The Pennsylvania State University

The Graduate School

College of Health and Human Development

A LIFESTYLE INTERVENTION PROMOTING WEIGHT LOSS WITH MODERATE OR STANDARD PROTEIN AND NON-FAT DAIRY INTAKE AND DAILY WALKING: IMPACT ON BODY COMPOSITION, METABOLIC PARAMETERS AND BONE HEALTH IN PREMENOPAUSAL WOMEN WITH OVERWEIGHT AND OBESITY

A Dissertation in

Nutritional Sciences

by

Julie D. Shlisky

Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Doctor of Philosophy

August 2012

The dissertation of Julie D. Shlisky was reviewed and approved* by the following:

Sharon M. Nickols-Richardson
Professor of Nutritional Sciences
Dissertation Advisor
Chair of Committee
Graduate Program Chair

Penny M. Kris-Etherton
Distinguished Professor of Nutritional Sciences

Terryl J. Hartman
Professor of Nutritional Sciences

Connie J. Rogers
Assistant Professor of Nutritional Sciences

Neil A. Sharkey
Professor of Kinesiology, Orthopedics and Rehabilitation

*Signatures are on file in the Graduate School.

ABSTRACT

Excess adiposity increases risk for the development of several co-morbid diseases. Body weight (BW) loss achieved by following an energy-restricted diet (ERD) promotes several metabolic benefits, and research suggests additive beneficial effects of certain dietary components within an ERD on weight-related outcomes including BW, body composition, insulin sensitivity and metabolic indicators of cardiovascular disease risk. However, maintenance of bone mineral density (BMD) may be compromised with BW loss, especially with continual cycles of BW loss and regain. Moderate protein (25-30% of kcal) and non-fat dairy (4-5 servings/d) within an ERD may increase the extent to which metabolic benefits are achieved. Similarly, an ERD including moderate dietary protein and non-fat dairy combined with daily exercise may help to maintain BMD with significant BW loss. This research project was conducted to evaluate the effects an ERD including moderate dietary protein and non-fat dairy on BW, body composition, metabolic parameters and inflammatory factors. Secondary measures included biomarkers of bone turnover and BMD. Additionally, a secondary analysis of data was performed to explore the relationship between change in dietary energy density (ED) in study participants during intervention and reductions in BW and fat mass (FM).

Premenopausal women, ages 20-45 years, with overweight/obesity [body mass index (BMI) $\geq$25 and <36 kg/m^2] were recruited for this 24-week parallel-arm randomized controlled trial. Women were randomized to an ERD including moderate protein (30% of kcal) and non-fat dairy (4-5 servings/d) combined with daily walking (YL group) or to an ERD including standard protein (16-17% of kcal) and non-fat dairy (3 servings/d) combined with daily walking (EXG group), for 12 weeks of weight loss (baseline through Week 12) followed by 12 weeks of weight-loss maintenance (Week 13–Week 24). The proportion of dietary carbohydrate was

approximately 45% and 59% of kcal for the YL group and EXG group, respectively. Dietary fat was kept constant comprising approximately 24-25% of total energy in both YL and EXG groups. These macronutrient distribution patterns were maintained throughout the intervention, and women in both YL and EXG groups walked at a moderate pace for 30 to 40 minutes per day for the duration of the study.

Dietary intake, BW, BMI, FM, fat-free soft tissue mass (FFSTM), body fat percentage (BF%), waist circumference, and hip circumference measurements were all primary outcome measures. Central abdominal tissue percentage (CAT%), selected vital signs, resting energy expenditure (REE), blood lipids, glucose, insulin, selected adipose-derived hormones, high-sensitivity (hs) C-reactive protein (hsCRP), biomarkers of bone turnover and BMD at select skeletal cites were secondary measures.

Baseline testing was completed by 123 women. Women enrolled at Week 2 (n=104) were included in an intention-to-treat (ITT) analysis, and an efficacy analysis was conducted including the 80 women who completed the 24-week intervention. Multivariate analysis of covariance with repeated measures on the time factor were performed to assess differences between YL and EXG groups. Independent or paired *t*-tests using Bonferroni adjustments for multiple comparisons were completed when significant group or group x time interactions were found. Tests were two-sided, and a probability of $p<0.01$ was considered statistically significant for primary analysis.

Women with compete dietary records (n=71) were separated into dietary ED change tertiles and included in an exploratory secondary analysis examining change in the primary outcomes of BW, body composition and anthropometrics. Tertile 1 included 23 women who had a small mean reduction in ED ($\leq$0.67 kcal/g), Tertile 2 (n=24) included women with a medium reduction in ED (>0.68 and <0.87 kcal/g), and Tertile 3 (n=24) included women with a large

reduction in energy density (≥0.92 kcal/g) from baseline to Week 12. A 3x3 analysis of covariance with repeated measures on the time factor were performed to assess differences between tertiles of ED change across three time intervals. Bonferroni adjustments were applied for multiple comparisons when significant group or group x time interactions were found. A probability of $p<0.05$ was considered statistically significant.

Using ITT analysis, estimated average energy intake was significantly reduced in both YL and EXG groups, respectively, from baseline by 25% and 30% at Week 12 and by 18% and 27% at Week 24 with no significant differences between groups. Protein intake did not reach 30% of total energy intake as designed in the YL group; however, estimated average protein intake was significantly greater in the YL group compared to EXG group at Week 12. No significant effect of time was observed for estimated energy expenditure from self-reported physical activity records.

There were no significant effects of diet group on body composition and anthropometric measurements. Both YL and EXG groups, respectively, had significant decreases in BW (-4.9±3.2 and -4.3±3.3 kg), BMI (-1.8±1.2 and -1.6±1.2 kg/m^2), FM (-3.0±2.2 and -2.3±2.3 kg), FFSTM (-1.5±1.6 and -1.7±2.1 kg), BF% (-1.7±1.7 and -1.2±2.0 %), waist circumference (-4.4±2.9 and -3.8±3.1 cm), hip circumference (-4.7±3.5 and -4.3±3.6 cm) and CAT% (-3.0±3.2 and -2.8±2.9 %), from baseline to Week 12. These losses were maintained through Week 24. Group x time interactions were not observed for any of the primary outcome variables. Similar results were found with analysis in study completers.

There were no significant effects of diet group on resting heart rate, systolic and diastolic blood pressure (BP) and REE with ITT analysis. There were no significant differences in resting heart rate or systolic and diastolic BP over time in either YL or EXG groups, and group x time

interactions were not observed for any of these measures. There was a significant reduction in REE from baseline to Week 12, which was maintained through Week 24 in both groups. Similar results were found with efficacy analysis, with the exception of a significant increase in diastolic BP between Weeks 12 and 24.

There were no significant effects of diet group for biochemical markers of health with ITT analysis, and women in both groups experienced a significant reduction in triglycerides and total cholesterol concentrations from baseline to Week 12. These reductions were maintained to Week 24. Serum low-density lipoprotein cholesterol significantly decreased at Week 12, but this reduction was no longer significant at Week 24. There were no other significant changes in groups over time, and group x time interactions were not observed in any of these secondary outcome measures. Results were similar with efficacy analysis.

Significant diet group effects were not found for serum leptin, resistin and adiponectin concentrations. Leptin concentration significantly decreased from baseline to Week 12, and this reduction was maintained through Week 24 in both groups. There were no significant changes in resistin concentration over time. There was a significant increase in adiponectin between Weeks 12 and 24 in both groups. Significant group x time interactions were not observed in these adipose-derived hormones. Results using efficacy analysis were similar to ITT analysis.

There were no significant differences in markers of bone turnover, including N-telopeptide of type I collagen and osteocalcin, between groups or over time. hsCRP decreased significantly from baseline to Week 12 in all women, and this reduction was maintained to Week 24. There were no significant differences in BMD at the total body, lumbar spine, total proximal femur and forearm between YL and EXG groups or over time. Results were similar with efficacy analysis.

In secondary analysis of ED change tertiles, women in Tertiles 1, 2 and 3 significantly reduced estimated energy intakes and ED; these reductions were maintained through Week 24. By design, Tertiles 2 and 3 had greater reductions in ED than Tertile 1, and Tertile 3 had a greater reduction in ED than Tertile 2.

Women in Tertiles 1, 2 and 3 significantly reduced BW, BMI, FM, FFSTM, waist and hip circumferences and CAT% from baseline to Week 12. Participants in Tertile 2 also reduced BF% from baseline to Week 12 and maintained this reduction to Week 24. The pattern of change in BW, BMI, FM, BF% and waist circumference was significantly different between tertiles. Women in Tertile 2 had significantly greater reductions in BW and BMI at Weeks 12 and 24 and greater reductions in FM, BF% and waist circumference at Week 24 compared to women in Tertile 1 but not compared to Tertile 3.

Free-living, premenopausal women with excess adiposity, facilitated by a weight-loss intervention including dietary modification within an ERD and moderate walking exercise significantly reduced BW and FM, triglycerides, total cholesterol, CRP and leptin across a 12-week weight-loss phase and maintained these changes across a 12-week weight-loss maintenance phase. The absence of detectable differences in primary outcome variables and markers of bone turnover and BMD between YL and EXG groups suggests that these two approaches, both meeting dietary guidelines for intake of at least three servings of dairy per day and recommendations for protein intake, may have been equally effective at inducing BW loss and maintaining BMD. In conclusion, free-living, premenopausal women with overweight/obesity who completed a 6-month lifestyle intervention with sufficient protein and non-fat dairy intake along with daily moderate walking achieved and maintained an approximate 6% loss of BW while reducing FM, CRP and leptin, and maintaining BMD.

Secondary analysis from this research project found that women who had a medium reduction in dietary ED, within a lifestyle intervention that included an ERD and daily walking, had greater reductions in BW, BMI, FM, BF% and waist circumference compared to women who had a small reduction in ED through Week 24. Thus, medium or large reductions of dietary ED best promoted BW and FM losses, suggesting a sustainable approach to weight-loss maintenance.

TABLE OF CONTENTS

List of Tables xi

List of Figures xiv

List of Acronyms xv

Acknowledgments xvi

Chapter 1. Introduction 1

Chapter 2. Literature Review 12

Chapter 3. An energy-restricted dietary pattern including moderate protein and non-fat dairy combined with walking promotes beneficial changes in body composition and metabolic parameters in premenopausal women with excess adiposity 77

Abstract 78

Introduction 80

Methods 83

Results 90

Discussion 93

Conclusions 99

References 100

Chapter 4. A lifestyle intervention promoting weight loss with moderate or standard protein and non-fat dairy intake and daily walking: impact on bone health in premenopausal women with overweight/obesity 115

Abstract 116

Introduction 117

Methods 119

Results 124

Discussion 127

References 132

Chapter 5. Medium reduction of dietary energy density within an energy-restricted dietary pattern benefits weight-loss outcomes 141

Abstract 142

Introduction 143

Methods 144

Results and Discussion 146

References 152

Chapter 6. Summary, Strengths, Limitations and Future Directions 158

Appendix A. Screening Forms and Informed Consent 168

IRB Approval Letter. 169

Informed Consent 170

Medical History Form 177

Screening Form 182

Health Care Provider Clearance Form 183

Appendix B. Diet-Related Materials 185

Meal plans by study phase for YL and EXG groups 186

LIST OF TABLES

Table 2.1 Summary of findings from moderate protein energy-restricted diet studies examining body composition, anthropometrics, and metabolic indicators of health in overweight/obese adults 72

Table 2.2 Summary of findings from energy-restricted diets with or without physical activity examining body weight loss and bone health related outcomes 75

Table 3.1 Estimated energy and macronutrient intakes and energy expenditure and physical activity of premenopausal women with overweight/obesity at baseline and Week 2, 12 and 24 in a study examining changes in body composition and metabolic parameters with an energy-restricted dietary pattern (ERD) including moderate protein and non-fat dairy (YL group) or ERD with standard protein and non-fat dairy (EXG group) combined with daily moderate walking 107

Table 3.2 Anthropometric and body composition measurements of premenopausal women with overweight/obesity at baseline and Week 2, 12 and 24 in a study examining changes in body composition and metabolic parameters with an energy-restricted dietary pattern (ERD) including moderate protein and non-fat dairy (YL group) or ERD with standard protein and non-fat dairy (EXG group) combined with daily moderate walking 109

Table 3.3 Resting heart rate, blood pressure and resting energy expenditure measurements of premenopausal women with overweight/obesity at baseline and Week 2, 12 and 24 in a study examining changes in body composition and metabolic parameters with an energy-restricted dietary pattern (ERD) including moderate protein and non-fat dairy (YL group) or ERD with standard protein and non-fat dairy (EXG group) combined with daily moderate walking 110

Table 3.4 Blood lipid, glucose and insulin concentrations of premenopausal women with overweight/obesity at baseline and Week 2, 12 and 24 in a study examining changes in body composition and metabolic parameters with an energy-restricted dietary pattern (ERD) including moderate protein and non-fat dairy (YL group) or ERD with standard protein and non-fat dairy (EXG group) combined with daily moderate walking 111

Table 3.5 Concentrations of adipose-derived hormones in premenopausal women with overweight/obesity at baseline and Week 12 and 24 in a study examining changes in body composition and metabolic parameters with an energy-restricted dietary pattern (ERD) including moderate protein and non-fat dairy (YL group) or ERD with standard protein and non-fat dairy (EXG group) combined with daily moderate walking 112

Table 4.1 Mean values ± standard deviation of dietary intake patterns at baseline and Weeks 12 and 24 in women with overweight/obesity participating in a weight-loss intervention including an energy-reduced diet and walking component with either moderate protein and non-fat dairy intake (YL) or standard protein and dairy intake (EXG) 137

Table 4.2 Mean values ± standard deviation of bone mineral density at the total body, lumbar spine, total proximal femur and forearm skeletal sites at baseline, Weeks 12 and 24 in women with overweight/obesity participating in a weight-loss intervention including an energy-reduced diet and walking component with either moderate protein and non-fat dairy intake (YL) or standard protein and non-fat dairy intake (EXG) 139

Table 4.3 Mean values ± standard deviation of N-telopeptide of type 1 collagen (NTx), osteocalcin and hsC-reactive protein (CRP) at baseline, Weeks 12 and 24 in women with overweight/obesity participating in a weight-loss intervention including an energy-reduced diet

and walking component with either moderate protein and non-fat dairy intake (YL) or standard protein and non-fat dairy intake (EXG). 140

Table 5.1 Characteristics of women at baseline and Weeks 12 and 24 by tertile of dietary energy density change from baseline to Week 12 of a lifestyle intervention, including an energy-restricted diet and moderate daily walking 155

LIST OF FIGURES

Figure 3.1 Diagram of recruitment, enrollment, randomization and attrition of participants in a study examining changes in body composition and metabolic parameters in premenopausal women with overweight/obesity following an energy-restricted dietary pattern (ERD) including moderate protein and non-fat dairy (YL group) or standard protein and non-fat dairy (EXG group) combined with daily moderate walking 106

Figure 3.2 Adipose-derived hormones (A) leptin, (B) resistin and (C) adiponectin in premenopausal women with overweight/obesity at baseline (Week 0), Week 12 and 24 in a study examining changes in body composition and metabolic parameters with an energy-restricted dietary pattern (ERD) including moderate protein and non-fat dairy (YL group) or standard protein and non-fat dairy (EXG group) combined with daily moderate walking 113

Figure 4.1 Intention to treat analysis (A; YL=52, EXG=52) and efficacy analysis (B; YL=43, EXG=37) of percentage body weight and percentage fat-free soft tissue mass (FFSTM) change in premenopausal women with overweight/obesity at Weeks 12 and 24 of a 24-week lifestyle intervention. 138

Figure 5.1 Percent change in dietary energy density at baseline and Weeks 12 and 24 by tertiles of energy density change from baseline to Week 12 of a lifestyle intervention including an energy-restricted diet and moderate daily walking 154

Figure 5.2 Percent change in body weight (BW), fat mass (FM), body fat percentage (BF%), and waist circumference (WC) at baseline and Weeks 12 and 24 by tertiles of dietary energy density change during a lifestyle intervention including an energy-restricted diet and moderate daily walking. 157

LIST OF ACRONYMS

AUC = area under the curve
BAP = bone-specific alkaline phosphatase
BF% = body fat percentage
BMC = bone mineral content
BMD = bone mineral density
BP = blood pressure
BW= body weight
Ca = calcium
CAT = central abdominal tissue
CRP = C-reactive protein
CTx = C-terminal telopeptide of type I collagen
dPYD = deoxypyridinoline
ED = energy density
EX = exercise
FA = forearm
FFSTM = fat-free soft tissue mass
FM = fat mass
HC = hip circumference
HDL-C = high-density lipoprotein
LDL-C = low-density lipoprotein
LS = lumbar spine
MP = moderate protein
NTx = N-terminal telopeptide of type I collagen
OC = osteocalcin
OPG = osteoprotegerin
P1NP = procollagen type 1 N-terminal propeptide
PYD = pyridinoline
SP = standard protein
TB = total body
TC = total cholesterol
TG = triglycerides
TPF = total proximal femur
WC = waist circumference

ACKNOWLEGMENTS

Sincere thanks to my faculty mentor, Sharon M. Nickols-Richardson, PhD, RD, for her consideration and mentoring. I also thank my dissertation committee members, Dr. Penny M. Kris-Etherton, Dr. Terry Hartman, Dr. Connie Rogers and Dr. Neil Sharkey for their guidance and support of my research goals. My lab group, including Carrie Durward, Catherine Metzgar, Sarah Nelson and Melissa Zack also supported of my academic pursuits. I have had the privilege of working with several undergraduate student research assistants, Tatiana Costellino, Collin Neal, Meghan Murry and Rachel Moyer who contributed greatly to success of this research.

I would like to thank the registered nurses who conducted our blood draws, the General Clinical Research Center staff who assisted with DXA scanning appointments, and the Unger and Kelleher labs for use of their lab equipment and much appreciated company. Additionally, I would like to thank the Department of Nutritional Sciences, our Department Head, Dr. Gordon Jensen, and the Nutrition Graduate Student Association for providing a dynamic work environment. Of course this research could not have been conducted without study participants who made this experience so memorable and the grant support from The Bell Institute of Health, General Mills Inc.

I am also grateful to the superb academic and professional mentors I have had the privilege of working with in my scientific career including, Dr. Ethan Solomon, Dr. Kurt Adams, Dr. Judy Levin and Dr. Mark Hiller. Finally, I would like to thank my husband, Josh Baxter for all of his encouragement and support, my parents, Matt and Susan, and my brother Paul for their confidence and understanding.

CHAPTER 1

INTRODUCTION

Study Rationale

Greater than two-thirds of individuals residing in the United States (US) are considered overweight or obese (1), conditions that increase the risk of type 2 diabetes, heart disease, hypertension and certain cancers (2–4). Reduction of excess body weight (BW) is recommended as it can reduce risk of future disease and improve quality of life (5–7). In the last several decades, many research groups have focused on the investigation of weight-loss approaches to aid in overweight/obesity treatment. Dietary weight-loss interventions have tested complex variations of dietary macronutrient composition (8–12) and inclusion of certain foods components (13–15) into an energy-restricted diet (ERD).

While metabolic health benefits of excess BW loss are numerous, BW loss is potentially detrimental to bone mineral density (BMD), a measure of the concentration of mineral in a defined section of bone used to estimate fracture risk. Higher BMD is associated with decreased risk in fracture incidence, while lower BMD is a risk factor for osteoporosis, a chronic condition that increases fracture risk (16). Overweight/obese individuals reducing BW may experience BMD loss (17–19) which may persist with BW regain (20); therefore, frequent dieters may be at greater risk for loss of BMD (21). Women are especially vulnerable to osteoporosis, making maintenance of BMD vital in this population during BW loss (22,23).

Modifiable lifestyle factors can attenuate weight-loss induced BMD loss. These factors include optimizing dietary intake of select nutrients vital to bone maintenance and formation within an ERD (24), engaging in physical activity to simulate bone formation (25) and reducing inflammatory factors believed to signal bone resorption (26). Research has demonstrated that moderate dietary protein (25-30% of total energy) and calcium and/or dairy intake within an ERD may favorably affect bone turnover biomarkers and BMD with BW loss (17,27–29).

Premenopausal women slowly losing moderate BW while consuming recommended amounts of protein and dairy/calcium (30,31) demonstrate that adverse changes in bone turnover or BMD can be attenuated or prevented (27–29,32). Moreover, weight-loss approaches including exercise in addition to an ERD may reduce BMD loss compared to ERD only approaches (33). Dietary interventions examining diets high in protein (>30% of total energy) combined with exercise have shown independent and additive effects of diet and exercise on body composition (34,35).

As an ERD approach including moderate dietary protein and calcium and/or dairy intake may improve bone health outcomes with BW loss, moderate dietary protein and calcium and/or dairy intake also may improve anthropometric and metabolic outcomes of BW loss (15,36–39). ERDs in which protein comprises greater than 30% of energy intake have been shown to enhance losses in BW (34–36,38) and fat mass (FM) (34,36–38,40). Moderate protein diets also may preserve fat-free soft tissue mass (FFSTM) during BW loss (37,39,41,42) which may contribute to BW loss maintenance and BMD maintenance (43,44).

Few studies have examined weight-loss strategies that incorporate the most favorable intervention components for bone health while still producing effective BW loss. Previous intervention studies that have examined the effect of various weight-loss approaches on bone loss often report only biomarkers of bone turnover (45,46) or are relatively short in duration, lasting less than six months (28,29,45–47). An intervention including free-living women aimed at optimizing BW loss and maintenance of weight loss while avoiding reductions in BMD may be more applicable to examine intervention effectiveness. This research project aimed to compare the effects of an ERD that incorporated either moderate (30% of kcal) protein intake and 4-5 servings of non-fat dairy per day combined with daily walking to an ERD with standard

protein (16-17% of kcal) and three servings of non-fat dairy per day combined with daily walking on BW loss, body composition, clinical and biochemical health outcomes and BMD.

Data from study participants were further analyzed to examine a relationship between changes in dietary energy density (ED), defined as the energy content of a food divided by its unit weight (kcal/g), and BW loss during the 6-month intervention. As several complex eating patterns have been investigated for effectiveness in randomized-control trials (8,9,38), ED is thought to be a simple feature of dietary intake that individuals can consider during food selection for overall reductions in energy intake. Short-term, laboratory-based studies have established that individuals consume less energy when presented with foods lower in ED than with similar foods that are higher in ED (48,49). Additionally, diets low in ED may help meet individual micronutrient recommendations (50). Therefore, the role of ED on BW loss in this research project was examined to explore success with BW loss and body composition changes.

Study Aims and Hypotheses

The primary objective of this research project was to examine the effect of an ERD with moderate protein and 4-5 servings of non-fat dairy intake per day combined with exercise as part of a 6-month comprehensive lifestyle intervention on BW, body mass index (BMI), FM, FFSTM, body fat percentage (BF%), waist and hip circumferences, central abdominal tissue percentage (CAT%) and metabolic parameters of health in premenopausal women with overweight or obesity. The second objective was to investigate if an ERD with moderate protein and 4-5 servings of non-fat dairy per day combined with exercise would improve the marker of inflammatory response, C-reactive protein (CRP), and mitigate loss of BMD during BW loss. The third objective of this research project was to explore changes in dietary ED and BW, BMI, FM, FFSTM, BF%, waist circumference, hip circumference, and CAT% in premenopausal

women with overweight or obesity during a 6-month comprehensive lifestyle intervention including an ERD and moderate daily walking component.

The first hypothesis was that premenopausal women with overweight or obesity following an ERD with moderate protein and 4-5 servings of non-fat dairy per day combined with 30-40 minutes of moderate-paced walking (i.e., treatment group) or an ERD with standard protein and 3 servings of non-fat dairy per day combined with walking (i.e., control group) could achieve significant BW loss and that women in the treatment group would have greater losses in BW and a greater proportion of BW loss from FM. Further, metabolic parameters were expected to improve in both groups with BW loss. The second hypothesis was that women in the treatment group would preserve BMD, while women in the control group would experience loss of BMD after the 6-month intervention. Further, hsCRP concentration would decrease in both groups with BW loss. The final hypothesis was that women with the greatest reduction in ED during the weight-loss portion of the intervention, regardless of intervention group, would have greater BW and FM losses than women who reduced ED less substantially.

Within this dissertation, Chapter 2 contains a literature review which provides an overview of dietary weight-loss approaches moderate in either protein content or dairy content designed to reduce BW and FM, preserve FFSTM, and reduce risk factors for chronic diseases such as hyperlipidemia, hypertriglyceridemia, hypertension and insulin resistance. Chapter 2 also discusses additional approaches to weight loss, including increased exercise and the use of meal replacements and reduced ED to mediate energy intake. Finally, Chapter 2 discusses contributors to skeletal health, including the effect of BW loss on BMD, physical activity, and emerging negative effects of excess adiposity and inflammation on BMD. Chapter 3 presents the main methods of the study and anthropometric, body composition and metabolic parameter changes

that occurred during the 24-week comprehensive lifestyle intervention in both diet conditions. Chapter 4 describes the effects of reduced energy intake and BW loss achieved by both dietary patterns on changes in inflammation, bone turnover biomarkers and BMD at several skeletal sites. Chapter 5 explores dietary ED change in study participants and anthropometric and soft tissue mass change. Chapter 6 summarizes results of this research project, discusses strengths and weakness of the study design and outlines potential ideas for future research. Supporting documents used in the implementation of this research project are contained in Appendix A and B.

Results of this research project provide information on the effects of a 6-month comprehensive lifestyle intervention including moderate protein and non-fat dairy intake combined with daily walking exercise in free-living premenopausal women with overweight or obesity on body composition, metabolic and bone health outcomes following BW loss. Additionally, modifications in dietary ED due to this 6-month comprehensive lifestyle intervention and changes in BW and body composition are explored in a secondary analysis.

REFERENCES

1. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA*. 2012;307(5):491–497.

2. Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. *BMC Public Health*. 2009;9:88.

3. Chang Y, Ryu S, Suh B-S, Yun KE, Kim C-W, Cho S-I. Impact of BMI on the incidence of metabolic abnormalities in metabolically healthy men. *Int J Obes (2005)*. 2011.

4. Dalton M, Cameron AJ, Zimmet PZ, et al. Waist circumference, waist-hip ratio and body mass index and their correlation with cardiovascular disease risk factors in Australian adults. *J. Intern. Med.* 2003;254(6):555–563.

5. Ringbäck Weitoft G, Eliasson M, Rosén M. Underweight, overweight and obesity as risk factors for mortality and hospitalization. *Scand J Public Health*. 2008;36(2):169–176.

6. Wille N, Erhart M, Petersen C, Ravens-Sieberer U. The impact of overweight and obesity on health-related quality of life in childhood – results from an intervention study. *BMC Public Health*. 2008;8(1):421.

7. Brown T, Avenell A, Edmunds LD, et al. Systematic review of long-term lifestyle interventions to prevent weight gain and morbidity in adults. *Obes Rev*. 2009;10(6):627–638.

8. Sacks FM, Bray GA, Carey VJ, et al. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *N Engl J Med*. 2009;360(9):859–873.

9. Gardner CD, Kiazand A, Alhassan S, et al. Comparison of the Atkins, Zone, Ornish, and LEARN diets for change in weight and related risk factors among overweight premenopausal women: the A TO Z Weight Loss Study: a randomized trial. *JAMA*. 2007;297(9):969–977.

10. Dansinger ML, Gleason JA, Griffith JL, Selker HP, Schaefer EJ. Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction: a randomized trial. *JAMA*. 2005;293(1):43–53.

11. Nordmann AJ, Nordmann A, Briel M, et al. Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. *Arch Intern Med*. 2006;166(3):285–293.

12. Abete I, Astrup A, Martínez JA, Thorsdottir I, Zulet MA. Obesity and the metabolic syndrome: role of different dietary macronutrient distribution patterns and specific nutritional components on weight loss and maintenance. *Nutr Rev*. 2010;68(4):214–231.

13. Champagne CM, Broyles ST, Moran LD, et al. Dietary intakes associated with successful weight loss and maintenance during the Weight Loss Maintenance trial. *J Am Diet Assoc*. 2011;111(12):1826–1835.

14. Mattes RD, Kris-Etherton PM, Foster GD. Impact of peanuts and tree nuts on body weight and healthy weight loss in adults. *J Nutr*. 2008;138(9):1741S–1745S.

15. Zemel MB, Teegarden D, Loan MV, et al. Dairy-rich diets augment fat loss on an energy-restricted diet: a multicenter trial. *Nutrients*. 2009;1(1):83–100.

16. Cummings DE, Weigle DS, Frayo RS, et al. Plasma ghrelin levels after diet-induced weight loss or gastric bypass surgery. *N Engl J Med.* 2002;346(21):1623–1630.

17. Ricci TA, Chowdhury HA, Heymsfield SB, Stahl T, Pierson RN Jr, Shapses SA. Calcium supplementation suppresses bone turnover during weight reduction in postmenopausal women. *J. Bone Miner Res.* 1998;13(6):1045–1050.

18. Jensen LB, Kollerup G, Quaade F, Sørensen OH. Bone minerals changes in obese women during a moderate weight loss with and without calcium supplementation. *J Bone Miner Res*. 2001;16(1):141–147.

19. Fogelholm GM, Sievänen HT, Kukkonen-Harjula TK, Pasanen ME. Bone mineral density during reduction, maintenance and regain of body weight in premenopausal, obese women. *Osteoporos Int*. 2001;12(3):199–206.

20. Hinton PS, Rector RS, Linden MA, et al. Weight-loss-associated changes in bone mineral density and bone turnover after partial weight regain with or without aerobic exercise in obese women. *Eur J Clin Nutr.* 2011.

21. Bacon L, Stern JS, Keim NL, Van Loan MD. Low bone mass in premenopausal chronic dieting obese women. *Eur J Clin Nutr*. 2004;58(6):966–971.

22. Hansen MA, Overgaard K, Riis BJ, Christiansen C. Role of peak bone mass and bone loss in postmenopausal osteoporosis: 12 year study. *BMJ*. 1991;303(6808):961–964.

23. Sirola J, Kröger H. Similarities in acquired factors related to postmenopausal osteoporosis and sarcopenia. *J Osteoporos*. 2011;2011:536735.

24. Esterle L, Sabatier J-P, Guillon-Metz F, et al. Milk, rather than other foods, is associated with vertebral bone mass and circulating IGF-1 in female adolescents. *Osteoporos Int*. 2008;20(4):567–575.

25. Borer KT. Physical activity in the prevention and amelioration of osteoporosis in women : interaction of mechanical, hormonal and dietary factors. *Sports Med*. 2005;35(9):779–830.

26. Cao JJ. Effects of obesity on bone metabolism. *J Orthop Surg Res*. 2011;6:30.

27. Thorpe MP, Jacobson EH, Layman DK, He X, Kris-Etherton PM, Evans EM. A diet high in protein, dairy, and calcium attenuates bone loss over twelve months of weight loss and maintenance relative to a conventional high-carbohydrate diet in adults. *J Nutr*. 2008;138(6):1096–1100.

28. Josse AR, Atkinson SA, Tarnopolsky MA, Phillips SM. Diets Higher in Dairy Foods and Dietary Protein Support Bone Health during Diet- and Exercise-Induced Weight Loss in Overweight and Obese Premenopausal Women. *The Journal of Clinical Endocrinology and Metabolism*. 2011.

29. Bowen J, Noakes M, Clifton PM. A high dairy protein, high-calcium diet minimizes bone turnover in overweight adults during weight loss. *J Nutr*. 2004;134(3):568–573.

30. Otten JJ, Hellwig JP, Meyers LD, National Academies Press (U.S.). *DRI, dietary reference intakes the essential guide to nutrient requirements*. Washington, D.C.: National Academies Press; 2006.

31. U.S. Department of Agriculture and U.S. Department of Health and Human Services. Dietary Guidelines for Americans, 2010. 7th Edition, Washington, DC: U.S. Government Printing Office, December 2010.

32. Riedt CS, Schlussel Y, von Thun N, et al. Premenopausal overweight women do not lose bone during moderate weight loss with adequate or higher calcium intake. *Am J Clin Nutr*. 2007;85(4):972–980.

33. Villareal DT, Fontana L, Weiss EP, et al. Bone mineral density response to caloric restriction-induced weight loss or exercise-induced weight loss: a randomized controlled trial. *Arch Intern Med*. 2006;166(22):2502–2510.

34. Layman DK, Evans E, Baum JI, Seyler J, Erickson DJ, Boileau RA. Dietary protein and exercise have additive effects on body composition during weight loss in adult women. *J Nutr*. 2005;135(8):1903–1910.

35. Meckling KA, Sherfey R. A randomized trial of a hypocaloric high-protein diet, with and without exercise, on weight loss, fitness, and markers of the Metabolic Syndrome in overweight and obese women. *Appl Physiol Nutr Metab*. 2007;32(4):743–752.

36. Layman DK, Evans EM, Erickson D, et al. A moderate-protein diet produces sustained weight loss and long-term changes in body composition and blood lipids in obese adults. *J Nutr*. 2009;139(3):514–521.

37. Layman DK, Boileau RA, Erickson DJ, et al. A Reduced Ratio of Dietary Carbohydrate to Protein Improves Body Composition and Blood Lipid Profiles during Weight Loss in Adult Women. *J Nutr*. 2003;133(2):411 –417.

38. Skov AR, Toubro S, Rønn B, Holm L, Astrup A. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. *Int J Obes Relat Metab. Disord.* 1999;23(5):528–536.

39. Farnsworth E, Luscombe ND, Noakes M, Wittert G, Argyiou E, Clifton PM. Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. *Am J Clin Nutr.* 2003;78(1):31–39.

40. Parker B, Noakes M, Luscombe N, Clifton P. Effect of a high-protein, high-monounsaturated fat weight loss diet on glycemic control and lipid levels in type 2 diabetes. *Diabetes Care*. 2002;25(3):425–430.

41. Brehm BJ, D'Alessio DA. Benefits of high-protein weight loss diets: enough evidence for practice? *Curr Opin Endocrinol Diabetes Obes*. 2008;15(5):416–421.

42. Leidy HJ, Carnell NS, Mattes RD, Campbell WW. Higher protein intake preserves lean mass and satiety with weight loss in pre-obese and obese women. *Obesity (Silver Spring)*. 2007;15(2):421–429.

43. Wing RR, Hill JO. Successful weight loss maintenance. *Annu Rev Nutr.* 2001;21:323–341.

44. Wang MC, Bachrach LK, Van Loan M, Hudes M, Flegal KM, Crawford PB. The relative contributions of lean tissue mass and fat mass to bone density in young women. *Bone*. 2005;37(4):474–481.

45. Wagner G, Kindrick S, Hertzler S, DiSilvestro RA. Effects of various forms of calcium on body weight and bone turnover markers in women participating in a weight loss program. *J Am Coll Nutr*. 2007;26(5):456–461.

46. Rector RS, Loethen J, Ruebel M, Thomas TR, Hinton PS. Serum markers of bone turnover are increased by modest weight loss with or without weight-bearing exercise in overweight premenopausal women. *Appl Physiol Nutr Metab*. 2009;34(5):933–941.

47. Van Loan MD, Johnson HL, Barbieri TF. Effect of weight loss on bone mineral content and bone mineral density in obese women. *Am J Clin Nutr.* 1998;67(4):734–738.

48. Bell EA, Castellanos VH, Pelkman CL, Thorwart ML, Rolls BJ. Energy density of foods affects energy intake in normal-weight women. *Am J Clin Nutr.* 1998;67(3):412–420.

49. Rolls BJ, Bell EA, Castellanos VH, Chow M, Pelkman CL, Thorwart ML. Energy density but not fat content of foods affected energy intake in lean and obese women. *Am J Clin Nutr*. 1999;69(5):863–871.

50. Ledikwe JH, Blanck HM, Khan LK, et al. Low-energy-density diets are associated with high diet quality in adults in the United States. *J Am Diet Assoc*. 2006;106(8):1172–1180.

CHAPTER 2

LITERATURE REVIEW

Obesity – A Growing National Burden

With two-thirds of individuals residing in the United States (US) classified as overweight or obese, treatment for this condition is a public health priority (1). Excess adiposity is associated with increased risk of type 2 diabetes, heart disease, hypertension and certain cancers (2–4). Body mass index (BMI), used to define overweight and obesity is calculated by dividing body mass in kilograms (kg) by height in meters squared (m^2). An individual with a BMI greater than 25 kg/m^2 is considered overweight, while a BMI greater than 30 kg/m^2 is considered obese (5). Currently, all 50 states within the US report that at least 20% of their adult population is obese (6). Excess adiposity is a considerable problem in developed nations such as the US, and globally, obesity has more than doubled since the 1980s (5). To improve overall health status, reduce the risk of future disease, improve quality of life and reduce risk for all-cause mortality, it is recommended that individuals with excess adiposity achieve and maintain body weight (BW) loss (7,8).

Weight-loss Interventions

Many health benefits are gained with the loss of excess adiposity. Notably, BW loss has been shown to reduce hyperglycemia, hypertension and hyperlipidemia, all of which are major risk factors for the development of chronic diseases (9,10). While overweight/obese individuals already exhibiting elevated cardiovascular risk factors can improve metabolic condition with the loss of BW (11), normolipidemic, non-diabetic, healthy individuals who are overweight/obese also may improve metabolic parameters by lowering BW. Studies examining metabolic risk indicators of disease such as fasting insulin and glucose, triglycerides (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) in healthy

adults report that improvements in these measures occur with BW loss of approximately 10% (12–14).

To evaluate the efficacy and effectiveness of weight-loss approaches, a variety of dietary interventions have been conducted in the last several decades with limited success. These interventions have tested dietary macronutrient composition (15–19) and inclusion of certain foods components (20–22) into an energy-restricted diet (ERD). Weight-loss approaches that manipulate macronutrient distribution within an ERD may enhance BW loss, benefit body composition changes, and improve markers of health status beyond those observed with traditional weight loss interventions.

High-protein Energy-restricted Dietary Interventions and Effects on Weight Loss and Body Composition

Optimal dietary macronutrient composition in the context of weight-loss regimens has been an area of debate. The ideal percentage of protein, carbohydrate and fat in a dietary pattern that best promotes BW loss and BW loss maintenance is equivocal (15–19). The Recommended Dietary Allowance (RDA) for protein is 0.8 g per kg BW, with an acceptable macronutrient distribution range of 10-35% of total energy for adults (23). In 1999, Smit et al. (24) reported that the US population consumes roughly 15% of dietary energy as protein. It is important to recognize that total protein (g) consumed during energy restriction may be substantially lower than total protein consumed during unrestricted intake, even though protein may comprise the same percentage of overall energy intake in restricted or unrestricted conditions. During energy restriction, g of protein intake per kg BW may fall below 0.8. This is of particular consequence as protein needs may increase during energy restriction and BW loss to avoid fat-free soft tissue mass (FFSTM) losses (25). A diet in which protein comprises 25-30% of total energy intake has

been defined as higher in protein in literature relevant to the examination of macronutrient distribution within an ERD (26–32). For the purposes of this review, diets in which protein comprises at least 20% of total energy, at the expense of dietary carbohydrate rather than fat, will be referred to as a moderate protein (MP) diet. Dietary approaches in which protein comprises 10-19% of total energy will be referred to as a standard-protein (SP) diet.

In weight-loss trials, MP ERDs have been shown to enhance losses in BW (30,31,33,34) fat mass (FM) (31,33–36) and central abdominal tissue (CAT) (26,35), compared to ERDs with SP. A MP ERD also may preserve FFSTM during BW loss (27,36–38). Beneficial changes in body composition outcomes with MP ERDs have been partially attributed to increased thermogenesis (39,40), increased satiety and reduced hunger (38,40–42) and maintenance of FFSTM with BW loss (36).

One potential mechanism by which increased dietary protein reportedly benefits BW loss is through increased thermogenesis. The thermic effect of a food is defined as the increase in energy expenditure above basal level following consumption and can be further defined as energy required for digestion, absorption, and disposal of ingested nutrients. Thermic effect of food is influenced by dietary composition. A systematic review examining 15 randomized studies consistently reported that dietary protein had a greater thermic effect than dietary carbohydrate or fat (43). A 4-day randomized crossover study using a respiration chamber to measure energy expenditure evaluated participants during consumption of two diets varying in protein (10 vs. 30% of kcal) and carbohydrate (60 vs. 40% of kcal) content with constant fat (30% of kcal) content. The 30% protein diet resulted in greater diet-induced thermogenesis, increased metabolic rate while sleeping, and greater self-rated satiety (40). A greater thermic effect (39,40,44) in addition to increased resting energy expenditure with MP meals (40,45) may

produce larger overall effects on daily energy expenditure than diets or meals lower in protein. Still, it is uncertain that an energy disparity caused by protein-induced increases in dietary thermogenesis would be sufficient to manifest statistically significant or clinically meaningful changes in BW and FM losses (37,46).

Several studies describe greater self-reported satiety and less hunger with MP ERDs than with standard-protein ERDs (37,38,46–48). Randomized clinical weight-loss trials in overweight/obese participants have used questionnaires to evaluate appetitive sensations. Leidy et al. (38) conducted a study assessing self-reported hunger, fullness and desire to eat using visual analog scales in women with overweight/obesity before and after a 12-week ERD. Leidy et al. (38) found that a decline in satiety was attenuated in the MP (30% of kcal) diet group during energy restriction relative to a SP (18% of kcal) diet group. Nickols-Richardson et al. (48) found that self-rated hunger decreased in overweight/obese premenopausal women consuming a MP (26% of kcal) ERD over six weeks, with no change in women consuming a SP (18% of kcal) ERD. However, fat content varied between the MP and SP group in this study, comprising 61 and 22% of total energy, respectively. Women in the MP group also lost relatively more BW than the SP group (48). Reduced hunger and greater satiety with MP intake during energy restriction may suggest that when consumed *ad libitum*, a MP diet would result in less energy intake than a SP diet. In fact, this is what Skov et al. (33) reported after a 6-month randomized dietary intervention comparing two *ad libitum* fat-reduced diets in healthy, overweight/obese men and women. Self-reported energy intake was 17% lower, and losses in BW, FM and CAT were significantly greater in the MP (25% of kcal) *ad libitum* group compared to the SP (12% of kcal) *ad libitum* group (33).

