^2)</td>
<td>60 (52%) (^2)</td>
<td>60 (52%) (^2)</td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

\(^1\) No differences between meals for any nutrient when meals were matched for total kcal. All fast day meals were consumed between 12.00 pm and 2.00 pm to ensure that each subject was undergoing the same duration of fasting.  
\(^2\) Percent of energy (kcal).
## TABLE III.II. SUBJECT CHARACTERISTICS AT BASELINE

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

Values reported as mean ± SEM. Intention to treat analysis. BMI: Body mass index, F: Female, M: Male.

<sup>1</sup> P-value between groups at baseline: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test)
# TABLE III. BODY WEIGHT AND BODY COMPOSITION DURING THE 12-WEEK TRIAL

<table>
<thead>
<tr>
<th>n</th>
<th>Intervention</th>
<th>Week 1</th>
<th>Week 12</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></td>
<td>Body weight (kg)</td>
<td>18 Combination</td>
<td>91 ± 6</td>
<td>85 ± 6</td>
<td>&lt;0.001</td>
<td>0.393</td>
<td>-6 ± 4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>25</td>
<td>ADF</td>
<td>94 ± 3</td>
<td>91 ± 3</td>
<td>&lt;0.001</td>
<td>0.393</td>
<td>-3 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Exercise</td>
<td>93 ± 2</td>
<td>92 ± 2</td>
<td>0.027</td>
<td>0.393</td>
<td>-1 ± 0&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Control</td>
<td>93 ± 5</td>
<td>93 ± 5</td>
<td>0.577</td>
<td>0.393</td>
<td>0 ± 0&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Body mass index (kg/m&lt;sup&gt;2&lt;/sup&gt;)</td>
<td>18 Combination</td>
<td>35 ± 1</td>
<td>33 ± 1</td>
<td>&lt;0.001</td>
<td>0.334</td>
<td>-2 ± 0&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>25</td>
<td>ADF</td>
<td>35 ± 1</td>
<td>34 ± 1</td>
<td>&lt;0.001</td>
<td>0.334</td>
<td>-1 ± 0&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Exercise</td>
<td>35 ± 1</td>
<td>34 ± 1</td>
<td>0.030</td>
<td>0.334</td>
<td>-1 ± 0&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Control</td>
<td>35 ± 1</td>
<td>35 ± 1</td>
<td>0.707</td>
<td>0.334</td>
<td>0 ± 0&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fat mass (kg)</td>
<td>18 Combination</td>
<td>45 ± 2</td>
<td>40 ± 2</td>
<td>&lt;0.001</td>
<td>0.054</td>
<td>-5 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>25</td>
<td>ADF</td>
<td>43 ± 2</td>
<td>41 ± 2</td>
<td>0.008</td>
<td>0.054</td>
<td>-2 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Exercise</td>
<td>46 ± 2</td>
<td>45 ± 2</td>
<td>0.182</td>
<td>0.054</td>
<td>-1 ± 0&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Control</td>
<td>43 ± 4</td>
<td>43 ± 4</td>
<td>0.570</td>
<td>0.054</td>
<td>0 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fat free mass (kg)</td>
<td>18 Combination</td>
<td>46 ± 2</td>
<td>46 ± 2</td>
<td>0.221</td>
<td>0.299</td>
<td>0 ± 1</td>
</tr>
<tr>
<td>25</td>
<td>ADF</td>
<td>51 ± 2</td>
<td>50 ± 2</td>
<td>0.031</td>
<td>0.299</td>
<td>-1 ± 1</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Exercise</td>
<td>48 ± 1</td>
<td>47 ± 1</td>
<td>0.321</td>
<td>0.299</td>
<td>-1 ± 0</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Control</td>
<td>50 ± 2</td>
<td>49 ± 2</td>
<td>0.693</td>
<td>0.299</td>
<td>-1 ± 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Waist circumference (cm)</td>
<td>18 Combination</td>
<td>96 ± 2</td>
<td>88 ± 1</td>
<td>&lt;0.001</td>
<td>0.310</td>
<td>-8 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>25</td>
<td>ADF</td>
<td>100 ± 2</td>
<td>95 ± 2</td>
<td>&lt;0.001</td>
<td>0.310</td>
<td>-5 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Exercise</td>
<td>98 ± 2</td>
<td>95 ± 2</td>
<td>&lt;0.001</td>
<td>0.310</td>
<td>-3 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Control</td>
<td>98 ± 3</td>
<td>97 ± 2</td>
<td>0.640</td>
<td>0.310</td>
<td>-1 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

Value reported as mean ± SEM. Intention to treat analysis. ADF: Alternate day fasting.

1 P-value between week 1 and week 12: Repeated-measures ANOVA.
2 P-value between groups at week 12: One-way ANOVA.
3 Absolute change between week 12 values.
4 P-value between groups for absolute change: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test)
TABLE III.IV. CORONARY HEART DISEASE RISK INDICATORS DURING THE 12-WEEK TRIAL

<table>
<thead>
<tr>
<th>n</th>
<th>Intervention</th>
<th>Week 1</th>
<th>Week 12</th>
<th>P-value</th>
<th>Change (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>^1</td>
<td>^2</td>
<td>^3</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>18</td>
<td>Combination</td>
<td>190 ± 10</td>
<td>186 ± 12</td>
<td>0.658</td>
<td>0.975</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>ADF</td>
<td>171 ± 8</td>
<td>183 ± 11</td>
<td>0.053</td>
<td>0.993</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>Exercise</td>
<td>181 ± 6</td>
<td>181 ± 8</td>
<td>0.921</td>
<td>-0.75</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>Control</td>
<td>185 ± 7</td>
<td>187 ± 10</td>
<td>0.784</td>
<td>-0.12</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dl)</td>
<td>18</td>
<td>Combination</td>
<td>125 ± 9</td>
<td>109 ± 11</td>
<td>0.043</td>
<td>0.660</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>ADF</td>
<td>113 ± 8</td>
<td>112 ± 9</td>
<td>0.917</td>
<td>-0.05</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>Exercise</td>
<td>113 ± 5</td>
<td>113 ± 7</td>
<td>0.947</td>
<td>-0.01</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>Control</td>
<td>119 ± 6</td>
<td>123 ± 8</td>
<td>0.586</td>
<td>0.02</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>18</td>
<td>Combination</td>
<td>50 ± 3</td>
<td>59 ± 4</td>
<td>0.041</td>
<td>0.194</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>ADF</td>
<td>49 ± 2</td>
<td>49 ± 3</td>
<td>0.807</td>
<td>-0.01</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>Exercise</td>
<td>51 ± 2</td>
<td>52 ± 3</td>
<td>0.457</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>Control</td>
<td>52 ± 3</td>
<td>56 ± 3</td>
<td>0.166</td>
<td>0.03</td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>18</td>
<td>Combination</td>
<td>77 ± 7</td>
<td>87 ± 8</td>
<td>0.161</td>
<td>0.267</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>ADF</td>
<td>81 ± 7</td>
<td>86 ± 8</td>
<td>0.341</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>23</td>
<td>Exercise</td>
<td>74 ± 6</td>
<td>79 ± 5</td>
<td>0.290</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>Control</td>
<td>97 ± 13</td>
<td>102 ± 11</td>
<td>0.452</td>
<td>0.02</td>
</tr>
<tr>
<td>Systolic BP (mm Hg) ^5</td>
<td>18</td>
<td>Combination</td>
<td>113 ± 3</td>
<td>111 ± 3</td>
<td>0.262</td>
<td>0.176</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>ADF</td>
<td>124 ± 3</td>
<td>120 ± 3</td>
<td>0.007</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Exercise</td>
<td>113 ± 2</td>
<td>115 ± 3</td>
<td>0.284</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>Control</td>
<td>122 ± 5</td>
<td>120 ± 6</td>
<td>0.603</td>
<td>0.02</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg) ^5</td>
<td>18</td>
<td>Combination</td>
<td>76 ± 2</td>
<td>76 ± 2</td>
<td>0.939</td>
<td>0.123</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>ADF</td>
<td>82 ± 2</td>
<td>80 ± 2</td>
<td>0.034</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>Exercise</td>
<td>76 ± 2</td>
<td>76 ± 2</td>
<td>0.976</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>Control</td>
<td>86 ± 2</td>
<td>84 ± 4</td>
<td>0.480</td>
<td>0.02</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>18</td>
<td>Combination</td>
<td>78 ± 2</td>
<td>76 ± 2</td>
<td>0.384</td>
<td>0.198</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>ADF</td>
<td>75 ± 2</td>
<td>75 ± 2</td>
<td>0.711</td>
<td>0.02</td>
</tr>
<tr>
<td>n</td>
<td>Intervention</td>
<td>Week 1</td>
<td>Week 12</td>
<td>P-value (^1)</td>
<td>P-value (^2)</td>
<td>Change (%) (^3)</td>
</tr>
<tr>
<td>----</td>
<td>--------------</td>
<td>----------</td>
<td>----------</td>
<td>----------------</td>
<td>----------------</td>
<td>-------------------</td>
</tr>
<tr>
<td>24</td>
<td>Exercise</td>
<td>71 ± 2</td>
<td>71 ± 2</td>
<td>0.925</td>
<td></td>
<td>0 ± 2</td>
</tr>
<tr>
<td>16</td>
<td>Control</td>
<td>76 ± 3</td>
<td>77 ± 3</td>
<td>0.763</td>
<td></td>
<td>1 ± 5</td>
</tr>
</tbody>
</table>

Fasting glucose (mg/dl)
11  | Combination  | 94 ± 2   | 92 ± 3 \(^a\) | 0.589          | 0.021          | -2 ± 4           | 0.461          |
20  | ADF          | 98 ± 5   | 95 ± 5 \(^a\) | 0.146          |               | -3 ± 2           |               |
18  | Exercise     | 92 ± 2   | 91 ± 2 \(^a,b\) | 0.862          |               | -1 ± 2           |               |
9   | Control      | 109 ± 7  | 111 ± 6 \(^b\) | 0.637          |               | 2 ± 4            |               |

Fasting insulin (μIU/ml)
11  | Combination  | 14 ± 2   | 11 ± 2   | 0.305          | 0.436          | -9 ± 15          | 0.559          |
20  | ADF          | 23 ± 8   | 21 ± 8   | 0.050          |               | -11 ± 6          |               |
18  | Exercise     | 11 ± 1   | 11 ± 1   | 0.666          |               | 0 ± 8            |               |
5   | Control      | 25 ± 4   | 21 ± 4   | 0.178          |               | -16 ± 9          |               |

HOMA-IR
11  | Combination  | 3 ± 1    | 3 ± 0    | 0.296          | 0.396          | 0 ± 17           | 0.589          |
20  | ADF          | 7 ± 3    | 7 ± 3    | 0.092          |               | 0 ± 7            |               |
18  | Exercise     | 3 ± 0    | 3 ± 0    | 0.782          |               | 0 ± 10           |               |
5   | Control      | 7 ± 2    | 7 ± 2    | 0.165          |               | 0 ± 11           |               |

C-reactive protein (mg/dl)
11  | Combination  | 0.5 ± 0.2 | 0.5 ± 0.2 | 0.488          | 0.943          | 0 ± 28           | 0.859          |
19  | ADF          | 0.5 ± 0.1 | 0.5 ± 0.1 | 0.344          |               | 0 ± 12           |               |
18  | Exercise     | 0.3 ± 0.1 | 0.3 ± 0.3 | 0.349          |               | 0 ± 21           |               |
5   | Control      | 0.8 ± 0.4 | 0.8 ± 0.2 | 0.823          |               | 0 ± 25           |               |

Value reported as mean ± SEM. Intention to treat analysis. ADF: Alternate day fasting, BP: Blood pressure, HOMA-IR: Homeostatic model assessment-Insulin resistance.

1 P-value between week 1 and week 12: Repeated-measures ANOVA.
2 P-value between groups at week 12: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test).
3 Percent change between week 1 and week 12 values.
4 P-value between groups for percent change: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test).
5 ANCOVA was performed with baseline values as a covariate.
### TABLE III.V. LDL PARTICLE SIZE DURING THE 12-WEEK TRIAL

<table>
<thead>
<tr>
<th>n</th>
<th>Intervention</th>
<th>Week 1</th>
<th>Week 12</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>LDL particle size (Å)</td>
<td>16 Combination</td>
<td>260 ± 1</td>
<td>264 ± 2</td>
<td>&lt;0.001</td>
<td>0.031</td>
<td>4 ± 1</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>16 ADF</td>
<td>261 ± 1</td>
<td>266 ± 1</td>
<td>&lt;0.001</td>
<td>0.010</td>
<td>5 ± 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 Exercise</td>
<td>261 ± 2</td>
<td>262 ± 2</td>
<td>0.426</td>
<td></td>
<td>1 ± 1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11 Control</td>
<td>259 ± 1</td>
<td>260 ± 2</td>
<td>0.884</td>
<td></td>
<td>0 ± 1</td>
<td></td>
</tr>
<tr>
<td>Large LDL particles (%)</td>
<td>16 Combination</td>
<td>38 ± 4</td>
<td>45 ± 5</td>
<td>0.142</td>
<td>0.014</td>
<td>7 ± 5</td>
<td>0.064</td>
</tr>
<tr>
<td></td>
<td>16 ADF</td>
<td>36 ± 3</td>
<td>51 ± 4</td>
<td>&lt;0.001</td>
<td></td>
<td>15 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 Exercise</td>
<td>39 ± 3</td>
<td>40 ± 4</td>
<td>0.792</td>
<td></td>
<td>1 ± 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11 Control</td>
<td>30 ± 3</td>
<td>31 ± 4</td>
<td>0.883</td>
<td></td>
<td>1 ± 4</td>
<td></td>
</tr>
<tr>
<td>Medium LDL particles (%)</td>
<td>16 Combination</td>
<td>37 ± 2</td>
<td>38 ± 2</td>
<td>0.845</td>
<td>0.301</td>
<td>1 ± 3</td>
<td>0.817</td>
</tr>
<tr>
<td></td>
<td>16 ADF</td>
<td>37 ± 1</td>
<td>35 ± 1</td>
<td>0.288</td>
<td></td>
<td>-2 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 Exercise</td>
<td>41 ± 3</td>
<td>40 ± 3</td>
<td>0.453</td>
<td></td>
<td>-1 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11 Control</td>
<td>41 ± 2</td>
<td>40 ± 2</td>
<td>0.717</td>
<td></td>
<td>-1 ± 2</td>
<td></td>
</tr>
<tr>
<td>Small LDL particles (%)</td>
<td>16 Combination</td>
<td>25 ± 3</td>
<td>18 ± 3</td>
<td>0.010</td>
<td>-7 ± 2</td>
<td>0.007</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16 ADF</td>
<td>27 ± 3</td>
<td>15 ± 3</td>
<td>&lt;0.001</td>
<td>0.023</td>
<td>-12 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>15 Exercise</td>
<td>21 ± 3</td>
<td>20 ± 4</td>
<td>0.972</td>
<td></td>
<td>-1 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>11 Control</td>
<td>29 ± 3</td>
<td>30 ± 3</td>
<td>0.776</td>
<td></td>
<td>1 ± 3</td>
<td></td>
</tr>
</tbody>
</table>

Value reported as mean ± SEM. Intention to treat analysis. ADF: Alternate day fasting. Large LDL particles (>260 Å), medium LDL particles (255-260 Å), and small LDL particles (<255 Å).

1 P-value between week 1 and week 12: Repeated-measures ANOVA.
2 P-value between groups at week 12: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test).
3 Absolute change between week 1 and week 12 values.
4 P-value between groups for absolute change: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test).
### TABLE III.VI. HDL PARTICLE SIZE DURING THE 12-WEEK TIRAL

<table>
<thead>
<tr>
<th>n</th>
<th>Intervention</th>
<th>Week 1</th>
<th>Week 12</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></td>
<td>Large HDL particles (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Combination</td>
<td>32 ± 3</td>
<td>34 ± 3</td>
<td>0.102</td>
<td>0.783</td>
<td>2 ± 1</td>
<td>0.106</td>
</tr>
<tr>
<td>16</td>
<td>ADF</td>
<td>34 ± 2</td>
<td>32 ± 2</td>
<td>0.157</td>
<td>-2 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Exercise</td>
<td>32 ± 2</td>
<td>31 ± 1</td>
<td>0.818</td>
<td>-1 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Control</td>
<td>34 ± 3</td>
<td>34 ± 3</td>
<td>0.777</td>
<td>0 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Medium HDL particles (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Combination</td>
<td>53 ± 2</td>
<td>54 ± 2</td>
<td>0.191</td>
<td>0.231</td>
<td>1 ± 1</td>
<td>0.904</td>
</tr>
<tr>
<td>16</td>
<td>ADF</td>
<td>52 ± 2</td>
<td>53 ± 1</td>
<td>0.885</td>
<td>1 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Exercise</td>
<td>55 ± 2</td>
<td>55 ± 2</td>
<td>0.747</td>
<td>0 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Control</td>
<td>49 ± 2</td>
<td>50 ± 2</td>
<td>0.423</td>
<td>1 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Small HDL particles (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Combination</td>
<td>15 ± 2</td>
<td>11 ± 1</td>
<td>0.007</td>
<td>0.134</td>
<td>-4 ± 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.006</td>
</tr>
<tr>
<td>16</td>
<td>ADF</td>
<td>13 ± 1</td>
<td>15 ± 2</td>
<td>0.136</td>
<td>2 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Exercise</td>
<td>13 ± 1</td>
<td>14 ± 1</td>
<td>0.709</td>
<td>1 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>Control</td>
<td>16 ± 2</td>
<td>16 ± 2</td>
<td>0.663</td>
<td>0 ± 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Value reported as mean ± SEM. Intention to treat analysis. ADF: Alternate day fasting. Large HDL particles (>88 Å), medium HDL particles (73-88 Å), and small HDL particles (<73 Å).

1 P-value between week 1 and week 12: Repeated-measures ANOVA.
2 P-value between groups at week 12: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test).
3 Absolute change between week 1 and week 12 values.
4 P-value between groups for absolute change: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test).
IV. MANUSCRIPT 2

Alternate day fasting with or without exercise: Effects on endothelial function and adipokines in obese humans

Bhutani Surabhi 1, MS, Klempel Monica C 1, MS, Kroeger Cynthia M 1, BS, Phillips SA 2, PhD, Norkeviciute Edita 2, Varady KA 1, PhD.

Author affiliation:

1 Department of Kinesiology and Nutrition, University of Illinois at Chicago, Chicago, IL
2 Department of Physical Therapy, University of Illinois at Chicago, Chicago, IL

Correspondence and reprint requests:

Surabhi Bhutani, M.S.
Kinesiology and Nutrition, University of Illinois at Chicago
1919 West Taylor Street, Room 103, Chicago, IL, 60612
Tel: 312-996-0354, Fax: 312-413-0319
Email: sbhuta2@uic.edu

Running title: Alternate day fasting and endothelial function

Funding: American Heart Association Pre-doctoral Fellowship, 12PRE835000; University of Illinois, Chancellor Discovery Fund

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

Objective: Alternate day fasting (ADF; which consists of an ad libitum “feed day” alternated with a 75% energy restriction “fast day”) combined with exercise improves several coronary heart disease (CHD) risk factors. However, the effect of this combination therapy on endothelial function, and the role that adipokines play in mediating this effect, is unknown. Accordingly, this study examined the effect of ADF combined with exercise on brachial artery flow-mediated dilation (FMD) and plasma adiponectin and leptin. Research Methods and Procedures: Sixty-four obese subjects were randomized to 1 of 4 groups: 1) combination (ADF + endurance exercise), 2) ADF, 3) exercise, or 4) control, for 12 weeks. Results: Body weight decreased (P < 0.05) in the combination (6 ± 4 kg), ADF (3 ± 1 kg) and exercise group (1 ± 0 kg). Fat mass decreased (P < 0.01) in the combination (5 ± 1 kg) and ADF (2 ± 1 kg) groups. FMD increased (P < 0.05) only in the ADF group (5 ± 1% to 10 ± 2%; 5% increase). Leptin decreased in the combination (34 ± 9 ng/ml, P < 0.001), ADF (10 ± 4 ng/ml, P < 0.05) and exercise group (11 ± 4 ng/ml, P < 0.05). Adiponectin was not changed by any intervention. Changes in FMD in the ADF group were not related to changes in leptin. Conclusions: These findings suggest that ADF alone is an effective intervention to improve vascular endothelial function. However, the role of adipokines in mediating this effect is still unclear.
B. Introduction

Alternate day fasting (ADF) is a novel dietary restriction strategy that has gained considerable popularity over the past decade. ADF consists of an ad libitum “feed day” alternated with a 75% energy restriction “fast day”. Recent evidence suggests that combining ADF with endurance exercise increases HDL cholesterol levels, decreases LDL cholesterol levels, and augments both LDL and HDL particle size (3, 113). These beneficial changes in plasma lipids suggest that this combination therapy may confer protection against coronary heart disease (CHD). Endothelial dysfunction is another gold standard prognostic indicator of future CHD, and most vascular disease risk factors are associated with reduced flow mediated dilation (FMD) (27). An important question that has yet to be tested is whether the combination of ADF plus endurance exercise can elicit added cardiovascular benefits by increasing FMD.

Adipose tissue acts as an endocrine organ in that it secretes biologically active hormones called adipokines. Recent evidence suggests that certain adipokines, such as adiponectin and leptin, may improve nitric oxide (NO) bioavailability and endothelial function. For instance, adiponectin induces the phosphorylation of endothelial nitric oxide synthase (eNOS), which results in increases in NO production and FMD (114) (115). Leptin, in contrast, is a pro-atherogenic hormone derived from adipocytes that has been shown to cause endothelial dysfunction in obese individuals (116). Hyperleptinemia in obesity increases the production of oxidative species that scavenge NO, resulting in a decline in FMD (116). Previous findings indicate that ADF and endurance exercise independently increase adiponectin while lowering leptin levels (2, 71). As such, combining these interventions may have an additive effect on the circulating concentrations of these adipokines, which may lead to more pronounced improvements in FMD.
Accordingly, the present study investigated the effect of ADF combined with endurance exercise on endothelial function, relative to ADF and exercise alone. The role of adipokines in mediating improvements in FMD was also investigated.
C. Materials and methods

Subjects and study design

As described previously (113), obese subjects were recruited from the University of Illinois at Chicago campus by advertisements. Key inclusion criteria were as follows: age 25 to 65 years; body mass index between 30 and 39.9 kg/m²; weight stable for 3 months prior to the beginning of the study (less than 5 kg weight loss or weight gain); non-diabetic; no history of cardiovascular disease; 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); non-smoker; no history of bariatric surgery; and not taking weight loss, lipid or glucose lowering medications; no polycystic ovarian syndrome (PCOS); not vegan or vegetarian; no food allergies. The experimental protocol was approved by the Office for the Protection of Research Subjects at the University of Illinois, Chicago, and all volunteers gave their written informed consent to participate in the trial. A 12-week, randomized, controlled, parallel-arm feeding trial was implemented to test the study objectives. Subjects were stratified on the basis of BMI, age, and sex, and then randomized into 1 of 4 groups: 1) combination group; 2) ADF group; 3) exercise group; 4) control group.

Diet protocol

The diet intervention has been previously described (113). Only the combination and ADF groups participated in the diet protocol. Briefly, the 12-week diet intervention consisted of two phases: 1) a controlled feeding phase (week 1-4), and 2) a self selected feeding phase (week 5-12). During the controlled feeding phase (week 1-4) participants consumed 25% of their baseline energy needs on the "fast day" (24 h) and consumed food ad libitum on each "feed day" (24 h). All fast day meals were provided to the subjects during the controlled feeding phase. The baseline energy requirements for the subjects were assessed by the Mifflin equation (87). Fast day meals were consumed between 12 pm and
2 pm. The macronutrient composition of the provided fast day meals was 25% kcal from fat, 20% kcal from protein, and 55% kcal from carbohydrates. During the self-selected feeding phase (week 5-12) subjects continued with the ADF regimen but no fast day food was provided to them. Instead, each subject met with a dietician at the beginning of each week to learn how to maintain the ADF regimen on his or her own at home. Control and exercise group subjects were not given any dietary counselling and maintained their regular eating habits.

Exercise protocol

Both the combination and exercise groups participated in a moderate intensity exercise intervention, 3 times/week, for 12 weeks. The supervised exercise sessions were performed at the research center using stationary bikes and elliptical machines. An age-predicted heart rate maximum (HRmax) equation [(209 – (0.7 × age))] (88) and a polar heart rate monitor (Polar USA, Inc., NY) were used to estimate exercise intensity. At the beginning of the study (weeks 1 to 4), each exercise session ran for 25 min duration and corresponded to 60% of the subject’s HRmax. Training duration and intensity increased incrementally at week 4, 7 and 10 by 5 min and 5% HRmax. As such, by week 10, each subject was exercising for a 40 min duration at an intensity of 75% HRmax. ADF and control subjects were asked to maintain their regular activity habits, and to refrain from joining an exercise class during the study.

Body weight and body composition assessment

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

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

Brachial artery FMD was assessed at week 1 and 12. Subjects did not exercise for 24 h prior to the FMD assessment. Ultrasound imaging of the brachial artery (MicroMaxx, Sonosite, Seattle, WA) was performed in a longitudinal plane at a site 1-3 cm proximal to the antecubital fossa, with the arm abducted approximately 80° from the body and the forearm supinated. The ultrasound probe (11 MHz) was positioned to visualize the anterior and posterior lumen-intima interfaces to measure diameter or central flow velocity (pulsed Doppler). The probe site was marked for accurate repositioning after exercise. After baseline images were recorded, a blood pressure cuff on the forearm was inflated to 200 mm Hg for 5 min. To assess FMD, 10 images were captured every second. A total of 10 seconds of images were recorded during the process. These images were taken at 30 seconds, 60 seconds and 120 seconds after cuff release. Baseline brachial flow velocity and peak velocity after cuff release were recorded. Images were digitally recorded using Brachial Imager (Medical Imaging, Iowa City, IA) and analyzed. Percent FMD was calculated using the averaged minimum mean brachial artery diameter at baseline compared to the largest mean values obtained after release of the forearm occlusion. Blood pressure was assessed in triplicated after a 10-min rest.

**Plasma adipokines**

Twelve-hour fasting blood samples were collected between 6.00 am and 10.00 am at week 1 and week 12. Subjects were instructed to avoid exercise, alcohol and coffee for 24 h before each visit. Blood was centrifuged for 10 minutes at 1000 g and 4°C to separate plasma from RBC and was stored at -80°C until analyzed. Plasma adiponectin and leptin were measured using high sensitivity enzymatic kits.
(R&D Systems, Minneapolis, MN). The intra-assay variances for the adiponectin and leptin ELISAs were 4.8 and 4.2%, respectively.

**Statistical analysis**

Results are presented as mean ± SEM. Differences between intervention groups at baseline were analyzed by a one-way ANOVA. When baseline differences were noted for a specific parameter, ANCOVA was performed with the baseline value as a covariate. Within-group differences were analysed using repeated-measures ANOVA. An intention-to-treat analysis was performed for all variables measured. A P value of < 0.05 was used as a criterion for statistical significance in all analyses. Data were analyzed using SPSS software (version 20.0 for Mac OSX; SPSS Inc, Chicago, IL, USA).
D. Results

Baseline characteristics and dropouts

Eighty-three subjects began the clinical trial (combination: n = 18, ADF: n = 25, exercise: n = 24, control: n = 16), and n = 16 subjects finished in each intervention group (total n = 64). Additional subjects were randomized to groups that had high dropout rates (i.e. the ADF and exercise group) to ensure that the total number of subjects would be the same in each group at the end of the study. At baseline, there were no differences in age (combination: 45 ± 5 y, ADF: 42 ± 2 y, exercise: 42 ± 2 y, control: 49 ± 2 y), sex (combination (F/M): 18/0, ADF: 24/1, exercise: 23/1, control: 15/1), and BMI (combination: 35 ± 1 kg/m², ADF: 35 ± 1 kg/m², exercise: 35 ± 1 kg/m², control: 35 ± 1 kg/m²) between groups. Moreover, there were no differences at baseline between groups for body weight, fat mass, waist circumference, and adipokines (Table IV.I). Systolic and diastolic blood pressures were higher in the ADF group at baseline versus the other groups.