Laboratory-based feeding studies using preload meals have examined the acute effects of MP on self-reported feelings of satiety and hunger as well as subsequent energy intake with less definitive results. Three studies (49–51) examined the satiety-producing effect of protein in a crossover design by varying protein content of foods and measuring self-reported hunger or satiety for several hours thereafter. Rolls et al. (49) reported that a MP preload in normal-weight women resulted in significantly lower ratings of hunger and increased ratings of satiety two hours after preload, compared to three other test preloads with the exception of a high starch preload. A similar crossover study conducted by Poppitt et al. (50) in lean women, offered *ad libitum* lunch on four occasions after preloads varying in macronutrient composition. Participants reported less hunger after the MP preload and consumed less of the *ad libitum* lunch (50). Effects of MP meals have not been consistently observed in feeding studies. A crossover study conducted by Blatt et al. (51) in normal weight women varied protein content by 5% increments (from 10 to 30% of kcal) in entrées consumed *ad libitum* by substituting animal protein for starchy ingredients, matching for fat content, palatability, and appearance. In this study, ratings of satiety and daily energy intake did not differ based on protein distribution of the diet (51). Caution should be taken in the interpretation of these results for application to weight-loss interventions as these trials were conducted in lean participants and were short-term. Overweight/obese individuals may have different physiologic responses to energy intake which may influence appetitive responses and energy balance (52). Additionally, short-term changes in appetitive hormones and energy intake may occur acutely in an unfamiliar, experimental setting and may be compensated for later (53), signifying the importance of more long-term studies.

Adherence to an ERD is a strong predictor of weight loss success (15,17,54,55). Increased satiety and decreased hunger within an ERD may promote BW loss by maintaining

dietary adherence. Though MP within an ERD may increase satiety, decrease hunger and reduce total energy intake, several studies have controlled energy intake between MP and SP diets (31,36), making any effect of protein on satiety, hunger or energy intake difficult to interpret. Additional research examining an effect or dose response of MP within an ERD or *ad libitum* eating condition on satiety, hunger and energy intake in overweight/obese individuals is warranted. Additional investigations including the measurement of orexigenic and anorexigenic hormones in overweight/obese adults may further support a mechanism for a protein-induced reduction in appetite.

Considerable evidence supports an effect of MP dietary patterns on improved BW loss during weight-loss interventions. In a 64-week study conducted by Clifton et al. (56), overweight/obese women reporting protein intake in the upper tertile (calculated from dietary records) had greater BW loss than women reporting protein intakes in the lower tertile. Clifton et al. (57) later examined participants with elevated baseline TG and found that adults in the MP (27% of kcal) ERD group lost significantly more BW, FM, and CAT than adults in the SP diet (16% of kcal) group. Similarly, a 1-year study by Keogh et al. (58) in free-living men and women with hyperinsulinemia and obesity found that protein intake as a percentage of total energy predicted BW loss after one year. Mecking et al. (30) reported that premenopausal women with overweight/obesity consuming a carbohydrate to protein ratio of 1.5:1 within an ERD, with and without exercise, experienced significantly more BW loss after 12 weeks than women consuming a standard 3:1 carbohydrate to protein ratio ERD, with and without exercise.

In addition to overall BW loss, MP ERDs may enhance FM loss. Josse et al. (26) reported that healthy premenopausal women with overweight/obesity consuming a MP (30% of kcal) ERD reduced FM and body fat percentage (BF%) to a greater extent than women consuming an

ERD including SP (15% of kcal). The MP group also lost significantly more CAT, even though all women lost similar amounts of BW throughout the 16-week study. A study by Lee et al. (59) reported that two ERDs including meal replacements (MR) resulted in significant reductions in BW and FM regardless of whether the MR was MP or SP. However, when only participants reporting greater than 70% dietary compliance over the 12-week study were analyzed, FM and CAT decreased more in the MP MR group (3.5 and 2.2 kg, respectively) than in the SP MR group (2.3 and 1.3 kg, respectively) (59). Finally, Due et al. (60) reported that overweight men and women consuming a MP (25% of kcal) ERD achieved greater BW (9.4 vs. 5.9 kg) and FM (7.6 vs. 4.3 kg) reductions after six months, compared to a SP (12% of kcal) ERD, respectively. These differences were no longer present after six months of weight-loss maintenance. Compared to the SP group, adults in the MP group reduced waist circumference and CAT to a greater extent after six months, and these reductions were maintained after one year (60).

Optimally, BW loss should be due to loss of FM and not FFSTM. Due to its high metabolic activity and effect on energy expenditure, preservation of FFSTM with BW loss may be essential for maintaining BW loss (61). A meta-analysis conducted by Krieger et al. (2006) identified dietary protein intake as a significant predictor of FFSTM retention during BW loss (62). Layman et al. (31) found that a MP (1.5 g protein/kg BW) ERD with and without exercise resulted in greater BW and FM losses compared to a SP (0.8 g protein/kg BW) ERD with and without exercise in overweight/obese women over four months. The combined effects of diet and exercise were additive for improving body composition, with the exercising MP intervention group exhibiting the greatest loss of FM with no significant loss of FFSTM (8.8 and 0.4 kg, respectively), and the SP ERD without exercise losing the least amount of FM and a significant amount of FFSTM (5.0 and 2.7 kg, respectively). Leidy et al. (38) found similar results in

FFSTM change when overweight/obese women consuming a MP (30% of kcal) ERD for 12 weeks lost significantly less FFSTM than women consuming a SP (18% of kcal) ERD (1.5 vs. 2.8 kg, respectively). Both groups lost significant and similar amounts of BW and FM.

Several interventions that support beneficial BW loss with MP ERDs may be difficult to generalize due to differing outcomes in men and women. In a study conducted in hyperinsulinemic men and women with obesity, Farnsworth et al. (27) found that energy intake, BW and FM losses after 12 weeks of weight loss followed by four weeks of weight maintenance were similar between a MP and SP (27% or 16% of kcal, respectively) ERD group. In women only, FFSTM was better preserved with the MP diet. Similarly, in a study conducted by Parker et al. (35) in adults with type 2 diabetes, women consuming a MP (28% of kcal) ERD lost significantly more BW (6.0 vs. 4.2 kg, respectively), FM (5.3 vs. 2.8 kg, respectively) and CAT (1.3 vs. 0.7 kg, respectively) over eight weeks of energy restriction followed by four weeks of energy balance than women on a SP (16% of kcal) ERD. Conversely, these endpoints improved in men consuming the SP ERD. Reported differences in BW and body composition outcomes by gender truly may be due to different responses in these populations. However, small sample sizes of men in the aforementioned studies (seven and ten men, respectively) may not have represented typical outcomes in males (35). Additionally, these studies were conducted in glucose intolerant adults who may respond differently to dietary perturbations compared to adults with normal glucose regulation. Additional studies with hyperinsulinemic and non-hyperinsulinemic participants, evaluated for sex differences, may be necessary to clarify reported outcomes in these populations.

Long-term maintenance of BW loss is not explored in many intervention trials. In studies that do measure BW maintenance, outcomes are typically similar between intervention groups

after one year or more (15,54,60,63,64). Brinkworth et al. (64) reported that obese men and women with type 2 diabetes consuming either a MP (30% of kcal) ERD or a SP ERD (15% of kcal) for eight weeks retained significant reductions in BW with no effect of diet after one year of maintenance. Overweight adults consumed one of four ERDs, with protein comprising either 15 or 25%, carbohydrate between 35 and 65%, and fat comprising 20 or 40% of total energy in a 2x2 factorial design (15). These macronutrient goals were not met at 6 or 24 months of intervention in any diet group. Still, all participants lost an average of 6 kg of BW by six months and began to regain BW by 12 months with similar weight-loss outcomes in all diet groups by 24 months (15). Similarly, a recent 6-month, multi-center weight-loss trial examined four ERDs differing in protein, carbohydrate and fat content and found significant FM and FFSTM losses with no differences between diet interventions in free-living participants (53). After 2 years, participants regained 40% of weight losses with no group differences. Study participants did not meet macronutrient goals during the intervention; protein comprised 18 and 22% of total energy intake in the SP and MP intervention groups, respectively (54). Gardner et al. (16) conducted a 1-year trial in premenopausal overweight/obese women to examine outcomes with four commercial weight-loss diets. At six months, diets ranged in protein, carbohydrate and fat content, respectively, and included the Ornish diet (18, 53, 29%), the LEARN diet (19, 49, 32%), the Zone diet (20, 44, 36%) and the Adkins diet (23, 30, 47%). Women in the Atkins group, with highest protein intake, lost more BW compared to all other diet groups at two and six months; however, at the end of one year, weight loss in the Atkins group remained significantly greater compared to the Zone diet but not to the Ornish or LEARN diets.

Overall, a MP ERD appears to promote BW loss and positive body composition changes in adults, compared to a SP ERD. Though an energy deficit is paramount for effective weight

loss, it seems that a diet higher in protein content may influence BW loss, as well as changes in body composition, resulting in proportionately greater loss of FM and preservation of FFSTM. This may be accomplished though various mechanisms including influence on hunger, satiety and the thermic effect of diet. Though maintenance of FFSTM achieved with MP ERDs may more effectively maintain BW loss, long-term studies do not appear to support beneficial weight-maintenance outcomes with MP diets. Beneficial effects of MP ERDs relative to SP ERDs may be especially evident in hyperglycemic populations (35,65); however, this appears to be primarily modulated by dietary carbohydrate content or percentage of total energy (66).

High-protein Energy-restricted Dietary Interventions and Effects on Metabolic Parameters

Improvements in select metabolic parameters with MP ERDs beyond those seen with SP ERDs have been observed (19,37,67), though evidence is mixed, with some studies showing no differences between MP and SP ERDs (38,56). Benefits of MP within an ERD on serum TG concentration have been most commonly reported (16,27,28,31,33,36,68,69), with mixed results for effects on HDL-C concentration (31,36,68,69). Observational studies and clinical trials suggest that increased protein intake also may reduce blood pressure (BP) (70,71), though this effect appears most substantial when the protein source is from plants rather than animals (72,73). Diets with MP content examining changes in metabolic health parameters, including serum TG, total cholesterol (TC), LDL-C and HDL-C, and BP and markers of glucose tolerance are briefly reviewed here.

Layman et al. (36) examined effects of a MP (30, 41 and 20% of kcal from protein, carbohydrate and fat, respectively) ERD and a SP (16, 58 and 26% of kcal from protein, carbohydrate and fat, respectively) ERD over 10 weeks. With similar energy intakes and reductions in BW and TC, fasting serum TG concentration was reduced by 20% from baseline in

the MP group with no change in the SP group. Additionally, the ratio of TG/HDL-C significantly decreased in the MP group, whereas the ratio did not change in the SP group. Glucose and insulin homeostasis also were examined; elevations in insulin response and reductions in postprandial blood glucose concentrations after test meals were found in the SP group but not MP group (74). In a similar study, Layman et al. (31) reported that after four months, women with overweight/obesity consuming a MP (1.5 g protein/kg BW) ERD with and without exercise had greater reductions in TG and maintained higher concentrations of HDL-C than women consuming a SP (0.8 g protein/kg BW) ERD with and without exercise. Participants in the SP ERD group also experienced larger reductions in TC and LDL-C. A randomized dietary intervention over six months conducted by Skov et al. (33) compared two *ad libitum* fat-reduced diets in healthy, overweight/obese men and women. TC and HDL-C decreased similarly in the MP (25% of kcal) and SP (12% of kcal) groups, but the MP diet group significantly decreased fasting plasma TG and free fatty acids.

Individuals with risk factors for certain chronic diseases common with obesity may fair particularly well with a MP ERD (28,57,68). Noakes et al. (28) found that after 12 weeks, overweight women consuming a MP (34% of kcal) ERD reduced TG concentration to a greater extent than participants consuming a SP (17% of kcal) ERD (0.30 vs. 0.10 mmol/L, respectively). Moreover, participants with elevated serum TG at baseline had even greater reductions in TG concentration (0.59 vs. 0.03 mmol/L, respectively), and lost more FM with the MP ERD compared to the SP ERD. Fasting LDL-C, HDL-C, glucose, insulin and free fatty acid concentrations decreased with BW loss regardless of diet.

MP ERDs also may benefit hypertensive individuals and those with metabolic syndrome. A MP (25% of kcal; one-half from plant sources) ERD produced greater reductions in TG, LDL-

C, HDL-C and systolic BP compared to a carbohydrate-rich ERD modeled after the Dietary Approaches to Stop Hypertension (DASH) diet (15% of kcal from protein) in a 6-week randomized crossover feeding study in men and women with pre-hypertension or stage 1 hypertension (68). Energy intake changed similarly between groups, and though this was not a weight-loss study, BW loss occurred and was similar between groups. The MP diet was especially effective at lowering systolic BP in those with hypertension (68). Muzio et al. (75) reported that both a MP (19% of kcal) and a SP (13% of kcal) ERD reduced BW, diastolic BP, TC, and blood glucose and insulin significantly after five months in obese adults diagnosed with metabolic syndrome using Adult Treatment Panel III criteria. While the SP ERD resulted in a significant decrease in LDL-C concentration, the MP ERD was associated with greater reduction in TG level and a reduced incidence of hypertension, mostly due to a significantly greater reduction in systolic BP in the MP group (75).

Insulin resistant individuals also may respond more positively to a MP ERD. A trial conducted by Parker et al. (35) in men and women with type 2 diabetes found that participants improved fasting and 2-hour plasma glucose and insulin concentrations, TG concentration and BP, with either a MP or SP ERDs. However, the MP ERD group had a significantly greater reduction in LDL-C compared to the SP diet group. Another study conducted in obese hyperinsulinemic men and women found that with similar energy intake, BW and FM losses between groups, participants following a 12-week MP ERD had a significantly greater reduction in serum TG concentration relative to participants following a 12-week SP ERD. Additionally, adults consuming the MP diet had a significantly lower glycemic response to meals, though there was no effect of diet on the reduction in postprandial serum insulin, after four additional weeks of weight maintenance or by week 16 (27).

Improvements in metabolic parameters are often observed with BW loss regardless of dietary protein intake (38,56). Differences in BW and FM losses between diet groups add difficulty to the interpretation of changes in metabolic health indicators. Reduction of BW and FM by any dietary approach may improve obesity-related metabolic risk factors in overweight/obese adults (76). As previously discussed, reduced BW and FM and preserved FFSTM are often observed with MP ERDs compared to SP ERDs, which may partially explain additional benefits in metabolic health markers observed with MP diets. Beyond these anthropometric changes, however, improvements in TG concentration are documented in studies in which similar BW (27,36,71) and FM losses (27,75) between MP and SP diet groups were included, suggesting other mechanisms of action for a MP ERD. MP ERDs may have the potential to reduce metabolic markers to a greater extent than SP ERDs. Additional research is needed to support definitive statements about effects of MP ERDs on metabolic indicators of health and disease so that evidence-based recommendations for dietary approaches to health promotion may be made (Table 1).

High-dairy Energy-restricted Dietary Interventions

With 8 g of protein per cup of fluid milk, dairy products are rich in complete protein, and non-fat dairy intake is associated with higher dietary quality (77,78). Scientific evidence indicates that consuming the recommended three daily servings of low-fat or fat-free (i.e., non-fat) dairy foods may help to reduce the risk for certain chronic diseases (79). Increased fluid milk and yogurt intake has been implicated in improving health status with and without corresponding BW loss (22,26,80–83). Both calcium and the branched-chain amino acid, leucine, found in dairy foods have been regarded as contributors to a proportional increase in FM loss and preservation of FFSTM during BW loss (84–86). Additionally, dairy foods are a good source of potassium

and magnesium which may aid in the prevention of chronic disease (77,87). As dairy foods contain nutrients and bioactive components that may contribute to health maintenance, studies have reported improvements in BP and glucose metabolism with diets high in dairy. Observational and randomized control trials support an inverse association between dairy food intake and BP (88–91). Indeed, low- and non-fat dairy is an important part of the DASH diet, designed to reduce hypertension and coronary heart disease risk (92–94). Low- and non-fat milk and yogurt also may improve glucose metabolism as several prospective studies report that diets higher in low-fat dairy reduce the risk of type 2 diabetes (95–97).

Preserving lean, metabolically active FFSTM during BW loss may be essential for the maintenance of BW loss (61). Better maintenance of FFSTM may be achieved with an ERD that is high in protein (27,36–38), and these results may be further extended with MP ERDs that include higher low- and non-fat milk and yogurt content (26,27,80). Josse et al. (26) reported that women with overweight/obesity consuming a MP, high-dairy (6-7 servings of low-fat milk or yogurt/day) ERD for 16 weeks reduced FM, BF% and CAT to a greater extent than women consuming an ERD with SP and recommended dairy (3-4 servings of low-fat milk or yogurt/day). The MP, high-dairy group also increased FFSTM from baseline, while women consuming the SP, standard-dairy diet maintained FFSTM, and women consuming a SP, low-dairy (<1 serving of low-fat milk or yogurt/day) diet lost FFSTM after 16 weeks. All women exercised daily. A study conducted by Farnsworth et al. (27) reported that women consuming a MP (30% of kcal) ERD with 45% of protein from non-fat milk and low-fat cheese and yogurt lost less FFSTM after 16 weeks than women in a SP (15% of kcal) ERD group where 18% of protein was from non-fat milk. Zemel et al. (80) found that obese adults who consumed three servings of non-fat yogurt a day within an ERD for 12 weeks lowered FM and CAT and

attenuated FFSTM loss significantly more than adults consuming one serving of dairy per day or less. Similar energy deficits and proportions of macronutrients, including 18% of energy from protein, were applied across group.

A suggested mechanism for FFSTM maintenance with increased dairy intake is leucine-regulated muscle protein synthesis during catabolic conditions (85). Circulating leucine may simulate protein synthesis, enhancing preservation of FFSTM during BW loss (46). However, a recent study found no increase in muscle protein synthesis in adults at rest after consuming 3.5 g of leucine compared to adults consuming 1.8 g of leucine (98). Both groups consumed a 10 g supplement of essential amino acids that contained the experimental leucine levels. These findings suggest that supplemental leucine within an essential amino acid mixture may be unnecessary for stimulation of muscle synthesis. In contrast, leucine intake during adherence to an ERD with energy expenditure exceeding energy intake may be necessary to induce beneficial effects of leucine. Alternatively, leucine may only yield positive effects within the context of the food (i.e., dairy) matrix.

Children and adults in the US do not consume recommended amounts of dairy foods (99). Inadequate dairy consumption is especially concerning during ERDs when limited energy intake coincides with lesser micronutrient intake; dairy products provide nutrients that are vital to skeletal and general health (100). The inclusion of recommended amounts of non-fat dairy in an ERD may improve diet quality and provide exceptional benefit to BW loss, BW maintenance, body composition changes and select metabolic outcomes (101,102). To better support BW loss, improving non-fat dairy intake may be an important target for public health and nutrition education during weight-loss intervention (103).

The Calcium Hypothesis

An inverse relationship between dietary calcium and adiposity has been reported in the literature (104,105). Likewise, there have been several studies documenting a relationship between high-dairy and/or high-calcium intake and energy balance (22,80,84,102,106). Some studies report that dairy intake or calcium supplementation may accelerate BW and FM loss in the context of an ERD in overweight/obese individuals. Zemel et al. (84) reported that adults assigned to an ERD including 1200 mg of calcium per day, consumed either as dairy foods or as calcium supplements, lost significantly more FM over six months than adults consuming an ERD including 400 mg of calcium from dairy and placebo supplements. Similarly, the same researchers reported that a group of adults consuming an ERD including 1100 mg of dietary calcium per day had a significantly greater loss of FM and CAT after 12 weeks than a group consuming an ERD with only 400-500 mg calcium intake per day (80). In both of these studies, individuals who were consuming one-half of the RDA for calcium (i.e., only 500 mg calcium/day) (107) were included. Because dairy or calcium-supplemented groups were compared to controls with habitual calcium intakes well below recommendations, these results are difficult to generalize to individuals consuming recommended or slightly inadequate levels of calcium.

In contrast, several studies have demonstrated no effect of calcium or dairy products on BW or FM losses. No significant differences in BW or FM change were observed in a study by Shapses et al. (108) in women with overweight/obesity supplemented with approximately 1500 mg of calcium per day or consuming approximately 600 mg of dietary calcium per day in a 25-week ERD trial. In a similar 12-week study by Wagner et al. (109) three ERD groups supplemented with approximately 1500 mg calcium each (as calcium lactate or calcium phosphate or dairy foods) had similar significant BW losses compared to a 750 mg dietary

calcium control ERD group. The 1500 mg dairy foods diet group, in fact, lost significantly less FM than the control diet, though all groups had similar energy intake. A study by Jensen et al. (110) reported similar BW loss over three months between a treatment group consuming 1800 mg of calcium and a control group consuming 800 mg of calcium. Finally, a study conducted by Gunther et al. (111) demonstrated no significant differences in BW or FM change after one year in normal-weight, low-calcium consuming women not actively pursuing weight loss who were randomly assigned to a control (approximately 700 mg calcium/day), medium-dairy (approximately 1000 mg calcium/day) or high-dairy (approximately 1100 mg calcium/day) intervention group.

Nevertheless, potential mechanisms for an effect of calcium intake on energy balance and adiposity have been proposed in the literature. Plasma calcium may be implicated in the suppression of calcitrophic hormones such as vitamin D, which are suggested to decrease calcium influx into the adipocyte, resulting in reduced expression of the fatty acid synthase complex (112). Suppression of this complex may decrease lipogenesis and stimulate lipolysis (112). Additionally, an increase in vitamin D may potentially reduce expression of uncoupling protein 2 (UCP2) (113). Increased expression of UCP2, expected when plasma calcium levels are normal and active vitamin D circulation is relativity low, may be implicated in increased adipocyte apoptosis (113). Reduced lipid absorption has also been proposed as a potential mechanism for the effect of calcium on energy balance. A diet high in calcium has been shown to increase lipid oxidation and reduce lipid absorption due to proposed calcium-fatty acid insoluble soap formation in the intestines (114).

Published literature suggests that the greatest benefit of additional calcium in an ERD occurs in individuals who report low calcium intakes at baseline (80,84,115) or when compared

to calcium-insufficient control conditions (80,84). This “calcium threshold” effect (115,116) suggests that in order to incur results from calcium supplementation during an ERD, an individual would have to have an habitual calcium intake below the threshold. Calcium supplementation may not have pronounced effects on weight loss for individuals consuming a typical daily amount that is more than the threshold (116). Systematically reviewing data from various trials for evidence of a threshold effect can be difficult as baseline calcium intakes are not always reported; however, it is possible to compare diets where supplementation of both groups is above a threshold. In the current review, studies citing an effect of calcium intake on BW included participants with baseline and control group calcium intakes of less than 500 mg (80,84). Meanwhile, studies reporting no effect of supplemental calcium on weight loss included control groups with calcium intakes at or above 600 mg (108–111). These results support a threshold effect of roughly 600 mg of calcium intake.

Summary of Energy-restricted Dietary Interventions

With examination of MP and high-dairy ERDs, it appears that this dietary pattern may produce significant improvements in BW and FM losses, with preservation of FFSTM and improvement in select markers of metabolic health in trials of less than 6 months. MP diets may have greater potential to decrease TG concentration. Production of an energy deficit is ultimately necessary for weight loss. Various mechanisms described with MP diets, including influence on satiety, hunger and the thermic effect of diet, may help to achieve an energy deficit. Maintenance of FFSTM achieved with MP, high-dairy ERDs also may be more effective in maintaining BW loss over time.

Ultimately, BW and FM losses seem to be the overriding factor for improvements in metabolic health outcomes. Several of the aforementioned studies show that ERDs of varying

macronutrient composition can be used to precipitate BW loss and confer metabolic health benefits. Due to variable blood lipid changes, certain populations such as adults with hypertriglyceridemia, hypertension and type 2 diabetes may benefit from increasing protein and reducing carbohydrate content within an ERD.

Energy Density and Weight Loss

Dietary energy density (ED) has been suggested in laboratory-based and randomized controlled trials to be a significant determinant of energy intake and, therefore, energy balance (117–119). ED is mainly influenced by the consumption of fruits and vegetables which contribute water and fiber to the diet and by fat which has a higher ED (9 kcal/g) than either carbohydrate or protein (each 4 kcal/g) (118,120). Short-term, laboratory-based studies have found that individuals consume less energy when presented with foods lower in ED than with similar foods with greater ED (117,118). A study conducted by Bell et al. (117) examined normal-weight women during three, 2-day test sessions. Women were presented with main entrée meals varying in ED from low, medium and high (0.8, 1.1 or 1.3 kcal/g, respectively) that were consumed *ad libitum* in addition to low-energy side dishes that were consumed in full (117). As women consumed a similar weight of food across all test conditions, significantly more energy was consumed during the high ED condition compared to the other two test conditions (117). In a separate study, Rolls et al. (118) examined both lean and obese women over four, 4-day test periods in which both ED and fat content of test items consumed in full were manipulated while entrées and side dishes were consumed *ad libitum.* With one test period serving as the control, remaining test periods included test items that were either low-fat (16% of kcal) and low-ED (1.1 kcal/g), low-fat and high-ED (1.6 kcal/g), or high-fat (36% of kcal) and high-ED. With no differences in palatability, both obese and lean participants reduced energy

intake of side dishes by 16% in the low-ED compared with the high-ED condition. Fat content had no significant effect on energy intake (118).

Long-term, randomized clinical trials indicate that a greater reduction in energy intake and BW may result with consumption of a weight-loss diet reduced in ED (119,121). Rolls et al. (121) observed adults during a 12-month randomized clinical intervention including six months of weight loss and six months of weight-loss maintenance. Women consumed 1-2 servings of low-ED soup or two servings of high-ED savory snacks daily within isocaloric diets (121). At one and two months, BW loss was correlated with the decrease in ED from baseline. A control group consuming no snacks and two soups per day lost significantly more BW than the savory snack group after one year (121). Similarly, Ledikwe et al. (119) examined the effect of reducing ED on energy intake and BW in a 6-month randomized controlled trial including behavioral-based interventions. Adults with elevated BP were randomized to either an advice group receiving one 30-minute counseling session, or to one of two treatment groups which included 18 behavioral weight-loss intervention sessions, with or without the implementation of the DASH diet. All groups reduced ED, though there were differences between groups. When participants were combined and analyzed by tertile of ED reduction from baseline, participants with the greatest reduction of ED from baseline lost more BW compared to participants in the other two tertiles. Participants in the highest and middle tertiles of ED reduction increased the weight of foods consumed while decreasing energy intake. These groups also had increased fruit and vegetable intake, favorably changing vitamin and mineral intakes (119).

As several complex eating patterns have been investigated for effectiveness in randomized clinical trials with a small degree of success (15,16,33), a simple message as part of a straight-forward dietary intervention may be more easily followed and maintained long term,

compared to dietary interventions which include complex and specific dietary goals. ED is a simple feature of dietary intake that individuals can consider during food and meal selection for overall reductions in energy intake. Altering the ED content of the diet does not necessarily require highly organized eating patterns or changes in dietary macronutrient distribution common in many dietary weight-loss interventions (16,122). Furthermore, diets that are low in ED via inclusion of increased fruit and vegetable consumption (120) may increase diet quality and help to meet individual micronutrient recommendations (123), further decreasing the need to carefully consider diet quality of weight-loss diets with modified macronutrient compositions.

Evidence suggests that food components of the diet should be considered, and beverages should be excluded in the analysis of ED and BW change (124,125). Epidemiological studies have indicated that inclusion of beverages in calculation of ED introduce large within-individual variance (124). Similarly, investigations into the contribution of caloric beverage to satiation have indicated that caloric beverages add a significant amount of energy intake within a meal without affecting satiation, thereby contributing to excess energy intake (126). As beverages may not be as satiating as low ED foods, such as soup and non-fat yogurt, these low ED foods which must be eaten may be a potent tool in the reduction of energy intake. The inclusion of low ED eaten foods may provide a greater impact on satiety while reducing overall dietary ED and energy intake. Therefore, the incorporation of non-fat dairy foods into an ERD may benefit diet quality and reduce ED and increase satiety, if consumed in a non-drinkable, yogurt state.

Physical Activity Interventions

The US Department of Health and Human Services suggest in the 2008 Physical Activity Guidelines for Americans that adults engage in 2.5 hours per week of moderate-intensity physical activity, such as brisk walking, for health promotion (127). Health benefits from

physical activity include maintaining or reducing BW, reducing FM and chronic disease risk factors, increasing FFSTM and improving bone mineral density (BMD) (128–130). Habitual physical activity is associated with decreased appetite and better long-term energy intake regulation (131). Additionally, physical activity is associated with reduced cardiovascular disease risk, decreased BP, reduced serum glucose concentration, greater level of HDL-C (132–135), and may lower markers of inflammation (136). However, experts suggest that physical activity without reduction in energy intake only modestly influences BW (137,138).

Coincident with an ERD, physical activity can provide additional benefits to BW and body composition beyond dietary strategies alone (26,30,139,140). Goodpaster et al. (141) found that obese adults (BMI=35-40) demonstrated significantly greater BW loss after six months of an ERD and physical activity intervention than with an ERD only intervention (10.9 vs. 8.2 kg, respectively)(141). Krieder et al. (140) reported that obese women participating in an ERD intervention with a supervised exercise component reduced BW, FM, and waist and hip circumferences to a greater extent after 10 weeks than obese women consuming an ERD without supervised exercise. A meta-analysis evaluating six ERD and exercise trials reported that ERD with exercise interventions produced a 20% greater initial BW loss and a 20% greater sustained BW loss after one year compared to ERD only interventions (142). Wycherley et al. (32) found that adults with type 2 diabetes who added resistance exercise to an ERD reduced BW and FM to a greater extent than adults who only changed dietary patterns. This effect of resistance exercise negated any protein-to-carbohydrate ratio effect also tested in this study (32). Specifically, MP ERD interventions including groups with and without exercise have revealed independent and additive effects of diet and exercise on body composition (30,31). Thus, it seems that increasing

daily exercise while adhering to an ERD is advantageous to BW loss and body composition outcomes.

Increased physical activity can potentially increase daily energy expenditure. Modified energy intake with increased energy expenditure may allow for adequate nutritional needs to be met while still achieving a net energy deficit. Maintaining some degree of physical activity after weight loss has been identified as the best predictor of BW loss maintenance (143,144). Increased physical activity in combination with an ERD may have the greatest potential to promote BW loss and improve body composition measurements.

Free-living vs. Controlled-feeding Approach to Energy-restricted Dietary Interventions

The efficacy of a specific dietary protocol in human participants can be examined in a controlled-feeding setting where intake quantity, food and dietary composition and timing is carefully managed and monitored (145,146). In this environment, compliance to dietary protocol can be measured and dietary intake recommendations strictly followed with provision of meals or key dietary components (145). These studies are in contrast to studies involving free-living participants who self-select food choices within intervention-directed guidelines, designed to mimic a "real world" setting (146). Studies with free-living participants test the effectiveness of a given protocol, when prescribed to an individual responsible for carrying out lifestyle modifications on his or her own. This fundamental difference between study designs is essential to the interpretation of weight-loss interventions with lifestyle modifications.

In a recent study by Krebs et al. (63) in free-living adults with type 2 diabetes, participants consumed a MP (30% of kcal) ERD or a SP ERD (15% of kcal) for 12 months, followed by 12 months of weight loss maintenance. Interventions were designed to be achievable in most healthcare systems, without providing meals but with dietary information and motivation

offered every two weeks to study groups of 8-12 participants. Adults in the MP ERD and SP ERD groups reduced BW and hip circumference and maintained these reductions to 24 months, with no differences between groups. The MP group was unable to reach intervention-prescribed dietary recommendations for protein intake in this free-living setting (63). A study by Campbell et al. (147), examining the effectiveness of three ERD with protein-to-carbohydrate ratios of 1:1, 1:2 or 1:4, found no differences among groups in BW loss and several metabolic parameters. In this study, the authors report that participants in the 1:1 and 1:4 protein-to-carbohydrate ratio groups reported difficulty following dietary plans and were unable to meet their protein intake goals. Participants in the 1:2 protein-to-carbohydrate ratio diet intervention reported ease of compliance and had greater improvements in FM reduction and preservation of FFSTM compared to the other two diets groups (147).

To minimize behavioral aspects of dietary compliance and focus on efficacy, several studies detailed in this review utilized highly controlled procedures to ensure excellent compliance to dietary treatments. Feeding procedures in these studies included providing daily meals or supplying the majority of food consumed (27,28,31,33,35,36,38,60,68,148). Several studies included only participants achieving significant BW loss or those participants defined as compliant in statistical analyses (34,59,67). The strong associations between MP or high-dairy ERDs and improved weight loss outcomes presented in many of these studies are generalizable only then to an extremely compliant population. Though it is important to understand the efficacy of a dietary protocol in inducing desired change, intervention effectiveness is more important to understanding the applicability of a dietary pattern to the general population. Controlled-feeding studies that report additional benefits of MP ERD beyond weight-loss set a foundation for the conduct of similar experiments including free-living individuals.

Meal Replacement Approach to Energy-restricted Diet Interventions

There is substantial evidence that MRs are effective tools for facilitating and maintaining BW loss. MRs are portion-controlled food(s), frequently of 200 to 300 total kcals and replace a conventional meal with considerably less energy. Commonly known MR products such as Slim-Fast® first entered the market in the 1970s, while other increasingly convenient forms of MR products, such as food bars, became available soon after. In general, these MR products are substituted for one conventional meal and sometimes also a snack, while being higher in protein and relativity low in energy (149). Many commercial MR products are usually fortified with vitamins and minerals in an effort to prevent long-term micronutrient deficiency potentially experienced during ERD adherence (150).

There are several suggested mechanisms for how MRs result in reduced daily energy intake. Many MRs utilize MP content, and as previously reviewed, protein may have a greater effect on hunger and satiety then other macronutrients (38,40). Additionally, the high-fiber content of some MRs may improve feelings of fullness (151). A recent study conducted by Levitsky et al. (149) suggests that the mechanism responsible for BW loss with MR use is reduced energy intake from controlled portion sizes and not the specific macronutrient composition of the MR. In this study, participants ate weighed meals provided from a buffet for two weeks. In the following two weeks, they were asked to select one item for lunch from six commercially available portion-controlled foods. All other meals and snacks were recorded and eaten *ad libitum*. Energy from the portion-controlled lunch was roughly 20% protein, 50% carbohydrate, and 30% fat, and none of the lunches were particularly high in any one nutrient. Participants experienced an approximate 250 kcal reduction in daily energy intake resulting in small but significant BW loss with no indication of caloric compensation (149).

Studies examining the effects of MRs as part of a weight-loss intervention report positive outcomes in BW, body composition and select metabolic parameters relative to weight-loss interventions without MRs. Heymsfield et al. (152) pooled analyses from six interventions of at least three months duration in overweight/obese adults. Included studies compared ERDs with one or two meals replaced daily by commercially available, energy-restricted products (i.e., MR) to participants randomized to a conventional ERD plan. Participants following a MR ERD plan had significantly greater BW loss compared to those in the conventional ERD group (152). In a 6-week weight-loss study conducted by Konig et al. (153), participants replacing two daily meals with a low-calorie, high soy-protein drink lost significantly more BW and FM (6.4 and 5.1 kg, respectively) than participants consuming a low-fat ERD without MR (3.1 and 2.8 kg, respectively). Participants in the MR group also had greater reductions in waist circumference and TG concentration (153).

Long-term studies examining the use of MRs in maintenance of BW loss have been promising. An assessment of the effectiveness of MR strategies for weight management conducted by Davis et al. (154) reported that obese men and women consuming an ERD that included a MR lost significantly more BW (12.3%) during a 16-week weight-loss intervention compared to a group consuming an ERD without a MR (6.9%). Though the MR group regained more weight during the following 24 weeks of weight maintenance, 62% of MR participants maintained BW loss of at least 5% at week 40 compared to only 30% in the control group (154). In a study by Vazquez et al. (155), adults who successfully lost at least 5% of BW during six months of a weight-loss phase were randomized into one of two weight-loss maintenance groups for an additional six months. The experimental group replaced dinner with a low-calorie diet formula, while the control group consumed a prescribed weight-maintenance ERD. After six

months of weight maintenance, 84% of participants in the MR group maintained BW or continued to lose BW, while 58% of the control group maintained BW loss (155). In a study conducted by Cheskin et al. (156), overweight/obese adults with type 2 diabetes who included a MR into an ERD for 34 weeks demonstrated greater BW loss and regained less BW after 1 year of weight-loss maintenance compared to adults consuming a standard maintenance diet. In addition to superior weight loss and maintenance, retention rate and self-reported ease of adherence were significantly higher throughout the study in the MR group. Both groups similarly improved metabolic parameters (156).

In conclusion, MRs may be a useful tool in moderating energy intake for the purpose of BW loss and BW maintenance, with potential benefit to diet quality within an ERD (157). Moreover, a MR approach to weight-loss intervention has been successfully implemented in persons with diabetes (150,158), metabolic syndrome (59) and low-income individuals (159). Potential advantages of MR use include broad availability of MR products and minimal need for specialist expertise, both serving to increase compliance and ease of use.

Skeletal Health

Importance of Bone Health Research

Direct medical costs due to osteoporosis are estimated at greater than 20 billion dollars in the US. Osteoporosis is a common disease, the prevalence of which is estimated to increase to more than 14 million people by 2020 (160). Osteoporosis manifests as fractures which may occur in many skeletal sites throughout the body causing significant morbidity and mortality. As the population of individuals greater than 50 years of age is growing, frailty and falls in the general population are expected to rise, increasing the burden of this disease. Because BMD is a measure of the concentration of mineral in a defined section of bone, it can be used to estimate

risk of fracture. Increased BMD is associated with decreased risk in fracture incidence (161). Several modifiable factors can be altered to attenuate age-related loss of BMD and prevent weight-loss induced BMD loss. These factors include optimizing dietary intake of select nutrients including protein, calcium and vitamin D, engaging in physical activity and reducing inflammatory status.

Bone Remodeling: A Continuous Process

Localized bone remodeling takes approximately six months in adults (162,163) and functions to maintain bone density and strength by replacing old and damaged bone with new bone (164). The constant balance of breakdown and formation makes up a complex process of bone remodeling. Bone renewal is successfully balanced when resorption and formation are perfectly coupled. In short, osteoclasts act to resorb existing mineralized bone, secreting enzymes to break down old bone. Osteoblasts form new bone by secreting collagen, non-collagenous proteins and hydroxyapatite crystals to construct an extracellular matrix that is subsequently mineralized.

Bone remodeling is initiated by osteoclast precursor cells becoming attracted to bone remodeling sites and differentiating into mature osteoclasts on the bone surface. Osteoclasts are multinucleated cells which, once activated, become tightly sealed to the bone surface with a specialized membrane so that the resorption area may be isolated from extracellular fluid (165). Into this sealed area, osteoclasts secrete acid hydrolases which solubilize the mineral phase of bone matrix and reveal the organic matrix for degradation by proteases activated by the acidic pH (165,166), and thereby, release calcium and phosphorous into the bloodstream (167). Osteoclasts also secrete factors that regulate osteoblasts (168). Bone formation follows this process with the tightly regulated cellular differentiation and maturation of mesenchymal

progenitor cells into osteoblasts. Osteoblasts synthesize type I collagen and secrete proteoglycans, osteocalcin, alkaline phosphatase and other matrix proteins (169). Once fully differentiated, osteoblasts form matrix vesicles which accumulate calcium and phosphate to form hydroxyapatite crystals. Osteocalcin controls mineralization of the bone matrix by regulating the deposition of hydroxyapatite into gaps once occupied by osteoclasts (169). Bone formation may proceed for weeks before osteoblasts undergo apoptosis and turn into osteocytes (170).

The bone remodeling process is regulated by several growth factors, signaling peptides and inflammatory factors. These include osteocalcin, osteoprotegerin, receptor activator of nuclear factor-κB (RANK) and RANK ligand (171). RANK ligand released from bone marrow stromal cells and immature osteoblasts binds RANK expressed on osteoclast precursor cells, triggering their differentiation into mature bone resorbing osteoclasts. When bone resorption nears completion, osteoprotegerin, a decoy receptor for RANK ligand which is secreted from osteoblasts, inhibits RANK ligand from binding its receptor. This prevents further bone resorption and allows diversion of cellular differentiation toward osteoblastic formation (167).

As a dynamic tissue taking cues from its immediate environment, bone is influenced by hormonal milieu and mechanical forces exerted upon it. Reductions in estrogen, such as those seen during menopause (172), poor dietary intake of nutrients vital to bone synthesis (173) and excess inflammation (174) are all factors, among others, causing imbalance in the bone remodeling process. Tumor necrosis factor-α (TNF-α) and interleukin (IL)-6, inflammatory cytokines elevated in obesity (175), enhance the ability of RANK ligand to bind to RANK, and thereby, support bone resorption (176). Alternatively, mechanical loading and stress on bone tissue from repeated physical activity results in signaling for bone to increase its size, shape or density in order to better resist mechanical stress and adapt to reduce risk of fracture (177).

Therefore, biochemical and mechanical factors exerted upon bone may impact the delicate balance of bone remodeling. Should the bone remodeling process be uncoupled with bone resorption exceeding bone formation, bone loss likely occurs.

Nutrition and Bone Health

Adequate nutrition is essential in early development and lifelong maintenance of bone. In addition to recommended intakes of calcium and vitamin D, dietary protein represents a key nutrient for bone health. Much of the volume of bone is made up of protein, and this bone protein matrix is constantly turning over and remodeling throughout the life span (178). As a result, a steady supply of dietary protein is necessary to maintain bone structure and integrity. However, effects of MP diets on bone are somewhat controversial. Several studies support a moderate benefit of MP diets on BMD, while some report no effect. Protein source is also believed to modulate the effect of protein on bone. Commonly cited mechanisms for how increased dietary protein positively affects BMD include improved calcium absorption in the gut (179–181), stimulation of insulin-like growth factor-1 secretion (179,182,183) and accrual or maintenance of FFSTM (184,185).