Body weight and body composition

Changes in body weight, fat mass and waist circumference are reported in Table IV.I. Body weight significantly decreased (P < 0.05) in all three intervention groups. The combination group showed the greatest (P < 0.001) weight loss (6 ± 4 kg), followed by the ADF (3 ± 1 kg) and exercise group (1 ± 0 kg). Fat mass decreased (P < 0.01) only in the combination (5 ± 1 kg) and ADF group (2 ± 1 kg) after 12 weeks. The decrease in waist circumference was higher (P < 0.001) in the combination group (8 ± 1 cm) compared to the ADF (5 ± 1 cm) and exercise group (3 ± 1 cm).
**Brachial artery flow mediated dilation (FMD)**

There were no differences in FMD between groups at baseline (Figure 4.1). By week 12, FMD improved ($P < 0.05$) in the ADF group relative to baseline ($5 \pm 1\%$ to $10 \pm 2\%$; 5% increase). There were no changes in FMD in the combination, exercise or control groups over the course of the trial. Systolic and diastolic blood pressure decreased ($P < 0.05$) only in the ADF group (Table IV.1).

**Plasma adipokines**

Adiponectin levels were not affected by any of the interventions after 12 weeks of treatment (Table IV.1). Leptin concentrations decreased ($P < 0.05$) by $34 \pm 9$ ng/ml, $10 \pm 4$ ng/ml, and $11 \pm 4$ ng/ml in the combination, ADF, and exercise group, respectively. There were no associations between FMD and adipokine concentrations at baseline or post-treatment in any intervention group.
E. Discussion

This study is the first to show that ADF is an effective intervention to improve endothelium-dependent flow mediated dilation. The improvements in FMD by ADF were not mediated by changes in adiponectin or leptin, however. Interestingly, exercise alone or in combination with ADF, had no effect on FMD.

In the present trial FMD increased in the ADF group only. Leptin levels were also decreased by the ADF intervention, however, no relationship was noted between lower leptin levels and increased FMD. It is possible that the correlation between leptin and FMD was not significant due to the small sample size of the ADF group. Contrary to previous reports (2, 16), adiponectin was not altered by 12 weeks of ADF. Seeing as adiponectin is only increased with substantial weight loss (>10% from baseline), the weight loss achieved here may not have been sufficient to augment levels of this adipokine (117). It is possible that FMD only improved in the ADF group because this was the only intervention to demonstrate decreases in blood pressure. In a review by Dharmashankar et al, the association between hypertension and endothelial dysfunction was assessed (118). Findings from this review indicate that high blood pressure increases oxidative stress and inflammation resulting in endothelium-dependent vasomotor dysfunction (118). Therefore, the change from the pre-hypertensive to normotensive state in the ADF group may have reduced the oxidative stress and inflammation, thus improving FMD. Since blood pressure was not altered by the exercise or combination intervention, this may explain why FMD did not increase in either of these groups. However, the physiological reason for this lack of effect remains to be determined.

As for endurance exercise, no change in FMD was observed after 12 weeks of treatment, despite reductions in leptin concentrations in this group. It is possible that the exercise intensity in the current
study (60%-75% HRmax) was not high enough to increase FMD. In a study by Tjonna et al, the effect of high intensity (90-95% HRmax) versus moderate intensity (75% HRmax) endurance exercise on FMD was examined (61). FMD improved to a greater extent in the high intensity group as compared to the moderate intensity group. The researchers speculated that the high intensity exercise produced greater sheer stress on the blood vessel walls, thus producing positive molecular changes (61).

Therefore, it is possible that a higher intensity of exercise (>90% HRmax) is required to improve endothelial function. Furthermore, a higher caloric expenditure with endurance exercise, which would result in greater weight loss, may also be required to improve endothelial function (119). These factors may explain why no changes in FMD were observed after 12 weeks of endurance training.

**Conclusion**

In summary, these findings suggest that ADF is an effective strategy to improve endothelium-dependent flow mediated dilation. However, the role of adipokines in mediating this effect is still not clear. We also show here that a moderate intensity exercise regimen may not be sufficient enough to improve FMD, thus explaining why the combination and exercise group demonstrated no change in this CHD risk parameter. Overall, this study contributes to the mounting evidence supporting role of ADF in the prevention of CHD in obese populations.
F. Acknowledgements

Surabhi Bhutani designed the study, conducted the clinical trial, analyzed the data, and wrote the manuscript. Monica C Klempel, and Cynthia M Kroeger assisted with clinical trial coordination. Edita Norkeviciute assisted with the data analysis. Shane A Phillips assisted with the data analysis. and wrote the manuscript. Krista A Varady designed the study and wrote the manuscript.
## TABLE IV.1. BODY WEIGHT, BLOOD PRESSURE, AND ADIPOKINES DURING THE 12-WEEK TRIAL

<table>
<thead>
<tr>
<th>n</th>
<th>Intervention</th>
<th>Week 1</th>
<th>Week 12</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></td>
<td>Body weight (kg)</td>
<td>91 ± 6</td>
<td>85 ± 6</td>
<td>&lt;0.001</td>
<td>0.393</td>
<td>-6 ± 4 ²</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>18</td>
<td>Combination</td>
<td>94 ± 3</td>
<td>91 ± 3</td>
<td>&lt;0.001</td>
<td>-3 ± 1 ²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>ADF</td>
<td>93 ± 2</td>
<td>92 ± 2</td>
<td>0.027</td>
<td>-1 ± 0 ²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Exercise</td>
<td>93 ± 5</td>
<td>93 ± 5</td>
<td>0.577</td>
<td>0 ± 0 ³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Control</td>
<td>45 ± 2</td>
<td>40 ± 2</td>
<td>&lt;0.001</td>
<td>0.054</td>
<td>-5 ± 1 ³</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>Fat mass (kg)</td>
<td>43 ± 2</td>
<td>41 ± 2</td>
<td>0.008</td>
<td>-2 ± 1 ³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>ADF</td>
<td>46 ± 2</td>
<td>45 ± 2</td>
<td>0.182</td>
<td>-1 ± 0 ³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Exercise</td>
<td>43 ± 4</td>
<td>43 ± 4</td>
<td>0.570</td>
<td>0 ± 1 ³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Control</td>
<td>96 ± 2</td>
<td>88 ± 1</td>
<td>&lt;0.001</td>
<td>0.310</td>
<td>-8 ± 1 ³</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>18</td>
<td>Waist circumference (cm)</td>
<td>113 ± 3</td>
<td>111 ± 3</td>
<td>0.262</td>
<td>0.176</td>
<td>-2 ± 2</td>
<td>0.254</td>
</tr>
<tr>
<td>25</td>
<td>ADF</td>
<td>124 ± 3</td>
<td>120 ± 3</td>
<td>0.007</td>
<td>-3 ± 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Exercise</td>
<td>113 ± 2</td>
<td>115 ± 3</td>
<td>0.284</td>
<td>2 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>Control</td>
<td>122 ± 5</td>
<td>120 ± 6</td>
<td>0.603</td>
<td>-2 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Systolic BP (mm Hg)</td>
<td>76 ± 2</td>
<td>76 ± 2</td>
<td>0.939</td>
<td>0.123</td>
<td>0 ± 3</td>
<td>0.570</td>
</tr>
<tr>
<td>18</td>
<td>Combination</td>
<td>120 ± 2</td>
<td>120 ± 2</td>
<td>0.034</td>
<td>-2 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>ADF</td>
<td>76 ± 2</td>
<td>76 ± 2</td>
<td>0.976</td>
<td>0 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Exercise</td>
<td>86 ± 2</td>
<td>84 ± 4</td>
<td>0.480</td>
<td>-2 ± 3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Control</td>
<td>11452 ± 1324</td>
<td>12084 ± 1470</td>
<td>0.369</td>
<td>0.101</td>
<td>632 ± 682</td>
<td>0.190</td>
</tr>
<tr>
<td></td>
<td>Diastolic BP (mm Hg)</td>
<td>82 ± 2</td>
<td>82 ± 2</td>
<td>0.034</td>
<td>-2 ± 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Exercise</td>
<td>9111 ± 1024</td>
<td>9813 ± 1196</td>
<td>0.221</td>
<td>0.221</td>
<td>702 ± 461</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Control</td>
<td>8234 ± 723</td>
<td>8943 ± 867</td>
<td>0.725</td>
<td>0.725</td>
<td>709 ± 417</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>Intervention</td>
<td>Week 1</td>
<td>Week 12</td>
<td>P-value(^1)</td>
<td>P-value(^2)</td>
<td>Change(^3)</td>
<td>P-value(^4)</td>
</tr>
<tr>
<td>----</td>
<td>--------------</td>
<td>---------</td>
<td>---------</td>
<td>----------------</td>
<td>----------------</td>
<td>--------------</td>
<td>--------------</td>
</tr>
<tr>
<td>18</td>
<td>Combination</td>
<td>63 ± 11</td>
<td>29 ± 7 (^a)</td>
<td>0.001</td>
<td>0.001</td>
<td>-34 ± 9 (^a)</td>
<td>0.015</td>
</tr>
<tr>
<td>24</td>
<td>ADF</td>
<td>41 ± 7</td>
<td>31 ± 6 (^a)</td>
<td>0.028</td>
<td>-10 ± 4 (^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Exercise</td>
<td>44 ± 9</td>
<td>33 ± 6 (^a)</td>
<td>0.025</td>
<td>-11 ± 4 (^b)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>Control</td>
<td>69 ± 8</td>
<td>73 ± 14 (^b)</td>
<td>0.760</td>
<td>4 ± 12 (^c)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values reported as mean ± SEM. Intention to treat analysis. ADF: Alternate day fasting, BP: Blood pressure.

1 P-value between week 1 and week 12: Repeated-measures ANOVA.
2 P-value between groups at week 12: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey test).
3 Absolute change between week 1 and week 12 values.
4 P-value between groups for absolute change: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey test).
5 ANCOVA was performed with baseline values as a covariate.
Figure 4.1. Flow mediated dilation (FMD) at baseline and post-treatment

Values reported as mean ± SEM. ADF: Alternate day fasting. *Week 1 value significantly different (P < 0.05) from week 12 value (Repeated-measures ANOVA).
V. MANUSCRIPT 3

Effect of exercising while fasting on eating behaviors and food intake

Surabhi Bhutani\textsuperscript{1}, Monica C. Klempel\textsuperscript{1}, Cynthia M. Kroeger\textsuperscript{1}, Eleanor Aggour\textsuperscript{1}, Yolian Calvo\textsuperscript{1}, Krista A. Varady\textsuperscript{1}

Author affiliation:
\textsuperscript{1}Department of Kinesiology and Nutrition, University of Illinois at Chicago, Chicago, IL, USA.

Correspondence and reprint requests:
Surabhi Bhutani, M.S.
Department of Kinesiology and Nutrition
University of Illinois at Chicago
1919 West Taylor Street, Room 103, Chicago, IL, 60612
Tel: 312-996-7897 Fax: 312-413-0319
Email: sbhuta2@uic.edu

Running head: Effect of exercise combined with fasting on eating behaviors

Funding source: American Heart Association 12PRE8350000; University of Illinois, Chicago, Departmental funding
A. **Abstract**

**Background:** Alternate day fasting combined with exercise is effective for weight loss. The aim of this study was to examine the behavioral adaptations that occur when ADF is combined with exercise, and to determine how these changes affect weight loss. **Method:** Obese subjects (n = 64) were randomized to 1 of 4 groups: 1) combination (ADF + endurance exercise), 2) ADF, 3) exercise, or 4) control, for 12 weeks. **Results:** Body weight decreased (P < 0.05) in the combination group (6 ± 4 kg), ADF (3 ± 1 kg) and exercise group (1 ± 0 kg). When given the choice, subjects chose to exercise the same amount (P = 0.790) on the fast days (48 ± 2%) as feed days (52 ± 2%). Percent of exercise sessions performed on fast day mornings (20 ± 6%) did not differ (P = 0.453) from fast day afternoons (28 ± 5%). Likelihood to cheat on the fast day was not higher if the subject exercised in the afternoon (17 ± 7%) versus the morning (10 ± 5%). Hunger decreased (P < 0.05) while satisfaction and fullness increased (P < 0.05) post-treatment in the ADF group only. Restrained eating increased (P < 0.05) and uncontrolled eating decreased (P < 0.05) in the combination and ADF groups. **Conclusion:** These findings suggest that endurance exercise is an excellent adjunct therapy to ADF, as it leads to positive behavioral changes that may contribute to long-term steady weight loss.
B. **Background**

Alternate day fasting (ADF) is a modified form of calorie restriction comprising a fast day (25% energy intake for 24 h) alternated with a feed day (ad libitum energy intake for 24 h) (3). Previous reports indicate that ADF is an effective strategy to reduce body weight (5% in 12 weeks) and improve body composition. More recently, it has been shown that combining ADF with exercise leads to greater weight loss (7% in 12 weeks) than what has been seen with ADF or exercise alone (113). Although these findings are promising, it is still unclear how this combination therapy affects eating behaviors, and how these behavioral changes enhance weight loss. Recent evidence suggests that weight loss in obese individuals is attributed to an increase in cognitive restraint (120-122), reduced disinhibition, lower hunger levels (121, 122) and decreased consumption of dietary fat (123). In view of these findings, key questions that have yet to be addressed in this field include: Are obese individuals able to exercise on the fast day? If so, does exercise increase hunger in a way that causes people to cheat on the fast day? What role does the timing of the exercise session play in determining whether or not the individual will cheat? Does ADF, with or without exercise, elicit positive behavioral changes that may contribute to long-term steady weight loss?

Therefore, the aim of this study was to examine the behavioral adaptations that occur when ADF is combined with endurance training, and to investigate how these changes affect weight loss.
C. **Materials and methods**

**Subjects**

As described previously (113), independently living subjects were recruited from the University of Illinois at Chicago campus by means of flyers. Of the 146 interested individuals, 83 were deemed eligible to participate according to a preliminary questionnaire and body mass index (BMI) assessment. Key inclusion criteria were as follows: age 25 to 65 years; BMI between 30 and 39.9 kg/m²; weight stable for 3 months prior to the beginning of the study (i.e. less than 5 kg weight loss or weight gain); non-diabetic; no history of cardiovascular disease; lightly active (i.e. <3 h/week of light intensity exercise at 2.5 to 4.0 metabolic equivalents (METs) for 3 months prior to the study); non-smoker; no history of bariatric surgery; no polycystic ovarian syndrome (PCOS); not vegan or vegetarian; no food allergies and not taking weight loss, lipid or glucose lowering medications. The experimental protocol was approved by the Office for the Protection of Research Subjects at the University of Illinois, Chicago. All volunteers gave informed consent to participate in the trial.