Several studies have found that dietary protein benefits BMD. A cohort study in elderly women conducted by Meng et al. (185) found greatest bone mineral content (BMC) in women with the highest tertile of protein consumption (on average 1.6 g protein/kg BW) compared to women in the other tertiles. Because participants consuming the highest protein also had the highest FFSTM among all study participants, authors suggested that effects of protein on bone may have been partly mediated by effects of protein on muscle (185). Similarly, Dawson-Hughes et al. (186) saw favorable changes in total body and femoral neck BMD in elderly men and women in the highest tertile of protein consumption who were supplemented with calcium and

vitamin D for three years. There was no association, however, with those in the highest tertile of protein consumption who were not receiving calcium and vitamin D supplementation (186). Hannan et al. (187) reported that elderly men and women enrolled in the Framingham Osteoporosis Study in the lowest quartile of protein intake (between 0.2 and 0.7 g of protein/kg BW) had the greatest bone loss at femoral and spinal BMD sites after four years, even after controlling for confounders known to effect bone. This suggests that sufficient protein intake is important in maintaining or minimizing bone loss. Offering further support, a meta-analysis by Darling et al. (188) suggested a small positive effect of protein on lumbar spine BMD when examining randomized placebo-controlled trials in adults. Though several studies suggest a protective effect of increased protein intake on BMD, it appears that favorable bone outcomes may rely upon the concurrent intake of recommended amounts of calcium and vitamin D (186).

Consensus in the literature may support that sufficient protein intake is beneficial to BMD; however, the effect of MP diets and protein type (animal vs. vegetable) remains controversial. Concerns that a MP diet may cause acidosis and subsequent hypercalciuria have been suggested. Acid production is thought to be greatest with consumption of sulfur-containing amino acids and phosphate, found in meat, grain and dairy (189). A high acid load is believed by some researchers to reduce extracellular pH which may directly enhance osteoclastic activity. Using the skeleton as a reservoir of alkali, bone resorption may occur (190). Currently, there is no description in the literature for a biologically plausible mechanism functioning at physiological pH for effects of dietary acid on calcium balance and bone health (191,192). Evidence from a recent meta-analysis by Fenton et al. (191) does not support a causal association between dietary acid load and bone disease. Fifty-five studies in healthy adults, including 22 randomized controlled trials that altered dietary acid load were examined for evidence of

compromised bone health, including urine calcium excretion, calcium balance, BMD changes and incidence of fractures (191). Though urinary calcium excretion rates were elevated in many studies, calcium balance did not demonstrate loss of whole body calcium with higher net acid excretion (189,191).

Similarly, this mechanism was not supported in a crossover study conducted by Kerstetter et al. (181) using calcium isotopes to track calcium kinetics in women consuming diets of varying amounts of protein. This technique allowed researchers to estimate urinary calcium derived from the diet and that derived from bone. Women spent 10 days on an experimental diet with one of two protein levels (1.0 g/kg BW or 2.1 g/kg BW), followed by a two-week wash-out period. In addition to protein composition, both diets were matched for micronutrient intake including 800 mg of calcium per day. When consuming 2.1 g of protein per kg BW, women had significantly greater diet-derived and less bone-derived calcium in the urine, in addition to significantly more intestinal calcium absorption than when consuming 1.0 g of protein per kg BW (181). The primary supplier of calcium in the experimental diets in this study was dairy foods. The calcium-to-phosphate ratio of dairy foods may help maintain calcium balance. Increased phosphate intake enhances calcium reabsorption in the kidney. In contrast, pharmaceutical calcium salt supplements provide large differences in concentrations of calcium and phosphate which may adversely affect bone health (193). Dairy foods also provide potassium and magnesium which may be vital to bone health (194), and the frequent intake of dairy foods has been associated with increased BMD (195).

Change in BMD may be easier to detect in an aging population who are undergoing rapid BMD change compared to younger cohorts with relatively stable BMD. In a recent cross-sectional study by Beasley et al. (196) more than 500 premenopausal women had similar mean

BMD across each tertile of protein intake. However, young adults may see accelerated bone loss during BW loss. Increased protein may be beneficial to BMD retention with loss of BW in young adults. In premenopausal women, studies of BMD and biomarkers of bone turnover with ERDs ranging in protein content have been conducted. Ultimately, increased dietary protein has proved important for mitigation of BMD loss during age-related change, and it appears to also be important during BW loss. Protein intake may improve preservation of FFSTM which increases mechanical loading on bone to enhance its strength. Additionally, increased protein intake during BW loss may enhance calcium absorption in the gut, improving circulating levels of this micronutrient. Effects of MP intake during weight-loss interventions on bone health conducted in premenopausal women are briefly reviewed below.

Body Weight and Bone Health

An unfortunate side effect of BW loss can be a reduction in FFSTM (38) and BMD (110). While excess adiposity has a negative effect on cardiovascular health and related risk factors, excess BW has been considered protective for skeletal health as a higher BW is associated with greater BMD (197,198). A greater BW increases mechanical loading on the skeleton during activity, while BW loss results in diminished mechanical loading and reduced need for bone to accumulate additional mass, resulting in possible bone loss (199). In clinical weight-loss studies, energy restriction and BW loss have been shown to reduce BMD (110,200). However, the notion that greater overall BW results in improved BMD has been debated recently, as some researchers believe that this association is driven by FFSTM rather than total BW (197). Likewise, as adipose tissue is increasingly thought of as an endocrine tissue with inflammatory capacity that may be detrimental to bone, the perception that increased total BW confers absolute benefit to BMD is beginning to change (201).

Weight-loss trials have shown significant BMD losses when individuals drastically reduce energy intake and lose BW rapidly (202). Additionally, ERDs with inadequate calcium may negatively affect bone (203). Individuals losing substantial BW quickly via bariatric surgery often have multiple micronutrient deficiencies and have been shown to consistently lose BMD (201,204). Also, it is believed that chronic dieting, with repeated loss and regain of BW, may be particularly detrimental to BMD (205,206). Alternatively, negative bone health outcomes with BW loss may be ameliorated under certain conditions as evidenced by several weight-loss interventions in premenopausal women which have resulted in little or no change in indicators of bone status (207–211). Premenopausal women slowly losing moderate BW while consuming adequate protein and calcium demonstrate that BMD losses or adverse changes in bone turnover can be attenuated or prevented (207,208,212,213).

ERDs with protein comprising at least 15% of total energy intake while meeting the RDA for calcium for premenopausal women (1000 mg/day) have maintained BMD with BW loss; however, MP ERDs meeting these criteria have improved BMD with BW loss. In a study by Riedt et al. (208), overweight premenopausal women did not lose BMD or experience changes in biomarkers of bone turnover after six months of moderate weight loss (7% BW loss) on a SP (15% of kcal) ERD with sufficient calcium intake (either 1000 or 1800 mg of calcium/day). Thorpe et al. (213) found that overweight men and women consuming a MP (30% of kcal) ERD including three daily servings of dairy (approximately 1100 mg of calcium/day) for four months achieved moderate weight loss (8% BW loss) and increased total body, lumbar spine and total hip BMD over the course of the study compared to participants consuming a SP (15% of kcal) ERD including two servings of dairy per day (approximately 800 mg of calcium/day). Weight loss and physical activity were similar in both groups, but women in the SP group did not meet

the RDA for calcium (213). Josse et al. (212) reported that a MP (30% of kcal) ERD including 6-7 servings of dairy per day improved several markers of bone health compared to diets with SP (15% of kcal) and three or fewer servings of dairy per day with moderate weight loss (5% BW loss) over 16 weeks. Women in all groups met the RDA for calcium intake (212).

Still, there are inconsistencies in the literature. Shapses et al. (211) reported that obese premenopausal women consuming a SP ERD with 1000 mg of calcium supplementation significantly increased BMD at the lumbar spine, while a placebo group consuming a SP ERD and approximately 500 mg of dietary calcium maintained BMD after six months. Conversely, in a study by Wagner et al. (109), three groups of premenopausal women consuming a SP ERD significantly increased markers of bone turnover after 12 weeks of weight loss, regardless of how they achieved 1500 mg of calcium intake (as calcium lactate or calcium phosphate supplements or as dairy intake). In these studies (109,211), weight loss was moderate with roughly 1-2% BW loss per month.

It is becoming more apparent that sufficient protein and calcium intake are beneficial to bone during weight loss. Furthermore, a MP ERD including recommended calcium and/or dairy intake may enhance bone health and increase BMD during BW loss (212,213). Regrettably, intervention studies examining the effect of various weight-loss approaches on bone loss often report only biomarkers of bone turnover (109,214) or are relatively short in duration, lasting less than six months (109,207,212,214). As bone changes take at least 6 months to occur and biomarkers of bone turnover are somewhat unreliable (215), these designs may be insufficient for the examination of true changes in bone. Future studies that examine actual change in BMD over time periods greater than six months may be more indicative of fracture risk in this population. Furthermore, a distinction has yet to be made between the effects of adequate

protein—that being at least 15% of energy intake—and MP on BMD. Currently, there is support that dietary patterns with protein comprising at least 15% of total energy intake serve to maintain BMD during BW loss (208). However, some studies suggest that BMD has the potential to increase during BW loss provided that dietary protein and dairy intakes are high (212,213) (Table 2).

Inflammation and Bone Health

Studies examining the anti-inflammatory potential of dietary components contained in an ERD suggest that BW loss drives the reduction in inflammatory status markers (59,216). However, one study has shown that dairy intake in weight-stable men and women reduced inflammatory factors (83). Overweight/obese adults consuming a dairy-supplemented, weight-maintenance diet for 28 days significantly lowered 8-isoprostane $F_{2\alpha}$, a marker of oxidative stress, and pro-inflammatory factors TNF-α, IL-6 and monocyte chemoattractant protein-1. Also, the adipocyte-derived anti-inflammatory factor adiponectin increased compared to a soy-supplemented, weight-maintenance diet in this randomized crossover study (83). Similarly, obese adults with metabolic syndrome suppressed TNF-α, IL-6 and monocyte chemoattractant protein-1 after consuming 3.5 servings of dairy per day for 12 weeks compared to participants who experienced no effect after consuming less than half a serving of dairy per day. Likewise, a corresponding increase in adiponectin was reported at 12 weeks in the high-dairy group (217).

Adipocytes as adipose tissue are recognized as acting like an endocrine organ. Compared to individuals of average FM, individuals with excess adiposity have higher levels of pro-inflammatory factors such as TNF-α, IL-1, IL-6, C-reactive protein (CRP) and leptin and lower levels of adiponectin (174). For this reason, obesity is considered a state of systemic low-grade inflammation by some clinicians. Pro-inflammatory cytokines elevated in obesity have effects on

the RANK/RANK ligand/osteoprotegerin pathway which may influence bone turnover (174). CAT is a particularly potent contributor to inflammation, known to effect insulin resistance, dyslipidemia, metabolic syndrome and hypertension (218). CAT has also been inversely associated with BMD at several bone sites in women and at the femoral neck in men (219).

Emerging research offers compelling evidence that excess adiposity inducing chronic systemic inflammation has the potential to adversely affect bone. A weight-loss intervention targeting FM and CAT may favorably reduce obesity-related inflammation (174,219). Similarly, the intake of dietary bioactives, such as dairy, may further reduce inflammation (83). With the potential detriment that BW loss has on loading the skeleton and thus BMD, optimizing the progression of BW loss to protect the skeleton while reducing FM and CAT-derived inflammation, and preserving FFSTM may be prudent.

Physical Activity and Bone Health

Bone is a dynamic tissue which is influenced biologically by mechanical demands. With the ability to sense mechanical loads, bones modify their structure accordingly (220). Weight-bearing exercises, during which additional load is applied to involved bones, increase bone strength and BMD (221). There are believed to be several principles needed in order for exercise to allow biological bone adaption to mechanical stress. Exercise should be dynamic and exceed threshold intensity and strain frequency (199). Additionally, exercise-induced adaption needs to be supported by sufficient energy, protein, calcium and vitamin D availability. Increased blood flow experienced with exercise may also help to supply bone with adequate nutrients, growth hormones and oxygen (199). Physical activity is known to protect against age-related bone loss (222) and reduce fracture risk (223). Additionally, activity early in life may help with maximal

BMD accrual as adults who were very physically active in young adulthood have greater BMD at several skeletal cites (224).

Many studies support the benefits of both resistance- and aerobic-based exercise on BMD during BW loss. Daly et al. (225) reported that gymnasium-based resistance training with an ERD maintained total body BMD in older adults after 12 months compared to adults undergoing only an ERD intervention who experienced significant decreases in total body BMD over the same time period. Silverman et al. (226) found that postmenopausal women incorporating 45-60 minutes of walking three days per week for six months had significant increases in BMD at the femoral neck compared to an ERD only group who had a reduction in BMD at the same site. A similar study conducted by Honsy et al. (227) in premenopausal women also supports a benefit of exercise on BMD during an ERD. Women randomized to an ERD in conjunction with treadmill walking for 40 minutes per day had significant increases in FFSTM and BMD at the hip and lumbar spine compared to a group with ERD alone who had significant reductions in BMD at both sites (227).

In contrast to these results, there are studies demonstrating no additional benefit of incorporating exercise into a weight-loss intervention on BMD. Rector et al. (214) found that premenopausal women with overweight following a moderate weight-loss diet while either jogging or cycling had similar increases in markers of bone turnover after six weeks compared to a non-exercising weight-loss control group. Nakata et al. (228) reported that premenopausal women experienced no additional benefit to BMD with the addition of three weekly 90-minute weight training sessions for 14 weeks compared to a control group not including resistance training. These trials were relatively short in duration, making the detection of significant change in BMD unlikely. Changes in serum measures of bone turnover as surrogate markers for BMD

can be measured instead (229) though these are not always consistent (215). Increased exercise for at least six months or more may be necessary to induce measurable change to BMD during energy restriction (164). A lack of observable change in BMD in premenopausal women after six months or more of exercise may be due to inadequate exercise intensity, duration or frequency, or life span stage where accumulation of BMD has peaked (199).

Walking is considered a weight-bearing activity, and it has been shown to produce enough ground reaction force to maintain and potentially generate bone accrual. Borer et al. (2007) examined postmenopausal women who engaged in 30 weeks of supervised walking four days per week. FFSTM and areal BMD in the legs and whole body were preserved and slightly increased with loads greater than 1.22 times BW (129). Overall, sufficient evidence suggests that increased exercise is beneficial to weight loss outcomes, including maintenance of FFSTM and BMD. With this in mind, incorporating exercise, including walking, into a weight-loss approach in conjunction with an ERD containing adequate protein and calcium should be recommended for protection of bone integrity.

Conclusions

Excess adiposity is a major public health concern and is associated with increased risk of type 2 diabetes, heart disease, hypertension, certain cancers and systemic inflammation. Reduction of adiposity can improve risk factors for chronic health conditions. Generation of a net energy deficit, via adherence to an ERD, with or without increasing energy expenditure, is necessary to induce BW loss. Though the condition of an energy deficit is paramount for effective weight loss, randomized clinical trials have demonstrated that MP ERDs may be superior to SP ERDs at inducing BW and FM losses and preserving FFSTM. Though beneficial changes in metabolic parameters are likely more influenced by degree of BW loss than dietary

macronutrient composition or specific dietary constituents in healthy populations, a greater reduction of TG concentration with MP ERDs seem to be evident in the literature.

A reduction in energy intake and BW loss without adequate nutrient intake can negatively impact bone integrity. It is of public health interest to attenuate bone loss during BW loss to reduce risk of osteoporosis. Sufficient protein intake has been consistently shown in the literature to benefit bone. Dairy foods are a rich source of protein with an ideal micronutrient profile which may reduce risk of BMD loss with BW loss. Additionally, as bone responds to change in mechanical loads, increases in physical activity may help offset reductions in loading from reduced BW. With sufficient protein and calcium intake as part of an ERD including physical activity, BMD can be maintained or even improved. Furthermore, obesity is associated with high concentrations of systemic pro-inflammatory factors which may stimulate bone resorption and lead to reductions in bone over time. Though weight-loss is often regarded as unfavorable to bone, reductions in circulating inflammatory factors observed with BW loss may benefit bone health overall.

In conclusion, moderate weight loss can be accomplished with a MP ERD including moderate dairy intake and physical activity. This macronutrient composition may reduce hunger, promote satiety and improve dietary adherence, while incorporation of regular physical activity may benefit weight-loss maintenance. The inclusion of adequate dairy intake will help meet goals for calcium intake and provide complete protein including the amino acid leucine. Few studies have incorporated all of these strategies to examine the most favorable intervention for bone health while still producing effective BW loss. Intervention studies examining the effect of various weight-loss approaches on bone loss often report only biomarkers of bone turnover or are relatively short in duration, lasting less than six months. Furthermore, an approach with free-

living women aimed at optimizing BW loss and BW-loss maintenance while avoiding reductions in BMD may be more applicable to examine intervention effectiveness.

REFERENCES

1. Flegal KM, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999-2008. *JAMA*. 2010;303(3):235–241.

2. Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. *BMC Public Health*. 2009;9:88.

3. Chang Y, Ryu S, Suh B-S, Yun KE, Kim C-W, Cho S-I. Impact of BMI on the incidence of metabolic abnormalities in metabolically healthy men. *Int J Obes (2005)*. 2011

4. Dalton M, Cameron AJ, Zimmet PZ, et al. Waist circumference, waist-hip ratio and body mass index and their correlation with cardiovascular disease risk factors in Australian adults. *J Intern. Med.* 2003;254(6):555–563.

5. Anon. WHO. Obesity and overweight. *WHO*. Available at: http://www.who.int/mediacentre/factsheets/fs311/en/. Accessed January 2, 2012.

6. Anon. Obesity and Overweight for Professionals: Data and Statistics: U.S. Obesity Trends | DNPAO | CDC. Available at: http://www.cdc.gov/obesity/data/trends.html. Accessed February 15, 2012.

7. Ringbäck Weitoft G, Eliasson M, Rosén M. Underweight, overweight and obesity as risk factors for mortality and hospitalization. *Scand J Public Health.* 2008;36(2):169–176.

8. Wille N, Erhart M, Petersen C, Ravens-Sieberer U. The impact of overweight and obesity on health-related quality of life in childhood--results from an intervention study. *BMC Public Health*. 2008;8:421.

9. Brown T, Avenell A, Edmunds LD, et al. Systematic review of long-term lifestyle interventions to prevent weight gain and morbidity in adults. *Obes Rev*. 2009;10(6):627–638.

10. Dombrowski SU, Avenell A, Sniehott FF. Behavioural interventions for obese adults with additional risk factors for morbidity: systematic review of effects on behaviour, weight and disease risk factors. *Obes Facts*. 2010;3(6):377–396.

11. Douketis JD, Macie C, Thabane L, Williamson DF. Systematic review of long-term weight loss studies in obese adults: clinical significance and applicability to clinical practice. *Int J Obes (Lond)*. 2005;29(10):1153–1167.

12. Lefevre M, Redman LM, Heilbronn LK, et al. Caloric restriction alone and with exercise improves CVD risk in healthy non-obese individuals. *Atherosclerosis*. 2009;203(1):206–213.

13. Coppola A, Marfella R, Coppola L, et al. Effect of weight loss on coronary circulation and adiponectin levels in obese women. *Int J Cardiol*. 2009;134(3):414–416.

14. Fontana L, Villareal DT, Weiss EP, et al. Calorie restriction or exercise: effects on coronary heart disease risk factors. A randomized, controlled trial. *Am J Physiol Endocrinol Metab.* 2007;293(1):E197 –E202.

15. Sacks FM, Bray GA, Carey VJ, et al. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *N Engl J Med.* 2009;360(9):859–873.

16. Gardner CD, Kiazand A, Alhassan S, et al. Comparison of the Atkins, Zone, Ornish, and LEARN diets for change in weight and related risk factors among overweight premenopausal women: the A TO Z Weight Loss Study: a randomized trial. *JAMA.* 2007;297(9):969–977.

17. Dansinger ML, Gleason JA, Griffith JL, Selker HP, Schaefer EJ. Comparison of the Atkins, Ornish, Weight Watchers, and Zone diets for weight loss and heart disease risk reduction: a randomized trial. *JAMA.* 2005;293(1):43–53.

18. Nordmann AJ, Nordmann A, Briel M, et al. Effects of low-carbohydrate vs low-fat diets on weight loss and cardiovascular risk factors: a meta-analysis of randomized controlled trials. *Arch Intern Med.* 2006;166(3):285–293.

19. Abete I, Astrup A, Martínez JA, Thorsdottir I, Zulet MA. Obesity and the metabolic syndrome: role of different dietary macronutrient distribution patterns and specific nutritional components on weight loss and maintenance. *Nutr Rev.* 2010;68(4):214–231.

20. Champagne CM, Broyles ST, Moran LD, et al. Dietary intakes associated with successful weight loss and maintenance during the Weight Loss Maintenance trial. *J Am Diet Assoc.* 2011;111(12):1826–1835.

21. Mattes RD, Kris-Etherton PM, Foster GD. Impact of peanuts and tree nuts on body weight and healthy weight loss in adults. *J Nutr.* 2008;138(9):1741S–1745S.

22. Zemel MB, Teegarden D, Loan MV, et al. Dairy-rich diets augment fat loss on an energy-restricted diet: a multicenter trial. *Nutrients.* 2009;1(1):83–100.

23. Otten JJ, Hellwig JP, Meyers LD, National Academies Press (U.S.). *DRI, dietary reference intakes the essential guide to nutrient requirements*. Washington, D.C.: National Academies Press; 2006.

24. Smit E, Nieto FJ, Crespo CJ, Mitchell P. Estimates of animal and plant protein intake in US adults: results from the Third National Health and Nutrition Examination Survey, 1988-1991. *J Am Diet Assoc.* 1999;99(7):813–820.

25. Layman DK. Protein Quantity and Quality at Levels above the RDA Improves Adult Weight Loss. *J Am Coll Nutr.* 2004;23(suppl 6):631S–636S.

26. Josse AR, Atkinson SA, Tarnopolsky MA, Phillips SM. Increased Consumption of Dairy Foods and Protein during Diet- and Exercise-Induced Weight Loss Promotes Fat Mass Loss and Lean Mass Gain in Overweight and Obese Premenopausal Women. *J Nutr.* 2011;141(9):1626–1634.

27. Farnsworth E, Luscombe ND, Noakes M, Wittert G, Argyiou E, Clifton PM. Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. *Am J Clin Nutr.*2003;78(1):31–39.

28. Noakes M, Keogh JB, Foster PR, Clifton PM. Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. *Am J Clin Nutr.*2005;81(6):1298–1306.

29. Luscombe-Marsh ND, Noakes M, Wittert GA, Keogh JB, Foster P, Clifton PM. Carbohydrate-restricted diets high in either monounsaturated fat or protein are equally effective at promoting fat loss and improving blood lipids. *Am J Clin Nutr.*2005;81(4):762–772.

30. Meckling KA, Sherfey R. A randomized trial of a hypocaloric high-protein diet, with and without exercise, on weight loss, fitness, and markers of the Metabolic Syndrome in overweight and obese women. *Appl Physiol Nutr Metab*. 2007;32(4):743–752.

31. Layman DK, Evans E, Baum JI, Seyler J, Erickson DJ, Boileau RA. Dietary protein and exercise have additive effects on body composition during weight loss in adult women. *J Nutr.* 2005;135(8):1903–1910.

32. Wycherley TP, Noakes M, Clifton PM, Cleanthous X, Keogh JB, Brinkworth GD. A high-protein diet with resistance exercise training improves weight loss and body composition in overweight and obese patients with type 2 diabetes. *Diabetes Care*. 2010;33(5):969–976.

33. Skov AR, Toubro S, Rønn B, Holm L, Astrup A. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. *Int J Obes Relat Metab. Disord.* 1999;23(5):528–536.

34. Layman DK, Evans EM, Erickson D, et al. A moderate-protein diet produces sustained weight loss and long-term changes in body composition and blood lipids in obese adults. *J Nutr.* 2009;139(3):514–521.

35. Parker B, Noakes M, Luscombe N, Clifton P. Effect of a high-protein, high-monounsaturated fat weight loss diet on glycemic control and lipid levels in type 2 diabetes. *Diabetes Care*. 2002;25(3):425–430.

36. Layman DK, Boileau RA, Erickson DJ, et al. A Reduced Ratio of Dietary Carbohydrate to Protein Improves Body Composition and Blood Lipid Profiles during Weight Loss in Adult Women. *J Nutr*. 2003;133(2):411 –417.

37. Brehm BJ, D'Alessio DA. Benefits of high-protein weight loss diets: enough evidence for practice? *Curr Opin Endocrinol Diabetes Obes*. 2008;15(5):416–421.

38. Leidy HJ, Carnell NS, Mattes RD, Campbell WW. Higher protein intake preserves lean mass and satiety with weight loss in pre-obese and obese women. *Obesity (Silver Spring)*. 2007;15(2):421–429.

39. Luscombe ND, Clifton PM, Noakes M, Parker B, Wittert G. Effects of energy-restricted diets containing increased protein on weight loss, resting energy expenditure, and the thermic effect of feeding in type 2 diabetes. *Diabetes Care*. 2002;25(4):652–657.

40. Lejeune MPGM, Westerterp KR, Adam TCM, Luscombe-Marsh ND, Westerterp-Plantenga MS. Ghrelin and glucagon-like peptide 1 concentrations, 24-h satiety, and energy and substrate metabolism during a high-protein diet and measured in a respiration chamber. *Am J Clin Nutr.*2006;83(1):89–94.

41. Eisenstein J, Roberts SB, Dallal G, Saltzman E. High-protein Weight-loss Diets: Are They Safe and Do They Work? A Review of the Experimental and Epidemiologic Data. *Nutr Rev*. 2002;60(7):189–200.

42. Veldhorst M, Smeets A, Soenen S, et al. Protein-induced satiety: effects and mechanisms of different proteins. *Physiol Behav.* 2008;94(2):300–307.

43. Halton TL, Hu FB. The Effects of High Protein Diets on Thermogenesis, Satiety and Weight Loss: A Critical Review. *J Am Coll Nutr*. 2004;23(5):373–385.

44. Johnston CS, Day CS, Swan PD. Postprandial thermogenesis is increased 100% on a high-protein, low-fat diet versus a high-carbohydrate, low-fat diet in healthy, young women. *J Am Coll Nutr*. 2002;21(1):55–61.

45. Mikkelsen PB, Toubro S, Astrup A. Effect of fat-reduced diets on 24-h energy expenditure: comparisons between animal protein, vegetable protein, and carbohydrate. *Am J Clin Nutr.*2000;72(5):1135–1141.

46. Devkota S, Layman DK. Protein metabolic roles in treatment of obesity. *Curr Opin Clin Nutr Metab Care*. 2010;13(4):403–407.

47. Johnston CS, Tjonn SL, Swan PD. High-protein, low-fat diets are effective for weight loss and favorably alter biomarkers in healthy adults. *J Nutr.* 2004;134(3):586–591.

48. Nickols-Richardson SM, Coleman MD, Volpe JJ, Hosig KW. Perceived hunger is lower and weight loss is greater in overweight premenopausal women consuming a low-carbohydrate/high-protein vs high-carbohydrate/low-fat diet. *J Am Diet Assoc*. 2005;105(9):1433–1437.

49. Rolls BJ, Hetherington M, Burley VJ. The specificity of satiety: The influence of foods of different macronutrient content on the development of satiety. *Physiol Behav*. 1988;43(2):145–153.

50. Poppitt SD, McCormack D, Buffenstein R. Short-term effects of macronutrient preloads on appetite and energy intake in lean women. *Physiol Behav.* 1998;64(3):279–285.

51. Blatt AD, Roe LS, Rolls BJ. Increasing the protein content of meals and its effect on daily energy intake. *J Am Diet Assoc*. 2011;111(2):290–294.

52. Zheng H, Lenard NR, Shin AC, Berthoud H-R. Appetite control and energy balance regulation in the modern world: reward-driven brain overrides repletion signals. *Int J Obes (Lond)*. 2009;33 Suppl 2:S8–13.

53. Timko CA, Juarascio A, Chowansky A. The effect of a pre-load experiment on subsequent food consumption. Caloric and macronutrient intake in the days following a pre-load manipulation. *Appetite*. 2012;58(2):747–753.

54. de Souza RJ, Bray GA, Carey VJ, et al. Effects of 4 weight-loss diets differing in fat, protein, and carbohydrate on fat mass, lean mass, visceral adipose tissue, and hepatic fat: results from the POUNDS LOST trial. *Am J Clin Nutr.* 2012.

55. Alhassan S, Kim S, Bersamin A, King AC, Gardner CD. Dietary adherence and weight loss success among overweight women: results from the A TO Z weight loss study. *Int J Obes (Lond)*. 2008;32(6):985–991.

56. Clifton PM, Keogh JB, Noakes M. Long-term effects of a high-protein weight-loss diet. *Am J Clin Nutr.*2008;87(1):23–29.

57. Clifton PM, Bastiaans K, Keogh JB. High protein diets decrease total and abdominal fat and improve CVD risk profile in overweight and obese men and women with elevated triacylglycerol. *Nutr Metab Cardiovasc Dis*. 2009;19(8):548–554.

58. Keogh JB, Luscombe-Marsh ND, Noakes M, Wittert GA, Clifton PM. Long-term weight maintenance and cardiovascular risk factors are not different following weight loss on carbohydrate-restricted diets high in either monounsaturated fat or protein in obese hyperinsulinaemic men and women. *Br J Nutr.* 2007;97(2):405–410.

59. Lee K, Lee J, Bae WK, Choi JK, Kim HJ, Cho B. Efficacy of low-calorie, partial meal replacement diet plans on weight and abdominal fat in obese subjects with metabolic syndrome: a double-blind, randomised controlled trial of two diet plans - one high in protein and one nutritionally balanced. *Int J Clin Pract.* 2009;63(2):195–201.

60. Due A, Toubro S, Skov AR, Astrup A. Effect of normal-fat diets, either medium or high in protein, on body weight in overweight subjects: a randomised 1-year trial. *Int J Obes Relat Metab Disord.* 2004;28(10):1283–1290.

61. Wing RR, Hill JO. Successful weight loss maintenance. *Annu Rev Nutr.* 2001;21:323–341.

62. Krieger JW, Sitren HS, Daniels MJ, Langkamp-Henken B. Effects of variation in protein and carbohydrate intake on body mass and composition during energy restriction: a meta-regression 1. *Am J Clin Nutr.*2006;83(2):260–274.

63. Krebs JD, Elley CR, Parry-Strong A, et al. The Diabetes Excess Weight Loss (DEWL) Trial: a randomised controlled trial of high-protein versus high-carbohydrate diets over 2 years in type 2 diabetes. *Diabetologia*. 2012.

64. Brinkworth GD, Noakes M, Parker B, Foster P, Clifton PM. Long-term effects of advice to consume a high-protein, low-fat diet, rather than a conventional weight-loss diet, in obese adults with type 2 diabetes: one-year follow-up of a randomised trial. *Diabetologia*. 2004;47(10):1677–1686.

65. Ebbeling CB, Leidig MM, Feldman HA, Lovesky MM, Ludwig DS. Effects of a low-glycemic load vs low-fat diet in obese young adults: a randomized trial. *JAMA*. 2007;297(19):2092–2102.

66. Cornier M-A, Donahoo WT, Pereira R, et al. Insulin sensitivity determines the effectiveness of dietary macronutrient composition on weight loss in obese women. *Obes Res.* 2005;13(4):703–709.

67. Layman DK, Clifton P, Gannon MC, Krauss RM, Nuttall FQ. Protein in optimal health: heart disease and type 2 diabetes. *Am J Clin Nutr.*2008;87(5):1571S–1575S.

68. Appel LJ, Sacks FM, Carey VJ, et al. Effects of protein, monounsaturated fat, and carbohydrate intake on blood pressure and serum lipids: results of the OmniHeart randomized trial. *JAMA*. 2005;294(19):2455–2464.

69. Foster GD, Wyatt HR, Hill JO, et al. A randomized trial of a low-carbohydrate diet for obesity. *N Engl J Med.* 2003;348(21):2082–2090.

70. He J, Wofford MR, Reynolds K, et al. Effect of dietary protein supplementation on blood pressure: a randomized, controlled trial. *Circulation*. 2011;124(5):589–595.

71. Appel LJ. The effects of protein intake on blood pressure and cardiovascular disease. *Curr Opin Lipidol.* 2003;14(1):55–59.

72. Wang YF, Yancy WS Jr, Yu D, Champagne C, Appel LJ, Lin PH. The relationship between dietary protein intake and blood pressure: results from the PREMIER study. *J Hum Hypertens.* 2008;22(11):745–754.

73. Altorf-van der Kuil W, Engberink MF, Brink EJ, et al. Dietary protein and blood pressure: a systematic review. *PLoS ONE*. 2010;5(8):e12102.

74. Layman DK, Shiue H, Sather C, Erickson DJ, Baum J. Increased dietary protein modifies glucose and insulin homeostasis in adult women during weight loss. *J Nutr.* 2003;133(2):405–410.

75. Muzio F, Mondazzi L, Harris WS, Sommariva D, Branchi A. Effects of moderate variations in the macronutrient content of the diet on cardiovascular disease risk factors in obese patients with the metabolic syndrome. *Am J Clin Nutr.*2007;86(4):946–951.

76. Pasanisi F, Contaldo F, de Simone G, Mancini M. Benefits of sustained moderate weight loss in obesity. *Nutr Metab Cardiovasc Dis.* 2001;11(6):401–406.

77. Nicklas TA, O'Neil CE, Fulgoni VL 3rd. The role of dairy in meeting the recommendations for shortfall nutrients in the American diet. *J Am Coll Nutr*. 2009;28 Suppl 1:73S–81S.

78. Fulgoni VL 3rd, Huth PJ, DiRienzo DB, Miller GD. Determination of the optimal number of dairy servings to ensure a low prevalence of inadequate calcium intake in Americans. *J Am Coll Nutr*. 2004;23(6):651–659.

79. U.S. Department of Agriculture and U.S. Department of Health and Human Services. Dietary Guidelines for Americans, 2010. 7th Edition, Washington, DC: U.S. Government Printing Office, December 2010.

80. Zemel MB, Richards J, Mathis S, Milstead A, Gebhardt L, Silva E. Dairy augmentation of total and central fat loss in obese subjects. *Int J Obes (Lond)*. 2005;29(4):391–397.

81. van Meijl LEC, Vrolix R, Mensink RP. Dairy product consumption and the metabolic syndrome. *Nutr Res Rev*. 2008;21(2):148–157.

82. Tremblay A, Gilbert J-A. Milk products, insulin resistance syndrome and type 2 diabetes. *J Am Coll Nutr*. 2009;28 Suppl 1:91S–102S.

83. Zemel MB, Sun X, Sobhani T, Wilson B. Effects of dairy compared with soy on oxidative and inflammatory stress in overweight and obese subjects. *Am J Clin Nutr*.2010;91(1):16–22.

84. Zemel MB, Thompson W, Milstead A, Morris K, Campbell P. Calcium and dairy acceleration of weight and fat loss during energy restriction in obese adults. *Obes Res*. 2004;12(4):582–590.

85. Layman DK, Walker DA. Potential Importance of Leucine in Treatment of Obesity and the Metabolic Syndrome. *J Nutr*. 2006;136(1):319S –323S.

86. Koopman R, Wagenmakers AJM, Manders RJF, et al. Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects. *Am J Physiol Endocrinol Metab*. 2005;288(4):E645–653.

87. Anon. The role of dairy products in diet. Introduction. *J Am Coll Nutr*. 2009;28 Suppl 1:69S–72S.

88. Ralston RA, Lee JH, Truby H, Palermo CE, Walker KZ. A systematic review and meta-analysis of elevated blood pressure and consumption of dairy foods. *J Hum Hypertens*. 2011.

89. Kris-Etherton PM, Grieger JA, Hilpert KF, West SG. Milk products, dietary patterns and blood pressure management. *J Am Coll Nutr*. 2009;28 Suppl 1:103S–19S.

90. Hilpert KF, West SG, Bagshaw DM, et al. Effects of dairy products on intracellular calcium and blood pressure in adults with essential hypertension. *J Am Coll Nutr*. 2009;28(2):142–149.

91. Appel LJ, Moore TJ, Obarzanek E, et al. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med*. 1997;336(16):1117–1124.

92. Obarzanek E, Sacks FM, Vollmer WM, et al. Effects on blood lipids of a blood pressure-lowering diet: the Dietary Approaches to Stop Hypertension (DASH) Trial. *Am J Clin Nutr.*2001;74(1):80–89.

93. Moore LL, Singer MR, Bradlee ML, et al. Intake of fruits, vegetables, and dairy products in early childhood and subsequent blood pressure change. *Epidemiology*. 2005;16(1):4–11.

94. Chen ST, Maruthur NM, Appel LJ. The effect of dietary patterns on estimated coronary heart disease risk: results from the Dietary Approaches to Stop Hypertension (DASH) trial. *Circ Cardiovasc Qual Outcomes*. 2010;3(5):484–489.

95. Liu S, Choi HK, Ford E, et al. A prospective study of dairy intake and the risk of type 2 diabetes in women. *Diabetes Care*. 2006;29(7):1579–1584.

96. Pittas AG, Dawson-Hughes B, Li T, et al. Vitamin D and calcium intake in relation to type 2 diabetes in women. *Diabetes Care*. 2006;29(3):650–656.

97. Pittas AG, Lau J, Hu FB, Dawson-Hughes B. The Role of Vitamin D and Calcium in Type 2 Diabetes. A Systematic Review and Meta-Analysis. *J Clin Endocrinol Metab*. 2007;92(6):2017 – 2029.

98. Glynn EL, Fry CS, Drummond MJ, et al. Excess leucine intake enhances muscle anabolic signaling but not net protein anabolism in young men and women. *J Nutr*. 2010;140(11):1970–1976.

99. Nicklas TA. Calcium intake trends and health consequences from childhood through adulthood. *J Am Coll Nutr*. 2003;22(5):340–356.

100. Webber KH, Lee E. The diet quality of adult women participating in a behavioural weight-loss programme. *J Hum Nutr Diet*. 2011;24(4):360–369.

101. Ranganathan R, Nicklas TA, Yang S-J, Berenson GS. The nutritional impact of dairy product consumption on dietary intakes of adults (1995-1996): the Bogalusa Heart Study. *J Am Diet Assoc*. 2005;105(9):1391–1400.

102. Astrup A, Chaput J-P, Gilbert J-A, Lorenzen JK. Dairy beverages and energy balance. *Physiol Behav*. 2010.

103. Nolan-Clark DJ, Neale EP, Probst YC, Charlton KE, Tapsell LC. Consumers' salient beliefs regarding dairy products in the functional food era: a qualitative study using concepts from the theory of planned behaviour. *BMC Public Health*. 2011;11:843.

104. McCarron DA, Morris CD, Henry HJ, Stanton JL. Blood pressure and nutrient intake in the United States. *Science*. 1984;224(4656):1392–1398.

105. Dougkas A, Reynolds CK, Givens ID, Elwood PC, Minihane AM. Associations between dairy consumption and body weight: a review of the evidence and underlying mechanisms. *Nutr Res Rev*. 2011:1–24.

106. Zemel MB, Shi H, Greer B, Dirienzo D, Zemel PC. Regulation of adiposity by dietary calcium. *FASEB J*. 2000;14(9):1132–1138.

107. Ross ACAC, Taylor CLCL, Yaktine ALAL, Del Valle HBHB eds. *Dietary Reference Intakes for Calcium and Vitamin D*. Washington (DC): National Academies Press (US); 2011.

108. Shapses SA, Heshka S, Heymsfield SB. Effect of Calcium Supplementation on Weight and Fat Loss in Women. *J Clin Endocrinol Metab*. 2004;89(2):632–637.

109. Wagner G, Kindrick S, Hertzler S, DiSilvestro RA. Effects of various forms of calcium on body weight and bone turnover markers in women participating in a weight loss program. *J Am Coll Nutr*. 2007;26(5):456–461.

110. Jensen LB, Kollerup G, Quaade F, Sørensen OH. Bone minerals changes in obese women during a moderate weight loss with and without calcium supplementation. *J Bone Miner Res*. 2001;16(1):141–147.

111. Gunther CW, Legowski PA, Lyle RM, et al. Dairy products do not lead to alterations in body weight or fat mass in young women in a 1-y intervention. *Am J Clin Nutr*. 2005;81(4):751–756.

112. Shi H, Norman AW, Okamura WH, Sen A, Zemel MB. 1alpha,25-Dihydroxyvitamin D3 modulates human adipocyte metabolism via nongenomic action. *FASEB J*. 2001;15(14):2751–2753.

113. Shi H, Norman AW, Okamura WH, Sen A, Zemel MB. 1alpha,25-dihydroxyvitamin D3 inhibits uncoupling protein 2 expression in human adipocytes. *FASEB J*. 2002;16(13):1808–1810.

114. Jacobsen R, Lorenzen JK, Toubro S, Krog-Mikkelsen I, Astrup A. Effect of short-term high dietary calcium intake on 24-h energy expenditure, fat oxidation, and fecal fat excretion. *Int J Obes (Lond)*. 2005;29(3):292–301.

115. Wennersberg MH, Smedman A, Turpeinen AM, et al. Dairy products and metabolic effects in overweight men and women: results from a 6-mo intervention study. *Am J Clin Nutr*.2009;90(4):960–968.

116. Thompson WG, Rostad Holdman N, Janzow DJ, Slezak JM, Morris KL, Zemel MB. Effect of energy-reduced diets high in dairy products and fiber on weight loss in obese adults. *Obes Res*. 2005;13(8):1344–1353.

117. Bell EA, Castellanos VH, Pelkman CL, Thorwart ML, Rolls BJ. Energy density of foods affects energy intake in normal-weight women. *Am J Clin Nutr*.1998;67(3):412–420.

118. Rolls BJ, Bell EA, Castellanos VH, Chow M, Pelkman CL, Thorwart ML. Energy density but not fat content of foods affected energy intake in lean and obese women. *Am J Clin Nutr*.1999;69(5):863–871.

119. Ledikwe JH, Rolls BJ, Smiciklas-Wright H, et al. Reductions in dietary energy density are associated with weight loss in overweight and obese participants in the PREMIER trial. *Am J Clin Nutr.*2007;85(5):1212–1221.

120. Rolls BJ, Drewnowski A, Ledikwe JH. Changing the energy density of the diet as a strategy for weight management. *J Am Diet Assoc*. 2005;105(5 Suppl 1):S98–103.

121. Rolls BJ, Roe LS, Beach AM, Kris-Etherton PM. Provision of foods differing in energy density affects long-term weight loss. *Obes Res.* 2005;13(6):1052–1060.

122. Appel LJ, Champagne CM, Harsha DW, et al. Effects of comprehensive lifestyle modification on blood pressure control: main results of the PREMIER clinical trial. *JAMA*. 2003;289(16):2083–2093.

123. Ledikwe JH, Blanck HM, Khan LK, et al. Low-energy-density diets are associated with high diet quality in adults in the United States. *J Am Diet Assoc*. 2006;106(8):1172–1180.

124. Ledikwe JH, Blanck HM, Khan LK, et al. Dietary energy density determined by eight calculation methods in a nationally representative United States population. *J Nutr.* 2005;135(2):273–278.

125. Ello-Martin JA, Roe LS, Ledikwe JH, Beach AM, Rolls BJ. Dietary energy density in the treatment of obesity: a year-long trial comparing 2 weight-loss diets. *Am J Clin Nutr.*2007;85(6):1465–1477.

126. DellaValle DM, Roe LS, Rolls BJ. Does the consumption of caloric and non-caloric beverages with a meal affect energy intake? *Appetite*. 2005;44(2):187–193.

127. U.S. Department of Health and Human Services: Office of Disease Prevention and Health Promotion--Healthy People 2010. *Nasnewsletter*. 2000;15(3):3.

128. Slentz CA, Bateman LA, Willis LH, et al. Effects of aerobic vs. resistance training on visceral and liver fat stores, liver enzymes, and insulin resistance by HOMA in overweight adults from STRRIDE AT/RT. *Am J Physiol Endocrinol Metab*. 2011;301(5):E1033 –E1039.

129. Borer KT, Fogleman K, Gross M, La New JM, Dengel D. Walking intensity for postmenopausal bone mineral preservation and accrual. *Bone*. 2007;41(4):713–721.

130. Asikainen T-M, Kukkonen-Harjula K, Miilunpalo S. Exercise for health for early postmenopausal women: a systematic review of randomised controlled trials. *Sports Med*. 2004;34(11):753–778.

131. Long SJ, Hart K, Morgan LM. The ability of habitual exercise to influence appetite and food intake in response to high- and low-energy preloads in man. *Br J Nutr.* 2002;87(5):517–523.

132. Luke A, Dugas LR, Durazo-Arvizu RA, Cao G, Cooper RS. Assessing physical activity and its relationship to cardiovascular risk factors: NHANES 2003-2006. *BMC Public Health.* 2011;11:387.

133. Reddigan JI, Ardern CI, Riddell MC, Kuk JL. Relation of physical activity to cardiovascular disease mortality and the influence of cardiometabolic risk factors. *Am J Cardiol.* 2011;108(10):1426–1431.

134. Warburton DER, Nicol CW, Bredin SSD. Health benefits of physical activity: the evidence. *CMAJ*. 2006;174(6):801–809.

135. Dunn AL, Marcus BH, Kampert JB, Garcia ME, Kohl HW 3rd, Blair SN. Reduction in cardiovascular disease risk factors: 6-month results from Project Active. *Prev Med*. 1997;26(6):883–892.

136. Colbert LH, Visser M, Simonsick EM, et al. Physical activity, exercise, and inflammatory markers in older adults: findings from the Health, Aging and Body Composition Study. *J Am Geriatr Soc*. 2004;52(7):1098–1104.

137. Jakicic JM, Davis KK. Obesity and physical activity. *Psychiatr Clin North Am.* 2011;34(4):829–840.

138. Donnelly JE, Blair SN, Jakicic JM, Manore MM, Rankin JW, Smith BK. American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. *Med Sci Sports Exerc*. 2009;41(2):459–471.

139. Avenell A, Broom J, Brown TJ, et al. Systematic review of the long-term effects and economic consequences of treatments for obesity and implications for health improvement. *Health Technol Assess*. 2004;8(21):iii–iv, 1–182.

140. Kreider RB, Serra M, Beavers KM, et al. A structured diet and exercise program promotes favorable changes in weight loss, body composition, and weight maintenance. *J Am Diet Assoc*. 2011;111(6):828–843.

141. Goodpaster BH, Delany JP, Otto AD, et al. Effects of diet and physical activity interventions on weight loss and cardiometabolic risk factors in severely obese adults: a randomized trial. *JAMA*. 2010;304(16):1795–1802.

142. Curioni CC, Lourenço PM. Long-term weight loss after diet and exercise: a systematic review. *Int J Obes (Lond)*. 2005;29(10):1168–1174.

143. Tate DF, Jeffery RW, Sherwood NE, Wing RR. Long-term weight losses associated with prescription of higher physical activity goals. Are higher levels of physical activity protective against weight regain? *Am J Clin Nutr.*2007;85(4):954–959.

144. Wing RR, Phelan S. Long-Term Weight Loss Maintenance. *Am J Clin Nutr*. 2005;82(1):222S–225S.

145. Hall DM, Most MM. Dietary adherence in well-controlled feeding studies. *J Am Diet Assoc*. 2005;105(8):1285–1288.

146. Most MM, Ershow AG, Clevidence BA. An overview of methodologies, proficiencies, and training resources for controlled feeding studies. *J Am Diet Assoc*. 2003;103(6):729–735.

147. Campbell DD, Meckling KA. Effect of the protein:carbohydrate ratio in hypoenergetic diets on metabolic syndrome risk factors in exercising overweight and obese women. *Br J Nutr*. 2012:1–14.

148. Shai I, Schwarzfuchs D, Henkin Y, et al. Weight loss with a low-carbohydrate, Mediterranean, or low-fat diet. *N Engl J Med.* 2008;359(3):229–241.

149. Levitsky DA, Pacanowski C. Losing weight without dieting. Use of commercial foods as meal replacements for lunch produces an extended energy deficit. *Appetite*. 2011;57(2):311–317.

150. Hamdy O, Zwiefelhofer D. Weight management using a meal replacement strategy in type 2 diabetes. *Curr Diab Rep.* 2010;10(2):159–164.

151. Samra RA, Anderson GH. Insoluble cereal fiber reduces appetite and short-term food intake and glycemic response to food consumed 75 min later by healthy men. *Am J Clin Nutr.*2007;86(4):972–979.

152. Heymsfield SB, van Mierlo CAJ, van der Knaap HCM, Heo M, Frier HI. Weight management using a meal replacement strategy: meta and pooling analysis from six studies. *Int. J Obes Relat Metab Disord.* 2003;27(5):537–549.

153. König D, Deibert P, Frey I, Landmann U, Berg A. Effect of meal replacement on metabolic risk factors in overweight and obese subjects. *Ann Nutr Metab*. 2008;52(1):74–78.

154. Davis LM, Coleman C, Kiel J, et al. Efficacy of a meal replacement diet plan compared to a food-based diet plan after a period of weight loss and weight maintenance: a randomized controlled trial. *Nutr J*. 2010;9:11.

155. Vázquez C, Montagna C, Alcaraz F, et al. Meal replacement with a low-calorie diet formula in weight loss maintenance after weight loss induction with diet alone. *Eur J Clin Nutr*. 2009;63(10):1226–1232.

156. Cheskin LJ, Mitchell AM, Jhaveri AD, et al. Efficacy of meal replacements versus a standard food-based diet for weight loss in type 2 diabetes: a controlled clinical trial. *Diabetes Educ*. 2008;34(1):118–127.

157. Ashley JM, Herzog H, Clodfelter S, Bovee V, Schrage J, Pritsos C. Nutrient adequacy during weight loss interventions: a randomized study in women comparing the dietary intake in a meal replacement group with a traditional food group. *Nutr J*. 2007;6:12.

158. Ditschuneit HH. Do meal replacement drinks have a role in diabetes management? *Nestle Nutr Workshop Ser Clin Perform Programme*. 2006;11:171–179; discussion 179–181.

159. Huerta S, Li Z, Li HC, Hu MS, Yu CA, Heber D. Feasibility of a partial meal replacement plan for weight loss in low-income patients. *Int J Obes Relat Metab Disord*. 2004;28(12):1575–1579.

160. Burge R, Dawson-Hughes B, Solomon DH, Wong JB, King A, Tosteson A. Incidence and economic burden of osteoporosis-related fractures in the United States, 2005-2025. *J Bone Miner Res*. 2007;22(3):465–475.

161. Cummings SR, Bates D, Black DM. Clinical Use of Bone Densitometry. *JAMA*. 2002;288(15):1889 –1897.

162. Nikander R, Sievänen H, Heinonen A, Daly RM, Uusi-Rasi K, Kannus P. Targeted exercise against osteoporosis: A systematic review and meta-analysis for optimising bone strength throughout life. *BMC Med*. 2010;8:47.

163. Datta HK, Ng WF, Walker JA, Tuck SP, Varanasi SS. The cell biology of bone metabolism. *J Clin Pathol*. 2008;61(5):577–587.

164. Robling AG, Castillo AB, Turner CH. Biomechanical and molecular regulation of bone remodeling. *Annu Rev Biomed Eng*. 2006;8:455–498.

165. Vääräniemi J, Halleen JM, Kaarlonen K, et al. Intracellular machinery for matrix degradation in bone-resorbing osteoclasts. *J Bone Miner Res*. 2004;19(9):1432–1440.

166. Boyle WJ, Simonet WS, Lacey DL. Osteoclast differentiation and activation. *Nature*. 2003;423(6937):337–342.

167. Murthy RK, Morrow PK, Theriault RL. Bone biology and the role of the RANK ligand pathway. *Oncology (Williston Park, N.Y.)*. 2009;23(14 Suppl 5):9–15.

168. Kular J, Tickner J, Chim SM, Xu J. An overview of the regulation of bone remodelling at the cellular level. *Clinical Biochemistry*. 2012.

169. Matsumoto T, Kuriwaka-Kido R, Kondo T, Endo I, Kido S. Regulation of osteoblast differentiation by interleukin-11 via AP-1 and Smad signaling. *Endocr J*. 2011.

170. Bianco P. Bone and the hematopoietic niche: a tale of two stem cells. *Blood*. 2011;117(20):5281 –5288.

171. Wada T, Nakashima T, Hiroshi N, Penninger JM. RANKL-RANK signaling in osteoclastogenesis and bone disease. *Trends Mol Med*. 2006;12(1):17–25.

172. D'Amelio P, Grimaldi A, Di Bella S, et al. Estrogen deficiency increases osteoclastogenesis up-regulating T cells activity: a key mechanism in osteoporosis. *Bone*. 2008;43(1):92–100.

173. Hamidi M, Tarasuk V, Corey P, Cheung AM. Association between the Healthy Eating Index and bone turnover markers in US postmenopausal women aged≥45 y. *Am J Clin Nutr*.2011;94(1):199–208.

174. Cao JJ. Effects of obesity on bone metabolism. *J Orthop Surg Res*. 2011;6:30.

175. Dandona P, Aljada A, Bandyopadhyay A. Inflammation: the link between insulin resistance, obesity and diabetes. *Trends Immunol*. 2004;25(1):4–7.

176. McLean RR. Proinflammatory cytokines and osteoporosis. *Curr Osteoporos Rep*. 2009;7(4):134–139.

177. Mosley JR. Osteoporosis and bone functional adaptation: mechanobiological regulation of bone architecture in growing and adult bone, a review. *J Rehabil Res Dev*. 2000;37(2):189–199.

178. Heaney RP. Protein intake and the calcium economy. *J Am Diet Assoc*. 1993;93(11):1259–1260.

179. Bonjour J-P. Dietary Protein: An Essential Nutrient For Bone Health. *J Am Coll Nutr*. 2005;24(suppl 6):526S–536S.

180. Hunt JR, Johnson LK, Fariba Roughead ZK. Dietary protein and calcium interact to influence calcium retention: a controlled feeding study. *Am J Clin Nutr*.2009;89(5):1357–1365.

181. Kerstetter JE, O'Brien KO, Caseria DM, Wall DE, Insogna KL. The impact of dietary protein on calcium absorption and kinetic measures of bone turnover in women. *J Clin Endocrinol Metab*. 2005;90(1):26–31.

182. Ammann P, Bourrin S, Bonjour JP, Meyer JM, Rizzoli R. Protein undernutrition-induced bone loss is associated with decreased IGF-I levels and estrogen deficiency. *J Bone Miner Res*. 2000;15(4):683–690.

183. Sukumar D, Ambia-Sobhan H, Zurfluh R, et al. Areal and volumetric bone mineral density and geometry at two levels of protein intake during caloric restriction: a randomized, controlled trial. *J Bone Miner Res*. 2011;26(6):1339–1348.

184. Gilbert J-A, Joanisse DR, Chaput J-P, et al. Milk supplementation facilitates appetite control in obese women during weight loss: a randomised, single-blind, placebo-controlled trial. *Br. J Nutr*. 2011;105(1):133–143.

185. Meng X, Zhu K, Devine A, Kerr DA, Binns CW, Prince RL. A 5-year cohort study of the effects of high protein intake on lean mass and BMC in elderly postmenopausal women. *J Bone Miner Res*. 2009;24(11):1827–1834.

186. Dawson-Hughes B, Harris SS. Calcium intake influences the association of protein intake with rates of bone loss in elderly men and women. *Am J Clin Nutr*.2002;75(4):773–779.

187. Hannan MT, Tucker KL, Dawson-Hughes B, Cupples LA, Felson DT, Kiel DP. Effect of dietary protein on bone loss in elderly men and women: the Framingham Osteoporosis Study. *J Bone Miner Res*. 2000;15(12):2504–2512.

188. Darling AL, Millward DJ, Torgerson DJ, Hewitt CE, Lanham-New SA. Dietary protein and bone health: a systematic review and meta-analysis. *Am J Clin Nutr.*2009;90(6):1674–1692.

189. Fenton TR, Lyon AW, Eliasziw M, Tough SC, Hanley DA. Meta-analysis of the effect of the acid-ash hypothesis of osteoporosis on calcium balance. *J Bone Miner Res.* 2009;24(11):1835–1840.

190. New SA. Nutrition Society Medal lecture. The role of the skeleton in acid-base homeostasis. *Proc Nutr Soc*. 2002;61(2):151–164.

191. Fenton TR, Tough SC, Lyon AW, Eliasziw M, Hanley DA. Causal assessment of dietary acid load and bone disease: a systematic review & meta-analysis applying Hill's epidemiologic criteria for causality. *Nutr J*. 2011;10:41.

192. Fenton TR, Lyon AW. Milk and acid-base balance: proposed hypothesis versus scientific evidence. *J Am Coll Nutr*. 2011;30(5 Suppl 1):471S–5S.

193. Bonjour J-P. Calcium and phosphate: a duet of ions playing for bone health. *J Am Coll Nutr*. 2011;30(5 Suppl 1):438S–48S.

194. Heaney RP. Dairy and bone health. *J Am Coll Nutr*. 2009;28 Suppl 1:82S–90S.

195. Esterle L, Sabatier J-P, Guillon-Metz F, et al. Milk, rather than other foods, is associated with vertebral bone mass and circulating IGF-1 in female adolescents. *Osteoporos Int*. 2008;20(4):567–575.

196. Beasley JM, Ichikawa LE, Ange BA, et al. Is protein intake associated with bone mineral density in young women? *Am J Clin Nutr.*2010;91(5):1311–1316.

197. Park J-H, Song Y-M, Sung J, et al. The association between fat and lean mass and bone mineral density: The Healthy Twin Study. *Bone*. 2012.

198. Albala C, Yáñez M, Devoto E, Sostin C, Zeballos L, Santos JL. Obesity as a protective factor for postmenopausal osteoporosis. *Int. J. Obes Relat Metab Disord.* 1996;20(11):1027–1032.

199. Borer KT. Physical activity in the prevention and amelioration of osteoporosis in women : interaction of mechanical, hormonal and dietary factors. *Sports Med*. 2005;35(9):779–830.

200. Riedt CS, Cifuentes M, Stahl T, Chowdhury HA, Schlussel Y, Shapses SA. Overweight postmenopausal women lose bone with moderate weight reduction and 1 g/day calcium intake. *J Bone Miner Res.* 2005;20(3):455–463.

201. Soleymani T, Tejavanija S, Morgan S. Obesity, bariatric surgery, and bone. *Curr Opin Rheumatol*. 2011;23(4):396–405.

202. Fogelholm GM, Sievänen HT, Kukkonen-Harjula TK, Pasanen ME. Bone mineral density during reduction, maintenance and regain of body weight in premenopausal, obese women. *Osteoporos Int*. 2001;12(3):199–206.

203. Bowen J, Noakes M, Clifton P. High dairy-protein versus high mixed-protein energy restricted diets - the effect on bone turnover and calcium excretion in overweight adults. *Asia Pac J Clin Nutr*. 2003;12 Suppl:S52.

204. Vilarrasa N, Gómez JM, Elio I, et al. Evaluation of bone disease in morbidly obese women after gastric bypass and risk factors implicated in bone loss. *Obes Surg*. 2009;19(7):860–866.

205. Bacon L, Stern JS, Keim NL, Van Loan MD. Low bone mass in premenopausal chronic dieting obese women. *Eur J Clin Nutr*. 2004;58(6):966–971.

206. Hinton PS, Rector RS, Linden MA, et al. Weight-loss-associated changes in bone mineral density and bone turnover after partial weight regain with or without aerobic exercise in obese women. *Eur J Clin Nutr*. 2011

207. Bowen J, Noakes M, Clifton PM. A high dairy protein, high-calcium diet minimizes bone turnover in overweight adults during weight loss. *J Nutr*. 2004;134(3):568–573.

208. Riedt CS, Schlussel Y, von Thun N, et al. Premenopausal overweight women do not lose bone during moderate weight loss with adequate or higher calcium intake. *Am J Clin Nutr*.2007;85(4):972–980.

209. Ricci TA, Chowdhury HA, Heymsfield SB, Stahl T, Pierson RN Jr, Shapses SA. Calcium supplementation suppresses bone turnover during weight reduction in postmenopausal women. *J Bone Miner Res*. 1998;13(6):1045–1050.

210. Redman LM, Rood J, Anton SD, Champagne C, Smith SR, Ravussin E. Calorie Restriction and Bone Health in Young, Overweight Individuals. *Arch Intern Med*. 2008;168(17):1859–1866.

211. Shapses SA, Von Thun NL, Heymsfield SB, et al. Bone Turnover and Density in Obese Premenopausal Women During Moderate Weight Loss and Calcium Supplementation. *J Bone Miner Res*. 2001;16(7):1329–1336.

212. Josse AR, Atkinson SA, Tarnopolsky MA, Phillips SM. Diets Higher in Dairy Foods and Dietary Protein Support Bone Health during Diet- and Exercise-Induced Weight Loss in Overweight and Obese Premenopausal Women *J Clin Endocrinol Metab*. 2011.

213. Thorpe MP, Jacobson EH, Layman DK, He X, Kris-Etherton PM, Evans EM. A diet high in protein, dairy, and calcium attenuates bone loss over twelve months of weight loss and maintenance relative to a conventional high-carbohydrate diet in adults. *J Nutr*. 2008;138(6):1096–1100.

214. Rector RS, Loethen J, Ruebel M, Thomas TR, Hinton PS. Serum markers of bone turnover are increased by modest weight loss with or without weight-bearing exercise in overweight premenopausal women. *Appl Physiol Nutr Metab*. 2009;34(5):933–941.

215. Cosman F, Nieves J, Wilkinson C, Schnering D, Shen V, Lindsay R. Bone density change and biochemical indices of skeletal turnover. *Calcif Tissue Int.* 1996;58(4):236–243.

216. Piehowski KE. Effects of incorporating dark chocolate and cocoa beverage into a weight-loss diet on inflammatory status and bone turnover. Unpublished dissertation, Pennsylvania State University, University Park, PA. 2011.

217. Stancliffe RA, Thorpe T, Zemel MB. Dairy attentuates oxidative and inflammatory stress in metabolic syndrome. *Am J Clin Nutr.*2011;94(2):422–430.

218. Lam YY, Mitchell AJ, Holmes AJ, et al. Role of the gut in visceral fat inflammation and metabolic disorders. *Obesity (Silver Spring)*. 2011;19(11):2113–2120.

219. Bhupathiraju SN, Dawson-Hughes B, Hannan MT, Lichtenstein AH, Tucker KL. Centrally located body fat is associated with lower bone mineral density in older Puerto Rican adults. *Am J Clin Nutr.*2011;94(4):1063–1070.

220. Martin RB, Burr DB, Sharkey NA. *Skeletal tissue mechanics*. Springer; 1998.

221. Vainionpää A, Korpelainen R, Leppäluoto J, Jämsä T. Effects of high-impact exercise on bone mineral density: a randomized controlled trial in premenopausal women. *Osteoporos Int*. 2005;16(2):191–197.

222. Herrmann D, Hebestreit A, Ahrens W. Impact of physical activity and exercise on bone health in the life course : A review. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz*. 2012;55(1):35–54.

223. Armstrong MEG, Spencer EA, Cairns BJ, et al. Body mass index and physical activity in relation to the incidence of hip fracture in postmenopausal women. *J Bone Miner Res.* 2011;26(6):1330–1338.

224. Rogers RS, Hinton PS. Bone loading during young adulthood predicts bone mineral density in physically active, middle-aged men. *Phys Sportsmed*. 2010;38(2):146–155.

225. Daly RM, Dunstan DW, Owen N, Jolley D, Shaw JE, Zimmet PZ. Does high-intensity resistance training maintain bone mass during moderate weight loss in older overweight adults with type 2 diabetes? *Osteoporos Int*. 2005;16(12):1703–1712.

226. Silverman NE, Nicklas BJ, Ryan AS. Addition of Aerobic Exercise to a Weight Loss Program Increases BMD, with an Associated Reduction in Inflammation in Overweight Postmenopausal Women. *Calcif Tissue Int*. 2009;84(4):257–265.

227. Hosny IA, Elghawabi HS, Younan WBF, Sabbour AA, Gobrial MAM. Beneficial impact of aerobic exercises on bone mineral density in obese premenopausal women under caloric restriction. *Skeletal Radiol.* 2011.

228. Nakata Y, Ohkawara K, Lee DJ, Okura T, Tanaka K. Effects of additional resistance training during diet-induced weight loss on bone mineral density in overweight premenopausal women. *J Bone Miner Metab*. 2008;26(2):172–177.

229. Romero Barco CM, Manrique Arija S, Rodríguez Pérez M. Biochemical markers in osteoporosis: usefulness in clinical practice. *Reumatol Clin*. 2011.

Table 2.1: Summary of findings from moderate protein energy-restricted diet studies examining body composition, anthropometrics, and metabolic indicators of health in overweight/obese adults.

Study	Population/Duration	Energy-restricted diet (Pro/Carb/Fat)	Physical Activity	Energy Intake	Body composition and anthropometrics	Metabolic endpoints	Insulin/Glucose	Other
Josse 2011(26)	Premenopausal overweight/obese women (n=90); 16 weeks	MP - 30/40/30%, 6-7 dairy svg; SP med dairy - 15/55/30%, 3-4 dairy svgs; SP low dairy - 15/55/30%, <1 dairy svg	All groups engaged in aerobic (5x/wk) and resistance (2x/wk) EX.	Similar between groups	Similar BW loss; MP group ↓FM week 8-16, ↓CAT compared to SP low dairy group. HP group ↑FFSTM while other groups maintained or lost.	MP and SP/low dairy ↓TC,↓LDL-C and ↓TG.	↓Insulin levels in SP med dairy group	↓CRP similarly between groups
Farnsworth 2003 (27)	Overweight/obese adults (n=57); 12 week weight loss, 4 week weight-loss maintenance.	MP - 30/40/30% (PRO 45% dairy); SP - 15/55/30% (PRO 18% dairy)	Participants were asked to maintain activity levels.	Similar between groups	No difference in BW, FM or FFSTM losses; a time x diet x sex interaction was present such that women on MP diet lost less FFSTM.	There was a greater ↓TG in the MP group; no effect of diet was observed in ↓TC or ↓LDL-C.	↓Fasting insulin with no effect of diet; ↓AUC for glucose after HP meal at baseline and week 16.	N/A
Noakes 2005 (28)	Overweight/obese women (n=100); 12 weeks	MP - 34/46/20% SP - 17/63/20%	N/A	Similar between groups	No differences in diet groups, but when participants with baseline TG >1.5mmol/L were examined, those on the MP diet had greater ↓BW, ↓FM and ↓CAT.	↓TC, ↓LDL-C and ↑HDL-C in all groups. ↓TG to a greater extent in the HP group, especially in those with high TGs were examined.	Reductions in glucose and insulin were similar between groups.	N/A
Meckling 2007 (30)	Overweight/obese women (n=44); 12 weeks	MP - 1g Pro:1g CHO SP - 1g Pro:3g CHO	Both groups examined interventions with and without EX; including circuit training 3x/wk.	Similar between groups	All groups lost BW but there was a main effect of MP diet and EX resulting in greater ↓BW.	↓BP in all groups, ↓TC in SP and MP-EX, ↓LDL-C in MP and ↓TG in MP-EX	N/A	N/A
Layman 2005 (31)	Overweight/obese women (n=48); 16 weeks	MP - 30/40/30% SP - 15/55/30%	Both groups examined interventions with and without EX, including aerobic (5x/wk) and resistance (2x/wk).	Similar between groups	MP group had greater ↓BW, ↓FM and ↓CAT. EX significantly ↓FM, and maintained FFSTM regardless of diet.	MP group had greater ↓TG. SP groups had greater ↓TC and LDL-C.	Reduced in response to BW loss	Greater ↑adiponectin in EX groups
Wycherley 2010 (32)	Overweight/obese adults with type 2 diabetes (n=83); 16 weeks	MP - 33/43/22 SP - 19/53/26	Both groups examined interventions with and without resistance training.	Similar between groups	Greatest ↓BW, ↓FM, ↓WC in MP EX group. Greater ↓BW in exercising groups compared to diet-only; more ↓FM in MP.	No differences between groups in BP, TG, TC, LDL-C or HLD-C.	No differences between groups in insulin concentrations.	N/A

Study	Population/Duration	Energy-restricted diet (Pro/Carb/Fat)	Physical Activity	Energy Intake	Body composition and anthropometrics	Metabolic endpoints	Insulin/Glucose	Other
Skov 1999 (33)	Overweight/obese adults (n=60); 24 weeks	Diets were ad libitum; there was a control group MP - 25/45/30% SP - 12/58/30%	N/A	Reported energy intake was lower in the MP group.	MP group had greater ↓BW, ↓FM and↓ CAT at 12 and 24 weeks.	↓TG in the MP and ↑TG in the SP at 12 weeks, but group difference did not remain at 24 weeks. Both groups ↓TC and HDL-C.		
Layman 2009 (34)	Overweight/obese adults (n=130); 8 month weight loss, 4 month weight-loss maintenance.	MP - 30/40/30% SP - 15/55/30%	N/A	Similar between groups	With similar BW loss, MP group had greater ↓FM at 12 months.	MP had greater↓ TG and ↑HDL-C at 4 and 12 months; the SP had ↓TC and ↓LDL-C at 4 months. These differences persisted after controlling for FM loss.	N/A	N/A
Parker 2002 (35)	Overweight/obese adults with type 2 diabetes (n=54); 8 week weight loss, 4 week weight-loss maintenance.	MP- 28/42/28% SP- 16/55/26%	Participants were asked to maintain activity levels.	Similar between groups	There was a diet x sex interaction present such that women on MP diet lost more BW, FM and CAT then men.	↓TC and LDL-C to a greater extent in the MP group. Both groups ↓TG and BP.	No effect of diet on insulin reductions.	N/A
Layman 2003 (36, 74)	Overweight/obese women (n=24); 10 weeks	MP - 30/40/30% SP - 15/55/30%	Participants were asked to maintain activity levels.	Similar between groups	With similar losses in BW, the MP group had a greater ratio of FM/FFSTM loss	MP group had greater ↓TG and ↓TG/HDL-C. All women ↓TC.	Women in SP group had ↑response to meals and postprandial hypoglycemia.	MP group reported ↑satiety
Leidy 2007 (38)	Overweight/obese women (n=46); 12 weeks	MP - 30/40/25% SP -18/57/25%	N/A	Similar between groups	Similar BW loss; MP lost less FFSTM	N/A	N/A	MP had less pronounced ↓satiety
Lee 2009 (59)	Overweight/obese adults with metabolic syndrome (n=67); 12 weeks	MP - 30/50/20% SP - 15/65/20%; both groups used a meal replacement 2x/d	N/A	Similar between groups	↓BW, ↓FM, ↓FFSTM, ↓WC and ↓CAT were similar; however, greater ↓FM in the MP group among subjects with ↑dietary compliance.	N/A	N/A	N/A

Study	Population/Duration	Energy-restricted diet (Pro/Carb/Fat)	Physical Activity	Energy Intake	Body composition and anthropometrics	Metabolic endpoints	Insulin/Glucose	Other
Due 2004 (60)	Overweight/obese adults (n=50); 6 month weight-loss, 24 month follow-up.	MP - 25/45/30% SP - 12/57/30%	N/A	Average energy intake was lower in MP group during the first 6 months.	MP group had greater ↓BW, ↓FM, ↓WC and ↓CAT at 6 months. Only difference in WC and CAT were significant at 12 months.	No difference observed between groups in TC, HDL-C or TG.	No difference observed between groups in insulin or glucose.	N/A
Bowen 2005 (103)	Overweight/obese adults (n=50); 12 week weight loss, 4 week weight-loss maintenance.	Both diets were 34/41/24%, and high in either dairy protein (64% of protein) or mixed protein sources.	N/A	Similar between groups	No difference in BW, FM or CAT losses.	↓TC, ↓LDL-C and ↓TG in both groups.	↓Fasting insulin and ↓AUC after glucose tolerance test.	N/A

MP = moderate protein
SP = standard protein
EX = exercise
BW= body weight
FM = fat mass
CAT = central abdominal tissue
FFSTM = fat-free soft tissue mass
TC = total cholesterol
LDL-C = low-density lipoprotein
TG = triglycerides
CRP = C-reactive protein
AUC = area under the curve
HDL-C = high-density lipoprotein
WC = waist circumference
BP = blood pressure

Table 2.2: Summary of findings from energy-restricted diets with or without physical activity examining body weight loss and bone health related outcomes.

Study	Population/Duration	Energy-restricted diet	Physical activity	Body composition	Biomarkers of bone turnover	Bone mineral density
Jenson 2001	Obese women (n=62); 6 months	Women consumed an energy-restricted diet containing 800 mg Ca and either: (1) placebo or (2) 1000 mg Ca supplement.	N/A	Similar BW loss between groups.	OC increased after 1 month in the placebo group.	After 1 month, both groups reduced TB BMC; the placebo group had reductions in LS at 3 and 6 months.
Riedt 2005	Postmenopausal, overweight women (n=66); 6 months	No macronutrient requirements in either of 2 Ca supplemented weight-loss groups; (1) 1000 mg Ca citrate, (2) 1700 mg Ca citrate.	Maintained activity levels.	Similar BW loss between groups.	Bone turnover markers (PYD and OC) were higher for the 1000 mg Ca compared with 1700 mg Ca group.	Women in 1000 mg Ca group reduced BMD at trochanter and LS over time, and trochanter BMD decreased more than 1700 mg Ca group.
Bowen 2004	Overweight/obese adults (n=60); 12 week weight loss, 4 week weight-loss maintenance.	Both diets were Pro 34/Cho 41/Fat 24%, and high in either dairy protein (2400 mg Ca/d) or mixed protein sources (500 mg Ca/d).	N/A	Similar BW loss between groups.	The mixed protein group had an increase in OC and dPYD.	No changes over time or between groups.
Riedt 2007	Premenopausal, overweight women (n=44); 6 months	No macronutrient requirements in either of 2 Ca supplemented weight-loss groups; (1) 1000 mg Ca citrate, (2) 1800 mg Ca citrate.	Maintained activity levels.	Similar BW loss between groups.	Markers of bone turnover (NTx, PYD, dPYD and OC) did not differ between groups or over time.	Women in the 1800 mg Ca group increased TB and FA BMD over time; FA BMD increased to a greater extent than the 1000 mg group.
Rector 2009	Premenopausal overweight/obese women (n=36); 6 weeks	Diets were self-selected in all three groups; however, protein comprised ~17% of kcal in (1) diet only and (2) weight-bearing (running) groups, and 21% in (3) nonweight-bearing (cycling) groups	Running and cycling groups participated in exercise 5x/wk for at least 45min.	Similar BW loss between groups; only exercising groups lost body fat%.	OC and CTx increased in all 3 groups; no change in BAP	N/A
Shapses 2001	Premenopausal, obese women (n=38); 6 months	No macronutrient requirements in either of 2 groups; (1) placebo group, (2) 1000 mg Ca citrate. A weight maintenance group was created from non-compliant placebo group.	Maintained activity levels.	Similar BW loss between groups.	No differences in markers of bone turnover between groups; dPYD increased over time in placebo group.	No change in TB BMD in any group; LS increased over time in the Ca citrate group and compared to other groups.
Thorpe 2008	Overweight/obese adults (n=130); 8 month weight loss, 4 month weight-loss maintenance.	MP - Pro 30/Cho 40/Fat 30%; 3 dairy svg/d, (1140 mg Ca/d) ; SP - Pro 15/Cho 55/Fat 30%; 2 dairy svg/d, (766 mg Ca/d).	Similar between groups.	Similar BW loss between groups	N/A	BMD was higher at TB, LS and TPF in MP compared to SP group.

Study	Population/Duration	Energy-restricted diet	Physical activity	Body composition	Biomarkers of bone turnover	Bone mineral density
Josse 2011	Premenopausal overweight/obese women (n=90); 16 weeks	MP - Pro 30/Cho 40/Fat 30%, 6-7 dairy svg; SP med dairy - Pro 15/ Cho 55/Fat 30%, 3-4 dairy svgs; SP low dairy - Pro 15/Cho 55/Fat 30%, <1 dairy svg	All groups engaged in aerobic (5x/wk) and resistance (2x/wk) exercise.	N/A	Markers of resorption (CTX, NTX, dPYD) increased in low dairy group; markers of formation (OC, P1NP) increased in the MP group, as did OPG, with a reduction in RANKL.	No change in TB BMD in any group
Wagner 2007	Premenopausal overweight/obese women (n=45); 12 weeks	Protein comprised ~15% of kcal in all 4 groups including 750 mg dietary Ca; (1)placebo group, (2) 800 mg Ca lactate, (3) 800 mg Ca phosphate, (4) low-fat milk (800 mg Ca).	Resistance and aerobic exercise 3x/week in all groups.	Similar BW loss between groups; milk group lost less FM than placebo.	All groups increased BAP. Ca lactate supplemented group reduced alpha helical peptide (resorption marker).	N/A

Ca = calcium
BW = body weight
OC = osteocalcin
TB = total body
BMC = bone mineral content
LS = lumbar spine
PYD = pyridinoline
BMD = bone mineral density
dPYD = deoxypyridinoline
CTx = C-terminal telopeptide of type I collagen
NTx = N-terminal telopeptide of type I collagen
FA = forearm
BAP = bone-specific alkaline phosphatase
MP = moderate protein
SP = standard protein
TPF = total proximal femur
P1NP = procollagen type 1 N-terminal propeptide
OPG = osteoprotegerin

CHAPTER 3

AN ENERGY-RESTRICTED DIETARY PATTERN INCLUDING MODERATE PROTEIN AND NON-FAT DAIRY COMBINED WITH WALKING PROMOTES BENEFICIAL CHANGES IN BODY COMPOSITION AND METABOLIC PARAMETERS IN PREMENOPAUSAL WOMEN WITH EXCESS ADIPOSITY

To be submitted to the *Journal of the Academy of Nutrition and Dietetics*

ABSTRACT

Background Moderate protein and non-fat dairy within an energy-reduced diet (ERD) may contribute to health benefits achieved from body weight (BW) loss.

Objective To examine the effectiveness of a weight-loss/weight-loss maintenance intervention including an ERD with moderate dietary protein and non-fat dairy including yogurt (YL) and daily walking in promoting beneficial changes in body composition and metabolic parameters compared to an ERD with standard protein and non-fat dairy (EXG) with daily walking.

Design Randomized clinical trial with free-living participants. Outcome measures were evaluated at baseline, Weeks 2, 12 and 24.

Participants/setting University-based intervention with 104 sedentary, healthy premenopausal women with overweight/obesity. Women engaged in a 24-week weight-loss/weight-loss maintenance intervention. Data collection extended from December 2009 to March 2011.

Intervention Women were randomized to YL or EXG groups and completed the intervention. Women attended weekly nutrition-related educational sessions.

Main outcome measures Dietary intake, BW, body mass index (BMI), fat mass (FM), fat-free soft tissue mass (FFSTM), body fat percentage (BF%), waist and hip circumference measurements.

Secondary outcome measures Selected vital signs, resting energy expenditure, blood lipids, glucose, insulin, and selected adipose-derived hormones.

Statistical analyses Multivariate analysis of covariance with repeated measures on the time factor.

Results There were no significant effects of diet group on outcome measures. Women in the YL and EXG groups, respectively, reduced BW (-4.9±3.2 and -4.3±3.3 kg) and FM (-3.0±2.2 and -

2.3±2.3 kg) during the weight-loss phase and maintained these losses to 24 weeks. Both groups also experienced significant decreases in BMI, FFSTM, BF%, waist and hip circumferences and serum triglycerides, total cholesterol and leptin.

Conclusions Healthy premenopausal women with excess adiposity effectively lost BW and FM and improved some metabolic risk factors with an ERD with either moderate protein and non-fat dairy or standard protein and non-fat dairy combined with moderate walking.

INTRODUCTION

Excess adiposity is associated with increased risk of developing type 2 diabetes, heart disease, hypertension and certain cancers (1–3). Successful prevention and treatment of overweight/obesity is paramount as economic and societal burdens of the obesity epidemic persist (4,5). Weight loss achieved by following an energy-restricted diet (ERD) promotes several metabolic benefits (6–8). In addition to favorable outcomes conferred by reduced body weight (BW), research suggests additive beneficial effects of certain dietary components on various weight-related outcomes including body composition, insulin sensitivity and risk factors for cardiovascular disease (9–11).

Dietary protein has been widely studied, with benefits beyond BW loss reported with other typical ERD interventions (12,13). Higher protein diets have resulted in enhancement of BW (14,15) and fat mass (FM) losses (12,16), preservation of fat-free soft tissue mass (FFSTM) (15,17) and improvement in blood lipids (14,16,18–21). These results have been partially attributed to increased thermic effect of food (22), increased satiety (22,23) and maintenance of FFSTM (18).

Similarly, increased dairy intake has been implicated in improved body composition with corresponding BW loss (24–26). Both dietary calcium (27) and the branched-chain amino acid leucine (28,29) contained in dairy products have been regarded as contributors to the reported link between dairy food consumption and weight-related outcomes.

Several clinical weight-loss interventions support the treatment of overweight/obesity with the incorporation of increased dietary protein and/or dairy. Healthy premenopausal women reduced FM and body fat percentage (BF%) to a greater extent when consuming an ERD with 30% of energy as protein and 6-7 servings of low-fat dairy per day, compared to women

consuming an ERD with adequate protein (15% of kcal) and 3-4 servings of low-fat dairy per day or adequate protein and <1 serving of low-fat dairy per day (26). Women consuming adequate-protein and <1 serving of low-fat dairy lost FFSTM; women consuming adequate-protein and 3-4 servings of low-fat dairy maintained FFSTM, and women consuming 30% of energy as protein and 6-7 servings of low-fat dairy gained FFSTM over 16 weeks (26). Women in all three groups exercised daily. In a separate study (30), premenopausal women consuming a carbohydrate to protein ratio of 1.5:1, with and without exercise, experienced significantly more BW loss after 12 weeks compared to women consuming a standard 3:1 ratio of carbohydrate to protein, with and without exercise. The group following the 1.5:1 ratio diet with exercise experienced the greatest reductions in BW and triglycerides (TG) of all intervention groups (30). Similarly, adults consuming roughly 30% of energy from protein over a 4-month weight-loss period lost more FM and maintained that loss over one year, compared to adults consuming a conventional weight-loss diet with lesser protein (16). The higher protein group also experienced a greater reduction in TG and elevation of high-density lipoprotein cholesterol (HDL-C) concentrations (16).

Additional weight-loss strategies demonstrating positive long-term outcomes include achieving early weight-loss success within an intervention (31,32) and use of portion-controlled meal replacements (MR) (33–35). Early response (i.e., within the first several weeks to 12 weeks) to weight-loss treatment has been identified as a positive predictor for improved overall BW loss and maintenance outcomes (31,36,37); however, this generally has been retrospectively identified in studies for which early response was not the intended outcome (32,37,38). It remains unclear why early response is an indicator of BW loss success. Randomized clinical trials are necessary to explore any specific effect of greater initial BW loss on further BW loss

and other intervention outcomes. Likewise, a MR approach to BW loss may support positive outcomes. For example, after six weeks, participants replacing two daily meals with a low-calorie, high-protein beverage lost significantly more BW and FM, respectively (6.4 and 5.1 kg), than participants in a control group consuming a low-fat ERD (3.1 and 2.8 kg, respectively). Participants in the MR group also had greater reductions in waist circumference and TG concentration (39). A separate study reported that adults with obesity consuming an ERD utilizing a MR lost significantly more BW (12.3%) during a 16-week weight-loss phase compared to a group consuming an ERD without MR (6.9%) (33). Although the MR group regained more BW during a subsequent weight maintenance period, 62% of MR participants maintained a BW loss of at least 5% compared to only 30% of participants in the control group (33).

Exercise and frequent contact with an interventionist have been shown to promote positive weight and health-related outcomes (30,40,41). A high-protein diet and exercise program produced independent and additive beneficial effects on body composition (19,30). Habitual exercise has been cited as a strong predictor of BW loss maintenance (42,43). Similarly, contact with and support from an interventionist has been associated with improved BW loss and weight-loss maintenance outcomes (36,41,44). Thus, it may be expected that an ERD that includes moderate dietary protein and non-fat dairy combined with daily exercise and weekly motivational sessions would be advantageous to reducing BW and producing additional positive health outcomes.

The current study evaluated effects of moderate dietary protein and non-fat dairy intake as part of an ERD within a 6-month comprehensive lifestyle intervention in premenopausal women with overweight/obesity. It was hypothesized that an ERD including moderate protein

and non-fat dairy combined with daily walking (YL group) would induce greater reductions in BW, body mass index (BMI), FM, BF%, waist circumference (WC) and hip circumference (HC) and maintenance of FFSTM measurements, compared to an ERD including standard protein and non-fat dairy (i.e., control diet) combined with daily walking (EXG group), during 12 weeks of weight loss followed by 12 weeks of weight-loss maintenance. It was further hypothesized that compared to EXG, the YL intervention would result in greater modification of resting heart rate (RHR) and blood pressure (BP), serum lipids, glucose and insulin concentrations and selected adipose-derived hormones. Additionally, the YL intervention examined the ability to achieve an accelerated initial BW loss through a twice daily MR.