**Experimental design and randomization**

A 12-week, randomized, controlled, parallel-arm feeding trial was implemented to test the effects of ADF, exercise, and ADF combined with exercise (combination group) on eating behaviors and weight loss. Eligible subjects were stratified on the basis of BMI, age, and sex, and then randomized into 1 of 4 groups: 1) combination group; 2) ADF group; 3) exercise group; 4) control group.

**Diet protocol**

As previously described (113) only the combination and ADF groups participated in the dietary intervention, which consisted of two periods: 1) a 4-week controlled feeding period, and 2) an 8-week
self-selected feeding period. During the controlled feeding period (week 1-4) participants consumed 25% of their baseline energy needs on the fast day (24 h) and consumed food ad libitum on each feed day (24 h). Baseline energy requirements were assessed by the Mifflin equation (87). The diet consisted of a 3-day rotating menu plan, and all fast day meals were prepared in the metabolic kitchen of the Human Nutrition Research Unit (HNRU). Fast day meals were consumed between 12.00 pm and 2.00 pm to ensure that each subject was undergoing the same duration of fasting. During the self-selected feeding period (week 8-12) subjects continued with the ADF regimen but no fast day food was provided to them. Instead, each subject met with a dietician at the beginning of each week to learn how to maintain the ADF regimen at home. Subjects were also taught how to monitor energy intake by reading food labels, reducing portion sizes, and choosing low fat meat and dairy options. Control and exercise group subjects were asked to maintain their regular food habits, and were not provided with any food or dietary counseling.

**Exercise protocol**

Only the combination and exercise groups participated the exercise intervention. These subjects participated in a moderate intensity exercise program 3 times per week under supervised conditions, for 12 weeks. Exercise was performed using stationary bikes and elliptical machines at the HNRU. Training intensity was estimated for each individual using an age-predicted heart rate maximum (HRmax) equation $[209 - (0.7 \times \text{age})]$ (88) and a Polar Heart Rate Monitor (Polar USA, Inc., NY). At the beginning of the study (weeks 1 to 4), each exercise session ran for 25 min duration and corresponded to 60% of the subject’s HRmax. Training duration and intensity increased incrementally at week 4, 7 and 10 by 5 min and 5% HRmax. As such, by week 10, each subject was exercising for a 40 min duration at an intensity of 75% HRmax. ADF and control subjects were asked to maintain their regular activity habits during the study.
Weight loss assessment

Body weight was measured weekly to the nearest 0.25 kg in the fasted state using a balance beam scale (HealthOMeter, Sunbeam Products, Boca Raton, FL). Waist circumference was measured by a flexible tape to the nearest 0.1 cm, midway between the lower costal margin and super iliac crest during a period of expiration.

Adherence to the ADF diet and exercise protocol

During the controlled feeding phase (week 1-4), subjects were instructed to eat only the fast day food provided, and to report any extra food item consumed using an “Extra food log”. The log was collected and reviewed by study personnel each week. If the log indicated that the subject ate an extra food item on a fast day, that day was labeled as “not adherent”. Exercise compliance was assessed by recording attendance at each supervised exercise session. If an exercise session was missed, the subject was required to make up for the missed session that same week.

Eating behavior assessment

A validated visual analog scale (VAS) (124) was used to measure hunger, fullness, and satisfaction with the ADF diet. The VAS scale was completed on each fast day (before bedtime). In brief, the VAS consisted of 100-mm lines, and subjects were asked to make a vertical mark across the line corresponding to their feelings from 0 (not at all) to 100 (extremely) for hunger, satisfaction, or fullness. Quantification was performed by measuring the distance from the left end of the line to the vertical mark. The three-factor eating questionnaire (TFEQ-R18) (125) was used to assess restrained eating (conscious restriction of food intake in order to control body weight), uncontrolled eating (tendency to eat more than usual due to a loss of control over intake), and emotional eating (inability to resist emotional eating cues). The TFEQ consists of 18 items on a 4-point response scale (definitely
true/mostly true/mostly false/definitely false). Responses to each of the 18 items are given a score between 1 and 4. The item scores are then summated into scale scores for restrained eating, uncontrolled eating, and emotional eating. The raw scale scores are transformed to a 0–100 scale \[((\text{raw score} - \text{lowest possible raw score})/\text{possible raw score range}) \times 100\]. Higher scores are indicative of greater restrained, uncontrolled, or emotional eating.

**Energy and macronutrient intake**

Each participant completed a 3-day food record on 2 feed days during the week, and on 1 feed day during the weekend, at each week of the 12-week trial. Thus, a total of 36 feed day food records were collected for each subject. At baseline, the dietician provided 15 min of instruction to each participant on how to complete the food records. These instructions included verbal information and detailed reference guides on how to estimate portion sizes and record food items in sufficient detail to obtain an accurate estimate of dietary intake. Subjects were instructed to record food items, in as much detail as possible, in the blank food diary provided. Any mixed foods were broken down to individual food items to be recorded one per line. Food records were collected at the weigh-in each week, and were reviewed by the dietician for accuracy and completeness. All dietary information from the food records was entered into the food analysis program, Nutritionist Pro (Axxya Systems). The program was used to calculate the total daily intake of energy, protein, carbohydrate, fat, cholesterol, and fiber.

**Statistical analysis**

Results are presented as mean ± SEM. Differences between intervention groups at baseline were analyzed by a one-way ANOVA. Within-group differences were analyzed using repeated-measures ANOVA. An intention-to-treat analysis was performed for all variables measured. A P value of < 0.05
was used as a criterion for statistical significance in all analyses. Data were analyzed using SPSS software (version 20.0 for Mac OSX; SPSS Inc, Chicago, IL, USA).
D. Results

Subject baseline characteristics and weight loss

As reported previously (113), 83 subjects began the clinical trial (combination: n = 18, ADF: n = 25, exercise: n = 24, control: n = 16), and sixteen subjects finished in each intervention group (total n = 64). Additional subjects were randomized to groups with high dropout rates, such as the ADF and exercise group, to ensure that the number of subjects would be the same in each group at the end of the trial. Dropouts were primarily due to scheduling conflicts. Baseline characteristics are reported in Table V.I. There were no between-group differences for age, sex, ethnicity, body weight, height, BMI, or waist circumference. Body weight decreased (P < 0.05) in the combination group (6 ± 4 kg), ADF (3 ± 1 kg) and exercise group (1 ± 0 kg), with no change in the control group (0 ± 0 kg). The decrease in waist circumference was greater (P < 0.001) in the combination group (8 ± 1 cm) compared to the ADF (5 ± 1 cm), exercise group (3 ± 1 cm), and control group (1 ± 1 cm).

ADF and exercise compliance

The combination group attended 95 ± 2% of the exercise sessions while the exercise group attended 94 ± 1% of the sessions. There was no difference (P = 0.83) in exercise compliance between groups. Adherence to the fast day diet remained high in the combination (81 ± 7%) and ADF group (80 ± 9%) throughout the course of the trial. No between-group differences were observed in fast day diet adherence when the combination group was compared to the ADF group (P = 0.23).

Timing of the fast day exercise session and impact on food intake

Subjects were given the option of scheduling their exercise sessions on feed days or fast days (morning or afternoon). Figure 5.1A portrays the percent of exercise sessions held on feed versus fast days.
Combination group subjects showed no preference \((P = 0.790)\) towards exercising on feed days \((52 \pm 2\%)\) versus fast days \((48 \pm 2\%)\). Furthermore, percent of exercise sessions performed on fast day mornings \((20 \pm 6\%)\) did not differ \((P = 0.453)\) from those performed on fast day afternoons \((28 \pm 5\%)\).

We also wanted to determine if subjects cheated more on the fast day (i.e. ate more than their prescribed amount of energy) if they exercised in the morning versus the afternoon. Results reveal that likelihood to cheat was not significantly higher if the subject chose to exercise in the afternoon \((17 \pm 7\%)\) versus the morning \((10 \pm 5\%)\) (Figure 5.1B).

Changes in eating behaviors

Changes in fast day eating behaviors are presented in Table V.II. No change in hunger, satisfaction or fullness was observed in the combination group post-treatment. However in ADF subjects, hunger decreased \((P < 0.05)\) while satisfaction and fullness increased \((P < 0.05)\) after 12 weeks of treatment. Restrained eating increased \((P < 0.05)\) by 16 \(\pm\) 6 and 10 \(\pm\) 2 points in the combination and ADF group, respectively, after 12 weeks. Uncontrolled eating decreased \((P < 0.05)\) in the combination \((14 \pm 4)\) and ADF group \((6 \pm 3)\) over the course of the trial. Emotional eating decreased \((P < 0.01)\) only in the combination group \((15 \pm 5)\). No changes were observed in eating behaviors in the exercise and control group.

Impact of the fast day exercise session on eating behaviors

Hunger did not increase if the subject exercised on a fast day \((week 1: 6 \pm 1, week 12: 4 \pm 2, P = 0.240)\).

Fullness did not decrease if the subject exercised on a fast day \((week 1: 4 \pm 2, week 12: 4 \pm 1, P = 0.653)\). Moreover, satisfaction with the ADF diet did not decrease if the subject exercised on a fast day \((week 1: 4 \pm 2, week 12: 4 \pm 1, P = 0.549)\).
Changes in energy and macronutrient intake on feed days

Dietary intake for each intervention group is reported in Table V.III. No changes were observed for energy, protein, carbohydrate, fat, cholesterol, and fiber after 12 weeks of treatment.
E. Discussion

Our findings show, for the first time, that endurance exercise can be easily incorporated into the ADF regimen. Specifically, subjects were able to exercise on the fast day, and this extra energy expenditure did not translate into increased hunger or extra food intake. We also show here that ADF combined with exercise improves several eating behaviors. For instance, after 12 weeks of treatment, restrained eating was increased while uncontrolled eating and emotional eating were decreased in obese individuals.

Our primary goal in this study was to see if subjects undergoing ADF can exercise on the fast day. All subjects exercised 3 times per week under supervised conditions. Throughout the study, subjects were allowed to choose the day (feed or fast day) and time (morning: 7.00-11.00 am, or afternoon: 3.00-7.00pm) of the exercise sessions. Results reveal that the combination group showed no preference towards exercising on feed days (52%) versus fast days (48%). Moreover, the percent of exercise sessions performed on fast day mornings (20%) did not differ from those performed on fast day afternoons (28%). We also wanted to see if the negative energy balance produced by the physical activity would lead to higher energy intake on the fast day. We hypothesized that the subjects exercising on fast day afternoons would be more likely to cheat (i.e. surpass their prescribed fast day energy goal) compared to subjects exercising in the morning. We assumed that cheating would be higher in the afternoon exercisers, as hunger peaks 30-40 minutes post workout (100). Since the morning exercisers would be able to eat their fast day meal shortly after their exercise session (12.00-2.00pm), they would be satisfied and less likely to cheat. In contrast, the afternoon exercisers would not have another meal to eat after their exercise session, which may lead them to consume extra food to suppress the post-workout hunger. Interestingly, the likeliness to cheat was not significantly higher in the afternoon exercisers (17%) compared to the morning exercisers (10%). However, it is possible that this difference was not significant due to small sample size (n = 16). Similar to our trial, Maraki et al. studied the acute
effect of one hour of morning (post breakfast) and afternoon (pre dinner) exercise on hunger and energy intake (126). Both morning and afternoon exercisers experienced increases in hunger, but did not exhibit increased energy intake. Our findings parallel those of Maraki et al. in that we also saw no increase in energy intake post-workout.

The effect of ADF with or without exercise on hunger, satisfaction and fullness was also tested. Hunger decreased while satisfaction and fullness increased after 12 weeks as compared to baseline values, only in the ADF group. The effect of ADF on eating behaviors was also tested by Heilbronn et al. Normal weight subjects participated in an ADF regimen (100% calorie restriction on the fast day) for 3 weeks. After this short intervention period, fullness increased, but there were no changes in the perception of hunger or satisfaction (13). The findings of Heilbronn et al. may have differed from ours because their study employed a true ADF regimen (complete fast on the fast day) whereas we used a modified ADF regimen (75% restriction on the fast day). Since the Heilbronn et al. subjects were not allowed to eat anything on the fast day, this may explain why hunger remained elevated throughout the course of the trial. In contrast to the ADF group, the combination group did not demonstrate any changes in hunger, satisfaction or fullness in the current study. The reason for this is not clear. Blundell et al. investigated the effect of medium term (7-16 d) moderate intensity endurance exercise on appetite stimulation. Findings reveal that hunger and food intake increased post-exercise in order to compensate for the negative energy balance achieved with training (127). In contrast, Guelfi et al. demonstrated that 12 weeks of 40-60 minutes of moderate intensity exercise (70-80 % HRmax) produced opposite results (77). Specifically, Guelfi et al. showed no change in perceived hunger, while levels of perceived fullness increased (77). In light of these contradictory findings, the impact of combination diet and exercise therapies on hunger and fullness warrant further investigation.
Changes in restrained eating, uncontrolled eating, and emotional eating were also examined. In both the ADF and combination groups, restrained eating increased while uncontrolled eating decreased. These positive changes in eating behaviors are most likely due to the subjects’ involvement in weekly dietary counseling (128). As for emotional eating, only the combination group experienced decreases in this parameter. It is possible that emotional eating was not decreased in the ADF group due to the lack of the exercise intervention. Positive changes in mood have been previously reported with short bouts of exercise (126, 129). Pendleton et al. designed a trial to study the effect of cognitive behavior therapy with or without exercise on binge eating in obese women. After 16 months, only the group that was exercising experienced improvements in mood, which resulted in decreased binge eating (130). Taken together, it is possible that the combination of ADF plus exercise may have better overall effects on these eating behaviors than each intervention alone.

We also wanted to examine the ability of our dietary counseling program to aid individuals in reducing energy intake. The combination and ADF subjects met with a dietician each week to learn how to ascertain the caloric content of foods, control portion sizes, read food labels, and avoid high fat foods. Dietary intake was measured using a 3-day food record that was completed each week (on feed days). After 12 weeks of treatment, energy intake decreased by approximately 300 kcal in the combination group and by 220 kcal in ADF group, though not significantly. These reported energy deficits are somewhat lower than expected given that the combination and ADF group lost 7 kg and 3 kg, respectively. These incongruences between weight loss and energy deficits are most likely due to reporting errors in the food records. In view of this, more robust measures to assess energy expenditure and intake, such as the doubly labeled water technique, should be used in future trials of ADF. The exercise and control group did not receive any dietary counseling.
This study has several limitations. First, the effect of exercise alone on hunger, fullness and satisfaction levels was not measured in this study. This makes it impossible to tease apart the effects of ADF versus exercise on these parameters. Secondly, the sample size employed (n = 16 per group) may have been too small to detect differences between groups for certain variables such as energy intake, and likelihood to cheat post-exercise. Thirdly, we implemented food records to measure energy intake, when we should have used a more accurate method, such as the doubly labeled water technique.

In summary, our results suggest that an endurance exercise program can be easily incorporated into the ADF regimen. Adding exercise to ADF does not increase the likeness to cheat on the fast day, which ensures that weight loss will be sizeable and consistent. We also show that the combination of ADF plus exercise increases restrained eating while decreasing uncontrolled and emotional eating. Taken together, endurance exercise is an excellent adjunct therapy to ADF, as it leads to positive behavioral changes that may contribute to long-term steady weight loss.

**Conflict of Interests**

The authors declare that they have no conflict of interests.
F. Acknowledgements

Surabhi Bhutani designed the experiment, conducted the clinical trial, analyzed the data, and wrote the manuscript. Monica C Klempel and Cynthia M Kroeger assisted with the conduction of the clinical trial. Elanour Aggour and Yolian Calvo assisted with the data analysis. Krista A Varady assisted with the design of the experiment, and wrote the manuscript.
Table V.I. Subject characteristics at baseline

<table>
<thead>
<tr>
<th></th>
<th>Combination</th>
<th>ADF</th>
<th>Exercise</th>
<th>Control</th>
<th>P-value $^1$</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>18</td>
<td>25</td>
<td>24</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>45 ± 5</td>
<td>42 ± 2</td>
<td>42 ± 2</td>
<td>49 ± 2</td>
<td>0.158</td>
</tr>
<tr>
<td>Sex (F/M)</td>
<td>18 / 0</td>
<td>24 / 1</td>
<td>23 / 1</td>
<td>15 / 1</td>
<td>0.266</td>
</tr>
<tr>
<td>Ethnicity (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>7</td>
<td>12</td>
<td>11</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>5</td>
<td>7</td>
<td>6</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>6</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>91 ± 6</td>
<td>94 ± 3</td>
<td>93 ± 2</td>
<td>93 ± 5</td>
<td>0.904</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160 ± 0</td>
<td>163 ± 0</td>
<td>162 ± 0</td>
<td>162 ± 1</td>
<td>0.896</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>35 ± 1</td>
<td>35 ± 1</td>
<td>35 ± 1</td>
<td>35 ± 1</td>
<td>0.934</td>
</tr>
<tr>
<td>Waist circumference</td>
<td>96 ± 2</td>
<td>100 ± 2</td>
<td>98 ± 2</td>
<td>99 ± 3</td>
<td>0.636</td>
</tr>
</tbody>
</table>

Values reported as mean ± SEM. Intention to treat analysis. BMI: Body mass index, F: Female, M: Male.

$^1$ P-value between groups at baseline: One-way ANOVA.
### TABLE V.II. CHANGES IN EATING BEHAVIORS DURING THE 12-WEEK STUDY

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Intervention</th>
<th>Week 1 (3 days)</th>
<th>Week 12 (3 days)</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>Hunger (cm)</td>
<td>14</td>
<td>Combination</td>
<td>5.7 ± 0.5</td>
<td>4.7 ± 0.7</td>
<td>0.185</td>
<td>0.941</td>
<td>-1.0 ± 0.7</td>
<td>0.495</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>ADF</td>
<td>6.3 ± 0.5</td>
<td>4.7 ± 0.7</td>
<td>0.034</td>
<td></td>
<td>-1.6 ± 0.7</td>
<td></td>
</tr>
<tr>
<td>Satisfaction (cm)</td>
<td>14</td>
<td>Combination</td>
<td>3.8 ± 0.8</td>
<td>4.1 ± 0.7</td>
<td>0.575</td>
<td>0.817</td>
<td>0.3 ± 0.5</td>
<td>0.240</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>ADF</td>
<td>3.2 ± 0.4</td>
<td>4.3 ± 0.6</td>
<td>0.031</td>
<td></td>
<td>1.1 ± 0.5</td>
<td></td>
</tr>
<tr>
<td>Fullness (cm)</td>
<td>14</td>
<td>Combination</td>
<td>3.7 ± 0.8</td>
<td>4.0 ± 0.7</td>
<td>0.564</td>
<td>0.967</td>
<td>0.3 ± 0.5</td>
<td>0.146</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>ADF</td>
<td>2.4 ± 0.4</td>
<td>4.0 ± 0.7</td>
<td>0.016</td>
<td></td>
<td>1.6 ± 0.6</td>
<td></td>
</tr>
<tr>
<td>Restrained eating score</td>
<td>14</td>
<td>Combination</td>
<td>40 ± 4</td>
<td>56 ± 7</td>
<td>0.029</td>
<td>0.207</td>
<td>16 ± 6 (^a)</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>ADF</td>
<td>52 ± 2</td>
<td>62 ± 3</td>
<td>0.006</td>
<td></td>
<td>10 ± 2 (^a)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Exercise</td>
<td>49 ± 3</td>
<td>49 ± 3</td>
<td>0.944</td>
<td></td>
<td>0 ± 2 (^b)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Control</td>
<td>47 ± 7</td>
<td>48 ± 6</td>
<td>0.828</td>
<td></td>
<td>1 ± 6 (^{a,b})</td>
<td></td>
</tr>
<tr>
<td>Uncontrolled eating score</td>
<td>14</td>
<td>Combination</td>
<td>44 ± 3</td>
<td>30 ± 4</td>
<td>0.007</td>
<td>0.050</td>
<td>-14 ± 4 (^a)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>ADF</td>
<td>35 ± 3</td>
<td>29 ± 3</td>
<td>0.023</td>
<td></td>
<td>-6 ± 3 (^{a,b})</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Exercise</td>
<td>40 ± 4</td>
<td>39 ± 5</td>
<td>0.783</td>
<td></td>
<td>-1 ± 2 (^b)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Control</td>
<td>23 ± 4</td>
<td>28 ± 6</td>
<td>0.152</td>
<td></td>
<td>5 ± 3 (^b)</td>
<td></td>
</tr>
<tr>
<td>Emotional eating score</td>
<td>14</td>
<td>Combination</td>
<td>57 ± 5</td>
<td>42 ± 6</td>
<td>0.002</td>
<td>0.063</td>
<td>-15 ± 5 (^a)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>ADF</td>
<td>38 ± 5</td>
<td>35 ± 5</td>
<td>0.428</td>
<td></td>
<td>-3 ± 3 (^b)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Exercise</td>
<td>58 ± 7</td>
<td>56 ± 7</td>
<td>0.447</td>
<td></td>
<td>-2 ± 3 (^b)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Control</td>
<td>38 ± 12</td>
<td>38 ± 11</td>
<td>0.584</td>
<td></td>
<td>0 ± 5 (^b)</td>
<td></td>
</tr>
</tbody>
</table>

Values reported as mean ± SEM. Intention to treat analysis. ADF: Alternate day fasting.

1 P-value between week 1 and week 12: Repeated-measures ANOVA.
2 P-value between groups at week 12: One-way ANOVA.
3 Absolute change between week 1 and 12 values.
4 P-value between groups for absolute change: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test)
## TABLE V.III. ENERGY AND MACRONUTRIENT INTAKE ON FEED DAYS DURING THE 12-WEEK STUDY

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Intervention</th>
<th>Week 1</th>
<th>Week 12</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><strong>Energy (kcal)</strong></td>
<td>11</td>
<td>Combination</td>
<td>1656 ± 265</td>
<td>1358 ± 123</td>
<td>0.278</td>
<td>0.854</td>
<td>-298 ± 260</td>
<td>0.897</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>ADF</td>
<td>1681 ± 155</td>
<td>1457 ± 204</td>
<td>0.228</td>
<td></td>
<td>-224 ± 173</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Exercise</td>
<td>1623 ± 145</td>
<td>1553 ± 135</td>
<td>0.739</td>
<td></td>
<td>-70 ± 203</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Control</td>
<td>1607 ± 307</td>
<td>1416 ± 207</td>
<td>0.360</td>
<td></td>
<td>191 ± 190</td>
<td></td>
</tr>
<tr>
<td><strong>Protein (g)</strong></td>
<td>11</td>
<td>Combination</td>
<td>70 ± 21</td>
<td>63 ± 14</td>
<td>0.903</td>
<td>0.958</td>
<td>-7 ± 23</td>
<td>0.581</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>ADF</td>
<td>65 ± 10</td>
<td>70 ± 10</td>
<td>0.115</td>
<td></td>
<td>5 ± 10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Exercise</td>
<td>60 ± 5</td>
<td>62 ± 8</td>
<td>0.467</td>
<td></td>
<td>-2 ± 8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Control</td>
<td>71 ± 9</td>
<td>68 ± 5</td>
<td>0.817</td>
<td></td>
<td>3 ± 12</td>
<td></td>
</tr>
<tr>
<td><strong>Carbohydrate (g)</strong></td>
<td>11</td>
<td>Combination</td>
<td>199 ± 35</td>
<td>164 ± 19</td>
<td>0.547</td>
<td>0.801</td>
<td>-35 ± 38</td>
<td>0.928</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>ADF</td>
<td>200 ± 19</td>
<td>161 ± 19</td>
<td>0.155</td>
<td></td>
<td>-39 ± 24</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Exercise</td>
<td>202 ± 25</td>
<td>177 ± 20</td>
<td>0.470</td>
<td></td>
<td>-25 ± 33</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Control</td>
<td>182 ± 34</td>
<td>140 ± 31</td>
<td>0.21</td>
<td></td>
<td>-42 ± 28</td>
<td></td>
</tr>
<tr>
<td><strong>Fat (g)</strong></td>
<td>11</td>
<td>Combination</td>
<td>64 ± 10</td>
<td>50 ± 7</td>
<td>0.454</td>
<td>0.793</td>
<td>-14 ± 11</td>
<td>0.983</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>ADF</td>
<td>69 ± 8</td>
<td>59 ± 13</td>
<td>0.327</td>
<td></td>
<td>-10 ± 9</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Exercise</td>
<td>64 ± 11</td>
<td>66 ± 6</td>
<td>0.717</td>
<td></td>
<td>2 ± 13</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Control</td>
<td>66 ± 16</td>
<td>65 ± 11</td>
<td>0.780</td>
<td></td>
<td>-1 ± 12</td>
<td></td>
</tr>
<tr>
<td><strong>Saturated fat (g)</strong></td>
<td>11</td>
<td>Combination</td>
<td>23 ± 3</td>
<td>19 ± 2</td>
<td>0.412</td>
<td>0.599</td>
<td>-4 ± 3</td>
<td>0.815</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>ADF</td>
<td>28 ± 2</td>
<td>26 ± 5</td>
<td>0.831</td>
<td></td>
<td>-2 ± 4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Exercise</td>
<td>23 ± 3</td>
<td>28 ± 3</td>
<td>0.700</td>
<td></td>
<td>5 ± 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Control</td>
<td>27 ± 7</td>
<td>26 ± 4</td>
<td>0.682</td>
<td></td>
<td>-1 ± 5</td>
<td></td>
</tr>
<tr>
<td><strong>Monounsaturated fat (g)</strong></td>
<td>11</td>
<td>Combination</td>
<td>25 ± 3</td>
<td>20 ± 3</td>
<td>0.375</td>
<td>0.975</td>
<td>-5 ± 4</td>
<td>0.716</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>ADF</td>
<td>24 ± 3</td>
<td>21 ± 6</td>
<td>0.969</td>
<td></td>
<td>-3 ± 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Exercise</td>
<td>24 ± 4</td>
<td>22 ± 2</td>
<td>0.118</td>
<td></td>
<td>-2 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Control</td>
<td>23 ± 5</td>
<td>24 ± 4</td>
<td>0.915</td>
<td></td>
<td>1 ± 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>Intervention</td>
<td>Week 1</td>
<td>Week 12</td>
<td>P-value 1</td>
<td>P-value 2</td>
<td>Change 3</td>
<td>P-value 4</td>
</tr>
<tr>
<td>--------------------------</td>
<td>-----</td>
<td>----------------</td>
<td>--------------</td>
<td>--------------</td>
<td>-----------</td>
<td>-----------</td>
<td>----------</td>
<td>-----------</td>
</tr>
<tr>
<td>Polyunsaturated fat (g)</td>
<td>11</td>
<td>Combination</td>
<td>16 ± 2</td>
<td>11 ± 2</td>
<td>0.309</td>
<td>0.725</td>
<td>-5 ± 3</td>
<td>0.930</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>ADF</td>
<td>17 ± 2</td>
<td>12 ± 2</td>
<td>0.452</td>
<td></td>
<td>-5 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Exercise</td>
<td>17 ± 3</td>
<td>16 ± 2</td>
<td>0.294</td>
<td></td>
<td>-1 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Control</td>
<td>16 ± 3</td>
<td>15 ± 3</td>
<td>0.926</td>
<td></td>
<td>-1 ± 4</td>
<td></td>
</tr>
<tr>
<td>Fiber (g)</td>
<td>11</td>
<td>Combination</td>
<td>18 ± 3</td>
<td>16 ± 2</td>
<td>0.609</td>
<td>0.280</td>
<td>-2 ± 4</td>
<td>0.657</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>ADF</td>
<td>16 ± 2</td>
<td>11 ± 2</td>
<td>0.078</td>
<td></td>
<td>-5 ± 2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Exercise</td>
<td>18 ± 2</td>
<td>12 ± 2</td>
<td>0.036</td>
<td></td>
<td>-6 ± 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Control</td>
<td>11 ± 3</td>
<td>10 ± 2</td>
<td>0.832</td>
<td></td>
<td>-1 ± 5</td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg)</td>
<td>11</td>
<td>Combination</td>
<td>245 ± 34</td>
<td>268 ± 47</td>
<td>0.744</td>
<td>0.868</td>
<td>23 ± 43</td>
<td>0.391</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>ADF</td>
<td>329 ± 83</td>
<td>225 ± 58</td>
<td>0.225</td>
<td></td>
<td>-104 ± 79</td>
<td></td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>Exercise</td>
<td>223 ± 49</td>
<td>227 ± 53</td>
<td>0.955</td>
<td></td>
<td>4 ± 69</td>
<td></td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>Control</td>
<td>380 ± 73</td>
<td>272 ± 25</td>
<td>0.120</td>
<td></td>
<td>-108 ± 57</td>
<td></td>
</tr>
</tbody>
</table>

Values reported as mean ± SEM. Intention to treat analysis. ADF: Alternate day fasting.
1 P-value between week 1 and week 12: Repeated-measures ANOVA.
2 P-value between groups at week 12: One-way ANOVA.
3 Percent change between week 1 and week 12 values.
4 P-value between groups for percent change: One-way ANOVA. Means not sharing a common superscript letter are significantly different (Tukey post-hoc test).
Figure 5.1 Timing of the fast day exercise session and impact on food intake

A. Percent of exercise sessions scheduled by subjects on feed days versus fast days (morning and afternoon). B. Percent of cheating on the fast day (i.e. eating more than the prescribed amount of energy) in relation to timing of the exercise session. No difference in cheating in relation to exercising in the morning versus the afternoon on the fast day (One-way ANOVA).
VI. DISCUSSION

The overarching goal of this study was to evaluate how changes in body weight and body composition by the combination, ADF, and exercise interventions mediate changes in CHD risk factors. The interactions between these variables are discussed in more detail below.