METHODS

Experimental approach

This randomized clinical trial was conducted from December 2009 to March 2011. Participants were stratified by baseline age, BMI and self-reported physical activity and then randomized into one of two interventions, including an ERD with moderate protein and non-fat dairy combined with walking (YL group) or an ERD with standard protein and non-fat dairy combined with walking (EXG group) (see further description of Dietary and walking intervention below). In this 24-week parallel-arm study, women completed testing sessions at baseline (pre-intervention, Week 0) and after two (Week 2), 12 (Week 12) and 24 (Week 24) weeks of the intervention. Primary outcome measures were dietary intake, BW, BMI, FM, FFSTM, BF%, WC and HC. Secondary outcome measures were central abdominal tissue (CAT), RHR, BP, and serum TG, total cholesterol (TC), HDL-C, low-density lipoprotein cholesterol (LDL-C), glucose and insulin concentrations and serum leptin, resistin and adiponectin levels. The intervention included three phases: 1) JumpStart phase, baseline to Week 2; 2) weight-loss

phase, Week 3 to Week 12 (total weeks of BW loss=12), and 3) weight-loss maintenance phase (Week 13 to Week 24).

Participants, screening, enrollment and informed consent

Participants were recruited by: newsletter, newspaper and radio advertisements; posted flyers; mailed advertisements; and by word-of-mouth. A total of 852 women responded to recruitment methods between July 2009 and September 2010. Of these, 465 women met pre-screening criteria (appropriate age, BMI and limited self-reported physical activity) and received screening materials, including a Medical History Form, Zung Self-Rating Depression Scale/Status Inventory and Informed Consent Form. A total of 321 women returned screening materials which were reviewed by investigators to further determine enrollment eligibility. Of these, 139 women were eligible and obtained medical clearance for participation from their health care providers. Women were then stratified and randomized to intervention group, and 123 women completed baseline (Week 0) testing. (Recruitment was conducted continuously, and four cohorts of women were enrolled in the study. One new cohort of women began the 24-week intervention approximately every three months.) Selection criteria included: women aged 20-45 years with a BMI of $\geq$25 and $\leq$36 kg/m^2; moderate physical activity ($\leq$2 hours of planned exercise/week); eumenorrhea; stable BW during the past six months; a score of <50 on the Zung Self-Rating Depression Scale/Status Inventory (45); absence of yogurt intolerance, aversion, or allergy; consumption of <24 oz. of yogurt per day, $\leq$16 oz. of caffeinated beverages per day; $\leq$1 alcoholic beverage per day (i.e., $\leq$12 fl. oz. of regular beer or $\leq$5 fl. oz. wine or $\leq$1.5 fl. oz. 80-proof distilled spirits); and a desire to lose weight. Exclusion criteria included current smoking; pregnancy; diagnosed metabolic or health conditions; use of medications or supplements for metabolic or health conditions or for BW loss; health or orthopedic conditions limiting

musculoskeletal activity; gastric bypass surgery; hysterectomy and ovariectomy without hormone replacement therapy; and/or oral contraceptive use of <2 years duration (if used).

One-hundred four women completed the JumpStart phase; 89 women completed the weight-loss phase, and 80 women completed the entire 24-week intervention (Figure 1). This study was approved by the Institutional Review Board for Research Involving Human Subjects at The Pennsylvania State University, University Park, PA. All participants provided implied consent for initial screening and written informed consent before review of complete eligibility screening materials.

Testing sessions

Testing sessions occurred at baseline (Week 0) and at Weeks 2, 12 and 24 between 0700 and 0930 hours in university-based laboratories. Women fasted for at least 12 hours and avoided strenuous physical activity and alcohol intake before each testing session. Women completed 4-day food and physical activity records in the days before each testing session. At all four testing sessions, women submitted 4-day food and physical activity records. Women then completed anthropometric, vital sign and body composition measurements. Fasting venous whole blood samples also were collected from women at Weeks 0, 2, 12 and 24. Whole blood was centrifuged at 4000 rpm for 10 minutes, and serum was aliquotted into cryovials and stored at -80°C for later analysis.

Dietary and walking intervention

Participants were randomized to the YL or EXG group for the 3-phase intervention. During the 2-week JumpStart phase, women in the YL group followed an ERD (1200 kcal/d) that included Yoplait Light® (General Mills, Inc., Minneapolis, MN) as part of a MR for two meals per day (breakfast and lunch). Each MR consisted of one non-fat yogurt choice, one cereal

or granola bar (General Mills, Inc., Minneapolis, MN) and one fruit serving. For dinner, women were instructed to consume foods based on an exchange system for selection of food intake. Women in the EXG group consumed a standard ERD during the JumpStart phase, limiting energy intake to 1500 or 1700 kcal per day, based on individual estimated energy needs closest to the individual's resting energy expenditure (REE; kcal/d) determined using the Mifflin-St. Jeor equation (46). Total energy was divided into food group exchanges, and women followed their exchange patterns during the JumpStart phase. Women in both groups could consume one additional fruit exchange and one additional vegetable exchange throughout the day, if needed during the first two weeks of the study. By design, the YL group intervention was expected to produce a slightly greater energy deficit compared to the EXG group during the JumpStart phase. The proportion of dietary protein recommended for the YL group was approximately 30% of kcal, whereas the proportion of dietary protein recommended for the EXG group was roughly 16-17% of kcal. Dietary fat and carbohydrate, respectively, comprised approximately 25% and 45% and roughly 24-25% and 59% of total energy for the YL and EXG group, respectively. These patterns of macronutrient distribution remained constant throughout the 24 weeks of the intervention.

The weight-loss phase lasted from Week 2 through 12 for a total of 12 weeks of BW loss. Women randomized to the YL group consumed an ERD (1500 or 1700 kcal/d) that included non-fat yogurt as a part of a MR for breakfast, and as a snack. Women in the YL group consumed two servings of non-fat yogurt each day and three additional servings of non-fat dairy per day. Women in the EXG group continued the ERD (1500 or 1700 kcal/d). Women in the EXG consumed three servings of non-fat dairy per day, not including yogurt. During the weight-loss phase, total energy intake was limited to 1500 or 1700 kcal per day, as previously described,

for women in both groups such that when combined with energy expenditure of walking, BW would decrease by approximately one pound per week.

The weight-loss maintenance phase lasted from Week 13 through 24 (total of 12 weeks). During this phase, women in the YL group consumed an energy-balanced diet, adjusted for current BW, which included non-fat yogurt twice each day along with three additional non-fat dairy servings per day. Women in the EXG group consumed an energy-balanced diet, adjusted for current BW, with three servings of non-fat dairy per day. Either a 1500 or 1700 kcal per day energy-balanced diet was required during Weeks 13-24 of the study to maintain BW losses achieved during the weight-loss phase. Women were provided with dietary patterns, sample menus and exchange list information. Women in the YL group also were provided with weekly supplies of non-fat yogurt and cereal and granola bars.

Women in both groups walked 30 to 40 minutes per day at a moderate pace (roughly three miles/hr) during the duration of the study (i.e., all three phases). Weekly informational and motivational sessions were conducted throughout the 6-month intervention and included lessons on basic nutrition knowledge, exchange pattern of eating, portion size and control, purchasing and preparing food and modifying recipes as well as outcome expectations, self-regulation and monitoring, problem-solving, lifestyle modification, emotional eating and motivation for walking. Throughout the 24-week intervention, women received incentives, such as insulated lunch bags, measuring cups and food storage containers, to encourage study continuation and motivation. These gift incentives were provided to women approximately one per month. Upon completion of the study, women were monetarily compensated for their time.

Dietary and physical activity assessment

Participants recorded their food and beverage intakes and physical activity for four days, including three weekdays and one weekend day. Diet records were reviewed with participants upon receipt and verified by a registered dietitian. Records were analyzed for estimated average energy and macronutrient intakes using Nutrition Data System for Research (NDS-R version 2010, Minneapolis, MN) by a registered dietitian blinded to intervention assignment.

Physical activity records were used to estimate time spent engaged in light, moderate, and vigorous physical activities, which corresponded to metabolic equivalent (MET) levels. Duration of activity, METs and participant BW were used to calculate total energy expended per day. Records were analyzed across all study phases by one research assistant blinded to intervention assignment.

Anthropometric and body composition measurements

Baseline height was recorded to the nearest 0.1 cm using a stadiometer (Seca 700, Seca North America East, Hanover, MD). At baseline and Weeks 2, 12 and 24, BW was measured to the nearest 0.1 kg on a calibrated digital scale (TBF-410GS, Tanita Corporation, Arlington Heights, IL). Participants wore lightweight clothing with bare feet during height and weight measurements, which were completed after a 12-hour overnight fast. BMI was calculated from BW and height measurements. Total body FM (kg), FFSTM (kg), BF% and CAT (%) were measured by dual-energy X-ray absorptiometry (Hologic QDR4500A, Bedford, MA). Total body scans were conducted by one investigator blinded to intervention assignment at baseline, Week 12 and Week 24. Test-retest reliability in 26 overweight/obese premenopausal women has produced coefficients of variation (CVs) of 1.87, 1.02, and 3.32% for FM, FFSTM, and CAT%, respectively. WC and HC were measured in duplicate (Gulik II tape measure, Country Technology, Gays Mills, WI), averaged, and recorded to the nearest 0.1 cm.

Resting heart rate, blood pressure and resting energy expenditure measurements

At baseline and Weeks 2, 12 and 24, RHR (beats/min) of the radial artery was measured by palpation, after a 5-minute rest period. Seated systolic and diastolic BP (mm Hg) was measured using a standard sphygmomanometer (Baumanometer® Desk Model, Copiague, NY) after a 5-minute rest period. Two measurements were taken for both RHR and BP with a 2- to 3-minute rest period between readings. Values were averaged. RHR and BP measurements were completed by a registered nurse blinded to intervention assignment. REE was estimated using the Mifflin-St. Jeor equation (46).

Biochemical markers of health

Fasting serum was analyzed for TG, TC, HDL-C, LDL-C, glucose, insulin, leptin, resistin and adiponectin concentrations. TG (mg/dL), TC (mg/dL) and HDL-C (mg/dL) concentrations were measured by spectrophotometry using standard assay kits (Stanbio Labs, Boerne, TX). LDL-C concentration (mg/dL) was calculated using the Friedewald equation: LDL-C = TC – HDL-C – (TG/5) (47). Serum glucose (mg/dL) was measured by spectrophotometry (Stanbio Labs, Boerne, TX). Serum insulin (μU/mL) was measured by ultra-sensitive enzyme-linked immunosorbent assay (ALPCO Diagnostics, Salem, NH). Serum leptin (ng/mL), resistin (ng/mL) and adiponectin (ng/mL) were measured by enzyme-linked immunosorbent assay (Bio-Rad, Hercules, CA). Duplicate serum samples were analyzed for each biomarker at corresponding study intervals. Intra- and inter-assay CVs were 2.9 and 6.2% for TG, 2.0 and 4.2% for TC and 4.1 and 14.2% for HDL-C, respectively. Intra- and inter-assay CVs were 2.5 and 11.2% for glucose and 4.3 and 12.0% for insulin, respectively. Intra- and inter-assay CVs were 9.3 and 18.7% for leptin, 3.2 and 27.0% for resistin and 8.4 and 20.9% and adiponectin.

Statistical analyses

Participants completing the JumpStart phase were included in intention-to-treat (ITT) analysis (n=104), while women completing all 24 weeks of the intervention were included in efficacy analysis (n=80). ITT analysis was conducted using the last observation carried forward approach. For efficacy analysis, any missing data were replaced with the value from the most recent observation. All baseline data were normally distributed. Related variables were analyzed by multivariate analysis of covariance with repeated measures on the time factor. Cohort and baseline values were used as covariates in all statistical models. Sample *t*-tests using Bonferroni adjustments for multiple comparisons were completed when significant group or group x time interactions were found. A probability of $p<0.01$ (two-tailed) was considered statistically significant. Data are reported as means ± standard deviation (SD), unless otherwise noted. Data analyses were conducted using the Statistical Package for the Social Sciences (version 19.0, 2010, SPSS Inc, Chicago, IL, USA). Power analysis using effect sizes within each group for BW and FM in the current study indicated that a sample size of 30 participants per group yielded >0.85 observed power

RESULTS

Baseline testing was completed by 123 healthy premenopausal women (n=98 Caucasian; n=11 African American; n=3 other; n=10 did not respond) with overweight/obesity. After the JumpStart phase, 104 (YL, n=52; EXG, n=52) women (age 33.8±7.5 years; BMI 29.3±3.1) remained and were included in ITT analysis. There were no significant differences in baseline age, BW or BMI between the 123 women who began the intervention and the 104 women included in ITT analysis, or the 80 women who completed the entire intervention. There were no significant differences in baseline age, BW or BMI for participants who completed the intervention (n=80) and participants who withdrew (n=43).

There were no significant differences in age, BW or BMI between groups at baseline or Week 2 for study completers (YL, n=43; EXG, n=37). Class attendance was 83% for the YL group and 78% for the EXG group. Non-fat yogurt intake was 73% of expected in the YL group. Approximately 60% of women completed the walking protocol on at least four days of each week for at least 19 of 24 weeks.

Dietary and physical activity assessments

Dietary intake data are presented in Table 1. Complete food records were available for 71 of 80 study completers. Compared to baseline, estimated average energy intake was reduced by 35.0 and 31.2% for women in the YL and EXG groups, respectively, at Week 2 and by 25.2 and 30.4% at Week 12 and by 18.4 and 27.0% at Week 24. As expected due to study design, estimated energy intake significantly differed between groups only at Week 2.

In the YL group, estimated total carbohydrate intake significantly decreased from Week 0 to Week 2, 12 and 24, while estimated carbohydrate intake as a percentage of total energy intake remained relatively constant between 49-52%. In the EXG group, estimated carbohydrate intake as a percentage of total energy intake significantly increased from baseline to Week 2, 12 and 24. Estimated protein intake as a percentage of total energy intake increased over time in both groups. Though protein intake did not reach 30% of total energy intake as designed in the YL group, estimated average percentage protein intake was significantly greater in the YL group compared to EXG group at Week 2 and 12. Both groups reduced estimated total fat intake and percentage of energy intake as dietary fat over time; the reduction in dietary fat as a percentage of total energy intake was significantly different between groups only at Week 2.

Energy expenditure from physical activity data are presented in Table 1. Complete physical activity records were available for 64 of 80 study completers. There was no effect of diet group or time on estimated energy expenditure from physical activity.

Anthropometric and body composition measurements

Table 2 displays ITT analysis for body composition and anthropometric measurements. There was no effect of diet group on body composition and anthropometric measurements. Women in both groups significantly reduced BW, BMI, WC and HC during the JumpStart phase. Both YL and EXG groups had significant decreases in BW (-4.9±3.2 and -4.3±3.3 kg), BMI (-1.8±1.2 and -1.6±1.2 kg/m^2), FM (-3.0±2.2 and -2.3±2.3 kg), FFSTM (-1.5±1.6 and -1.7±2.1 kg), BF% (-1.7±1.7 and -1.2±2.0 %), WC (-4.4±2.9 and -3.8±3.1 cm), HC (-4.7±3.5 and -4.3±3.6 cm) and CAT% (-3.0±3.2 and -2.8±2.9 %), respectively, from baseline to Week 12. These losses were maintained from Week 12 through Week 24. A group x time interaction was not observed for any of these outcome variables. Similar results were found with efficacy analysis (study completers) (data not shown).

Resting heart rate, blood pressure and resting energy expenditure measurements

There was no significant effect of diet group on RHR, systolic and diastolic BP and REE with ITT analysis (Table 3). There were no significant differences in RHR or systolic and diastolic BP over time in either group, and a group x time interaction was not observed for any of these measures. There was a significant reduction in REE from baseline to Week 12, and this reduction was maintained through Week 24. A group x time interaction was not found for REE. Similar results were found with efficacy analysis, with the exception of a significant increase in diastolic BP between Weeks 12 and 24 (data not shown).

Biochemical markers of health

There were no significant diet group effects for biochemical markers of health with ITT analysis (Table 4). Women in the YL and EXG groups experienced a significant reduction in TG and TC concentrations at Week 12 that were maintained to Week 24. Serum LDL-C significantly decreased at Week 12, but this reduction was no longer significant at Week 24. There were no other significant changes in groups over time, and a group x time interaction was not observed in any of these secondary outcome measures. Using efficacy analysis, results were similar to ITT analysis (data not shown).

A significant diet group effect was not found for serum leptin, resistin and adiponectin concentrations (Table 5). Leptin concentration significantly decreased at Week 12, and this reduction was maintained through Week 24 (Figure 2). There were no significant changes in resistin concentration over time. There was a significant increase in adiponectin between Weeks 12 and 24. Significant group x time interactions were not observed in these adipose-derived hormones. Results using efficacy analysis were similar to ITT analysis (data not shown).

DISCUSSION

Sedentary premenopausal women with excess adiposity, facilitated by an intervention including diet modification and moderate walking exercise, significantly reduced BW and FM as well as TG and TC across a 12-week JumpStart+weight-loss phase and maintained these changes across a 12-week weight-loss maintenance phase. The lack of detectable differences in primary outcome variables between diet groups at Week 12 suggests that these two approaches to BW loss may have been equally effective. Both intervention groups met dietary guidelines for intake of at least three servings of dairy per day (48) and recommendations for protein intake within the acceptable macronutrient distribution range (49). Although protein comprised a greater proportion of total energy in the YL group, estimated dietary protein intake did not reach the

goal of 30% of total energy and fell within the range of 0.89-0.96 g of protein per kg BW per day. Estimated protein intake in the EXG group was within the range of 0.78-0.87 g of protein per kg BW per day. Absolute protein (g) consumed during energy restriction is often less than the amount consumed during energy balance, though protein may comprise the same percentage of overall energy intake. During energy restriction, g of protein intake per kg BW may fall below 0.8. Within the context of an ERD (i.e., lowered total energy intake), dietary protein as a percentage of total energy should increase to meet a minimally recommended amount (49).

In order to maintain FFSTM, protein intake should increase or at least be maintained as BW declines due to reduced caloric intake (50). Interventions citing improved body composition and blood lipid outcomes within experimental high-protein diet groups often include dietary protein at 1.3-1.6 g of protein per kg BW per day (approximately 30% of total energy consumed) (17–19,26,30). This level of protein intake was not met in the current study, likely due to the study design involving free-living women. Studies that provide meals or utilize controlled feedings may better achieve a strict dietary protein goal.

Participants in both groups similarly improved TG and TC irrespective of dietary intervention, indicating overall improvement in these metabolic parameters with energy restriction, moderate walking and BW loss (51). This is in contrast to other studies in which larger reductions in TG concentrations were found in participants with greater protein intake (14,16,18–21). However, these previous studies compared wider extremes of dietary protein intake than in the current study. Protein as a percentage of total energy intake differed by approximately 12% between intervention groups in previous studies (14,16,18–21), while the current study achieved an approximate 5% difference in dietary protein intake between groups.

Caution should be taken when interpreting the small increase in diastolic BP between Weeks 12 and 24 with efficacy analysis. Although statistically significant, this increase may be within the range of error. Systolic BP remained unchanged, and diastolic BP remained well within normal BP ranges (52).

Initial BW loss (within the first 5-12 weeks) predicts overall BW loss (31,36) and maintenance of BW loss over time (31,36–38). In adult women with obesity, overall BW loss occurring during a 9-month physician-led weight-loss approach including an ERD and behavior therapy was significantly predicted by BW loss within the first five weeks of intervention (37). However, rate of early weight loss was associated with self-selected diet type, such that women who selected very-low-calorie diets (500-700 kcal/d) showed more BW loss within the first five weeks than women who selected low-calorie diets (1000-1200 kcal/d). The factor most strongly associated with overall BW loss was early change in uncontrolled eating, measured using the Larocque Obesity Questionnaire (37). In the current study, the JumpStart phase was designed to accelerate BW loss within the YL group with use of MR at both breakfast and lunch as part of a 1200 kcal per day meal plan. As designed, estimated energy intake was significantly lower in the YL group compared to the EXG group, and though not significant at $p<0.01$ in the context of the entire intervention, BW and BMI also were reduced to a greater extent ($p<0.01$) in the YL group than the EXG group at Week 2 (2.4±1.0 vs. 1.7±1.1 kg), when evaluated as an independent 2-week interval. The 2-week JumpStart phase may not have been sufficient in length to induce significant differences in BW loss (at $p<0.01$) between groups as part of the entire 12-week weight-loss phase plus 12-week weight-loss maintenance phase (31,36,37). With the differences observed between groups at Week 2, it did not appear that an early reduction in energy intake gave advantage to the YL group in reducing or maintaining BW in the overall intervention.

Evidence from randomized interventions support that a greater initial weight loss is positively related to long-term weight maintenance provided that early weight loss is not induced by intervention protocol, but rather when early weight loss is inherent (32,53). A study by Toubro and colleagues (53) designed to compare effects of initial BW loss on long-term outcomes randomized adults with obesity to either eight weeks on a very-low-calorie diet or 17 weeks of a conventional ERD both aimed at producing similar BW loss by the end of intervention. With similar BW loss between groups at the end of each respective intervention, no effect of rate of initial BW loss was observed on BW loss maintenance after six and 12 months (53). In the current study and the study by Toubro and colleagues (53), randomization of participants into a greater initial weight-loss group examined whether an induced greater initial BW loss could generate overall increases in BW loss and improvements in BW loss maintenance. Yet, neither the current study nor the previous study (53) was designed to examine early change in eating behaviors or patterns, which have been shown to predict both improved initial and overall BW loss (37,54). The current study does not support the effect of greater initial energy reduction or weight loss *per se* on overall weight-loss success; instead, it assumes factors leading to improved initial success on an individual basis also result in improved overall weight-loss outcome.

As part of the JumpStart phase during which ERDs between groups were not isocaloric, women in the YL group consumed MRs for lunch and dinner as part of a lower energy diet, resulting in significantly less energy intake compared to the EXG group during this phase. Alternatively, there was no difference in energy intake between groups during the remaining 22 weeks of intervention as designed, and additionally no difference in BW between groups at any time. The suggested mechanism responsible for BW loss with MR use is reduced energy intake from controlled portion sizes (55). The MR may have been advantageous to women in YL group

during the Jumpstart phase in achieving their designed greater energy deficit. With similar reductions in energy intake at Week 12 and 24, YL and EXG groups had similar BW loss regardless of means of achieving a reduced energy intake (i.e., inclusion of a MR). It did not appear that a MR approach to reducing energy intake, used in the last 22 weeks of intervention for breakfast and as a snack in the YL group, enhanced ERD compliance or in any other way accelerated weight loss.

Mechanisms for improved metabolic condition with BW loss may be partially explained by beneficial changes in select adipose-derived hormones (56). Leptin, resistin and adiponectin originate predominately in adipose tissue (57,58) and are known to contribute to a pro-inflammatory milieu (59). While leptin and resistin are positively correlated with BMI (60,61), low adiponectin levels are associated with obesity (62). In their dysregulated states, these adipose-derived hormones may negatively impact other body systems. Indeed, adipose tissue has been implicated in the progression of cardiometabolic disease (63). In the current study, reductions in leptin and increases in adiponectin are consistent with other studies demonstrating BW and FM loss (64–66). A review of adipose-derived hormone response to randomized control ERDs reports that FM losses of <10% may be insufficient to reduce circulating resistin and increase adiponectin levels; however, studies examining resistin response to BW and FM loss are limited (58). Leptin is particularly responsive to ERD-induced weight loss (58), responding to both short term alterations in energy intake (67) and reductions in FM. Further insight into the function of these adipose-derived hormones and their part in the improvement of metabolic factors observed during BW and FM loss should be a research priority.

Strengths of the current study include a research design including free-living women, designed to test a “real-world” situation (68). Additionally, this study included a daily moderate

walking component in both groups. As an accepted promoter of BW and FM losses with energy restriction (69), increased physical activity was not among study variables but a component of the overall intervention strategy. Similarly, all women attended weekly nutrition-related educational sessions that did not differ between groups, as attendance at group sessions during weight-loss intervention has been strongly associated with weight loss (41). This study employed a control group following a dietary pattern adequate in both protein and dairy/calcium, rather than inadequate in dairy/calcium as evaluated in past studies (24,27). The current study employed a control group meeting protein and dairy/calcium recommendations for comparison with the experimental diet, thereby examining effects of protein and dairy/calcium intake beyond recommendations. Studies reporting that dairy intake or calcium supplementation may accelerate BW and FM loss in the context of an ERD often begin with participants who are calcium deficient or use a low-calcium ERD control group (24,26,27). Recruitment in the current study was not limited to women who were deficient in either protein or dairy, making this design more generalizable. Additionally, this study used highly conservative statistical methods, limiting the likelihood of type 1 error. However, highly conservative statistical approaches may inflate the rate of false negatives.

Though free-living participants examine a more realistic experience with intervention components (68), the lack of feeding control in this study limits its ability to address diet efficacy. Protein intake goals for the YL group were not achieved, likely due to the study design involving free-living women. Generalizations from this study may be limited to only healthy, premenopausal and primarily Caucasian women as that was the study population. Finally, this study design may not have been optimized to provide the most potent effect with the least resources (70). As this design was rather complex, it provided little information on which

intervention components were most effective. Recent work suggests that a simpler design, including the best set of several potential intervention components previously identified through screening trials, would likely be more effective and efficient (70,71).

CONCLUSIONS

In conclusion, an ERD including moderate protein and non-fat dairy combined with walking exercise resulted in significant losses in BW and FM along with other beneficial changes in anthropometric measurements and some metabolic biomarkers in healthy premenopausal women with excess adiposity. An ERD that meets dietary protein (0.8 g protein/kg BW) and dairy intake (3 servings/d) recommendations is as effective as an ERD that includes greater protein and more servings of non-fat dairy intake per day in producing BW and FM losses and maintaining these beneficial changes over time. Additionally, the current study did not demonstrate any benefit of early and initial BW loss on longer-term BW loss and maintenance of that BW loss.

REFERENCES

1. Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL, Anis AH. The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. *BMC Public Health*. 2009;9:88.

2. Chang Y, Ryu S, Suh B-S, Yun KE, Kim C-W, Cho S-I. Impact of BMI on the incidence of metabolic abnormalities in metabolically healthy men. *Int J Obes. (2005)*. 2011.

3. Dalton M, Cameron AJ, Zimmet PZ, et al. Waist circumference, waist-hip ratio and body mass index and their correlation with cardiovascular disease risk factors in Australian adults. *J Intern Med*. 2003;254(6):555–563.

4. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA*. 2012;307(5):491–497.

5. Wang YC, McPherson K, Marsh T, Gortmaker SL, Brown M. Health and economic burden of the projected obesity trends in the USA and the UK. *Lancet*. 2011;378(9793):815–825.

6. Lefevre M, Redman LM, Heilbronn LK, et al. Caloric restriction alone and with exercise improves CVD risk in healthy non-obese individuals. *Atherosclerosis*. 2009;203(1):206–213.

7. Coppola A, Marfella R, Coppola L, et al. Effect of weight loss on coronary circulation and adiponectin levels in obese women. *Int J Cardiol*. 2009;134(3):414–416.

8. Fontana L, Villareal DT, Weiss EP, et al. Calorie restriction or exercise: effects on coronary heart disease risk factors. A randomized, controlled trial. *Am J Physiol Endocrinol Metab*. 2007;293(1):E197 –E202.

9. Layman DK, Shiue H, Sather C, Erickson DJ, Baum J. Increased dietary protein modifies glucose and insulin homeostasis in adult women during weight loss. *J Nutr*. 2003;133(2):405–410.

10. Appel LJ, Champagne CM, Harsha DW, et al. Effects of comprehensive lifestyle modification on blood pressure control: main results of the PREMIER clinical trial. *JAMA*. 2003;289(16):2083–2093.

11. Parker B, Noakes M, Luscombe N, Clifton P. Effect of a high-protein, high-monounsaturated fat weight loss diet on glycemic control and lipid levels in type 2 diabetes. *Diabetes Care*. 2002;25(3):425–430.

12. Layman DK, Clifton P, Gannon MC, Krauss RM, Nuttall FQ. Protein in optimal health: heart disease and type 2 diabetes. *Am J Clin Nutr*. 2008;87(5):1571S–1575S.

13. Abete I, Astrup A, Martínez JA, Thorsdottir I, Zulet MA. Obesity and the metabolic syndrome: role of different dietary macronutrient distribution patterns and specific nutritional components on weight loss and maintenance. *Nutr Rev*. 2010;68(4):214–231.

14. Skov AR, Toubro S, Rønn B, Holm L, Astrup A. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. *Int J Obes Relat Metab Disord.* 1999;23(5):528–536.

15. Brehm BJ, D'Alessio DA. Benefits of high-protein weight loss diets: enough evidence for practice? *Curr Opin Endocrinol Diabetes Obes*. 2008;15(5):416–421.

16. Layman DK, Evans EM, Erickson D, et al. A moderate-protein diet produces sustained weight loss and long-term changes in body composition and blood lipids in obese adults. *J Nutr.* 2009;139(3):514–521.

17. Leidy HJ, Carnell NS, Mattes RD, Campbell WW. Higher protein intake preserves lean mass and satiety with weight loss in pre-obese and obese women. *Obesity (Silver Spring)*. 2007;15(2):421–429.

18. Layman DK, Boileau RA, Erickson DJ, et al. A Reduced Ratio of Dietary Carbohydrate to Protein Improves Body Composition and Blood Lipid Profiles during Weight Loss in Adult Women. *J Nutr*. 2003;133(2):411 –417.

19. Layman DK, Evans E, Baum JI, Seyler J, Erickson DJ, Boileau RA. Dietary protein and exercise have additive effects on body composition during weight loss in adult women. *J Nutr.* 2005;135(8):1903–1910.

20. Noakes M, Keogh JB, Foster PR, Clifton PM. Effect of an energy-restricted, high-protein, low-fat diet relative to a conventional high-carbohydrate, low-fat diet on weight loss, body composition, nutritional status, and markers of cardiovascular health in obese women. *Am J Clin Nutr.* 2005;81(6):1298–1306.

21. Farnsworth E, Luscombe ND, Noakes M, Wittert G, Argyiou E, Clifton PM. Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. *Am J Clin Nutr.* 2003;78(1):31–39.

22. Lejeune MPGM, Westerterp KR, Adam TCM, Luscombe-Marsh ND, Westerterp-Plantenga MS. Ghrelin and glucagon-like peptide 1 concentrations, 24-h satiety, and energy and substrate metabolism during a high-protein diet and measured in a respiration chamber. *Am J Clin Nutr.* 2006;83(1):89–94.

23. Veldhorst M, Smeets A, Soenen S, et al. Protein-induced satiety: effects and mechanisms of different proteins. *Physiol Behav.* 2008;94(2):300–307.

24. Zemel MB, Richards J, Mathis S, Milstead A, Gebhardt L, Silva E. Dairy augmentation of total and central fat loss in obese subjects. *Int J Obes (Lond)*. 2005;29(4):391–397.

25. Zemel MB, Teegarden D, Loan MV, et al. Dairy-rich diets augment fat loss on an energy-restricted diet: a multicenter trial. *Nutrients.* 2009;1(1):83–100.

26. Josse AR, Atkinson SA, Tarnopolsky MA, Phillips SM. Increased Consumption of Dairy Foods and Protein during Diet- and Exercise-Induced Weight Loss Promotes Fat Mass Loss and Lean Mass Gain in Overweight and Obese Premenopausal Women. *J Nutr.* 2011;141(9):1626–1634.

27. Zemel MB, Thompson W, Milstead A, Morris K, Campbell P. Calcium and dairy acceleration of weight and fat loss during energy restriction in obese adults. *Obes Res.* 2004;12(4):582–590.

28. Layman DK, Walker DA. Potential Importance of Leucine in Treatment of Obesity and the Metabolic Syndrome. *J Nutr.* 2006;136(1):319S –323S.

29. Koopman R, Wagenmakers AJM, Manders RJF, et al. Combined ingestion of protein and free leucine with carbohydrate increases postexercise muscle protein synthesis in vivo in male subjects. *Am J Physiol Endocrinol Metab.* 2005;288(4):E645–653.

30. Meckling KA, Sherfey R. A randomized trial of a hypocaloric high-protein diet, with and without exercise, on weight loss, fitness, and markers of the Metabolic Syndrome in overweight and obese women. *Appl Physiol Nutr Metab*. 2007;32(4):743–752.

31. Guaraldi F, Pagotto U, Pasquali R. Predictors of weight loss and maintenance in patients treated with antiobesity drugs. *Diabetes Metab Syndr Obes.* 2011;4:229–243.

32. Astrup A, Rössner S. Lessons from obesity management programmes: greater initial weight loss improves long-term maintenance. *Obes Rev*. 2000;1(1):17–19.

33. Davis LM, Coleman C, Kiel J, et al. Efficacy of a meal replacement diet plan compared to a food-based diet plan after a period of weight loss and weight maintenance: a randomized controlled trial. *Nutr J*. 2010;9:11.

34. Vázquez C, Montagna C, Alcaraz F, et al. Meal replacement with a low-calorie diet formula in weight loss maintenance after weight loss induction with diet alone. *Eur J Clin Nutr*. 2009;63(10):1226–1232.

35. Cheskin LJ, Mitchell AM, Jhaveri AD, et al. Efficacy of meal replacements versus a standard food-based diet for weight loss in type 2 diabetes: a controlled clinical trial. *Diabetes Educ*. 2008;34(1):118–127.

36. Wadden TA, Foster GD, Wang J, et al. Clinical correlates of short- and long-term weight loss. *Am J Clin Nutr.* 1992;56(1 Suppl):271S–274S.

37. Stotland SC, Larocque M. Early treatment response as a predictor of ongoing weight loss in obesity treatment. *Br J Health Psychol*. 2005;10(Pt 4):601–614.

38. Jeffery RW, Wing RR, Mayer RR. Are smaller weight losses or more achievable weight loss goals better in the long term for obese patients? *J Consult Clin Psychol*. 1998;66(4):641–645.

39. König D, Deibert P, Frey I, Landmann U, Berg A. Effect of meal replacement on metabolic risk factors in overweight and obese subjects. *Ann Nutr Metab*. 2008;52(1):74–78.

40. Kreider RB, Serra M, Beavers KM, et al. A structured diet and exercise program promotes favorable changes in weight loss, body composition, and weight maintenance. *J Am Diet Assoc*. 2011;111(6):828–843.

41. Sacks FM, Bray GA, Carey VJ, et al. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *N Engl J Med*. 2009;360(9):859–873.

42. Wing RR, Hill JO. Successful weight loss maintenance. *Annu Rev Nutr*. 2001;21:323–341.

43. Tate DF, Jeffery RW, Sherwood NE, Wing RR. Long-term weight losses associated with prescription of higher physical activity goals. Are higher levels of physical activity protective against weight regain? *Am J Clin Nutr*. 2007;85(4):954–959.

44. Wadden TA, Butryn ML, Byrne KJ. Efficacy of lifestyle modification for long-term weight control. *Obes Res*. 2004;12 Suppl:151S–62S.

45. Zung WW. The Depression Status Inventory: an adjunct to the Self-Rating Depression Scale. *J Clin Psychol*. 1972;28(4):539–543.

46. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr*. 1990;51(2):241–247.

47. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem*. 1972;18(6):499–502.

48. U.S. Department of Agriculture and U.S. Department of Health and Human Services. Dietary Guidelines for Americans, 2010. 7th Edition, Washington, DC: U.S. Government Printing Office, December 2010

49. A Report of the Panel on Macronutrients, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. Washington, D.C.: The National Academies Press; 2005.

50. Layman DK. Protein Quantity and Quality at Levels above the RDA Improves Adult Weight Loss. *J Am Coll Nutr*. 2004;23(suppl 6):631S–636S.

51. Blumenthal JA, Babyak MA, Sherwood A, et al. Effects of the dietary approaches to stop hypertension diet alone and in combination with exercise and caloric restriction on insulin sensitivity and lipids. *Hypertension*. 2010;55(5):1199–1205.

52. Goldstein LB, Bushnell CD, Adams RJ, et al. Guidelines for the primary prevention of stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke*. 2011;42(2):517–584.

53. Toubro S, Astrup A. Randomised comparison of diets for maintaining obese subjects' weight after major weight loss: ad lib, low fat, high carbohydrate diet v fixed energy intake. *BMJ*. 1997;314(7073):29–34.

54. Hart CN, Jelalian E, Raynor HA, et al. Early patterns of food intake in an adolescent weight loss trial as predictors of BMI change. *Eat Behav*. 2010;11(4):217–222.

55. Levitsky DA, Pacanowski C. Losing weight without dieting. Use of commercial foods as meal replacements for lunch produces an extended energy deficit. *Appetite*. 2011;57(2):311–317.

56. Jung SH, Park HS, Kim K-S, et al. Effect of weight loss on some serum cytokines in human obesity: increase in IL-10 after weight loss. *J Nutr Biochem.* 2008;19(6):371–375.

57. Rajala MW, Scherer PE. Minireview: The adipocyte--at the crossroads of energy homeostasis, inflammation, and atherosclerosis. *Endocrinology*. 2003;144(9):3765–3773.

58. Klempel MC, Varady KA. Reliability of leptin, but not adiponectin, as a biomarker for diet-induced weight loss in humans. *Nutr Rev.* 2011;69(3):145–154.

59. Ma K, Jin X, Liang X, Zhao Q, Zhang X. Inflammatory mediators involved in the progression of metabolic syndrome. *Diabetes Metab Res Rev*. 2012.

60. Friedman JM. The function of leptin in nutrition, weight, and physiology. *Nutr Rev.* 2002;60(10 Pt 2):S1–14; discussion S68–84, 85–87.

61. Pagano C, Marin O, Calcagno A, et al. Increased serum resistin in adults with prader-willi syndrome is related to obesity and not to insulin resistance. *J Clin Endocrinol Metab.* 2005;90(7):4335–4340.

62. Weyer C, Funahashi T, Tanaka S, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab.* 2001;86(5):1930–1935.

63. Allende-Vigo MZ. Pathophysiologic mechanisms linking adipose tissue and cardiometabolic risk. *Endocr Pract*. 2010;16(4):692–698.

64. Josse AR, Atkinson SA, Tarnopolsky MA, Phillips SM. Diets Higher in Dairy Foods and Dietary Protein Support Bone Health during Diet- and Exercise-Induced Weight Loss in Overweight and Obese Premenopausal Women. *J Clin Endocrinol Metab.* 2011.

65. Miller LE, Volpe JJ, Coleman-Kelly MD, Gwazdauskas FC, Nickols-Richardson SM. Anthropometric and leptin changes in women following different dietary approaches to weight loss. *Obesity (Silver Spring)*. 2009;17(1):199–201.

66. Varady KA, Tussing L, Bhutani S, Braunschweig CL. Degree of weight loss required to improve adipokine concentrations and decrease fat cell size in severely obese women. *Metab Clin Exp.* 2009;58(8):1096–1101.

67. Sartorio A, Agosti F, Resnik M, Lafortuna CL. Effects of a 3-week integrated body weight reduction program on leptin levels and body composition in severe obese subjects. *J Endocrinol Invest.* 2003;26(3):250–256.

68. Most MM, Ershow AG, Clevidence BA. An overview of methodologies, proficiencies, and training resources for controlled feeding studies. *J Am Diet Assoc*. 2003;103(6):729–735.

69. Jakicic JM, Davis KK. Obesity and physical activity. *Psychiatr Clin North Am.* 2011;34(4):829–840.

70. Collins LM, Baker TB, Mermelstein RJ, et al. The multiphase optimization strategy for engineering effective tobacco use interventions. *Ann Behav Med.* 2011;41(2):208–226.

71. Dziak JJ, Nahum-Shani I, Collins LM. Multilevel factorial experiments for developing behavioral interventions: Power, sample size, and resource considerations. *Psychol Methods.* 2012.

Figure 3.1 Diagram of recruitment, enrollment, randomization and attrition of participants in a study examining changes in body composition and metabolic parameters in premenopausal women with overweight/obesity following an energy-restricted dietary pattern (ERD) including moderate protein and non-fat dairy (YL group) or ERD with standard protein and non-fat dairy (EXG group) combined with daily moderate walking. Primary reasons for study withdrawal included lack of time (n=22), undisclosed (n=16) and no return for subsequent testing (n=7).