1.1 Association between changes in body weight, body composition and plasma lipids

In the present study, we observed a decrease in body weight, fat mass and waist circumference in the combination and ADF group after 12 weeks of intervention. Simultaneously, a decrease in LDL cholesterol was observed in both these intervention groups. The combination group subjects also reported a significant increase in HDL cholesterol levels post intervention. There are several mechanisms that may link changes in body weight to changes in plasma lipids. Lipoprotein lipase (LPL) plays an important role in the metabolism of lipoproteins. More specifically, this enzyme hydrolyzes triglycerides in lipoproteins, such as those found in chylomicrons and very low-density lipoproteins (VLDL), into two free fatty acids and one monoacylglycerol molecule. It is also involved in promoting the cellular uptake of chylomicron remnants, cholesterol-rich lipoproteins, and free fatty acids. An accumulation of abdominal fat impairs LPL activity, leading to impaired hydrolysis of triglycerides and chylomicrons, resulting in increased triglyceride concentrations (131). With exercise induced weight loss and abdominal fat loss, LPL activity is increased in muscle and adipose tissue. This results in increased HDL2, peak LDL particle diameter and decreases in small LDL and VLDL concentrations (132). Similarly, with diet induced weight loss, adipose tissue LPL activity increases, causing chylomicron and VLDL particles to be catalyzed and cleared more rapidly (132). Increased abdominal adiposity is also associated with increased binding of HDL cholesterol to the fat cell membrane. This leads to disproportionate uptake of HDL cholesterol ester by large abdominal fat cells, reducing plasma HDL cholesterol levels. Reduction in body weight and abdominal fat has been reported to decrease HDL
binding to abdominal adipose tissue and increase plasma HDL cholesterol levels (133). Greater decreases in body weight and waist circumference by the combination group may have therefore played a role in increasing circulating HDL cholesterol levels.

1.2 Association between changes in body weight, body composition, blood pressure, FMD and insulin resistance

Our study also reported improvements in systolic blood pressure, diastolic blood pressure, and FMD, but only in the ADF group. A trend towards increased insulin sensitivity was also noted in this group. Excess body weight is a key risk factor for hypertension (134). In obese humans, weight gain results in increased cardiac output and heart rate, leading to chronic high blood pressure. Several mediators play a role in increasing blood pressure (Figure 6.1). Rapid weight gain affects renal tubular reabsorption and water retention due to the physical pressure exerted with fat accumulation around the kidney (135). Additionally, the sympathetic nervous system (SNS) is activated in the kidney and skeletal muscles, leading to sodium and water retention (135). This sodium and water retention increases total blood volume causing high blood pressure (135, 136). Therefore, decreases in body weight by ADF may have resulted in lower sodium and water retention, which may have in turn reduced blood pressure.

Carrying extra weight, particularly in the abdominal area, also puts individuals at greater risk for insulin resistance. An accumulation of visceral fat results in greater free fatty acid (FFA) turnover and increased flux of FFA to the liver. High FFA concentrations are associated with a reduction in glucose utilization (through glucose fatty acid cycle) causing insulin resistance. Insulin resistance may also be linked to hypertension (137). Insulin resistance is associated with the increased production of inflammatory cytokines, leading to the formation reactive oxygen species (ROS). ROS acts as a quencher for endothelium-derived NO (a potent vasodilator), causing vascular dysfunction and abnormal FMD (138).
Insulin resistance also activates angiotensin II, increasing the production of NADH and NADPH oxidase, leading to increased ROS production and decreased NO production (138). Taken together, it can be speculated that the decreases in body weight and visceral fat mass by ADF may have improved insulin sensitivity and decreased SNS activation as well as angiotensin II levels. This may have played a role in reducing blood pressure and ROS, thereby increasing FMD (Figure 6.1).

1.3 Association between changes in leptin, blood pressure and FMD

Decreases in leptin concentrations were also observed in the ADF group, and these reductions may be linked to decreased blood pressure and increased FMD. Serum leptin levels are elevated in obesity due to enlarged adipose tissue, which is the main source of the hormone. A number of studies have found leptin to be positively correlated with SBP and DBP (139-141). In a systematic review, Hall et al. showed that chronic hyperleptinemia increased renal sympathetic activity, therefore causing water and sodium retention and thus high blood pressure (142). Up-regulation of the Na/K-ATPase pump, especially in renal medulla in obese individuals, also plays a role in sodium retention. Iannello et al. showed that systemic hyperleptinemia could modulate renal Na/K-ATPase through multiple indirect mechanisms such as increased activation of the renin-angiotensin system (143). Hyperleptinemia also increases endothelial generation of NO, although not shown at physiological levels (144). Endogenously produced NO inhibits renal sodium reabsorption in most of the tubule segments, apparently through a decrease in the activity of apical transporters and basolateral Na/K-ATPases (144). However, a chronic hyperleptinemic state in obesity also increases systemic and intrarenal oxidative stress, leading to NO deficiency and vascular dysfunction (145). Thus, decreases in leptin by ADF after 12 weeks, may have played a role in decreasing blood pressure and improving vascular function.
Figure 6.1. Associations between weight loss and improved CHD risk by ADF
ADF: Alternate day fasting; FFA: Free fatty acid; CO: Cardiac output; SNS: Sympathetic nervous system; RAAS: Renin angiotensin aldosterone system; IR: Insulin resistance; ROS: Reactive oxygen species
### TABLE VI.I. SAMPLE SIZE NEEDED FOR COMBINATION GROUP TO ACHIEVE GREATER IMPROVEMENTS THAN ADF BY WEEK- 12

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Change in combination group at week 12</th>
<th>Change in ADF group at week 12</th>
<th>Difference between groups at week 12</th>
<th>SD</th>
<th>α</th>
<th>1-β</th>
<th>n/group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>-2%</td>
<td>7%</td>
<td>-9%</td>
<td>17</td>
<td>0.05</td>
<td>0.80</td>
<td>61</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>19%</td>
<td>8%</td>
<td>11%</td>
<td>46</td>
<td>0.05</td>
<td>0.80</td>
<td>186</td>
</tr>
<tr>
<td>Fasting glucose</td>
<td>-2%</td>
<td>-3%</td>
<td>1%</td>
<td>12</td>
<td>0.05</td>
<td>0.80</td>
<td>125</td>
</tr>
<tr>
<td>Fasting insulin</td>
<td>-9%</td>
<td>-11%</td>
<td>2%</td>
<td>49</td>
<td>0.05</td>
<td>0.80</td>
<td>56</td>
</tr>
<tr>
<td>Systolic BP</td>
<td>-2%</td>
<td>-3%</td>
<td>1%</td>
<td>9</td>
<td>0.05</td>
<td>0.80</td>
<td>90</td>
</tr>
<tr>
<td>Diastolic BP</td>
<td>0%</td>
<td>-2%</td>
<td>2%</td>
<td>12</td>
<td>0.05</td>
<td>0.80</td>
<td>93</td>
</tr>
<tr>
<td>Heart rate</td>
<td>-2%</td>
<td>0%</td>
<td>-2%</td>
<td>7</td>
<td>0.05</td>
<td>0.80</td>
<td>176</td>
</tr>
<tr>
<td>Adiponectin</td>
<td>8%</td>
<td>-3%</td>
<td>11%</td>
<td>34</td>
<td>0.05</td>
<td>0.80</td>
<td>37</td>
</tr>
<tr>
<td>FMD</td>
<td>1%</td>
<td>5%</td>
<td>-4%</td>
<td>3</td>
<td>0.05</td>
<td>0.80</td>
<td>44</td>
</tr>
</tbody>
</table>

ADF: Alternate day fasting, BP: blood pressure, FMD: Flow mediated dilation, SD: Standard deviation
VII. FUTURE DIRECTIONS

It will be of interest in future studies in this field to examine how changes in ADF meal timing affect body weight and CHD risk. In the present study, all subjects were required to consume the fast day meal at lunchtime (12.00-2.00pm). 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, to a dinnertime meal, or three small meals throughout the day, on dietary adherence, weight loss, and CHD risk reduction, is an important question that has yet to be addressed.
VIII. SUMMARY OF FINDINGS AND GENERAL CONCLUSION

A. Summary of findings

This study is the first to show that the combination of ADF and exercise produces superior changes in body weight, body composition, and lipid indicators of CHD risk, when compared to ADF or exercise alone. More specifically, we report here that the combination group lost more weight (6 kg), and experienced greater decreases in fat mass (5 kg), when compared to individual interventions after 12 weeks. The combination therapy also elicited retention in lean mass, however, this preservation of lean mass was also noted in the ADF and exercise groups. As for CHD risk indicators, the combination of ADF plus exercise decreased LDL cholesterol (12% from baseline) while increasing HDL cholesterol (18% from baseline); a change that was not noted for any other intervention. The combination group also experienced an increase in LDL particle size, and a reduction in the proportion of small LDL and HDL particles. The study also showed for the first time that ADF is an effective intervention to improve endothelium-dependent flow mediated dilation. The improvements in FMD by ADF may not be mediated by changes in adiponectin or leptin, however. Interestingly, exercise alone or in combination with ADF, had no effect on FMD. In addition, results reveal that endurance exercise can be easily incorporated into the ADF regimen. Specifically, subjects were able to exercise on the fast day, and this extra energy expenditure did not translate into increased hunger or extra food intake. We also show here that ADF combined with exercise improves several eating behaviors. For instance, after 12 weeks of treatment, restrained eating was increased while uncontrolled eating and emotional eating were decreased in obese individuals.
B. Conclusion

In conclusion, the results of this study suggest that the combination of ADF plus endurance training results in greater body composition and lipid altering effects than that of each intervention alone. For instance, the combination therapy produced greater weight loss and fat loss than the ADF-only and exercise-only group, while eliciting retention of lean mass. This lifestyle regimen also favourably altered LDL and HDL cholesterol concentrations, and decreased the proportion of small LDL and HDL particles. The effect of this combination therapy on FMD is still not clear, however, and requires more research. As for the effect of this lifestyle regimen on eating behaviors, we found that endurance exercise is an excellent adjunct therapy to ADF, as it leads to positive behavioral changes that may contribute to long-term steady weight loss. In conclusion, the combination of ADF plus exercise may be implemented as a viable lifestyle intervention to help obese individuals lose weight, retain lean mass, and lower their risk of CHD.
Appendix A

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

Alternate day fasting combined with exercise for weight loss and cardio-protection in obese adults

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

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

Why am I being asked?

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

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

Approximately 100 subjects may be involved in this research at UIC.

What is the purpose of this research?

This research is being done to better understand how alternate day reductions in calorie intake combined with exercise can help a person lose weight and reduce their risk of heart disease.

What procedures are involved?

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

You will need to come to the study site 15 times over the next 12 weeks. Each of those visits will take about 20 minutes. However the first and last visit may take up to 45 minutes.
The study procedures are:

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

- **Body weight assessment:** You will be weighed during the screening visit, and if you do not fall in the range of obese, you will not be eligible to participate.
- **Pregnancy Screening:** If you are a premenopausal woman, you will be asked if you may be pregnant, and undergo a urine pregnancy test.
- **Blood pressure:** Your blood pressure will be measured during the screening visit, and if your blood pressure does not fall between 120-139 mm Hg (systolic blood pressure) and 80-89 mm Hg (diastolic blood pressure), you will not be eligible to participate.

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. **Combination (alternate day fasting + exercise) group:** If you fall in this category you will be asked to consume food we provide to you on the "fast day", and eat whatever you wish to eat on each "feed day". You will also be asked to come to the research center 3 times per week to participate in an exercise session.
2. **Exercise group:** If you fall in this category you will be asked to come to the research center 3 times per week to participate in an exercise session, and you will not have to change your diet in any way.
3. **Alternate day fasting group:** If you fall in this category you will be asked to consume food we provide to you on the "fast day", and eat whatever you wish to eat on each "feed day", and you will not have to participate in the exercise sessions.
4. **Control group/ No treatment group:** If you fall into this category you will be asked not to change your eating or exercise habits during the time that you are participating in the research. You will have to come to the research center every week to have all measurements taken. At each weekly visit you will also be asked to submit your food records and pedometer readings for review by a study team member. You should opt not to participate in the study if any changes to diet or exercise habits are planned.

You will take part in a study that is 12 weeks long, and at each visit you will be asked to do the following:

**Week 1 visit:** During your first visit, you will go to Human Nutrition Research Unit to have a blood draw (about 4 tablespoons of blood). You will also have your blood pressure taken and your body weight / percent fat measured. You will also have an ultrasound done on your arm, and a full-body x-ray (DEXA scan) to determine the distribution of fat in your body. You will be given a pedometer (little counter that measures the amount of steps you take per day) and instructed how and when to wear it during the study. At this visit, you will also be given food records (forms to record your food intake) to fill out on 2 days of that week, and 3 questionnaires to fill out, which will determine your level of hunger on fast days and your general eating behaviors. If you are in the combination group or alternate day fasting group, you will be given your “fast day” food for the week.

If you are a premenopausal women, at every visit you will be asked if you are using acceptable method of birth control (e.g. low dose contraceptives, Nuvaring, Mirena IUD, Paragard IUD, barrier methods (such as condoms) combined with spermicide or diaphragm) and if you might be pregnant (i.e. if you have missed a period). If you have a reason to believe that you might be pregnant, a pregnancy test will be performed. If you become pregnant during the study, you will not be allowed to continue.