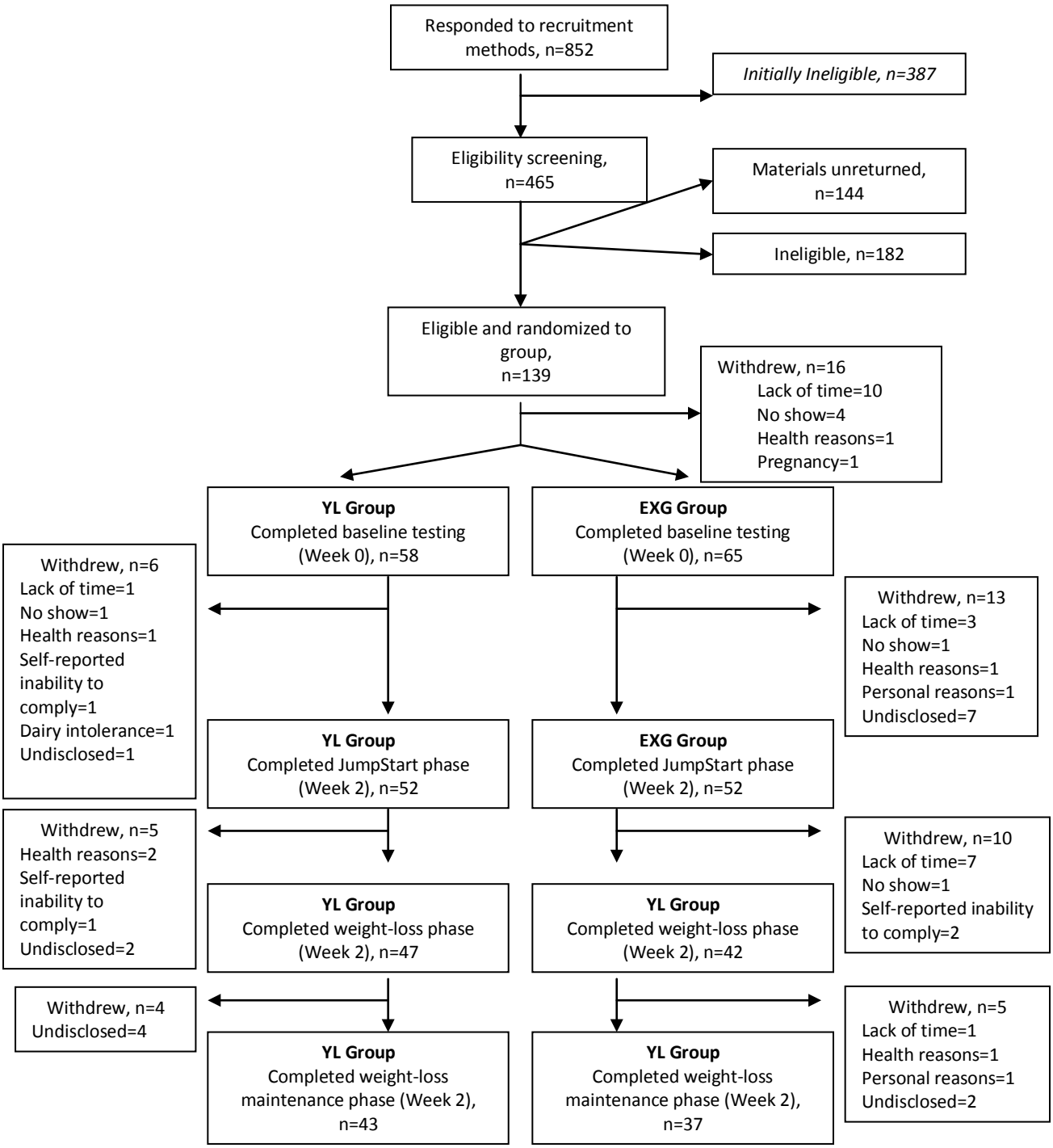


Table 3.1 Estimated energy and macronutrient intakes and energy expenditure from physical activity of premenopausal women with overweight/obesity at baseline and Week 2, 12 and 24 in a study examining changes in body composition and metabolic parameters with an energy-restricted dietary pattern (ERD) including moderate protein and non-fat dairy (YL group) or ERD with standard protein and non-fat dairy (EXG group) combined with daily moderate walking

Variable	Group	Baseline (Week 0)	Week 2	Week 12	Week 24	*p*-value
	←		mean ± standard deviation		→	
Energy intake (kcal/d)	YL (n=37) EXG (n=34)	1757 ± 387 1974 ± 441	1142 ± 182[a] 1358 ± 246[a,c]	1315 ± 308[a,b] 1374 ± 269[a]	1433 ± 309[a,b] 1442 ± 366[a]	**Group < 0.01** **Time < 0.001** G x T = 0.03
Total carbohydrate (g/d)[a]	YL (n=37) EXG (n=34)	219 ± 51 232 ± 57	165 ± 27 198 ± 43	177 ± 42 194 ± 43	188 ± 35 195 ± 47	Group = 0.013 **Time < 0.001** G x T = 0.17
Percentage carbohydrate (%kcal/d)	YL (n=37) EXG (n=34)	49.2 ± 6.4 46.2 ± 4.6	52.2 ± 5.8[a] 54.5 ± 5.8[a]	52.2 ± 5.9 54.5 ± 5.8[a]	51.5 ± 6.4 53.4 ± 6.6[a]	Group = 0.97 **Time < 0.001** **G x T < 0.01**
Total protein (g/d)	YL (n=37) EXG (n=34)	65.1 ± 14.0 75.0 ± 18.2	72.7 ± 14.5 62.8 ± 12.5[a]	75.7 ± 18.6 66.8 ± 11.8	71.9 ± 16.5 62.8 ± 14.3	Group = 0.52 Time = 0.15 **G x T < 0.001**
Percentage protein (%kcal/d)	YL (n=37) EXG (n=34)	15.3 ± 2.8 15.6 ± 2.3	26.1 ± 4.0[a] 18.6 ± 2.8[a,c]	23.9 ± 4.6[a] 19.6 ± 2.6[a,c]	20.8 ± 4.3[a,b,d] 17.8 ± 2.8	**Group < 0.001** **Time < 0.001** **G x T < 0.001**
Total fat (g/d)	YL (n=37) EXG (n=34)	69 ± 20 82 ± 21	24 ± 10[a] 38 ± 11[a,c]	36 ± 15[a,b] 40 ± 14[a]	44 ± 17[a,b] 46 ± 19[a]	**Group = 0.01** **Time < 0.001** **G x T = 0.01**
Percentage fat (%kcal/d)	YL (n=37) EXG (n=34)	34.0 ± 5.4 36.2 ± 3.5	17.7 ± 5.5[a] 24.4 ± 5.7[a,c]	22.9 ± 5.6[a,b] 24.9 ± 5.7[a]	25.9 ± 5.6[a,b] 27.0 ± 6.3[a]	**Group = 0.01** **Time < 0.001** G x T = 0.10
Energy expenditure (kcal/d)	YL (n=32) EXG (n=32)	556 ± 425 457 ± 475	600± 399 553 ± 415	580 ± 456 575 ± 442	544. ± 488 467± 400	Group = 0.90 Time = 0.57 G x T = 0.15

Dietary intake records analyzed using Nutrition Data System for Research (NDS-R, version 2010, Minnesota, MN) and reported for 71 women who provided full records and completed the 24-week study. Energy expenditure estimated using metabolic equivalent level calculations, duration of activity and participant body weight. Energy expenditure is reported for 64 women who provided full records and completed the 24-week study.

[a] different from baseline; [b] different from Week 2; [c] different between groups; [d] different from Week 12, using $p<0.01$ for statistical significance; *p*-values from multivariate analysis of covariance with repeated measures on the time factor.

G x T=group x time interaction.

Table 3.2 Anthropometric and body composition measurements of premenopausal women with overweight/obesity at baseline and Week 2, 12 and 24 in a study examining changes in body composition and metabolic parameters with an energy-restricted dietary pattern (ERD) including moderate protein and non-fat dairy (YL group) or ERD with standard protein and non-fat dairy (EXG group) combined with daily moderate walking

Variable	Group	Baseline (Week 0)	Week 2	Week 12	Week 24	*p*-value
	← mean ± standard deviation →					
Body weight (kg)[a,b]	YL (n=52)	84.1 ± 12.5	81.7 ± 12.5	79.2 ± 12.9	79.2 ± 13.1	Group = 0.28
	EXG (n=52)	80.8 ± 10.2	79.1 ± 9.9	76.5 ± 9.5	76.1 ± 9.5	**Time < 0.001**
						G x T = 0.49
Body mass index (kg/m^2)[a,b]	YL (n=52)	30.3 ± 3.2	29.4 ± 3.2	28.5 ± 3.2	28.5 ± 3.4	Group = 0.88
	EXG (n=52)	29.9 ± 3.2	29.2 ± 3.1	28.3 ± 3.2	28.2 ± 3.3	**Time < 0.001**
						G x T = 0.51
Fat mass (kg)[a]	YL (n=52)	30.9 ± 7.4	—	28.0 ± 7.6	28.2 ± 7.8	Group = 0.80
	EXG (n=52)	29.7 ± 6.1	—	27.4 ± 6.4	27.2 ± 6.6	**Time < 0.001**
						G x T = 0.42
Fat-free soft tissue mass (kg)[a]	YL (n=52)	49.0 ± 5.7	—	47.5 ± 5.8	47.3 ± 5.5	Group = 0.05
	EXG (n=52)	47.2 ± 5.3	—	45.5 ± 4.7	45.4 ± 4.7	**Time = < 0.001**
						G x T = 0.67
Body fat percentage (%)[a]	YL (n=52)	37.2 ± 4.1	—	35.4 ± 4.3	35.7 ± 4.5	Group = 0.38
	EXG (n=52)	37.2 ± 4.4	—	36.1 ± 5.0	35.9 ± 5.3	**Time < 0.001**
						G x T = 0.43
Waist circumference (cm)[a,b]	YL (n=52)	89.2 ± 8.7	87.1 ± 8.6	84.8 ± 8.4	84.7 ± 8.4	Group = 0.78
	EXG (n=52)	87.7 ± 7.9	86.2 ± 7.8	84.0 ± 7.5	83.6 ± 7.9	**Time < 0.001**
						G x T = 0.55
Hip circumference (cm)[a,b]	YL (n=52)	113.7 ± 8.6	111.8 ± 8.2	109.0 ± 8.5	108.8 ± 8.8	Group = 0.60
	EXG (n=52)	112.2 ± 8.0	110.7 ± 7.9	107.9 ± 7.9	107.8 ± 7.8	**Time < 0.001**
						G x T = 0.84
Central abdominal tissue percentage (%)[a]	YL (n=52)	36.9 ± 6.3	—	33.9 ± 7.0	34.1 ± 7.2	Group = 0.77
	EXG (n=52)	36.1 ± 5.2	—	33.3 ± 5.9	33.2 ± 6.7	**Time < 0.001**
						G x T = 0.77

Fat mass, fat-free soft tissue mass, body fat percentage and central abdominal tissue percentage analyzed by dual-energy X-ray absorptiometry at baseline and Week 12 and 24 only.
[a] different from baseline at Weeks 12 and 24; [b] different from Week 2 at baseline, Weeks 12 and 24, using $p<0.01$ for statistical significance; *p*-values from intention-to-treat analysis, using multivariate analysis of covariance with repeated measures on the time factor.
G x T=group x time interaction.

Table 3.3 Resting heart rate, blood pressure and resting energy expenditure measurements of premenopausal women with overweight/obesity at baseline and Week 2, 12 and 24 in a study examining changes in body composition and metabolic parameters with an energy-restricted dietary pattern (ERD) including moderate protein and non-fat dairy (YL group) or ERD with standard protein and non-fat dairy (EXG group) combined with daily moderate walking

Variable	Group	Baseline (Week 0)	Week 2	Week 12	Week 24	*p*-value
	← mean ± standard deviation →					
Resting heart rate (bpm)	YL (n=52) EXG (n=52)	71.4 ± 7.5 72.7 ± 7.2	72.4 ± 8.0 72.1 ± 7.0	71.5 ± 8.6 73.1 ± 8.1	73.0 ± 9.5 75.2 ± 9.7	Group = 0.39 Time = 0.23 G x T = 0.79
Resting systolic blood pressure (mm Hg)	YL (n=52) EXG (n=52)	116.4 ± 9.0 116.5 ± 8.3	116.7 ± 8.7 115.6 ± 9.3	115.7 ± 10.4 114.7 ± 10.6	117.4 ± 9.2 117.8 ± 12.6	Group = 0.85 Time = 0.24 G x T = 0.94
Resting diastolic blood pressure (mm Hg)	YL (n=52) EXG (n=52)	73.7 ± 7.8 72.9 ± 8.0	73.5 ± 6.7 72.1 ± 8.0	73.2 ± 7.5 72.3 ± 7.6	75.4 ± 7.6 75.1 ± 9.0	Group = 0.43 Time = 0.02 G x T = 0.99
Resting energy expenditure (kcal/d)[a,b]	YL (n=52) EXG (n=52)	1549 ± 168 1508 ± 122	1525 ± 168 1491 ± 120	1499 ± 174 1465 ± 115	1499 ± 175 1461 ± 116	Group = 0.25 **Time < 0.001** G x T = 0.49

[a] different from baseline at Weeks 12 and 24; [b] different from Week 2 at baseline, Weeks 12 and 24, using $p<0.01$ for statistical significance; *p*-values from intention-to-treat analysis, using multivariate analysis of covariance with repeated measures on the time factor.
G x T=group x time interaction.

Table 3.4 Blood lipid, glucose and insulin concentrations of premenopausal women with overweight/obesity at baseline and Week 2, 12 and 24 in a study examining changes in body composition and metabolic parameters with an energy-restricted dietary pattern (ERD) including moderate protein and non-fat dairy (YL group) or ERD with standard protein and non-fat dairy (EXG group) combined with daily moderate walking

Variable	Group	Baseline (Week 0)	Week 2	Week 12	Week 24	*p*-value
		← mean ± standard deviation →				
Triglycerides (mg/dL)[a,b]	YL (n=52)	104.5 ± 48.4	—	84.3 ± 42.0	84.9 ± 35.1	Group = 0.30
	EXG (n=52)	107.8 ± 52.8	—	91.3 ± 46.1	91.1 ± 44.0	**Time < 0.001**
						G x T = 0.52
Total cholesterol (mg/dL)[a,b]	YL (n=52)	185.2 ± 34.4	—	177.3 ± 33.5	176.2 ± 29.8	Group = 0.73
	EXG (n=52)	188.2 ± 29.0	—	173.3 ± 28.9	177.5 ± 27.1	**Time < 0.001**
						G x T = 0.38
High-density lipoprotein cholesterol (mg/dL)	YL (n=52)	49.1 ± 12.5	—	49.6 ± 12.8	50.1 ± 12.9	Group = 0.42
	EXG (n=52)	53.4 ± 15.8	—	51.2 ± 14.9	53.8 ± 14.3	Time = 0.13
						G x T = 0.14
Low-density lipoprotein cholesterol (mg/dL)[a]	YL (n=52)	115.1 ± 30.0	—	110.7 ± 28.8	109.1 ± 26.0	Group = 0.67
	EXG (n=52)	113.2 ± 32.2	—	103.4 ± 28.3	105.0 ± 26.1	**Time < 0.01**
						G x T = 0.52
Glucose (mg/dL)	YL (n=52)	82.8 ± 10.6	81.6 ± 9.9	80.9 ± 12.7	81.1 ± 9.4	Group = 0.45
	EXG (n=52)	81.1 ± 11.6	79.7 ± 9.4	81.1 ± 7.5	79.9 ± 7.4	Time = 0.25
						G x T = 0.80
Insulin (μIU/mL)	YL (n=52)	12.7 ± 10.2	12.1 ± 14.0	9.5 ± 5.5	10.4 ± 8.8	Group = 0.42
	EXG (n=52)	11.0 ± 6.8	9.9 ± 5.0	9.0 ± 5.2	9.4 ± 4.4	Time = 0.02
						G x T = 0.38

Fasting serum used to measure biomarker concentrations; for triglycerides, total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol, samples were analyzed at baseline and Week 12 and 24 only.

[a] different from baseline at Week 12, [b] different from baseline at Week 24, using $p<0.01$ for statistical significance; *p*-values from intention-to-treat analysis, using multivariate analysis of covariance with repeated measures on the time factor.

G x T=group x time interaction.

Table 3.5 Concentrations of adipose-derived hormones in premenopausal women with overweight/obesity at baseline and Week 12 and 24 in a study examining changes in body composition and metabolic parameters with an energy-restricted dietary pattern (ERD) including moderate protein and non-fat dairy (YL group) or ERD with standard protein and non-fat dairy (EXG group) combined with daily moderate walking

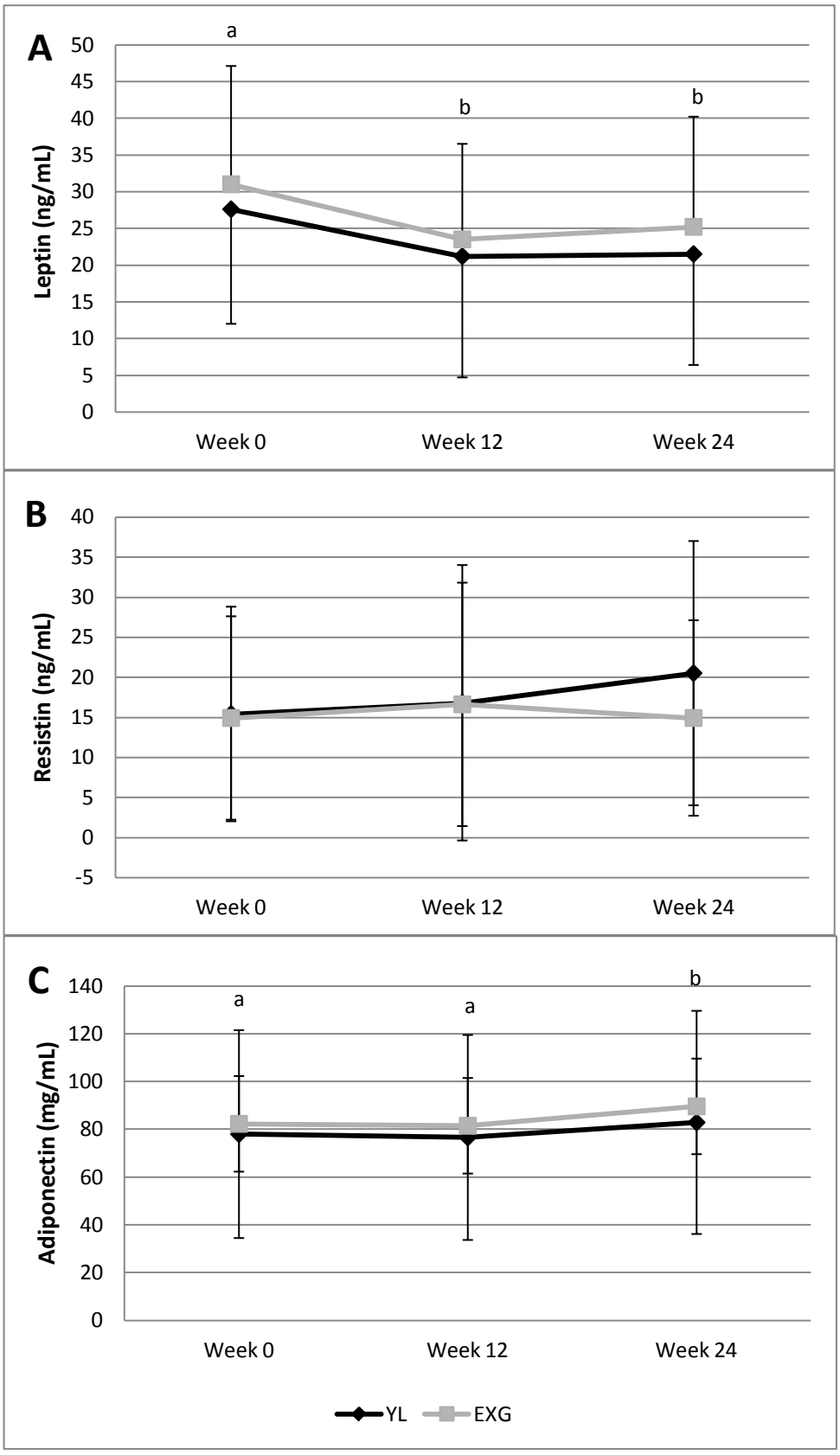
Variable	Group	Baseline (Week 0)	Week 12	Week 24	*p*-value
		← mean ± standard deviation →			
Leptin (ng/mL)[a]	YL (n=52)	27.6 ± 15.6	21.2 ± 16.5	21.5 ± 15.1	Group = 0.12
	EXG (n=52)	31.0 ± 16.1	23.5 ± 13.0	25.2 ± 15.0	**Time < 0.001**
					G x T = 0.58
Resistin (ng/mL)	YL (n=52)	15.4 ± 13.4	16.8 ± 17.2	20.5 ± 16.5	Group = 0.42
	EXG (n=52)	14.9 ± 12.7	16.6 ± 15.2	14.9 ± 12.2	Time = 0.35
					G x T = 0.08
Adiponectin (μg/mL)[b]	YL (n=52)	7.8 ± 4.4	7.7 ± 4.3	8.3 ± 4.7	Group = 0.57
	EXG (n=51)	8.2 ± 4.2	8.2 ± 4.1	9.0 ± 4.3	**Time < 0.01**
					G x T = 0.66

Fasting serum used to measure adipose-derived hormone concentrations; data missing from one participant in the EXG group.

[a] different from baseline at Weeks 12 and 24, [b] baseline and Week 12 different from Week 24 , using $p<0.01$ for statistical significance; *p*-values from intention-to-treat analysis, using multivariate analysis of covariance with repeated measures on the time factor.

G x T=group x time interaction.

Figure 3.2 Adipose-derived hormones (A) leptin, (B) resistin and (C) adiponectin in premenopausal women with overweight/obesity at baseline (Week 0), Week 12 and 24 in a study examining changes in body composition and metabolic parameters with an energy-restricted dietary pattern (ERD) including moderate protein and non-fat dairy (YL group) or ERD with standard protein and non-fat dairy (EXG group) combined with daily moderate walking. Fasting serum was used to measure adipose-derived hormone concentrations; data missing from one participant in the EXG group. Values with different superscripted letters were significantly different, using $p<0.01$ for statistical significance; p-values from intention-to-treat analysis, using multivariate analysis of covariance with repeated measures on the time factor.


A
Leptin (ng/mL)
50
45
40
35
30
25
20
15
10
5
0
a
b
b
Week 0
Week 12
Week 24
B
Resistin (ng/mL)
40
35
30
25
20
15
10
5
0
-5
Week 0
Week 12
Week 24
C
Adiponectin (mg/mL)
140
120
100
80
60
40
20
0
a
a
b
Week 0
Week 12
Week 24
YL
EXG

CHAPTER 4

A LIFESTYLE INTERVENTION PROMOTING WEIGHT LOSS WITH MODERATE OR STANDARD PROTEIN AND NON-FAT DAIRY INTAKE AND DAILY WALKING: IMPACT ON BONE HEALTH IN PREMENOPAUSAL WOMEN WITH OVERWEIGHT/OBESITY

To be submitted to *Osteoporosis International*

ABSTRACT

Summary Maintenance of bone mineral density (BMD) may be compromised during body weight (BW) loss, notably through repeated cycles of BW loss and regain. An energy-restricted diet (ERD) including moderate dietary protein and non-fat dairy along with daily moderate exercise may help maintain BMD during weight loss. *Introduction* This parallel-arm randomized controlled trial examined effects of a lifestyle intervention including an ERD with moderate protein and non-fat dairy intake and daily moderate walking on bone health. *Methods* Baseline testing was completed by 123 healthy premenopausal women with overweight/obesity; after two weeks of intervention, 104 women remained (85%) and were included in an intention-to-treat analysis. The 24-week intervention included two ERD groups with either moderate protein comprising 30% of energy and 4-5 servings of non-fat dairy per day (YL group) or standard protein comprising 16-17% of total energy and three servings of non-fat dairy per day [EXG (i.e., control) group]. Both interventions included daily moderate walking. BW, fat-free soft tissue mass percentage and site-specific BMD measurements were assessed at baseline, Week 12 and Week 24. Blood samples were analyzed for serum N-telopeptide of type I collagen, osteocalcin and C-reactive protein (CRP). *Results* Compared to baseline, there were significant reductions in BW and CRP concentration at Week 12 which were maintained at Week 24 in YL and EXG groups. Significant changes in bone biomarkers or site-specific BMD measurements were not found. *Conclusions* An ERD with moderate or standard protein and at least three servings of non-fat dairy per day plus daily moderate walking may be sufficient to prevent BMD loss during BW loss in premenopausal women with overweight/obesity.

INTRODUCTION

While metabolic health benefits of excess body weight (BW) loss are numerous (1), BW loss is potentially detrimental to bone mineral density (BMD). In individuals with overweight/obesity, reducing BW may accelerate bone turnover (2–4) and result in BMD loss (5–7). Moreover, BMD loss accompanying BW loss may persist with weight regain (4), setting up a dangerous paradigm for frequent dieters (8). Maintenance of BMD is vital to delay declines in bone heath that are associated with aging, particularly in women, and to protect against osteoporosis(9,10).

Bone turnover is a highly controlled process, orchestrated by an array of chemical messengers and physical stimuli (11), and is commonly measured by biochemical markers of bone resorption [e.g., N-telopeptide of type 1 collagen (NTx)] and formation [e.g., osteocalcin (OC)]. Along with non-modifiable factors, such as age and genetics, modifiable factors, such as diet and physical activity, influence BMD (12). Mechanisms suggested to influence bone during energy restriction and BW reduction include lowered and limited intake of energy, protein and calcium (3,4,13,14), diminished skeletal loading (15) and alterations in osteogenic hormones such as estrogen and leptin (16,17). Maintenance of BMD during BW loss may rely upon optimization of nutrition and physical activity within an energy-restricted diet (ERD).

With high rates of obesity in the United States (18), various weight-loss approaches have been evaluated to determine their effectiveness for lowering BW in populations with overweight/obesity; most approaches have shown limited long-term success (19–21), and many individuals regain BW. Moderate dietary protein, calcium and/or dairy intake have been tested for their effects on BW loss as well as impact on biomarkers of bone turnover and BMD during BW loss (5,14,22). Additionally, research has demonstrated that moderate dietary protein (i.e.,

25-30% of total energy as dietary protein), calcium and/or dairy intake as part of an ERD may improve anthropometric and metabolic outcomes of BW loss (21,23–26). Higher dietary protein, particularly dairy protein which is rich in the branched-chain amino acid leucine, has been implicated in the sparing of fat-free soft tissue mass (FFSTM) during diet-induced BW loss (27). Increased intake of non-fat fluid milk also may suppress oxidative and inflammatory stress associated with overweight and obesity (28)

BW loss, particularly FFSTM loss, results in decreased mechanical loading on weight-bearing bones, reducing bone strength and BMD (29). Weight-loss approaches that include exercise in addition to an ERD may attenuate BMD loss when compared to only ERD interventions (2). Weight-loss studies including both resistance and aerobic exercise have demonstrated favorable outcomes in biomarkers of bone turnover and BMD (2,14,30) or no difference in bone-related outcome measures compared to diet-only approaches to BW loss (3,4). Mechanical demands of added physical activity may offset reduced skeletal loading resulting from BW, namely FFSTM losses. Greater preservation of FFSTM, often observed in weight-loss interventions that include exercise (14,30,31), may enhance the BMD-preserving effects of exercise (32).

Reductions in leptin coinciding with energy restriction and BW loss may exert a negative effect on BMD (17,33). However, the reduction in inflammatory factors resulting from BW loss may have a beneficial effect on bone. Increased circulating pro-inflammatory cytokines common with obesity may promote bone-resorbing osteoclast activation through the receptor activator of nuclear factor kappa-B (RANK)/RANK ligand pathway (34). Therefore, a weight loss-induced reduction in circulating inflammatory factors may mitigate bone resorption and preserve BMD (34,35).

Few studies have incorporated all of these components—moderate dietary protein and non-fat dairy combined with moderate exercise—to examine the most favorable intervention for bone health while still producing effective BW loss. Intervention studies examining the effect of various weight-loss approaches on bone health often report only biomarkers of bone turnover (3,36) or are relatively short in duration, lasting less than six months (3,14,22,36,37). Few studies have evaluated the impact of BW loss while specifically attempting to preserve BMD in free-living women with overweight/obesity. The goal of the present study was to examine the impact of BW loss achieved with an ERD including either moderate protein and non-fat dairy intake (YL group) or standard protein and non-fat dairy intake [EXG (i.e., control) group] along with daily moderate walking within a 6-month comprehensive lifestyle intervention on bone health. It was hypothesized that the YL intervention would induce an equivalent reduction in BW and C-reactive protein (CRP), while maintaining FFSTM percentage and BMD at the total body (TB), lumbar spine (LS), total proximal femur (TPF) and forearm (FA) compared to the EXG intervention.

METHODS

Experimental design

This parallel-arm, randomized controlled trial included healthy, but overweight/obese premenopausal women. Inclusion criteria included women aged 20-45 years with a BMI of ≥25 and ≤36 kg/m^2; moderate physical activity (≤2 hours of planned exercise/week); eumenorrhea; stable BW during the past six months; and a desire to lose weight. Exclusion criteria included current smoking; pregnancy or attempting to become pregnant; diagnosed metabolic or health conditions; diagnosed osteopenia or osteoporosis; use of medications known to affect bone (e.g., steroid or thyroid hormones, bisphosphonates, anticonvulsants, glucocorticoids); health or

orthopedic conditions limiting musculoskeletal activity; hysterectomy and ovariectomy without hormone replacement therapy; and/or oral contraceptive use of <2 years duration (if used). Women were stratified by baseline age, body mass index (BMI) and self-reported physical activity and then randomized to either the YL or EXG intervention group for 24 weeks. Details on participant recruitment have been reported elsewhere (38). This study was approved by the Institutional Review Board for Research Involving Human Subjects at The Pennsylvania State University (University Park, PA, USA). Each woman provided written informed consent before participating in this study.

Dietary and walking intervention

Women were randomized to an ERD that included moderate protein and non-fat dairy (YL group) or to an ERD that included standard protein and non-fat dairy (EXG group) for 12 weeks of weight loss followed by 12 weeks of weight-loss maintenance. Briefly, women randomized to the YL group followed an ERD with moderate protein (i.e., 30% of total energy as dietary protein). As part of the YL dietary pattern, women consumed Yoplait Light® (General Mills, Inc., Minneapolis, MN, USA) twice daily in addition to three more servings of non-fat dairy per day. Women randomized to the EXG group followed an ERD with standard protein (i.e., 16-17% of total energy as dietary protein) as well as three servings of non-fat dairy per day. Dietary fat was constant between YL and EXG groups at approximately 24-25% of total energy, with dietary carbohydrate comprising the remaining proportion of total energy in the ERD dietary patterns. These macronutrient distribution patterns remained constant throughout the 24-week intervention.

The intervention was separated into three phases, including a 2-week JumpStart phase, a 10-week weight-loss phase and a 12-week weight-loss maintenance phase. During the JumpStart

phase (Weeks 0-2), women in the YL group followed their ERD (1200 kcal/day) pattern that included non-fat yogurt intake at breakfast and lunch, while women in the EXG group consumed their ERD pattern (1500 or 1700 kcal/day based on individual resting energy expenditure) (39). From Weeks 3-12 (weight-loss phase), women consumed their ERD (all at 1500 or 1700 kcal/day) pattern (YL or EXG). Dietary patterns combined with daily walking were established to promote a 1-pound BW loss per week. During the weight-loss maintenance phase (Weeks 13-24), women consumed an energy-balanced diet, adjusted for current BW, specific to YL or EXG group.

Daily walking for 30 to 40 minutes at moderate pace was completed by women in both intervention groups for the duration of the study. Women walked at approximately three miles per hour. Additional information regarding dietary patterns, meal replacements, weekly nutrition education and motivation sessions and participant compensation have been reported elsewhere (38). Main methods and complete anthropometric and metabolic measurement results from this 24-week trial have been previously reported (38).

Calcium and vitamin D supplementation

Calcium and vitamin D intakes were normalized across all ERD patterns, using the YL group of 1700 kcal per day as the standard pattern. This ERD pattern included 1500 mg of calcium and 6.25 μg of vitamin D per day. Women across all other ERD patterns consumed supplemental calcium and vitamin D in addition to their dietary intakes to match the target of 1500 mg of calcium and 10-20 μg of vitamin D per day. Nutrient supplements (Wegmans Calcium Citrate + Vitamin D, Wegmans Food Market, Rochester, NY, USA) were dispensed weekly by investigators, and participants self-reported compliance at weekly educational sessions.

Testing sessions

Testing sessions took place at baseline and after the weight-loss (Week 12) and weight-loss maintenance (Week 24) phases. Dietary and physical activity records were submitted by participants at each testing session. In addition, whole blood samples were collected by venipuncture performed by a registered nurse, between 0700 and 0930 hours, after a 12-hour fast. Women were instructed to avoid strenuous physical activity, alcohol and food or beverage intake (except for water) during the 12-hour fast. Anthropometric measurements also were completed at testing sessions. At baseline and after the weight-loss phase and weight-loss maintenance phase, women completed dual-energy X-ray absorptiometry (DXA) scans.

Dietary and physical activity assessment

Food and beverage intakes and physical activity were self-recorded for four days, including three weekdays and one weekend day, in the week preceding each testing session. Diet records were reviewed with participants upon receipt at baseline and at Weeks 12 and 24, verified by a registered dietitian and analyzed for estimated average energy (kcal/day), macronutrients (g/day of protein, carbohydrate and fat) and calcium (mg/day) and vitamin D (µg/day) content using Nutrition Data System for Research (NDS-R version 2010, Minneapolis, MN, USA). Dietary analysis did not include supplemental calcium and vitamin D intakes, and only dietary sources of calcium and vitamin D are reported here.

Physical activity records were used to quantify time spent engaged in light, moderate, and vigorous physical activities. Corresponding metabolic equivalents (40) were applied to estimate total energy expenditure per day.

Anthropometric and body composition measurements

Height was recorded at baseline to the nearest 0.1 cm using a stadiometer (Seca 700, Seca North America East, Hanover, MD, USA). At baseline and Weeks 12 and 24, BW was measured to the nearest 0.1 kg using an electronic scale (TBF-410GS, Tanita Corporation, Arlington Heights, IL, USA). Women wore lightweight clothing and were shoeless during these measurements.

FFSTM (%) and BMD (g/cm^2) of the TB, LS (L_1-L_4), TPF (non-dominant) and FA (non-dominant) were measured by DXA (version 12.7.3.1, QDR 4500A, Hologic Inc., Bedford, MA, USA) at baseline and Weeks 12 and 24, using standard procedures (41,42). Test-retest reliability in 26 premenopausal women with overweight/obesity using this DXA and the same technician produced coefficients of variation (CVs) of 1.02 and 0.97% for FFSTM and TB BMD, respectively, and CVs of 0.88, 1.56, and 1.03% for LS, TPF and FA BMD, respectively. One technician completed all DXA scans throughout the 24-week study.

Biomarkers of bone turnover and inflammation

Serum NTx (nM BCE) (Wampole Labs, Princeton, NJ, USA), serum OC (ng/mL) (Alpco Immunoassays, Salem, NH, USA), and serum high-sensitivity (hs) CRP (ng/mL) (Alpco Immunoassays) were measured by enzyme-linked immunosorbent assay at baseline and after the weight-loss and weight-loss maintenance phases. Whole blood was centrifuged at 4000 rpm for 10 minutes. Serum was pipetted into cryovials and stored at -80°C until analyzed. Samples were analyzed in duplicate for each bioassay. Intra- and inter-assay CVs were 9.6 and 8.7% for NTx, 6.1 and 25.5% for OC and 11.6 and 31.3% for hsCRP, respectively.

Statistical analysis

Women who remained enrolled in the study after Week 2 (n=104) were included in an intention-to-treat model. The last-observation-carried-forward approach was used to populate

missing values for women who did not complete all 24 weeks of intervention. An efficacy analysis also was conducted and included only women who completed the 24-week intervention (n=80). Last-observation-carried-forward was used for missing data points in the efficacy analysis.

Data are presented as means ± standard deviation (SD), unless otherwise indicated. Six participants reported acute illness at the time of blood collection, resulting in hsCRP levels greater than three SD of the mean; data from these women were not included in hsCRP analysis. hsCRP values were not normally distributed; hence, values were logarithmically transformed for analysis. For interpretation, actual hsCRP values are presented. All other variables were normally distributed. A 2 x 3 multivariate analysis of covariance with repeated measures on the time factor was performed to assess differences between intervention groups using baseline values as covariates. Independent (for group interactions) and paired (for group x time interactions) *t*-tests using Bonferroni adjustments for multiple comparisons were completed when significant interactions were found. Data were analyzed using the Statistical Package for the Social Sciences (SPSS; version 19.0, 2010, SPSS Inc., Chicago, IL, USA). All tests were two-sided and a probability of $p<0.01$ was considered statistically significant.

RESULTS

Baseline testing was completed by 123 sedentary, premenopausal women (80% Caucasian, 9% African-American, 3% other, 8% did not respond) with overweight/obesity (age 33.6±7.5 years; height 165.6±6.3 cm; BW 82.4±11.4 kg; BMI 30.0±3.1 kg/m^2), and 104 women (age 33.8±7.5 years; height 165.4±6.5 cm; weight 82.5±11.5 kg; BMI 30.1±3.2 kg/m^2) remained after two weeks (YL, n=52; EXG, n=52). There were no significant differences observed in baseline age, height, BW or BMI between the 104 women remaining after Week 2 and women

who withdrew (n=19). Significant differences were not found between the 80 women who completed the 24-week study (baseline age 34.6±7.3 years; height 166.0±6.3 cm; weight 83.3±11.3 kg; BMI 30.1±3.0 kg/m^2) and women who withdrew (n=43) from the study (baseline age 31.9 ±7.6 years; height 164.8±6.2 cm; BW 80.9±11.7 kg; BMI 29.6±3.3 kg/m^2). Adherence to calcium and vitamin D supplementation intake was approximately 63% of expected.

Dietary assessment and physical activity

Dietary intake data are presented in Table 1. No significant group effect or group x time interaction was observed; however, there was a significant decrease in estimated energy intake over time in both YL and EXG groups. Estimated average energy intake was 75 and 70% of baseline intake at Week 12, and was 82 and 73% of baseline intake at Week 24 for the YL and EXG group, respectively. There was a different pattern of change in estimated total protein intake between groups evidenced by a significant group x time interaction ($p<0.01$). Mean total protein intake did not change from baseline in either group, though change in protein intake from baseline was significantly different between groups at both Weeks 12 and 24. Protein comprised approximately 24% and 21% of energy in the YL group and 20% and 18% of energy in the EXG group, at Weeks 12 and 24, respectively. Mean total carbohydrate intake significantly decreased compared to baseline at Weeks 12 and 24 in YL and EXG groups. Carbohydrate was maintained at approximately 52% and 54% of total energy in the YL and EXG groups for the duration of the study. Total fat significantly decreased over time in YL and EXG groups at Weeks 12 and 24. Total fat comprised approximately 23-26% of total energy in the YL and 25-27% of total energy in the EXG group. Mean calcium and vitamin D intakes significantly increased from baseline to Weeks 12 and 24 in the YL group. Mean vitamin D intake significantly increased from baseline to Week 12 in the EXG group. There was a different pattern of change for calcium and vitamin

D intakes between groups, evidenced by a significant group x time interaction ($p<0.01$ for both). Calcium from baseline to Week 24 and vitamin D from baseline to Week 12 changed to a greater extent in the YL group. No significant group effect, time effect or group x time interaction was observed for estimated energy expenditure from physical activity. Energy expenditure from physical activity increased by approximately 50 kcal per day from baseline to Week 12; however, this change was not maintained at Week 24.

Anthropometric and body composition measurements

Significant differences in the changes in BW percentage and FFSTM percentage were not observed between YL and EXG groups. Women significantly reduced BW percentage (-6.0±3.7 and -5.2±4.0% in YL and EXG groups, respectively), from baseline to Week 12 (Figure 1). Women significantly increased FFSTM percentage (1.5±1.6 and 1.0±2.0% in the YL and EXG groups, respectively), from baseline to Week 12. These changes were maintained from Week 12 to Week 24. Significant group x time interactions were not observed. Similar results were found with efficacy analysis (study completers; Figure 1).

BMD measurements of the TB, LS, TPF and FA are presented in Table 2. There were no significant effects of group or time and no significant group x time interactions in BMD at any skeletal site. Similar results were found with efficacy analysis (Table 2).

Biomarkers of bone turnover and inflammation

There were no significant group or time effects, and no group x time interactions were found for serum NTx or OC (Table 3). There was no group effect for hsCRP and no group x time interaction. However, hsCRP decreased significantly from baseline to Week 12 in YL and EXG groups, and this reduction was maintained to Week 24. Results were similar with efficacy analysis.

DISCUSSION

The hypothesis tested in this study was partially supported; both interventions examined resulted in significant reductions in BW and CRP after 12 weeks, which were maintained through 12 weeks of weight-loss maintenance. The lack of observable changes in BMD and biomarkers of bone turnover over time in both groups suggests that either approach to BW loss may have been equally effective in preventing BMD loss with significant BW loss. The current study demonstrates that healthy premenopausal women with overweight/obesity consuming an ERD with either moderate or standard dietary protein and at least three servings of non-fat dairy per day within a lifestyle intervention including daily moderate walking can lose significant BW while maintaining BMD.

In the current study, women in both groups approximated the most current Recommended Dietary Allowance (RDA) for calcium (1000 mg/day) and vitamin D (15 µg/day) (43). Dietary restriction (44) and insufficient protein intake (45) have been associated with reduced intestinal calcium absorption. As a good source of high quality protein and calcium, consuming at least the three recommended servings of non-fat (46) dairy may be an effective way of preserving BMD during BW loss. Both groups maintained recommended intakes of dietary protein of at least 0.8 g of protein per kg BW per day (47), despite lowered total energy intake.

Bone is a dynamic tissue that undergoes a constant remodeling process, continually breaking down and rebuilding. Lack of change in biomarkers of bone turnover may suggest that bone turnover was balanced during the 6-month intervention, with bone formation coupled to bone resorption. This study supports previous findings in which women with overweight/obesity consuming recommended amounts of protein and calcium within an ERD lost significant BW

and maintained BMD (48). Increased dietary protein without the recommended calcium intake may be insufficient to prevent bone loss induced by BW loss (22). In one study, women demonstrating moderate BW loss with low calcium intake did not exhibit bone loss; however, LS BMD increased in women supplemented with calcium, in spite of BW loss (49).

Josse and colleagues reported that ERDs containing calcium and protein beyond RDAs improved some markers of bone health compared to diets with protein comprising 15% of total energy and recommended (3-4 servings/day) or low (<1 serving/day) low-fat dairy intake (14). These investigators also demonstrated that women consuming an ERD with protein comprising 30% of energy plus 6-7 servings of dairy per day could increase FFSTM over 16 weeks, while women consuming an ERD with protein comprising 15% of energy and 3-4 or less than one serving of dairy per day maintained and lost FFSTM, respectively (50). As a percent of total body mass, FFSTM increased over time in the present study; however, there was no difference between intervention groups. To maintain FFSTM as BW decreases, protein intake should increase or be maintained (51).

Significant BW loss was achieved at a modest pace (approximately 2%/month) and was maintained during the weight-loss maintenance phase of this study. It has been observed that rapid weight loss may stimulate increased bone loss (7). Moderate BW loss, occurring slowly, may facilitate maintenance of bone mass during weight loss (5,48,49,52). This may be due to the smaller caloric deficit that is imposed on the body during moderate and gradual BW loss, as opposed to during large and rapid BW loss, allowing intake of nutrients vital to bone health and maintenance, such as protein and calcium, to fall within recommendations (53).

Several interventions have examined the role of both resistance and aerobic exercise during weight loss on bone health outcomes (3,4,14,30). Energy expended during exercise may

offset the degree to which energy intake must be reduced to achieve weight loss; however, additional mechanical loading on the skeleton is likely a greater contributor to the benefit of exercise on BMD (54). Mechanical loading applied to the skeleton during weight-bearing exercises, such as walking, benefits involved bones by increasing strength and BMD (55). The current study did not find increased energy expenditure during the course of the intervention. This may suggest that women who included daily walks were compensating by forgoing other planned or spontaneous activity. Still, women in both intervention groups maintained BMD, despite BW loss. Previous studies have demonstrated increases in BMD during BW loss achieved with an ERD combined with increased exercise. Premenopausal women with obesity following an ERD with aerobic exercise over three months showed significantly greater BW loss with increases in BMD at the LS and TPF compared to a group only following an ERD (30). However, exercise without intake of calcium and/or protein meeting recommendations within an ERD may be insufficient to prevent or reduce unbalanced bone turnover and maintain BMD during moderate BW loss (3,4,14).

Although obesity has historically been associated with increased bone mass (56), recent evidence suggests that the greater BMD in individuals with obesity may be more accurately correlated to greater amounts of FFSTM rather than fat mass (57,58). In addition, inflammatory factors and cytokines play a role in the regulation of bone resorption and formation (59), and a chronically inflamed environment caused by excess adiposity may have deleterious effects on BMD via the RANK/RANK ligand pathway (34). Fat mass loss, especially in the abdominal region, is associated with improvements in markers of inflammation including CRP (60), and the significant reduction of CRP with BW loss observed in the present study is likely indicative of reduced systemic inflammation (60).

Limitations of this study include potentially insufficient power to detect changes in BMD, NTx or OC. Sample size in this study was calculated for the primary purpose of detecting differences in BW and fat mass losses. Though biomarkers used to detect bone turnover are notoriously variable, they were not considered in sample size estimations. However, similar studies using smaller samples sizes have observed significant changes in bone turnover markers or BMD over time (37,49). Though the current study was longer than some previous studies examining changes in bone (3,14,22,36,37), it was still relatively short in terms of the bone remodeling process.

Finally, reductions in BMD that accompany BW loss may persist even with the subsequent regain of BW (4), adding detriment to serial weight-loss efforts. As recovery of BMD lost during BW reduction in adult women is unlikely, preservation of BMD during BW reduction may be critical to the retention of peak bone mass and prevention of osteoporosis. Likewise, BW loss induced via sustainable lifestyle changes and conducive to BW loss maintenance may reduce the need for repeated weight-loss attempts. Though three months is not considered long-term, women in both diet intervention groups were able to maintain BW losses achieved during the weight-loss phase throughout the weight-loss maintenance phase. An ideal ERD would facilitate successful BW loss and maintenance without reductions in BMD, and sufficient dietary calcium and protein intake may further improve weight-loss outcomes (26,50,61,62).

In conclusion, free-living, premenopausal women with overweight/obesity who completed a 6-month lifestyle intervention with moderate or standard protein and sufficient non-fat dairy intake along with daily moderate walking were able to achieve and maintain an approximate 6% BW loss while maintaining BMD at several skeletal sites. Results of the current study demonstrate that moderate weight loss with retention of BMD is feasible in women with

overweight/obesity when dietary protein and dairy are sufficiently consumed and combined with daily moderate physical activity. Current recommendations for protein and dairy/calcium may be sufficient to maintain BMD with significant, moderate BW loss in premenopausal women. Future research should examine the long-term weight loss sustainability of combined weight loss and physical activity interventions. Following participants for one year or more would offer additional insights regarding changes in bone with acute BW loss and further information about the impact of weight-loss maintenance on bone turnover and BMD.

REFERENCES

1. Brown T, Avenell A, Edmunds LD, et al. Systematic review of long-term lifestyle interventions to prevent weight gain and morbidity in adults. *Obes Rev*. 2009;10(6):627–638.

2. Villareal DT, Fontana L, Weiss EP, et al. Bone mineral density response to caloric restriction-induced weight loss or exercise-induced weight loss: a randomized controlled trial. *Arch Intern Med.* 2006;166(22):2502–2510.

3. Rector RS, Loethen J, Ruebel M, Thomas TR, Hinton PS. Serum markers of bone turnover are increased by modest weight loss with or without weight-bearing exercise in overweight premenopausal women. *Appl Physiol Nutr Metab*. 2009;34(5):933–941.

4. Hinton PS, Rector RS, Linden MA, et al. Weight-loss-associated changes in bone mineral density and bone turnover after partial weight regain with or without aerobic exercise in obese women. *Eur J Clin Nutr.* 2011.

5. Ricci TA, Chowdhury HA, Heymsfield SB, Stahl T, Pierson RN Jr, Shapses SA. Calcium supplementation suppresses bone turnover during weight reduction in postmenopausal women. *J. Bone Miner Res.* 1998;13(6):1045–1050.

6. Jensen LB, Kollerup G, Quaade F, Sørensen OH. Bone minerals changes in obese women during a moderate weight loss with and without calcium supplementation. *J Bone Miner Res.* 2001;16(1):141–147.

7. Fogelholm GM, Sievänen HT, Kukkonen-Harjula TK, Pasanen ME. Bone mineral density during reduction, maintenance and regain of body weight in premenopausal, obese women. *Osteoporos Int*. 2001;12(3):199–206.

8. Bacon L, Stern JS, Keim NL, Van Loan MD. Low bone mass in premenopausal chronic dieting obese women. *Eur J Clin Nutr*. 2004;58(6):966–971.

9. Hansen MA, Overgaard K, Riis BJ, Christiansen C. Role of peak bone mass and bone loss in postmenopausal osteoporosis: 12 year study. *BMJ*. 1991;303(6808):961–964.

10. Sirola J, Kröger H. Similarities in acquired factors related to postmenopausal osteoporosis and sarcopenia. *J Osteoporos*. 2011;2011:536735.

11. Robling AG, Castillo AB, Turner CH. Biomechanical and molecular regulation of bone remodeling. *Annu Rev Biomed Eng*. 2006;8:455–498.

12. Guthrie JR, Dennerstein L, Wark JD. Risk factors for osteoporosis: A review. *Medscape Womens Health*. 2000;5(4):E1.

13. Palacios C. The role of nutrients in bone health, from A to Z. *Crit Rev Food Sci Nutr*. 2006;46(8):621–628.

14. Josse AR, Atkinson SA, Tarnopolsky MA, Phillips SM. Diets Higher in Dairy Foods and Dietary Protein Support Bone Health during Diet- and Exercise-Induced Weight Loss in Overweight and Obese Premenopausal Women. *J Clin Endocrinol Metab*. 2011.

15. Mosley JR. Osteoporosis and bone functional adaptation: mechanobiological regulation of bone architecture in growing and adult bone, a review. *J Rehabil Res Dev*. 2000;37(2):189–199.

16. Reid IR. Relationships between fat and bone. *Osteoporos Int*. 2008;19(5):595–606.

17. Gómez-Ambrosi J, Rodríguez A, Catalán V, Frühbeck G. The bone-adipose axis in obesity and weight loss. *Obes Surg*. 2008;18(9):1134–1143.

18. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA*. 2012;307(5):491–497.

19. Sacks FM, Bray GA, Carey VJ, et al. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *N Engl J Med*. 2009;360(9):859–873.

20. Gardner CD, Kiazand A, Alhassan S, et al. Comparison of the Atkins, Zone, Ornish, and LEARN diets for change in weight and related risk factors among overweight premenopausal women: the A TO Z Weight Loss Study: a randomized trial. *JAMA*. 2007;297(9):969–977.

21. Skov AR, Toubro S, Rønn B, Holm L, Astrup A. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. *Int J Obes Relat Metab Disord*. 1999;23(5):528–536.

22. Bowen J, Noakes M, Clifton PM. A high dairy protein, high-calcium diet minimizes bone turnover in overweight adults during weight loss. *J Nutr*. 2004;134(3):568–573.

23. Layman DK, Evans EM, Erickson D, et al. A moderate-protein diet produces sustained weight loss and long-term changes in body composition and blood lipids in obese adults. *J Nutr*. 2009;139(3):514–521.

24. Layman DK, Boileau RA, Erickson DJ, et al. A Reduced Ratio of Dietary Carbohydrate to Protein Improves Body Composition and Blood Lipid Profiles during Weight Loss in Adult Women. *J Nutr*. 2003;133(2):411 –417.

25. Farnsworth E, Luscombe ND, Noakes M, Wittert G, Argyiou E, Clifton PM. Effect of a high-protein, energy-restricted diet on body composition, glycemic control, and lipid concentrations in overweight and obese hyperinsulinemic men and women. *Am J Clin Nutr*. 2003;78(1):31–39.

26. Zemel MB, Teegarden D, Loan MV, et al. Dairy-rich diets augment fat loss on an energy-restricted diet: a multicenter trial. *Nutrients*. 2009;1(1):83–100.

27. Devkota S, Layman DK. Protein metabolic roles in treatment of obesity. *Curr Opin Clin Nutr Metab Care*. 2010;13(4):403–407.

28. Zemel MB, Sun X, Sobhani T, Wilson B. Effects of dairy compared with soy on oxidative and inflammatory stress in overweight and obese subjects. *Am J Clin Nutr.* 2010;91(1):16–22.

29. Ehrlich PJ, Lanyon LE. Mechanical strain and bone cell function: a review. *Osteoporos Int*. 2002;13(9):688–700.

30. Hosny IA, Elghawabi HS, Younan WBF, Sabbour AA, Gobrial MAM. Beneficial impact of aerobic exercises on bone mineral density in obese premenopausal women under caloric restriction. *Skeletal Radiol.* 2011.

31. Borer KT, Fogleman K, Gross M, La New JM, Dengel D. Walking intensity for postmenopausal bone mineral preservation and accrual. *Bone*. 2007;41(4):713–721.

32. Wang MC, Bachrach LK, Van Loan M, Hudes M, Flegal KM, Crawford PB. The relative contributions of lean tissue mass and fat mass to bone density in young women. *Bone*. 2005;37(4):474–481.

33. Blain H, Vuillemin A, Guillemin F, et al. Serum leptin level is a predictor of bone mineral density in postmenopausal women. *J Clin Endocrinol Metab.* 2002;87(3):1030–1035.

34. Cao JJ. Effects of obesity on bone metabolism. *J Orthop Surg Res*. 2011;6:30.

35. Rosen CJ, Bouxsein ML. Mechanisms of Disease: is osteoporosis the obesity of bone? *Nat Clin Pract Rheumatol.* 2006;2(1):35–43.

36. Wagner G, Kindrick S, Hertzler S, DiSilvestro RA. Effects of various forms of calcium on body weight and bone turnover markers in women participating in a weight loss program. *J Am Coll Nutr*. 2007;26(5):456–461.

37. Van Loan MD, Johnson HL, Barbieri TF. Effect of weight loss on bone mineral content and bone mineral density in obese women. *Am J Clin Nutr.* 1998;67(4):734–738.

38. Shlisky JD. A lifestyle intervention promoting weight loss with moderate or standard protein and non-fat dairy intake and daily walking: impact on body composition, metabolic parameters and bone health in premenopausal women with overweight and obesity. Unpublished manuscript, Pennsylvania State University, University Park, PA. 2012.

39. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr.* 1990;51(2):241–247.

40. Ainsworth BE, Haskell WL, Herrmann SD, et al. 2011 Compendium of Physical Activities: a second update of codes and MET values. *Med Sci Sports Exerc*. 2011;43(8):1575–1581.

41. Miller LE, Nickols-Richardson SM, Ramp WK, Gwazdauskas FC, Cross LH, Herbert WG. Bone mineral density in postmenopausal women: does exercise training make a difference? *Phys Sportsmed*. 2004;32(2):18–24.

42. Nickols-Richardson SM, Miller LE, Wootten DF, Ramp WK, Herbert WG. Concentric and eccentric isokinetic resistance training similarly increases muscular strength, fat-free soft tissue mass, and specific bone mineral measurements in young women. *Osteoporos Int*. 2007;18(6):789–796.

43. Ross AC, Taylor CL, Yaktine AL, Del Valle HB eds. *Dietary Reference Intakes for Calcium and Vitamin D*. Washington (DC): National Academies Press (US); 2011.

44. Cifuentes M, Riedt CS, Brolin RE, Field MP, Sherrell RM, Shapses SA. Weight loss and calcium intake influence calcium absorption in overweight postmenopausal women. *Am J Clin Nutr.* 2004;80(1):123–130.

45. Hunt JR, Johnson LK, Fariba Roughead ZK. Dietary protein and calcium interact to influence calcium retention: a controlled feeding study. *Am J Clin Nutr.* 2009;89(5):1357–1365.

46. U.S. Department of Agriculture and U.S. Department of Health and Human Services. Dietary Guidelines for Americans, 2010. 7th Edition, Washington, DC: U.S. Government Printing Office, December 2010.

47. A Report of the Panel on Macronutrients, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. Washington, D.C.: The National Academies Press; 2005.

48. Riedt CS, Schlussel Y, von Thun N, et al. Premenopausal overweight women do not lose bone during moderate weight loss with adequate or higher calcium intake. *Am J Clin Nutr.* 2007;85(4):972–980.

49. Shapses SA, Von Thun NL, Heymsfield SB, et al. Bone turnover and density in obese premenopausal women during moderate weight loss and calcium supplementation. *J Bone Miner Res.* 2001;16(7):1329–1336.

50. Josse AR, Atkinson SA, Tarnopolsky MA, Phillips SM. Increased Consumption of Dairy Foods and Protein during Diet- and Exercise-Induced Weight Loss Promotes Fat Mass Loss and Lean Mass Gain in Overweight and Obese Premenopausal Women. *J Nutr*. 2011;141(9):1626–1634.

51. Layman DK. Protein Quantity and Quality at Levels above the RDA Improves Adult Weight Loss. *J Am Coll Nutr.* 2004;23(suppl 6):631S–636S.

52. Redman LM, Rood J, Anton SD, Champagne C, Smith SR, Ravussin E. Calorie Restriction and Bone Health in Young, Overweight Individuals. *Arch Intern Med*. 2008;168(17):1859–1866.

53. Webber KH, Lee E. The diet quality of adult women participating in a behavioural weight-loss programme. *J Hum Nutr Diet*. 2011;24(4):360–369.

54. Borer KT. Physical activity in the prevention and amelioration of osteoporosis in women : interaction of mechanical, hormonal and dietary factors. *Sports Med*. 2005;35(9):779–830.

55. Vainionpää A, Korpelainen R, Leppäluoto J, Jämsä T. Effects of high-impact exercise on bone mineral density: a randomized controlled trial in premenopausal women. *Osteoporos Int*. 2005;16(2):191–197.

56. Felson DT, Zhang Y, Hannan MT, Anderson JJ. Effects of weight and body mass index on bone mineral density in men and women: the Framingham study. *J Bone Miner Res.* 1993;8(5):567–573.

57. Bogl LH, Latvala A, Kaprio J, Sovijärvi O, Rissanen A, Pietiläinen KH. An investigation into the relationship between soft tissue body composition and bone mineral density in a young adult twin sample. *J Bone Miner Res.* 2011;26(1):79–87.

58. Blum M, Harris SS, Must A, et al. Leptin, body composition and bone mineral density in premenopausal women. *Calcif Tissue Int.* 2003;73(1):27–32.

59. Silverman NE, Nicklas BJ, Ryan AS. Addition of Aerobic Exercise to a Weight Loss Program Increases BMD, with an Associated Reduction in Inflammation in Overweight Postmenopausal Women. *Calcif Tissue Int.* 2009;84(4):257–265.

60. Fisher G, Hyatt TC, Hunter GR, Oster RA, Desmond RA, Gower BA. Effect of diet with and without exercise training on markers of inflammation and fat distribution in overweight women. *Obesity (Silver Spring)*. 2011;19(6):1131–1136.

61. Zemel MB, Thompson W, Milstead A, Morris K, Campbell P. Calcium and dairy acceleration of weight and fat loss during energy restriction in obese adults. *Obes Res*. 2004;12(4):582–590.

62. Rodríguez-Rodríguez E, Perea JM, López-Sobaler AM, Ortega RM. An adequate calcium intake could help achieve weight loss in overweight/obese women following hypocaloric diets. *Ann Nutr Metab*. 2010;57(2):95–102.

Table 4.1 Mean values ± standard deviation of dietary intake patterns at baseline and Weeks 12 and 24 in women with overweight/obesity participating in a weight-loss intervention including an energy-reduced diet and walking component with either moderate protein and non-fat dairy intake (YL) or standard protein and dairy intake (EXG).

	YL (n=37)			EXG (n=34)		
Variable	Baseline	Change from Baseline to Week 12	Change from Baseline to Week 24	Baseline	Change from Baseline to Week 12	Change from Baseline to Week 24
Energy (kcal/d)	1757±387	-442±348*	-323±341*	1974±441	-600±437*	-532±479*
Protein (g/d)	65±14	+11±18[#]	+7±18[#]	75.0±18.2	-8±21	-12±19
Carbohydrate (g/d)	219±51	-43±45*	-31±42*	232±57	-38±50*	-37±59*
Fat (g/d)	69±20	-33±20*	-25±20*	82±21	-42±23*	-36±26*
Calcium (mg/d)	718±205	+419±326*	+331±302*[#]	929±315	+133±464	+42±340
Vitamin D (µg/d)	2.9±2.0	+6.8±3.6*[#]	+5.1±3.8*	3.9±2.6	+3.0±3.2*	+2.2±3.5

Complete 4-day food records were available for 71 women at all time points. Improbable reports of <1000 and >3200kcal were not included in analysis.

*P<0.01 compared with baseline.

[#] P<0.01 between groups.

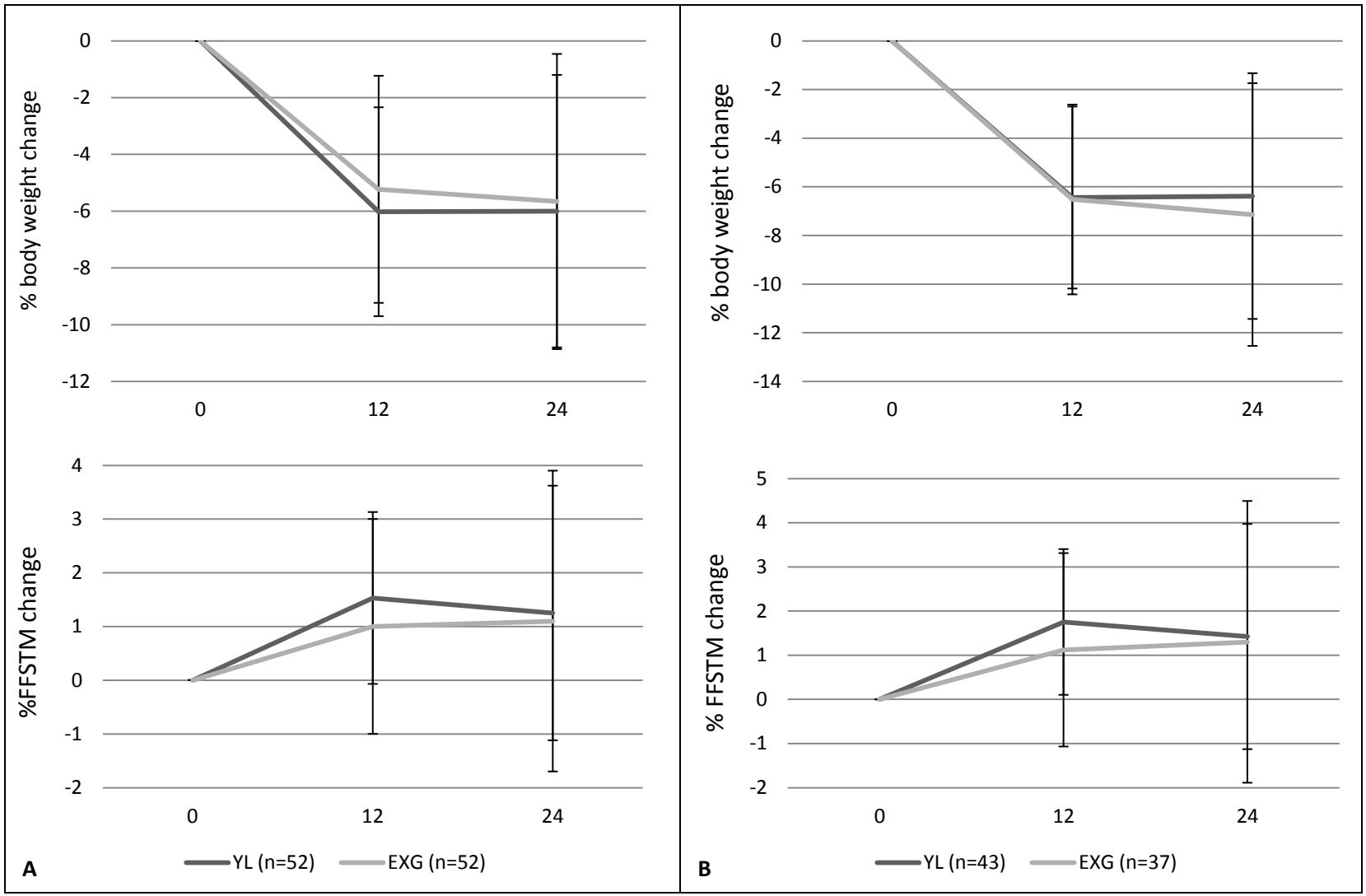


Fig. 4.1 Intention to treat analysis (A; YL=52, EXG=52) and efficacy analysis (B; YL=43, EXG=37) of percentage body weight and percentage fat-free soft tissue mass (FFSTM) change in premenopausal women with overweight/obesity at Weeks 12 and 24 of a 24-week lifestyle intervention.

Table 4.2 Mean values ± standard deviation of bone mineral density at the total body, lumbar spine, total proximal femur and forearm skeletal sites at baseline, Weeks 12 and 24 in women with overweight/obesity participating in a weight-loss intervention including an energy-reduced diet and walking component with either moderate protein and non-fat dairy intake (YL) or standard protein and non-fat dairy intake (EXG).

	Intention-to-treat model (n=104)					Efficacy model (n=80)				
Bone mineral density	Group	Baseline	Week 12	Week 24	p-value	Group	Baseline	Week 12	Week 24	p-value
Total body (g/cm^2)					Group=0.41					Group=0.36
	YL (n=52)	1.23±0.09	1.23±0.09	1.23±0.09	Time=0.18	YL (n=43)	1.24±0.09	1.24±0.09	1.24±0.09	Time=0.11
	EXG (n=52)	1.22±0.10	1.22±0.09	1.22±0.10	G x T =0.60	EXG (n=37)	1.23±0.10	1.23±0.09	1.23±0.09	G x T=0.90
Lumbar spine (g/cm^2)					Group=.06					Group=0.38
	YL (n=52)	1.09±0.11	1.09±0.10	1.09±0.11	Time=0.05	YL (n=43)	1.10±0.12	1.10±0.11	1.10±0.12	Time=0.012
	EXG (n=51)	1.05±0.09	1.06±0.10	1.06±0.10	G x T =0.64	EXG (n=36)	1.07±0.10	1.08±0.10	1.08±0.10	G x T =0.07
Total proximal femur (g/cm^2)					Group=0.15					Group=0.25
	YL (n=52)	1.05±0.10	1.05±0.10	1.05±0.11	Time=0.28	YL (n=43)	1.04±0.10	1.05±0.10	1.05±0.10	Time=0.48
	EXG (n=52)	1.03±0.08	1.03±0.08	1.03±0.08	G x T =0.17	EXG (n=37)	1.04±0.08	1.03±0.08	1.03±0.09	G x T =0.11
Forearm (g/cm^2)					Group=0.54					Group=0.36
	YL (n=52)	0.58±0.04	0.59±0.03	0.59±0.04	Time=0.09	YL (n=43)	0.59±0.04	0.59±0.03	0.59±0.04	Time=0.63
	EXG (n=52)	0.58±0.04	0.58±0.04	0.58±0.04	G x T =0.49	EXG (n=37)	0.58±0.04	0.59±0.04	0.59±0.04	G x T =0.94

Data missing from one woman in the EXG group.
All variables analyzed by dual-energy X-ray absorptiometry, using $p<0.01$ for statistical significance; *p*-values from intention-to-treat analysis, using multivariate analysis of covariance with repeated measures on the time factor.
G x T group by time interaction

Table 4.3 Mean values ± standard deviation of N-telopeptide of type 1 collagen (NTx), osteocalcin and hsC-reactive protein (CRP) at baseline, Weeks 12 and 24 in women with overweight/obesity participating in a weight-loss intervention including an energy-reduced diet and walking component with either moderate protein and non-fat dairy intake (YL) or standard protein and non-fat dairy intake (EXG).

	Intention to treat model (n=104)					Efficacy model (n=80)				
Characteristic	Group	Baseline	Week 12	Week 24	p-value	Group	Baseline	Week 12	Week 24	p-value
NTx (nM BCE)	YL (n=52) EXG (n=52)	13.3±4.2 13.4±6.0	13.3±3.7 12.8±4.9	13.7±4.1 12.5±3.7	Group=0.27 Time=0.80 G x T =0.52	YL (n=43) EXG (n=37)	12.8±4.1 13.8±6.9	12.8±3.3 13.0±5.5	13.3±3.9 12.6±3.9	Group=0.86 Time=0.73 G x T =0.66
Osteocalcin (ng/mL)	YL (n=52) EXG (n=52)	13.3±5.2 13.4±5.0	13.1±5.6 12.9±4.3	12.9±5.0 12.7±5.3	Group=0.15 Time=0.42 G x T =0.73	YL (n=43) EXG (n=37)	12.4±4.9 13.3±5.4	12.2±5.5 12.9±4.4	11.9±4.6 12.7±5.7	Group=0.07 Time=0.13 G x T =0.80
hsCRP(mg/L)[*#]	YL (n=49) EXG (n=49)	15.9±48.4 3.5±4.0	4.6±10.6 3.6±8.3	4.6±7.4 3.7±8.4	Group=0.31 **Time=0.001** G x T =0.12	YL (n=40) EXG (n=35)	11.5±29.4 3.5±3.1	5.0±11.5 2.6±3.0	5.0±7.9 2.6±3.5	Group=0.26 **Time=0.005** G x T =0.61

Fasting serum used to measure biomarker concentrations; CRP data were log transformed for analysis.

*different baseline to Week 12,[#]different baseline to Week 24, using $p<0.01$ for statistical significance; *p*-values from intention-to-treat analysis, using multivariate analysis of covariance with repeated measures on the time factor.

G x T=group x time interaction

CHAPTER 5

MEDIUM REDUCTION OF DIETARY ENERGY DENSITY WITHIN AN ENERGY-RESTRICTED DIETARY PATTERN BENEFITS WEIGHT-LOSS OUTCOMES

To be submitted to the *Appetite*

ABSTRACT

Consuming foods low in energy density (ED) may reduce caloric intake short term. A few clinical trials report a relationship between dietary ED change during body weight (BW) loss and BW loss success. Consuming a diet low in ED may be a simple approach to effectively reduce hunger and promote BW loss. In a secondary analysis of data collected from a randomized clinical weight loss/weight-loss maintenance trial, the present study explored changes in ED and BW, body composition and anthropometric measurements. Participants included 71 free-living premenopausal women with overweight/obesity who completed in a 24-week lifestyle intervention that included an energy-restricted diet and daily moderate walking. Women were categorized into ED change tertiles; outcome measurements were compared across tertile groups. Change in ED was different between tertiles as designed ($p<0.001$), with ED changing the least in Tertile 1 and most in Tertile 3. Women in Tertile 2 (43.0% reduction in ED) experienced greater reductions in BW ($p<0.01$) and body mass index ($p<0.01$) compared to Tertile 1 (26.3% reduction in ED) but not to Tertile 3 (56.3% reduction in ED) at Week 12. These improvements extended to Week 24, along with greater reductions in fat mass ($p<0.01$), body fat percentage ($p<0.05$) and waist circumference ($p<0.05$). A medium reduction in dietary ED may facilitate and sustain positive changes in anthropometric and body composition measurements in premenopausal women with overweight/obesity. Additional research is needed to identify if ED is an appropriate simple approach to promote BW loss and weight-loss maintenance.

INTRODUCTION

With the prevalence of obesity in the United States greater than 35% among adults (1), identification of a simple yet effective strategy for body weight (BW) loss, weight-loss maintenance and the prevention of weight gain/regain is urgent. To date, several complex eating patterns have been investigated for their effectiveness via randomized clinical weight-loss trials with limited success (2–4). Adherence to an energy-restricted diet (ERD) intervention is a strong predictor of BW loss, regardless of the intervention components (4–6). Therefore, a simple message as part of a straight-forward dietary pattern may be more easily adhered to and incorporated into overall lifestyles so that weight-loss may be achieved and maintained long term.

One simple strategy reported to impact energy intake is manipulation of dietary energy density (ED) (7–11). ED is the energy content of a food divided by the food's unit weight (kcal/g). Increasing fruit and vegetable intake, which contributes water and fiber to the diet, and reducing fat intake, which has a higher ED (9 kcal/g) than either carbohydrate (4 kcal/g) or protein (4 kcal/g), have substantial influences on dietary ED (7,11). Intake of a greater weight of food that contains less energy may enhance satiation while reducing overall energy intake (7,12,13). Short-term, laboratory-based studies have found that individuals consume less total energy when presented with foods lower in ED than with similar foods having a greater ED (7,14). Furthermore, a small number of longitudinal studies suggest that reduction in ED may be a foundational principle for achieving reduced energy intake during ERD weight-loss interventions (8–10).

Relative to complex diet strategies suggested for BW loss, ED is a simple feature of dietary patterns that individuals can consider during food and meal selection for overall

reductions in energy intake. Furthermore, diets that are low in ED via inclusion of increased fruit and vegetable consumption (11,15) may increase diet quality and help meet individual micronutrient recommendations (16), decreasing the need for careful attention to diet quality of an ERD. The current study explored changes in ED in 71 free-living premenopausal women with overweight/obesity who participated in a 24-week lifestyle intervention that included an ERD combined with daily moderate walking. It was hypothesized that women characterized by a large reduction in dietary ED during the intervention would exhibit the greatest reductions in energy intake, BW, body mass index (BMI), fat mass (FM), body fat percentage (BF%), central abdominal tissue percentage (CAT%), waist circumference (WC) and hip circumference (HC), compared to small or medium reduction in dietary ED.

METHODS

Participants

Data from a randomized clinical weight-loss intervention in premenopausal women, ages 20-45 years, with overweight/obesity (BMI $\geq$25 to $\leq$36 kg/m^2) was used for this secondary analysis. Exclusion criteria included greater than 5% weight fluctuation in the 6-month period before study enrollment and current pregnancy or lactation and/or the presence of chronic diseases. The primary study was approved by the Institutional Review Board for Research Involving Human Subjects at The Pennsylvania State University, University Park, PA, and each woman provided written informed consent before participation.

Primary weight-loss intervention

Women were stratified by baseline age, BMI and self-reported physical activity and then randomized into one of two ERD groups. Both groups used a food exchange system as the basis of the ERD to meet macronutrient recommendations. Macronutrient content varied between

diets, though both diets were within Acceptable Macronutrient Distribution Ranges (17) and had similar fat content, comprising 24-25% of total energy intake. Women in both ERD groups walked at a moderate pace for 30-40 minutes daily for the entirety of the study. The intervention included 12 weeks of weight loss followed by 12 weeks of weight-loss maintenance.

Daily energy needs were estimated for each participant using the Mifflin-St. Jeor equation (18). A total energy deficit of 500 kcal per day was designed to induce an approximate 1-pound BW loss per week. Each week, participants attended a nutrition education session, which focused on basic nutrition information, such as following an exchange pattern of eating, purchasing and preparing foods and modifying recipes. Additional details regarding study methods have been presented elsewhere (19). Briefly, women in both ERD groups were combined for one sample, and the 71 women included in this secondary analysis had a mean ± standard deviation age of 35.0 ±7.3 years, height of 166.2±6.2 cm, BW of 83.2±11.3 kg, BMI of 30.0±3.0 kg/m^2 and were primarily Caucasian (91%).

Energy density calculation

Participants recorded their food and beverage intakes over four consecutive days, including three weekdays and one weekend day before baseline and during Week 12 and Week 24. Review and verification of dietary records was conducted by a registered dietitian upon receipt. Dietary records were analyzed using the Nutrition Data System for Research (NDS-R version 2010, Minneapolis, MN). Food energy and food weight data were averaged at each time point. ED values were calculated as the ratio of food only energy (kcal) to food only weight (g), with the exclusion of all beverages per recommended protocol (9,20).

Anthropometric and body composition measurements

At baseline, Week 12 and Week 24, BW was measured to the nearest 0.1 kg using an electronic scale (TBF-410GS, Tanita Corporation, Arlington Heights, IL) after a 12-hour fast. Baseline height was recorded to the nearest 0.1 cm using a stadiometer (Seca 700, Seca North America East, Hanover, MD). Women were measured without shoes and wearing lightweight clothing. BMI (kg/m^2) was calculated from BW and height measurements. Dual-energy X-ray absorptiometry (Hologic QDR4500A, Bedford, MA) was used for measurement of total body FM (kg), fat-free soft tissue mass (FFSTM) (kg), BF% and CAT% at baseline, Week 12 and Week 24. Dual-energy X-ray absorptiometry scans were performed by one investigator. Test-retest reliability in 26 overweight/obese premenopausal women has produced coefficients of variation (CVs) of 1.87, 1.02, and 3.32% for FM, FFSTM, and CAT%, respectively. WC and HC were measured to the nearest 0.1 cm in duplicate (Gulik II tape measure, Country Technology, Gays Mills, WI) and then averaged and recorded at baseline, Week 12 and Week 24.

Statistical analyses

Participants with complete dietary data at baseline, Week 12 and Week 24 were included in analyses. Any missing anthropometric or body composition data were replaced using the last-observation-carried-forward technique. All data were normally distributed. Women were equally divided into tertiles based on the magnitude of change in estimated dietary ED from baseline to Week 12. Each outcome variable was analyzed by analysis of covariance with repeated measures on the time factor, using baseline value as a covariate. Paired (time effect) and independent (group x time interaction) sample *t*-tests using Bonferroni adjustments for multiple comparisons were completed when significant effects were found. A probability of $p<0.05$ (two-tailed) was considered statistically significant. Data are reported as means ± standard deviation (SD), unless

otherwise noted. Data analyses were conducted using the Statistical Package for the Social Sciences (version 19.0, 2010, SPSS Inc, Chicago, IL, USA).

RESULTS AND DISCUSSION

Significant reductions in BW, BMI, FM, FFSTM, BF%, CAT%, WC and HC were observed over time in the primary study for both intervention groups; however, there were no significant differences in these measures between groups (19). Baseline variables for women in each of the three ED tertiles were not significantly different, except for baseline dietary ED (Table 1). Women in Tertile 1 (n=23) made the smallest change in ED (≤0.67 kcal/g) from baseline to Week 12. Average estimated ED was reduced by 26.3% from baseline to Week 12 in Tertile 1. Women in Tertile 2 (n=24) had a medium reduction in ED (>0.68 and <0.87 kcal/g). In Tertile 2, average estimated ED was reduced by 43.0% from baseline to Week 12. Women in Tertile 3 (n=24) made the largest reduction in ED (≥0.92 kcal/g), with a reduction of 56.3% from baseline to Week 12 (Figure 1).

From baseline to Week 12, women in all tertiles significantly reduced estimated energy intake from food, and these reductions were maintained through Week 24 (Table 1). There was a significantly different pattern of change in energy intake between groups. Between baseline and Week 12, women in Tertile 3 had a significantly greater reduction in estimated energy intake compared to Tertile 1 but not to Tertile 2. There was no significant effect of group or group x time interaction for food weight. There was a significant increase in food weight in all three groups over time from baseline to Week 12, but this increase was not maintained through Week 24. Dietary ED was significantly reduced in all groups from baseline to Week 12, and these reductions were maintained through Week 24. There was a significantly different pattern of change in ED between groups. Tertiles 2 and 3 had significantly greater reductions in ED from

baseline to Week 12 and Week 24 than Tertile 1, and Tertile 3 had a significantly greater reduction in ED from baseline to Week 12 and Week 24 than Tertile 2 (Table 1).

Women in Tertiles 1, 2 and 3, respectively, significantly reduced BW, BMI, FM, FFSTM, CAT%, WC and HC from baseline to Week 12. Tertile 2 significantly reduced BF% from baseline to Week 12, and this reduction was maintained to Week 24. There was a significantly different pattern of change in BW, BMI, FM, BF% and WC between groups. Women in Tertile 2 had significantly greater reductions in BW and BMI at Week 12 and Week 24 and significantly greater reductions in FM, BF% and WC at Week 24 compared to Tertile 1 but not Tertile 3 (Table 1/Figure 2).

A medium to high reduction in dietary ED within an ERD intervention may be beneficial to modifying anthropometric and body composition measurements. Women in Tertile 2, who had a medium reduction in dietary ED, experienced greater reductions in BW, BMI, FM, and WC compared to Tertile 1 but not Tertile 3. Additionally, Tertile 2 was the only group to significantly reduce BF%. Notably, these improvements persisted through Week 24. Thus, greater reductions of dietary ED in this intervention were associated with enhanced BW and FM losses. These results suggest that a medium reduction in ED during an ERD may increase sustainability of BW loss.

With the largest estimated reduction in energy intake at Week 12 and the greatest estimated reduction in dietary ED at both Week 12 and Week 24, Tertile 3 did not improve any outcome measure beyond those observed in Tertile 1 or 2. This suggests that extreme reductions in ED and energy intake are not required to produce positive change in anthropometric and body composition measurements. Moreover, it is possible that Tertile 3 captured the majority of implausible reporters. These implausible reports in dietary intake may be reflected in the

relatively high estimated dietary ED at baseline and the relatively low estimated ED observed at Week 12 in Tertile 3.

Population-based data have suggested that a healthy BMI is associated with low dietary ED (21,16) and that consumption of a diet low in ED may protect against future BW gain (22). The current study and others suggest that a reduction in ED may be an important method for reduced dietary energy intake and BW loss in randomized clinical weight-loss interventions (8–10). Rolls and colleagues observed that reduction in ED predicted weight loss during the first several months of a weight-loss intervention in overweight/obese adults (8). In this primary study of dietary components that varied in ED, a standard diet control group and an intervention group consuming two daily servings of low energy-dense soup lost more weight than a group consuming two high energy-dense daily snacks (8). Similarly, a secondary analysis conducted by Ledikwe and colleagues of a weight-loss trial including behavioral based interventions found that adults with the greatest reduction in ED from baseline had the greatest BW loss (10). Adults with elevated BP were randomized to either an advice group who received one counseling session, or to one of two treatment groups who attended 18 behavioral weight-loss sessions with or without implementation of the Dietary Approaches to Stop Hypertension diet. When participants were combined and analyzed by tertiles of ED reduction, participants in the highest and middle tertiles decreased energy intake while increasing the weight of foods consumed and fruit and vegetable intake, favorably changing micronutrient intakes (10).

Diets low in ED also may support long-term weight loss. Ello-Martin and colleagues reported that obese women counseled to reduce dietary fat and increase foods rich in water (e.g., soup, fruits and vegetables) reduced ED to a greater extent and lost more BW after one year compared to a group counseled to reduce fat intake only (9). Secondary analysis of a separate

study also reported that a diet low in ED may be important for weight-loss maintenance (15). Raynor and colleagues investigated the self-reported eating patterns of overweight adults, normal-weight adults and successful weight-loss maintainers from previously conducted studies. Dietary ED was significantly lower in the weight-loss maintainers group, who consumed more vegetables, fiber and whole grains and less fat than both normal and overweight groups (15). The current study is consistent with evidence that a medium reduction in ED may help maintain beneficial changes to BW long term. At Week 24, women who had a medium reduction in ED (Tertile 2) continued to experience improvements in BW and body composition beyond those seen in women who minimally reduced ED.

There are several limitations to the current study. As a secondary analysis, these data were from an intervention not designed for ED measurement. Self-reported dietary intake data may have contributed to inaccuracies in ED calculation. Furthermore, arbitrary limits set by division of participants into three equal groups by ED change may have obscured some other feature important to outcomes. Inaccurate dietary reporters may have been grouped into Tertile 3, in which reductions in ED may have been exaggerated, further complicating the tertile grouping strategy. ED calculated using food only intake is reported here as this method may provide the most meaningful measure of dietary ED (20,23). However, this does not allow for comparison with investigations that included beverages into calculations of ED.

In conclusion, women who had a medium reduction in dietary ED within a 24-week lifestyle intervention that included an ERD and daily walking component had greater reductions in BW, BMI, FM, BF% and WC compared to women who had a small reduction in ED. Adopting an eating pattern lower in ED than the customary diet may allow an individual to consume fewer calories without reducing the weight of food consumed. Moreover, a diet plan

focused on ED is also relatively open, allowing an individual to include favorite foods when desired. This may enhance dietary satisfaction and promote long-term adherence and weight-loss maintenance. A medium reduction in ED may also be more sustainable compared to a drastic change in dietary pattern. Future research should be conducted to examine the ED of successful weight loss approaches. Furthermore, interventions based on dietary advice to simply reduce ED should be examined.

REFERENCES

1. Flegal KM, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA*. 2012;307(5):491–497.

2. Gardner CD, Kiazand A, Alhassan S, et al. Comparison of the Atkins, Zone, Ornish, and LEARN diets for change in weight and related risk factors among overweight premenopausal women: the A TO Z Weight Loss Study: a randomized trial. *JAMA*. 2007;297(9):969–977.

3. Skov AR, Toubro S, Rønn B, Holm L, Astrup A. Randomized trial on protein vs carbohydrate in ad libitum fat reduced diet for the treatment of obesity. *Int J Obes Relat Metab Disord.* 1999;23(5):528–536.

4. Sacks FM, Bray GA, Carey VJ, et al. Comparison of weight-loss diets with different compositions of fat, protein, and carbohydrates. *N Engl J Med.* 2009;360(9):859–873.

5. Del Corral P, Chandler-Laney PC, Casazza K, Gower BA, Hunter GR. Effect of dietary adherence with or without exercise on weight loss: a mechanistic approach to a global problem. *J Clin Endocrinol Metab.* 2009;94(5):1602–1607.

6. Williamson DA, Anton SD, Han H, et al. Early behavioral adherence predicts short and long-term weight loss in the POUNDS LOST study. *J Behav Med*. 2010;33(4):305–314.

7. Rolls BJ, Bell EA, Castellanos VH, Chow M, Pelkman CL, Thorwart ML. Energy density but not fat content of foods affected energy intake in lean and obese women. *Am J Clin.Nutr*. 1999;69(5):863–871.

8. Rolls BJ, Roe LS, Beach AM, Kris-Etherton PM. Provision of foods differing in energy density affects long-term weight loss. *Obes Res.* 2005;13(6):1052–1060.

9. Ello-Martin JA, Roe LS, Ledikwe JH, Beach AM, Rolls BJ. Dietary energy density in the treatment of obesity: a year-long trial comparing 2 weight-loss diets. *Am J Clin Nutr*. 2007;85(6):1465–1477.

10. Ledikwe JH, Rolls BJ, Smiciklas-Wright H, et al. Reductions in dietary energy density are associated with weight loss in overweight and obese participants in the PREMIER trial. *Am J Clin Nutr.* 2007;85(5):1212–1221.

11. Rolls BJ, Drewnowski A, Ledikwe JH. Changing the energy density of the diet as a strategy for weight management. *J Am Diet Assoc*. 2005;105(5 Suppl 1):S98–103.

12. Rolls BJ, Castellanos VH, Halford JC, et al. Volume of food consumed affects satiety in men. *Am J Clin Nutr.* 1998;67(6):1170–1177.

13. Blatt AD, Roe LS, Rolls BJ. Hidden vegetables: an effective strategy to reduce energy intake and increase vegetable intake in adults. *Am J Clin Nutr.* 2011;93(4):756–763.

14. Bell EA, Castellanos VH, Pelkman CL, Thorwart ML, Rolls BJ. Energy density of foods affects energy intake in normal-weight women. *Am J Clin Nutr.* 1998;67(3):412–420.

15. Raynor HA, Van Walleghen EL, Bachman JL, Looney SM, Phelan S, Wing RR. Dietary energy density and successful weight loss maintenance. *Eat Behav*. 2011;12(2):119–125.

16. Ledikwe JH, Blanck HM, Khan LK, et al. Low-energy-density diets are associated with high diet quality in adults in the United States. *J Am Diet Assoc*. 2006;106(8):1172–1180.

17. A Report of the Panel on Macronutrients, Subcommittees on Upper Reference Levels of Nutrients and Interpretation and Uses of Dietary Reference Intakes, and the Standing Committee on the Scientific Evaluation of Dietary Reference Intakes. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients)*. Washington, D.C.: The National Academies Press; 2005.

18. Mifflin MD, St Jeor ST, Hill LA, Scott BJ, Daugherty SA, Koh YO. A new predictive equation for resting energy expenditure in healthy individuals. *Am J Clin Nutr.* 1990;51(2):241–247.

19. Shlisky JD. A lifestyle intervention promoting weight loss with moderate or standard protein and non-fat dairy intake and daily walking: impact on body composition, metabolic parameters and bone health in premenopausal women with overweight and obesity. Unpublished manuscript, Pennsylvania State University, University Park, PA. 2012.

20. Ledikwe JH, Blanck HM, Khan LK, et al. Dietary energy density determined by eight calculation methods in a nationally representative United States population. *J Nutr.* 2005;135(2):273–278.

21. Kant AK, Graubard BI. Energy density of diets reported by American adults: association with food group intake, nutrient intake, and body weight. *Int J Obes (Lond)*. 2005;29(8):950–956.

22. Bes-Rastrollo M, van Dam RM, Martinez-Gonzalez MA, Li TY, Sampson LL, Hu FB. Prospective study of dietary energy density and weight gain in women. *Am J Clin Nutr.* 2008;88(3):769–777.

23. Johnson L, Wilks DC, Lindroos AK, Jebb SA. Reflections from a systematic review of dietary energy density and weight gain: is the inclusion of drinks valid? *Obes Rev*. 2009;10(6):681–692.

Figure 5.1 Percent change in dietary energy density from baseline to Week 12 by tertile of energy density change for women who participated in a lifestyle intervention including an energy-restricted diet and moderate daily walking.

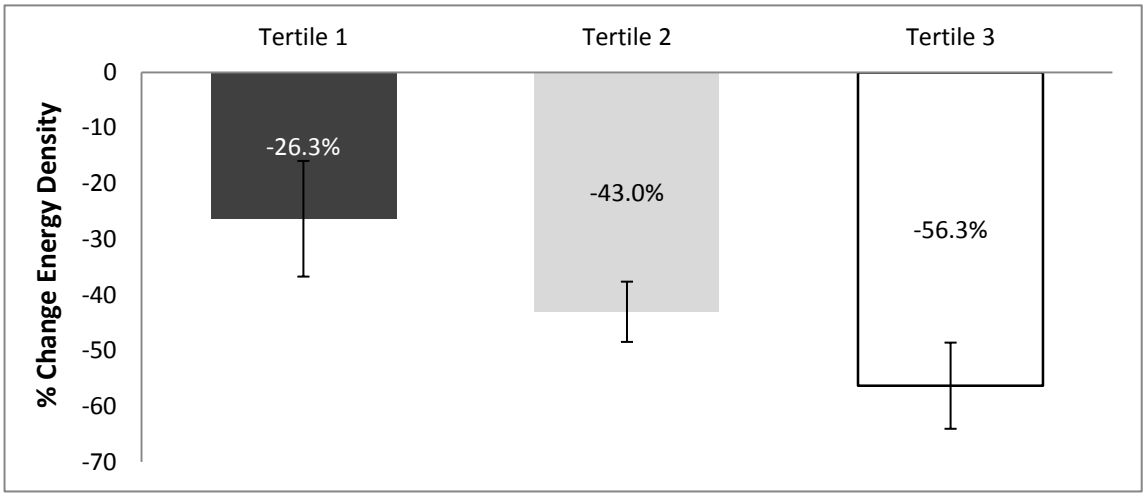


Table 5.1 Characteristics of women at baseline and Weeks 12 and 24 by tertile of dietary energy density change from baseline to Week 12 of a lifestyle intervention including an energy-restricted diet and moderate daily walking

	Tertile 1 (small ↓ED; n=23)		Tertile 2 (medium ↓ED; n=24)		Tertile 3 (large ↓ED; n=24)		Group x Time effect
Outcome measure	Value	Change from Baseline	Value	Change from Baseline	Value	Change from Baseline	p-value
Age (years)	34.2±7.2	–	35.9±7.4	–	34.8±7.6	–	NS
Food energy (kcal/d)							
Baseline	1635±307	–	1688±350	–	1752±460	–	
Week 12	1257±303[a]	-378±303	1161±269[a]	-527±327	1016±192[a]	-736±418[c]	0.009
Week 24	1341±274[a]	-294±303	1236±335[a]	-451±405	1152±247[a]	-600±457	
Food weight (g/d)							
Baseline	999±197	–	935±225	–	820±224	–	
Week 12	1045±240[a]	46±245	1139±268[a]	204±236	1051±253[a]	231±241	NS
Week 24	1016±213	16±217	1048±281	113±257	962±252	142±326	
Food energy density (kcal/g)							
Baseline	1.69±0.26	–	1.85±0.23[c]	–	2.25±0.32[c,d]	–	
Week 12	1.25±0.29[a]	-0.44±0.18	1.07±0.24[a]	-0.79±.06[c]	0.97±0.19[a]	-1.28±0.32[c,d]	<0.001
Week 24	1.38±0.30[a]	-0.31±0.26	1.23±0.24[a]	-0.63±0.24[c]	1.26±0.37[a,b]	-0.98±0.47[c,d]	
Body weight (kg)							
Baseline	81.1±9.7	–	84.3±10.8	–	84.013.2	–	
Week 12	76.6±9.1[a]	-4.4±2.9	77.4±11.0[a]	-6.9±2.9[c]	78.3±13.6[a]	-5.7±3.2	0.006
Week 24	76.6±9.1[a]	-4.5±3.1	76.1±11.4[a]	-8.2±4.2[c]	78.5±13.5[a]	-5.5±4.7	
Body mass index (kg/m^2)							
Baseline	29.7±2.9	–	30.4±3.1	–	30.0±3.1	–	
Week 12	28.1±2.9[a]	-1.6±1.0	27.8±3.1[a]	-2.5±1.1[c]	27.9±3.5[a]	-2.1±1.1	0.006
Week 24	28.1±2.9[a]	-1.6±1.1	27.4±3.3[a]	-3.0±1.6[c]	27.9±3.7[a]	-2.1±1.8	
Fat mass (kg)							
Baseline	28.7±5.2	–	31.3±6.9	–	31.1±8.4	–	
Week 12	25.9±5.5[a]	-2.8±2.2	27.3±6.2[a]	-4.0±2.0	28.1±9.0[a]	-3.0±2.2	0.007

Week 24	26.6±6.0	-2.1±2.9	26.2±6.9[a]	-5.0±3.1[c]	28.1±8.9[a]	-2.9±3.5	
Fat-free soft tissue mass (kg)							
Baseline	48.5±5.6	–	48.9±5.6	–	48.7±5.1	–	
Week 12	46.7±5.3[a]	-1.8±1.8	46.4±4.9[a]	-2.5±1.8	46.5±5.1[a]	-2.2±2.0	NS
Week 24	46.1±4.8[a]	-2.4±1.7	46.3±5.1[a]	-2.6±1.6	46.6±5.0[a]	-2.2±2.1	
Body fat percentage (%)							
Baseline	35.9±3.8	–	37.7±3.2	–	37.3±5.0	–	
Week 12	34.3±4.4	-1.6±2.0	35.5±3.9[a]	-2.1±1.9	35.8±5.4	-1.5±1.9	0.012
Week 24	35.1±4.8	-0.8±2.6	34.5±4.9[a]	-3.2±3.2[c]	35.8±5.1	-1.5±2.6	
Central abdominal tissue (%)							
Baseline	35.7±5.1	–	36.1±4.8	–	37.6±6.9	–	
Week 12	32.2±5.4[a]	-3.5±2.5	32.4±6.1[a]	-4.4±3.3	34.1±7.6[a]	-3.4±3.5	NS
Week 24	32.6±6.9[a]	-3.2±3.2	31.5±6.8[a]	-5.3±4.5	34.5±7.7[a]	-3.1±3.9	
Waist circumference (cm)							
Baseline	88.0±6.7	–	89.3±7.1	–	89.5±9.5	–	
Week 12	84.2±6.3[a]	-3.8±2.5	83.5±6.4[a]	-5.8±2.5	84.0±8.8[a]	-5.5±3.3	0.011
Week 24	83.9±6.5[a]	-4.1±2.5	82.4±6.9[a]	-7.0±3.3[c]	84.2±8.9[a]	-5.4±3.9	
Hip circumference (cm)							
Baseline	110.2±7.3	–	114.3±7.9	–	113.1±7.6	–	
Week 12	106.5±8.0[a]	-4.5±2.7	108.3±8.1[a]	-6.1±3.9	108.4±9.4[a]	-5.5±2.8	NS
Week 24	106.7±7.9[a]	-4.0±3.3	107.3±8.4[a]	-7.1±4.8	108.5±9.5[a]	-5.4±3.8	

Dietary intake records analyzed using Nutrition Data System for Research (NDS-R, version 2010, Minnesota, MN) and reported for 71 women who provided full records. Dietary energy density included food only, excluding beverages.
[a] different from baseline; [b] different from Week 12; [c] different from Tertile 1; [d] different from Tertile 2, using $p<0.05$ for statistical significance; p-values from analysis of variance with repeated measures on the time factor.

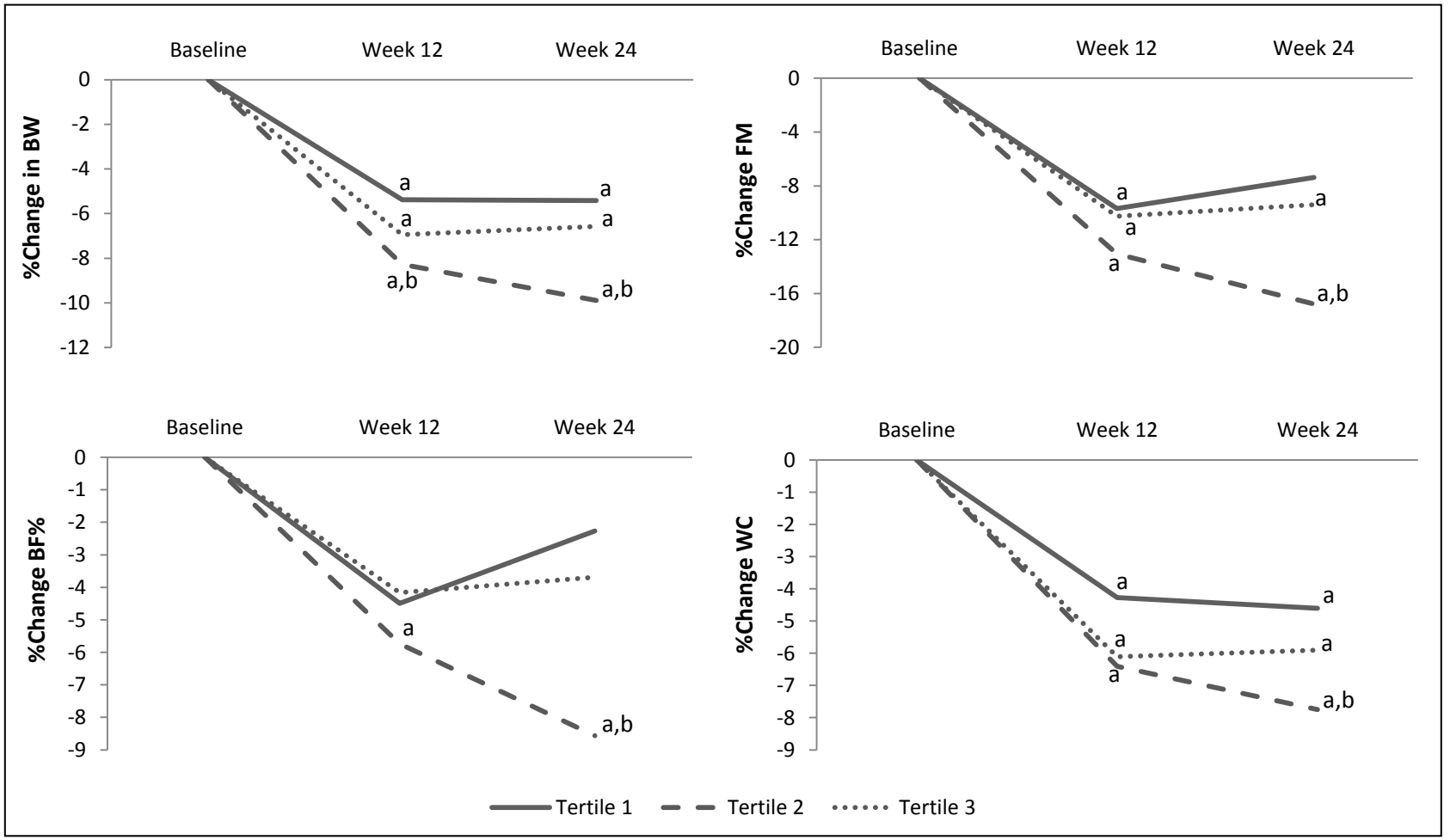


Figure 5.2 Percent change in body weight (BW), fat mass (FM), body fat percentage (BF%), and waist circumference (WC) at baseline and Weeks 12 and 24 by tertiles of dietary energy density change during a lifestyle intervention including an energy-restricted diet and moderate daily walking.

[a] different from baseline; [b] different from Tertile 1; using $p<0.05$ for statistical significance; p-values from analysis of variance with repeated measures on the time factor.

CHAPTER 6

SUMMARY, STRENGTHS, LIMITATIONS AND FUTURE DIRECTIONS

Effective and sustainable approaches to induce body weight (BW) loss and BW loss maintenance have never been more crucial than with the current high prevalence of obesity in the United States. Contributing to risk for chronic disease, obesity comes at a great cost to individual health, translating into a large economic burden to the United States healthcare system. BW and fat mass (FM) losses can ameliorate risk factors for chronic disease and are recommended for health improvement. Several studies have investigated the association between moderate protein and non-fat dairy intake to find that within an energy-restricted diet (ERD), these dietary components provide additional benefits to BW and FM losses. Moderate protein and non-fat dairy within an ERD have been shown to produce greater losses in FM, specifically visceral fat, than ERD with standard protein and dairy. Additionally, ERDs with moderate protein content have been implicated in the improvement of several metabolic parameters. A potentially negative effect of BW loss is bone mineral density (BMD) loss. Moderate protein and non-fat dairy intake have also been associated in the proportional preservation of fat-free soft tissue mass (FFSTM) and BMD with increased FM loss.

While numerous weight-loss approaches have been investigated and recommended to individuals with overweight/obesity, many of these are composed of complex and restrictive dietary patterns that may reduce participant compliance with intervention protocols. Adherence to an intervention is a strong determinate of weight-loss success; therefore, it is imperative that intervention components include sustainable lifestyle changes, which promote long-term maintenance of BW reduction. A simple intervention designed to reduce energy intake while making sustainable dietary changes may be more appropriate for long-term dietary adherence.

This research project examined premenopausal women with overweight/obesity in a randomized controlled trial consuming an ERD with moderate protein content (30% of kcal) and

4-5 servings of non-fat dairy per day combined with walking exercise (i.e., treatment group) or an ERD with standard protein (16-17% of kcal) and three servings of non-fat dairy per day combined with walking exercise (i.e., control group) as part of a 24-week comprehensive lifestyle intervention. This 24-week ERD and exercise intervention tested the three main hypotheses proposed in this research project. The first hypothesis was that women in the treatment group and women in the control group would achieve significant BW loss; however, the treatment group would have greater losses in BW, with a greater proportion of weight coming from FM instead of FFSTM. Additionally, it was expected that metabolic parameters would improve in both groups with BW loss. The second hypothesis stated that women in both groups would show a reduction in C-reactive protein (CRP) with BW loss, but that women in the treatment group would preserve BMD while those in the control group would see reductions in BMD after the 6-month intervention. The final hypothesis was that when categorized into ED change tertiles during the weight-loss portion of the intervention, women with the largest reduction in ED would have greater BW and FM losses than women with smaller reductions in ED.

The first and second hypotheses from this research project were partially supported. An ERD that was moderate in protein and non-fat dairy as part of a weight-loss/weight-loss maintenance intervention including walking promoted beneficial changes in body composition and metabolic parameters similarly to an ERD with standard protein and non-fat dairy with daily walking. All women reduced BW and FM, improved anthropometric and metabolic measures of health, including triglycerides, total cholesterol and leptin concentrations, with no difference between groups. Likewise, women in both groups maintained BMD throughout the intervention with no significant changes in biomarkers of bone turnover. hsCRP was reduced similarly in both

groups with no group differences. Although the predicated beneficial changes were observed in both groups, a proportional increase in FM loss and preservation of FFSTM was not observed in the treatment group. Additionally, the treatment group did not experience an observable benefit to markers of bone turnover or greater preservation of BMD during the 6-month intervention.

In support of the third hypothesis, women with medium reductions in ED during the first 12 weeks of weight-loss demonstrated greater reductions in BW, BMI, FM, body fat percentage (BF%) and waist circumference compared to women who reported small reductions in ED during the same time period. Somewhat incongruous with the initial hypothesis was that the group of women that reported the largest reductions in ED during the first 12 weeks of weight-loss intervention did not experience greater reductions in BW or FM relative to the other two groups with medium and small changes in ED. Instead, the group that reported medium reductions in ED was the only group to significantly reduce BF% over time. Also, the medium ED reduction group had greater reductions in BW and BMI at Weeks 12 and 24 and greater reductions in FM, BF% and waist circumference at Week 24 compared to women with the smallest changes in ED.

The overall findings of this research project demonstrate that premenopausal women with excess adiposity can significantly reduce BW and FM as well as triglycerides, total cholesterol, hsCRP and leptin across a 12-week weight-loss phase and maintain these changes across a 12-week weight-loss maintenance phase, facilitated by an intervention including diet modification within an ERD and moderate walking exercise. As no differences were detected in body composition, metabolic parameters, and markers bone turnover and BMD between intervention groups, these two approaches may have been equally effective. Both intervention groups were meeting daily recommendations for protein, dairy, calcium and vitamin D intake. In conclusion, free-living, premenopausal women with overweight/obesity who completed a 24-week lifestyle

intervention with sufficient protein and non-fat dairy intake along with daily moderate walking achieved and maintained a statistically significant and clinically meaningful loss of BW while reducing FM, hsCRP and leptin, and maintaining BMD.

Furthermore, exploratory analyses of change in ED during the weight-loss intervention examined in this research project found that women with medium reductions in ED had greater reductions in BW, BMI, FM, BF% and waist circumference compared to women who reported small reductions in ED. This supports a roll for the reduction of dietary ED in enhanced BW and FM losses, which can be maintained through to 24 weeks. These data may suggest that medium reductions in ED during an ERD may increase participant adherence to an ERD, resulting in reduced energy intake and sustainability of BW loss.

Strengths of this research project include a research design with free-living women, designed to examine the effectiveness of the intervention. A free-living study population allows for examination of intervention implementation. The ultimate goal of randomized clinical trials that examine lifestyle interventions is to find what may work effectively when applied in a free-living setting. These trials are necessary to examine if protocols can be successfully implemented and carried out by study participants who are proxies for individuals in the population or in a healthcare setting.

This study included several components in both intervention groups that have been shown in research studies to be beneficial for weight-loss and weight-loss maintenance. Both groups participated in a daily moderate walking component. Physical activity was a component of the overall intervention strategy as a promoter of weight loss combined with energy restriction and a commonly reported facilitator of weight-loss maintenance. Similarly, all women attended weekly nutrition-related educational and motivational sessions. Attendance at group sessions during

weight-loss intervention has been associated with weight loss. Women in both intervention groups had equal access to interventionists, with similar contact time between groups.

This research project includes a control group (standard protein and three servings of non-fat dairy) with a dietary pattern meeting recommendations for both dietary protein and dairy/calcium. Because the control group met protein and dairy/calcium recommendations, this research project was able to examine effects of protein and dairy/calcium intake beyond recommendations. This is an important distinction as some studies that report an effect of non-fat dairy or calcium supplementation on weight loss in the context of an ERD recruit participants who are calcium deficient. This research project recruited healthy women and was not limited to women who were deficient in either protein or dairy. Finally, a strength of this research project is that the first two hypotheses were examined using highly conservative statistical methods which limit the likelihood of reporting false positives.

Free-living participants offer benefits in the examination of a more realistic experience with intervention components; however, this comes at the price of potentially poorer intervention adherence and loss of dietary and physical activity record integrity. A more controlled feeding environment may have resulted in the moderate protein group meeting dietary recommendations, specifically, a dietary pattern with protein comprising 30% of total energy intake. The lack of feeding control in this research design limits its ability to address diet efficacy. Furthermore, it is curious that no significant effect of time was observed in energy expenditure when reportedly sedentary women began including 30 - 40 minutes of moderate walking each day. This also may have resulted from a free living environment where women compensated for their daily walks by reducing other daily activities. As the study population included only healthy, premenopausal women who were primarily Caucasian, generalizations from this study also may be limited.

The highly conservative statistical approach used in these analyses may have inflated the rate of false negatives. Additionally, there may not have been sufficient power to detect change in BMD, and in particular, the two biomarkers of bone turnover used in these analyses. This research project was powered to detect changes in primary outcome measures including BW and FM. Biomarkers used to detect bone turnover are rather variable. Additionally, this study measured change in bone within a short time period relative to the bone remodeling process. Bone may take at least six months to remodel a measurable amount, and there is a degree of error associated with dual-energy X-ray absorptiometry measurement of BMD. However, studies have observed significant change in biomarkers of bone turnover and BMD using smaller samples sizes over similar and shorter lengths of time.

Finally, this study design may not have been optimized to provide the most potent effect with the least resources. As this design was rather complex, it provided little information on which intervention components were most effective. Recent work suggests that a simpler design, including the best set of several potential intervention components previously identified through screening trials, would likely be more effective and efficient.

Future Directions

Given that bone remodeling is a slow and continuous process, assessment of study participants at a follow-up one year or more after study completion may give a better indication of true change in bone. This follow-up would also allow for more information on BW loss maintenance to be collected. Information from study participants one year after study completion would allow for identification of study components that remained part of the lifestyle changes made by successful weight-loss maintainers. Investigation of successful weight-loss maintenance approaches may be an important avenue of research in the study of BMD maintenance with BW

loss. Repeated cycles of weight loss and regain are detrimental to bone health and should be avoided with sustained BW loss. Moreover, maintenance of BW loss and prevention of BW gain are increasingly important to the population at large. Information gained from this intervention and other research projects may be applied to lifestyle interventions to mitigate excess BW gain, support BW loss and healthy BW maintenance.

Previous research has demonstrated that time spent with interventionists predicts weight-loss success; while the current research project found that the most commonly cited reason for discontinuing the intervention was lack of time. A lifestyle intervention implemented in the work environment, where individuals spend a large portion of time may be an ideal place for intervention delivery. As part of the current research project, increased daily exercise in the form of a 30-40 minute unsupervised walk may have been unsuccessful in promotion of increased energy expenditure. Previous research suggests that supervised activity may result in better compliance and achievement of activity goals. A work environment offering means of passively increasing physical activity (e.g. standing social areas, etc.) while also providing onsite gyms or exercise centers might increase daily energy expenditure. These efforts would be complimented further with efforts from an onsite food service provider. Modification of recipes to be lower in ED, and the adoption of healthy default side dishes within the menu, such as fruit, salads or soups, may reduce daily energy intake. Support from the organization or worksite would be imperative for the implementation of this type of intervention. This may be possible by underlining the benefits such interventions could have on productivity in the work environment and health insurance costs.

A separate, optimized intervention study could also be designed using information learned from this research project and other similar lifestyle interventions aimed at reducing BW

and maintaining BW losses long term. A study utilizing a factorial design would allow for several study components (i.e. dietary, physical activity, etc.) and the interactions between these components to be investigated efficiently and simultaneously. A novel weight-loss intervention may include three components in a factorial study design: 1) an energy-reduced eating plan focused on using the concept of ED to consume fewer calories (ED); 2) enhanced daily physical activity and moderate daily exercise (PA); and 3) counseling to improve sleep behaviors such as reducing exposure to lit environments and stress reduction (SB). Therefore, a factorial design with these three components would result in eight intervention groups: Control group (no intervention), ED only, PA only, SB only, ED+PA, ED+SB, PA+SB and ED+PA+SB.

Findings from the present research study and others show no difference in weight loss and metabolic health outcomes long-term with dietary regimens that very in macronutrient composition in healthy, free-living, overweight/obese adults. In fact, long-term modulation of macronutrient composition may not be feasible as many studies report participants eventually revert to baseline macronutrient compositions. The complexity of dietary recommendations to increase/reduce select macronutrients may prove tedious and restrictive for some individuals, reducing adherence. Therefore, a simple, flexible approach to reducing energy intake may be prudent for long term adaptation within a lifestyle intervention. An intervention with the positive message of including foods like fruits and vegetables rather than a restrictive message of foods to avoid may allow more freedom to customize diets based on preference.

Coincident with an ERD, physical activity may provide additional benefits to BW loss and body composition beyond dietary strategies alone. However, it is important to assess if physical activity, combined with an ERD, bring added benefit to BW loss for the additional effort invested. The lack of change in energy expenditure over time in the current research project

questions whether efforts to increase energy expenditure in this free-living population were effective. A factorial design can investigate the strength of an additional benefits observed with a physical activity component in addition to an effect had by an ERD.

A lifestyle component not commonly targeted within a weight-loss intervention is sleeping behavior. Though evidence continues to support a link between elevated BMI and partial sleep deprivation, it is unclear if a lifestyle intervention targeting sleep would benefit BW outcomes alone or within an ERD and exercise intervention. By assessing sleep counseling alongside two relatively well tested intervention components, the degree of benefit from sleep counseling can be assessed in several conditions. Overall, this method of combining intervention components will help determine which has the biggest return on investment.

In conclusion, this randomized clinical intervention examined the effect of an ERD with either moderate protein and non-fat dairy combined with daily exercise or standard protein and non-fat dairy combined with daily exercise on BW loss, body composition, and subsequent effects on markers of metabolic health and BMD. Women in both groups were able to reduce BW and FM, experience metabolic benefits and reduce CRP and leptin. In addition, neither group exhibited changes in biomarkers of bone turnover or changes in BMD. There was no effect of diet group on any outcome measure. Finally, in all study participants, a medium change in dietary ED from baseline to Week 12 of intervention was associated with greater BW loss and BW maintenance relative to those reducing ED to a lower extent.

APPENDIX A

SCREENING FORMS AND INFORMED CONSENT

Date: November 1, 2009

From: Tracie L. Kahler, IRB Administrator

To: Sharon M. Nickols-Richardson

Subject: Research Proposal - Modification **(IRB #30404)**
Approval Expiration Date: February 18, 2010
(Note: This date reflects the anniversary date of the actual submission approval date.)

"Yoplait Light Can Be Included in a Healthy Lifestyle Plan that Facilitates Short- and Long-Term Weight and Fat Mass Losses and Maintenance of Weight Loss and Bone Health in Premenopausal Women"

The revision(s) to the above-referenced study has been reviewed and approved by the Institutional Review Board (IRB). You may proceed with your study. Please continue to notify the IRB of any further changes to your study.

Comment: Approval, as per the modification request received on 10/14/09, is for the following: 1) addition of 1 personnel (K. Piehowski); 2) change from use of Lunar iDXA at Noll to use of Hologic DXA at the GCRC and subsequent addition of the GCRC as a research site; 3) change age of female participants from 25-45 to 20-45; 4) change of BMI of female participants from 27-35 to 25-35; 5) change the exclusion of women with elevated blood lipids; 6) revision of recruitment materials to reflect changes and inclusion of radio advertisements; 7) inclusion of 6-month gym memberships for participants so required walking protocol can be completed indoors during winter months; 8) revision of consent form to reflect changes; 9) revision of instruments to reflect changes.

Attached is the revised and dated, IRB-approved informed consent to be used when enrolling participants for this research. Participants must receive a **copy** of the approved informed consent form to keep for their records.

On behalf of the IRB and the University, thank you for your efforts to conduct research in compliance with the federal regulations that have been established for the protection of human participants.

Please Note: The ORP encourages you to subscribe to the ORP listserv for protocol and research-related information. Send a blank email to: L-ORP-Research-L-subscribe-request@lists.psu.edu

TLK/tlk
Attachment
cc: Susan L. Eberly

ORP OFFICE USE ONLY
DO NOT REMOVE OR MODIFY
IRB#30404 Doc. #1
The Pennsylvania State University
Office for Research Protections
Institutional Review Board
Approval Date: 02/12/10 S. Krout
Expiration Date: 02/11/11 S. Krout

Informed Consent Form for Biomedical Research
The Pennsylvania State University

Title of Project: Yoplait Light Can Be Included in a Healthy Lifestyle Plan that Facilitates Short- and Long-Term Weight and Fat Mass Losses and Maintenance of Weight Loss and Bone Health in Premenopausal Women

Principal Investigator: Sharon M. (Shelly) Nickols-Richardson, PhD, RD
Department of Nutrition Sciences
323 Chandlee Laboratory
University Park, PA 16802
814-863-2920
E-mail: smn13@psu.edu

1. Purpose of the study: The purpose of this research is to compare two different weight loss interventions ("diets"), both of which include a moderate walking program, on various measures of health including body fat, markers of inflammation, cholesterol levels, blood pressure and bone mineral density in women between the ages of 20 and 45 years. A total of 120 premenopausal women, with a body mass index (BMI) between 25 and 35, will participate in this study. This study is being funded by General Mills, Bell Institute of Health & Nutrition; however, General Mills will have no influence on the study results.

2. Procedures to be followed: As a participant, you will be asked to complete screening forms and seek physician, or gynecologist or physician assistance or nurse practitioner or registered nurse clearance to participate; if enrolled in the study, you will then attend two informational meetings, five blood collection sessions, daily walking with supervised walking sessions four times per week and weekly diet education sessions for this 24 week study. During this study, you will complete several procedures:

Informational Meeting – 1 hour of your time.

You will attend an initial informational meeting where the details of the study and the different diet groups will be explained. You will have plenty of time to ask questions of the investigators and will have up to 2 weeks after the meeting to decide not to be in the study. No further measurements will be taken until we receive your final agreement or consent to be in the study.

At the informational meeting, you will:

1) Review your signed Informed Consent Form and reaffirm or agree again your desire and willingness to be in the study (within two weeks of the meeting);
2) Ask questions about the study; and
3) Be instructed on how to use a pedometer to count your steps, and properly complete a 4-day food record and a 4-day physical activity record.

After the informational meeting and your agreement to be in the study, you will undergo baseline testing. After this testing, you will be randomized into a diet group. This means that you will be put into one of the two diet groups by chance. Once you are assigned in a diet group, you will attend another meeting

specifically for your diet assignment. At this meeting that will take 1 hour of your time, we will discuss the diet guidelines, answer any of your questions, and give you a schedule for your testing sessions, weekly diet meetings, and the date on which to start your diet. You will stop using or taking any vitamin and mineral supplements or eating yogurt until the end of the study, unless these supplements or products are part of your assigned diet. We will tell you if you are supposed to eat yogurt or not or use calcium and vitamin D supplements or not.

Diet Groups

The two diet groups include: 1) a weight loss diet with exercise plus Yoplait Light (**YL**) group; and 2) a weight loss diet control (**CON**) group. There will be no yogurt in the CON diet, and you will not consume yogurt if you are on the CON diet.

Both diets will go through three different phases over a six-month period: the *"**jumpstart phase",*** the "***weight loss phase"*** and the "***weight loss maintenance phase."***

The "jumpstart phase" (2 weeks)

YL group: You will consume a low calorie diet (about 1200 calories per day) that includes Yoplait Light as a "meal replacement" for two meals (breakfast and lunch) per day plus exercise for two weeks. Each of your "meal replacements" will consist of 1 Yoplait Light choice, a cereal or cereal bar choice and one fruit choice. You may consume 1 additional fruit choice and 1 additional vegetable choice throughout the day if needed during the jumpstart phase. Handouts containing yogurt, cereal and fruit choices will be given to you.

CON group: You will consume a low calorie diet plus exercise for two weeks. We will set calorie goals for you at 1,500 or 1,700 calories per day based on your energy needs. We will instruct you on your diet, with the help of exchange lists and portion sizes for servings per day from various food groups. Handouts with food choices, menus, and other guidelines will be given to you.

The "weight loss phase" (10 weeks)

YL group: You will consume a low calorie diet that includes intake of Yoplait Light two times per day (as a "meal replacement" for the breakfast meal and as a snack) plus exercise.

CON group: You will continue the standard weight loss diet plus exercise.

The "weight loss maintenance phase" (12 weeks)

YL: You will consume a calorie-balanced diet which includes intake of Yoplait Light two times per day along with exercise. This diet will help you maintain the body weight you have achieved after the 12 weeks of weight loss.

CON: You will consume a calorie-balanced diet which includes exercise. This diet will help you maintain the body weight you have achieved after the 12 weeks of weight loss.

If you are in the YL group, yogurt and cereal products for the "meal replacement" meals will be provided to you at specific phases of the study.

Calcium and vitamin D supplementation

If you are in the YL group and depending on your calorie level, you may or may not receive a daily calcium and vitamin D supplement. If you are in the CON group, you will consume a daily calcium and vitamin D supplement. We will provide the calcium and vitamin D supplement if it is part of your diet. We will dispense the supplements weekly during the jumpstart phase and monthly during the following two phases.

Testing Sessions

Test Session 1 at Baseline, Week 12, and Week 24 – 2 hours of your time at each of these weeks (6 hours total).
During this test session, you will need to:

1) Arrive in Chandlee Lab on the campus of the Pennsylvania State University (University Park) at your scheduled appointment day and time;
2) Turn in 4-day food record, step counts and 4-day physical activity record;
3) Have your height, weight, body fat percentage, waist circumference, and hip circumference measured;
4) Complete the current illness survey, a general symptoms questionnaire, and the SF-36 health status questionnaire;
5) Complete the D-SAT questionnaire, Zung Scale, the Eating Inventory, and Body Figure Rating scale;
6) Have your blood pressure, resting energy expenditure and resting heart rate measured;
7) Have 30 mL (about 2 Tablespoons) of whole blood drawn from your arm by a registered nurse who is trained in drawing blood from people;
8) Eat breakfast foods and beverages if desired;
9) Provide a urine sample for pregnancy testing; and
10) Undergo a peripheral quantitative computed tomography (pQCT) scan of your tibia (lower leg bone).

Test Session 2 at Baseline, Week 12, and Week 24 – 30 minutes of your time at each of these weeks (1.5 hours total.
During this test session, you will need to:

1) Arrive in the General Clinical Research Center on the campus of the Pennsylvania State University (University Park) at your scheduled appointment day and time;
2) Provide a urine sample for pregnancy testing; and
3) Undergo a dual-energy X-ray absorptiometry (DXA) scan of your whole body, spine, hip and forearm.

After test session 2, you will begin your assigned diet. This will require that you spend time each day thinking about the food that you eat, purchasing foods that fit with your assigned diet, and preparing foods that fit with your assigned diet.

Test Session at Week 2 – 1.5 hours of your time.
During this test session, you will need to:

1) Arrive in Chandlee Lab on the campus of the Pennsylvania State University (University Park) at your scheduled appointment day and time;
2) Turn in 4-day food record, step counts and 4-day physical activity record;
3) Have your height, weight, body fat percentage, waist circumference, and hip circumference measured;
4) Complete the current illness survey, a general symptoms questionnaire, and the SF-36 health status questionnaire;
5) Complete the D-SAT questionnaire, Zung Scale, the Eating Inventory, and Body Figure Rating Scale;
6) Have your blood pressure, resting energy expenditure and resting heart rate measured;
7) Have 30 mL (about 2 Tablespoons) of whole blood drawn from your arm by a registered nurse who is trained in drawing blood from people; and

8) Eat breakfast foods and beverages if desired.

Test Session at Weeks 4, 8, 10, and 18 – 30 minutes of your time at each of these weeks (2 hours total).
During this test session, you will need to:

1) Arrive in Chandlee Lab on the campus of the Pennsylvania State University (University Park) at your scheduled appointment day and time; and
2) Have your height, weight, body fat percentage, waist circumference, and hip circumference measured.

Test Session a Week 6 – 1 hour of your time.
During this test session, you will need to:

1) Arrive in Chandlee Lab on the campus of the Pennsylvania State University (University Park) at your scheduled appointment day and time;
2) Have your height, weight, body fat percentage, waist circumference, and hip circumference measured;
3) Have your blood pressure, resting energy expenditure and resting heart rate measured;
4) Have 30 mL (about 2 Tablespoons) of whole blood drawn from your arm by a registered nurse who is trained in drawing blood from people; and
5) Eat breakfast foods and beverages if desired.

Exercise intervention – 30-40 minutes of your time per day.
For both the YL and CON groups, exercise will consist of 30- or 40-minutes per day of walking at a comfortable pace. You may choose the time of day during which you will complete your walking. Your walking session will be supervised by study personnel for 4 of the 7 days per week. The exercise intervention will not change during the 6-month intervention. You will be instructed on how to "warm-up" and "cool-down" before and after walking, and how to maintain your pace during walking sessions. You may complete your walking at a gym. The gym membership will be paid for by the study.

Weekly Diet and Nutrition Education Sessions – 1 hour of your time each week.

At these sessions, we will discuss food purchasing and preparation, eating in restaurants, recipe modification, basic nutrition knowledge and exercise strategies. We will provide ways to problem-solve in difficult diet situations, and we will help you stay motivated to lose weight and continue the study. Your sessions will only be attended by other women in your diet group, although topics will be the same for both groups. At these weekly sessions, we will provide yogurt and cereal products, if you are in the YL group. We will measure your compliance with diet and exercise interventions weekly by counting your food (yogurt and cereal) containers.

Follow-up Meeting – 1 hour of your time.

Once everyone has finished the whole study, we will have one last meeting. At this meeting, you will be given your study results, and we will provide nutrition education to help you continue your diet, if you wish. Any snack and drink mix products provided during the study will no longer be given to you, once the study is finished.

Bone Density Scans and Weight Loss during Pregnancy

Any woman who is pregnant or planning to become pregnant should not participate in this study. Pregnancy is not a time to go on a diet to lose weight. Therefore, if you are pregnant or planning to become

pregnant during the study, you should inform the Primary Investigator immediately.

Clothing during Test Sessions

You should wear a short-sleeved or loose-fitting shirt for the blood draw and elastic-waisted pants for the waist and hip circumference measurements. Do not wear expensive jewelry or many clothing accessories to the test sessions. During bone density scans, you will have to take any jewelry off of your body and remove any metal zippers or snaps, metal buttons, or other metal materials.

Length of Time for Study Procedures

Your appointments may take more or less time than estimated to complete each procedure. You will be given plenty of time to complete measurements and blood draws and to understand your diet.

3. Discomforts and risks: There are two potential risks: 1) blood draws, and 2) radiation exposure from DXA and pQCT (bone density scans). There is minimal risk involved in blood draws. A bruise or a little bleeding may result from blood collection procedures with no known harmful effects to your health or well-being. In order to minimize bruising and bleeding, a registered nurse trained to do blood draws will draw all blood samples. You may become slightly lightheaded or nauseous during blood draws, but you may sit or recline for as long as you need to minimize discomfort. After each blood draw, you will be provided with breakfast foods and beverages. Two attempts to draw blood (or two needle sticks) will be allowed. If a second attempt is unsuccessful, no further tries for blood collection will be performed. Universal blood precautions will be taken by research personnel during handling of all blood samples.

This research project involves a sequence of total body, spine, hip, and forearm dual-energy X-ray absorptiometry (DXA) scans, and a pQCT leg scan. These bone density procedures will expose you to a small amount of radiation where the X-ray beam crosses the body. This radiation exposure is not necessary for your medical care and is for research purposes only. These series of scans will be repeated a total of 3 times over the course of this study. The dose for one set of scans is equivalent to a whole body radiation exposure of 4.5 millirem. The total dose for all three sets of scans would be 13.5 millirem (mrem). A millirem is