**Week 2 visit:** During this visit you will have your blood pressure taken, and your body weight/percent fat measured. You will also be given food records and hunger questionnaires to complete that week. If you are in the
combination group or alternate day fasting group, you will be given your “fast day” food for the rest of the week. 

**Week 3 visit:** During this visit you will have your blood pressure taken, and your body weight/percent fat measured. You will also be given food records and hunger questionnaires to complete that week. If you are in the combination group or alternate day fasting group, you will be given your “fast day” food for the rest of the week.

**Week 4 visit:** During this visit you will have your blood pressure taken, and your body weight/percent fat measured. You will also be given food records and hunger questionnaires to complete that week. If you are in the combination group or alternate day fasting group, you will be given your “fast day” food for the rest of the week.

**Week 5 visits (2 visits at the beginning of the week):** During these visits you will have your blood drawn (about 4 tablespoons), your blood pressure taken, and your body weight/percent fat measured. You will also be given food records and hunger questionnaires to complete that week. In addition, if you are in the combination group or alternate day fasting group, you will meet with a nutritionist for a brief dietary counseling session to learn how to maintain the diet at home.

**Week 6 visit:** During this visit you will have your blood pressure taken, and your body weight/percent fat measured. You will also be given food records and hunger questionnaires to complete that week.

**Week 7 visit:** During this visit you will have your blood pressure taken, and your body weight/percent fat measured. You will also be given food records and hunger questionnaires to complete that week.

**Week 8 visit:** During this visit you will have your blood pressure taken, and your body weight/percent fat measured. You will also be given food records and hunger questionnaires to complete that week.

**Week 9 visits (2 visits at the beginning of the week):** During these visits you will have your blood drawn (about 4 tablespoons), your blood pressure taken, and your body weight/percent fat measured. You will also be given food records and hunger questionnaires to complete that week.

**Week 10 visit:** During this visit you will have your blood pressure taken, and your body weight/percent fat measured. You will also be given food records and hunger questionnaires to complete that week.

**Week 11 visit:** During this visit you will have your blood pressure taken, and your body weight/percent fat measured. You will also be given food records and hunger questionnaires to complete that week.

**Week 12 (2 visits at the end of the week):** During these visits you will have your blood drawn (about 4 tablespoons), your blood pressure taken, and your body weight/percent fat measured. You will also be given eating behavior questionnaires to complete during the visit. You will also have an ultrasound done on your arm, and a full-body x-ray to determine the distribution of fat in your body.

**Note:** All subjects in the combination and exercise groups will visit the Human Nutrition Research Unit three additional times per week to participate in the exercise sessions. Exercise will be performed using stationary bikes and elliptical machines starting with 25 min and increasing to 45 min/session by week 12. **PLEASE SEE TABLES AT THE END OF THE DOCUMENT FOR AN OVERVIEW OF EACH GROUP’S STUDY SCHEDULE**

**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 will be drawn during the study.

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

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

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

What are the reproductive risks?

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

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

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

Are there benefits to taking part in the research?

Previous studies of alternate day fasting and exercise show that these interventions may help people lose weight and lower heart disease risk. However, because individuals respond differently to therapy, no one can know in advance if it will be helpful in your particular case. If you are assigned to the no treatment group, you are not expected to directly benefit from participating in this research.

What other options are there?

If you decide not to enter this study, there is other care available to you, such as losing weight by reducing your daily energy intake and exercising 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 312-355-0542 to talk to her about your illness or injury. You can also contact the study clinician: Karen Vuckovic, A.P.N. at 312-996-1042

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

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

Will I be reimbursed for any of my expenses or paid for my participation in this research?
You will not be offered payment for being in this study.

Can I withdraw or be removed from the study?
If you decide to participate, you are free to withdraw your consent and discontinue participation at any time without affecting your future care at UIC. You have the right to leave a study at any time without penalty. For your safety, however, you should consider the investigator’s advice about how to leave the study. If you leave the study before the final planned study visit, the investigator may ask you to complete the final steps.

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

Who should I contact if I have questions?
Contact the researchers Dr. Krista Varady (312-996-7897) or Surabhi Bhutani (312-355-0542)
• If you have any questions about this study or your part in it,
• If you feel you have had a research-related injury (or a bad reaction to the study treatment), and/or
• If you have questions, concerns or complaints about the research.

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

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

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

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

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

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

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

**Signature of Subject or Legally Authorized Representative**

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

_____________________________  ________________________
Signature                      Date

_____________________________
Printed Name

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

_____________________________
Printed Name of Person Obtaining Consent
## STUDY SCHEDULE FOR CONTROL GROUP

<table>
<thead>
<tr>
<th>WEEK</th>
<th>Body Weight</th>
<th>Body fat % (BIA)</th>
<th>Waist Circumference</th>
<th>BP</th>
<th>Blood draw</th>
<th>DEXA</th>
<th>Ultrasound (BA FMD)</th>
<th>Pedometer</th>
<th>Food Record</th>
<th>Hunger Form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wk 1</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 2</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 3</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 4</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 5</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 6</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 7</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 8</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 9</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 10</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 11</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td></td>
<td></td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 12</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
</tbody>
</table>
# STUDY SCHEDULE FOR ALTERNATE DAY FASTING GROUP

<table>
<thead>
<tr>
<th>WEEK</th>
<th>Body Weight</th>
<th>Body fat % (BIA)</th>
<th>Waist Circumference</th>
<th>BP</th>
<th>Blood draw</th>
<th>DEXA</th>
<th>Ultrasound (BA FMD)</th>
<th>Pedometer</th>
<th>Food Record</th>
<th>Hunger Form</th>
<th>Food Pickup</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wk 1</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Wk 2</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Wk 3</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Wk 4</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Wk 5</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Wk 6</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Wk 7</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Wk 8</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Wk 9</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Wk 10</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Wk 11</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
<tr>
<td>Wk 12</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
<td>√</td>
</tr>
</tbody>
</table>
## STUDY SCHEDULE FOR ALTERNATE DAY FASTING + EXERCISE GROUP

<table>
<thead>
<tr>
<th>WEEK</th>
<th>Body Weight</th>
<th>Body fat %</th>
<th>Waist Circumference</th>
<th>BP</th>
<th>Blood draw</th>
<th>DEXA</th>
<th>Ultrasound (BA FMD)</th>
<th>Pedometer</th>
<th>Food Record</th>
<th>Hunger form</th>
<th>Food Pickup</th>
<th>Exercise</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wk 1</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 2</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 3</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 4</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 5</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 6</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 7</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 8</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 9</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 10</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 11</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>Wk 12</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
<td>✔</td>
</tr>
<tr>
<td>WEEK</td>
<td>Body Weight</td>
<td>Body fat % (BIA)</td>
<td>Waist Circumference</td>
<td>BP</td>
<td>Blood draw</td>
<td>DEXA</td>
<td>Ultrasound (BA FMD)</td>
<td>Pedometer</td>
<td>Food Record</td>
<td>Hunger form</td>
<td>Exercise</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>-------------</td>
<td>------------------</td>
<td>---------------------</td>
<td>----</td>
<td>-------------</td>
<td>------</td>
<td>---------------------</td>
<td>-----------</td>
<td>--------------</td>
<td>------------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>Wk 1</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Wk 2</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Wk 3</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Wk 4</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Wk 5</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Wk 6</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Wk 7</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Wk 8</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Wk 9</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Wk 10</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Wk 11</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
<tr>
<td>Wk 12</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
</tr>
</tbody>
</table>
Volunteers needed for a Weight Loss Study

Volunteers are needed for a 12-week research study of the effects of alternate-day food restriction combined with exercise for weight loss and heart disease prevention.

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

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

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

Alternate day fasting combined with exercise for weight loss
Version 1, December 1 2009
Appendix C

Screening questionnaire

Date of screening: _________________________

First name ______________________   Middle initial: ____   Last: ___________________________

Phone: ______________________________    Email: _________________________________

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

Weight: ______ Height: ______  BMI: _______ (BMI must be 30-39.9kg/m², NOT OVER 300lb)

_______________________________________________________

Do you smoke?   yes    no  (If yes, disqualify)

Currently Dieting:   yes    no  (If yes, disqualify because weight is not stable)

Weight gain/loss in past 3 months (>5 kg):   yes    no  (If yes, disqualify)

If yes, explain: ______________________________________________________

Vegan or vegetarian:    yes    no    Food allergies (Nuts, soy, etc) _______________________

Can you commit to an alternate day fasting regimen for 12 weeks?   yes    no

Do you exercise?    yes    no    If yes...  What kind of exercise: ____________________________

No. days per week: _______  No. of min /exercise session: _______  Total hours/week: _______

How long have you been on this exercise regimen/routine? ____________________________

_______________________________________________________

Do you have any health issues and/or health problems:   yes    no

If yes, explain: ________________________________________________________

(Disqualify if diabetic, history of heart disease or stroke, or cancer)

Are you on any medication:   yes    no    If yes:

What kind: _________________________  Taken for: ____________________________

Dosage: _________________________  Started meds when: ______________________

What kind: _________________________  Taken for: ____________________________

Dosage: _________________________  Started meds when: ______________________

Subject ID: 006 –
What kind: _________________________  Taken for: _____________________________
Dosage: ______________________  Started meds when: ________________________
(Must not be taking glucose lowering, weight loss, HRT, or psychotropic medications)

Are you perimenopausal? (3-6 missed menstrual cycles in 12 mo)  yes  no (If yes, disqualify)

Are you pregnant or trying to become pregnant?  yes  no (If yes, disqualify)

Have you undergone weight loss surgery in the past?  yes  no (If yes, disqualify)
Appendix D

Alternate day fasting – Take home meal instructions

General instructions:

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

When eating the “feed day” meal:

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

When eating the “fast day” meal:

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

Zero calorie items that you can eat at any time:

• We recommend you DRINK 6-8 glasses of water each day.
• You may not drink other beverages except those listed here:
  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.
Appendix E

Appetite Assessment

Study #: _____  Subject: ___  Date: ________________  Time: ________  Week: ________

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 F

Three Factor Eating Questionnaire

Subject ID: ______________________  Date: ____________________  Study week: ______

1. When I smell a sizzling steak or juicy piece of meat, I find it very difficult to keep from eating, even if I have just finished a meal.
   
   Definitely true (4)   Mostly true (3)   Mostly false (2)   Definitely false (1)

2. I deliberately take small helpings as a means of controlling my weight.
   
   Definitely true (4)   Mostly true (3)   Mostly false (2)   Definitely false (1)

3. When I feel anxious, I find myself eating.
   
   Definitely true (4)   Mostly true (3)   Mostly false (2)   Definitely false (1)

4. Sometimes when I start eating, I just can’t seem to stop.
   
   Definitely true (4)   Mostly true (3)   Mostly false (2)   Definitely false (1)

5. Being with someone who is eating often makes me hungry enough to eat also.
   
   Definitely true (4)   Mostly true (3)   Mostly false (2)   Definitely false (1)

6. When I feel blue, I often overeat.
   
   Definitely true (4)   Mostly true (3)   Mostly false (2)   Definitely false (1)

7. When I see a real delicacy, I often get so hungry that I have to eat right away.
   
   Definitely true (4)   Mostly true (3)   Mostly false (2)   Definitely false (1)
8. I get so hungry that my stomach often seems like a bottomless pit.
   Definitely true (4)  Mostly true (3)  Mostly false (2)  Definitely false (1)

9. I am always hungry so it is hard for me to stop eating before I finish the food on my plate.
   Definitely true (4)  Mostly true (3)  Mostly false (2)  Definitely false (1)

10. When I feel lonely, I console myself by eating.
    Definitely true (4)  Mostly true (3)  Mostly false (2)  Definitely false (1)

11. I consciously hold back at meals in order not to gain weight.
    Definitely true (4)  Mostly true (3)  Mostly false (2)  Definitely false (1)

12. I do not eat some foods because they make me fat.
    Definitely true (4)  Mostly true (3)  Mostly false (2)  Definitely false (1)

13. I am always hungry enough to eat at any time.
    Definitely true (4)  Mostly true (3)  Mostly false (2)  Definitely false (1)

14. How often do you feel hungry?
    Only at meal times (1)  Sometimes between meals (2)  Often between meals (3)  Almost always (4)

15. How frequently do you avoid "stocking up" on tempting foods?
    Almost never (1)  Seldom (2)  Usually (3)  Almost always (4)

16. How likely are you to consciously eat less than you want?
    Unlikely (1)  Slightly likely (2)  Moderately likely (3)  Very likely (4)
17. Do you go on eating binges though you are not hungry?

Never (1)  Rarely (2)  Sometimes (3)  At least once a week (4)

18. On a scale of 1 to 8 where:
1 means no restraint in eating (eating whatever you want, whenever you want it)
8 means total restraint (constantly limiting food intake and never "giving in")

What number would you give yourself?
Appendix G

JOHN WILEY AND SONS LICENSE
TERMS AND CONDITIONS

Mar 21, 2013

This is a License Agreement between Surabhi Bhutani ("You") and John Wiley and Sons ("John Wiley and Sons") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by John Wiley and Sons, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

<table>
<thead>
<tr>
<th>License Number</th>
<th>3113740379091</th>
</tr>
</thead>
<tbody>
<tr>
<td>License date</td>
<td>Mar 21, 2013</td>
</tr>
<tr>
<td>Licensed content publisher</td>
<td>John Wiley and Sons</td>
</tr>
<tr>
<td>Licensed content publication</td>
<td>Obesity</td>
</tr>
<tr>
<td>Licensed content title</td>
<td>Alternate day fasting and endurance exercise combine to reduce body weight and favorably alter plasma lipids in obese humans</td>
</tr>
<tr>
<td>Licensed copyright line</td>
<td>Copyright © 2013 The Obesity Society</td>
</tr>
<tr>
<td>Licensed content author</td>
<td>Surabhi Bhutani, Monica C. Klempel, Cynthia M. Kroeger, John F. Trepanowski, Krista A. Varady</td>
</tr>
<tr>
<td>Licensed content date</td>
<td>Feb 14, 2013</td>
</tr>
<tr>
<td>Start page</td>
<td>n/a</td>
</tr>
<tr>
<td>End page</td>
<td>n/a</td>
</tr>
<tr>
<td>Type of use</td>
<td>Dissertation/Thesis</td>
</tr>
<tr>
<td>Requestor type</td>
<td>Author of this Wiley article</td>
</tr>
<tr>
<td>Format</td>
<td>Print and electronic</td>
</tr>
<tr>
<td>Portion</td>
<td>Full article</td>
</tr>
<tr>
<td>Will you be translating?</td>
<td>No</td>
</tr>
<tr>
<td>Order reference number</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.00 USD</td>
</tr>
</tbody>
</table>

Terms and Conditions

TERMS AND CONDITIONS

This copyrighted material is owned by or exclusively licensed to John Wiley & Sons, Inc. or one of its group companies (each a "Wiley Company") or a society for whom a Wiley Company has exclusive publishing rights in relation to a particular journal (collectively WILEY). By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the billing and payment terms and conditions established by the Copyright Clearance Center Inc., ("CCC’s Billing and Payment terms...
Terms and Conditions

1. The materials you have requested permission to reproduce (the "Materials") are protected by copyright.

2. You are hereby granted a personal, non-exclusive, non-sublicensable, non-transferable, worldwide, limited license to reproduce the Materials for the purpose specified in the licensing process. This license is for a one-time use only with a maximum distribution equal to the number that you identified in the licensing process. Any form of republication granted by this licence must be completed within two years of the date of the grant of this licence (although copies prepared before may be distributed thereafter). The Materials shall not be used in any other manner or for any other purpose. Permission is granted subject to an appropriate acknowledgement given to the author, title of the material/book/journal and the publisher. You shall also duplicate the copyright notice that appears in the Wiley publication in your use of the Material. Permission is also granted on the understanding that nowhere in the text is a previously published source acknowledged for all or part of this Material. Any third party material is expressly excluded from this permission.

3. With respect to the Materials, all rights are reserved. Except as expressly granted by the terms of the license, no part of the Materials may be copied, modified, adapted (except for minor reformatting required by the new Publication), translated, reproduced, transferred or distributed, in any form or by any means, and no derivative works may be made based on the Materials without the prior permission of the respective copyright owner. You may not alter, remove or suppress in any manner any copyright, trademark or other notices displayed by the Materials. You may not license, rent, sell, loan, lease, pledge, offer as security, transfer or assign the Materials, or any of the rights granted to you hereunder to any other person.

4. The Materials and all of the intellectual property rights therein shall at all times remain the exclusive property of John Wiley & Sons Inc or one of its related companies (WILEY) or their respective licensors, and your interest therein is only that of having possession of and the right to reproduce the Materials pursuant to Section 2 herein during the continuance of this Agreement. You agree that you own no right, title or interest in or to the Materials or any of the intellectual property rights therein. You shall have no rights hereunder other than the license as provided for above in Section 2. No right, license or interest to any trademark, trade name, service mark or other branding ("Marks") of WILEY or its licensors is granted hereunder, and you agree that you shall not assert any such right, license or interest with respect thereto.

5. NEITHER WILEY NOR ITS LICENSORS MAKES ANY WARRANTY OR REPRESENTATION OF ANY KIND TO YOU OR ANY THIRD PARTY, EXPRESS, IMPLIED OR STATUTORY, WITH RESPECT TO THE MATERIALS OR THE ACCURACY OF ANY INFORMATION CONTAINED IN THE MATERIALS, INCLUDING, WITHOUT LIMITATION, ANY IMPLIED WARRANTY OF MERCHANTABILITY, ACCURACY, SATISFACTORY QUALITY, FITNESS FOR A PARTICULAR PURPOSE, USABILITY, INTEGRATION OR NON-INFRINGEMENT AND ALL SUCH WARRANTIES ARE HEREBY EXCLUDED BY WILEY AND ITS LICENSORS AND WAIVED BY YOU.

6. WILEY shall have the right to terminate this Agreement immediately upon breach of this Agreement by you.

7. You shall indemnify, defend and hold harmless WILEY, its Licensors and their respective directors, officers, agents and employees, from and against any actual or threatened claims, demands, causes of action or proceedings arising from any breach of this Agreement by you.

8. IN NO EVENT SHALL WILEY OR ITS LICENSORS BE LIABLE TO YOU OR ANY OTHER PARTY OR ANY OTHER PERSON OR ENTITY FOR ANY SPECIAL, CONSEQUENTIAL, INCIDENTAL, INDIRECT, EXEMPLARY OR PUNITIVE DAMAGES, HOWEVER CAUSED, ARISING OUT OF OR IN CONNECTION
WITH THE DOWNLOADING, PROVISIONING, VIEWING OR USE OF THE MATERIALS REGARDLESS OF THE FORM OF ACTION, WHETHER FOR BREACH OF CONTRACT, BREACH OF WARRANTY, TORT, NEGLIGENCE, INFRINGEMENT OR OTHERWISE (INCLUDING, WITHOUT LIMITATION, DAMAGES BASED ON LOSS OF PROFITS, DATA, FILES, USE, BUSINESS OPPORTUNITY OR CLAIMS OF THIRD PARTIES), AND WHETHER OR NOT THE PARTY HAS BEEN ADVISED OF THE POSSIBILITY OF SUCH DAMAGES. THIS LIMITATION SHALL APPLY NOTWITHSTANDING ANY FAILURE OF ESSENTIAL PURPOSE OF ANY LIMITED REMEDY PROVIDED HEREIN.

9. Should any provision of this Agreement be held by a court of competent jurisdiction to be illegal, invalid, or unenforceable, that provision shall be deemed amended to achieve as nearly as possible the same economic effect as the original provision, and the legality, validity and enforceability of the remaining provisions of this Agreement shall not be affected or impaired thereby.

10. The failure of either party to enforce any term or condition of this Agreement shall not constitute a waiver of either party’s right to enforce each and every term and condition of this Agreement. No breach under this agreement shall be deemed waived or excused by either party unless such waiver or consent is in writing signed by the party granting such waiver or consent. The waiver by or consent of a party to a breach of any provision of this Agreement shall not operate or be construed as a waiver of or consent to any other or subsequent breach by such other party.

11. This Agreement may not be assigned (including by operation of law or otherwise) by you without WILEY’s prior written consent.

12. Any fee required for this permission shall be non-refundable after thirty (30) days from receipt.

13. These terms and conditions together with CCC’s Billing and Payment terms and conditions (which are incorporated herein) form the entire agreement between you and WILEY concerning this licensing transaction and (in the absence of fraud) supersedes all prior agreements and representations of the parties, oral or written. This Agreement may not be amended except in writing signed by both parties. This Agreement shall be binding upon and inure to the benefit of the parties’ successors, legal representatives, and authorized assigns.

14. In the event of any conflict between your obligations established by these terms and conditions and those established by CCC’s Billing and Payment terms and conditions, these terms and conditions shall prevail.

15. WILEY expressly reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions and (iii) CCC’s Billing and Payment terms and conditions.

16. This Agreement will be void if the Type of Use, Format, Circulation, or Requestor Type was misrepresented during the licensing process.

17. This Agreement shall be governed by and construed in accordance with the laws of the State of New York, USA, without regards to such state’s conflict of law rules. Any legal action, suit or proceeding arising out of or relating to these Terms and Conditions or the breach thereof shall be instituted in a court of competent jurisdiction in New York County in the State of New York in the United States of America and each party hereby consents and submits to the personal jurisdiction of such court, waives any objection to venue in such court and consents to service of process by registered or certified mail, return receipt requested, at the last known address of such party.

**Wiley Open Access Terms and Conditions**

All research articles published in Wiley Open Access journals are fully open access: immediately
freely available to read, download and share. Articles are published under the terms of the Creative Commons Attribution Non Commercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes. The license is subject to the Wiley Open Access terms and conditions: Wiley Open Access articles are protected by copyright and are posted to repositories and websites in accordance with the terms of the Creative Commons Attribution Non Commercial License. At the time of deposit, Wiley Open Access articles include all changes made during peer review, copyediting, and publishing. Repositories and websites that host the article are responsible for incorporating any publisher-supplied amendments or retractions issued subsequently. Wiley Open Access articles are also available without charge on Wiley's publishing platform, Wiley Online Library or any successor sites.

Use by non-commercial users

For non-commercial and non-promotional purposes individual users may access, download, copy, display and redistribute to colleagues Wiley Open Access articles, as well as adapt, translate, text-and data-mine the content subject to the following conditions:

- The authors' moral rights are not compromised. These rights include the right of "paternity" (also known as "attribution" - the right for the author to be identified as such) and "integrity" (the right for the author not to have the work altered in such a way that the author's reputation or integrity may be impugned).
- Where content in the article is identified as belonging to a third party, it is the obligation of the user to ensure that any reuse complies with the copyright policies of the owner of that content.
- If article content is copied, downloaded or otherwise reused for non-commercial research and education purposes, a link to the appropriate bibliographic citation (authors, journal, article title, volume, issue, page numbers, DOI and the link to the definitive published version on Wiley Online Library) should be maintained. Copyright notices and disclaimers must not be deleted.
- Any translations, for which a prior translation agreement with Wiley has not been agreed, must prominently display the statement: "This is an unofficial translation of an article that appeared in a Wiley publication. The publisher has not endorsed this translation."

Use by commercial "for-profit" organisations

Use of Wiley Open Access articles for commercial, promotional, or marketing purposes requires further explicit permission from Wiley and will be subject to a fee. Commercial purposes include:

- Copying or downloading of articles, or linking to such articles for further redistribution, sale or licensing;
- Copying, downloading or posting by a site or service that incorporates advertising with such content;
- The inclusion or incorporation of article content in other works or services (other than normal quotations with an appropriate citation) that is then available for sale or licensing, for a fee (for example, a compilation produced for marketing purposes, inclusion in a sales pack);
- Use of article content (other than normal quotations with appropriate citation) by for-profit
organisations for promotional purposes
● Linking to article content in e-mails redistributed for promotional, marketing or educational purposes;
● Use for the purposes of monetary reward by means of sale, resale, licence, loan, transfer or other form of commercial exploitation such as marketing products
● Print reprints of Wiley Open Access articles can be purchased from: corporatesales@wiley.com

Other Terms and Conditions:

BY CLICKING ON THE "I AGREE..." BOX, YOU ACKNOWLEDGE THAT YOU HAVE READ AND FULLY UNDERSTAND EACH OF THE SECTIONS OF AND PROVISIONS SET FORTH IN THIS AGREEMENT AND THAT YOU ARE IN AGREEMENT WITH AND ARE WILLING TO ACCEPT ALL OF YOUR OBLIGATIONS AS SET FORTH IN THIS AGREEMENT.

v1.7

If you would like to pay for this license now, please remit this license along with your payment made payable to "COPYRIGHT CLEARANCE CENTER" otherwise you will be invoiced within 48 hours of the license date. Payment should be in the form of a check or money order referencing your account number and this invoice number RLNK500982632. Once you receive your invoice for this order, you may pay your invoice by credit card. Please follow instructions provided at that time.

Make Payment To:
Copyright Clearance Center
Dept 001
P.O. Box 843006
Boston, MA 02284-3006

For suggestions or comments regarding this order, contact RightsLink Customer Support: customercare@copyright.com or +1-877-622-5543 (toll free in the US) or +1-978-646-2777.

Gratis licenses (referencing $0 in the Total field) are free. Please retain this printable license for your reference. No payment is required.
CITED LITERATURE


68. Marcell TJ, McAuley KA, Traustadottir T, Reaven PD. Exercise training is not associated with improved levels of C-reactive protein or adiponectin. Metabolism. 2005;54(4):533-41.


VITA

NAME: Surabhi Bhutani

EDUCATION:  
- B.Sc., Home Science (Honors), University of Delhi, India 2003  
- M.Sc., Home Science (Food and Nutrition), University of Delhi, India 2005  
- Ph.D., Kinesiology, Nutrition, and Rehabilitation, University of Illinois at Chicago, Chicago, Illinois, 2013

TEACHING EXPERIENCE:  
- Teaching Assistant, Department of Kinesiology and Nutrition  
  Aug 2008 – May 2010  
- Lecturer, Department of Kinesiology and Nutrition  
  Aug 2008 – May 2010

RESEARCH EXPERIENCE:  
- Clinical Coordinator, Department of Kinesiology and Nutrition, University of Illinois, Chicago,  
  Jan 2008-June 2008  
  Supervisor: Dr. Krista Varady, Ph.D.  
- Research Assistant – Department of Kinesiology and Nutrition, University of Illinois, Chicago  
  Sep 2007 – Dec 2007  
  Supervisor: Dr. Krista Varady, Ph.D.  
- Research Dietician – Diabetes Foundation (India), New Delhi,  
  Jul 2006-Oct 2006  
  Supervisor: Dr. Anoop Misra, M.D.  
- Research Dietician – All India Institute of Medical Sciences, New Delhi,  
  Supervisor: Ms. Rekha Sharma, R.D., Chief Dietician  
- Dietetic Internship, All India Institute of Medical Sciences, New Delhi,  
  Dec 2004 - Jan 2005

HONORS AND AWARDS:  
- Central Society for Clinical and Translational Research: Trainee Travel Award 2013, CSCR/MWAFMR Combined Annual Meeting, Chicago, Illinois
Kamath Award for most productive PhD student in Nutrition 2010, Department of Kinesiology and Nutrition, University of Illinois, Chicago

Saraswati Anand Memorial Prize for Best Student in Therapeutic Nutrition 2005, Institute of Home Economics, University of Delhi, India

Distinction Award – Master of Science in Foods and Nutrition (Final year) 2005, Institute of Home Economics, University of Delhi, India

Distinction Award – Master of Science in Foods and Nutrition (First year) 2004, Institute of Home Economics, University of Delhi, India

FELLOWSHIP: Predoctoral Fellowship – American Heart Association, Jan 2012-Dec 2013 Alternate day fasting combined with exercise for weight loss

CERTIFICATIONS: Certified Phlebotomist, Certificate 2009, Medtexx Medical Corporation

Human Research Ethics Certification 2009, CITI Collaborative Institutional Training Initiative, Reference # 2368633

National Eligibility Test For Lecturership 2005, University Grants Commission, Govt. of India. UGC ref. no. 4198/ (NET DEC, 2005)

PROFESSIONAL MEMBERSHIP: Member of the Obesity Society since 2008

Member of the American Society for Nutritional Sciences since 2008

PEER REVIEW ACTIVITIES: Journal reviewer for Applied Physiology, Nutrition and Metabolism

ABSTRACTS:


13. Varady KA, Bhutani S, Klempel MC. Effects of alternate day modified fasting on LDL particle size and distribution in obese adults. Federation of


PUBLICATIONS:


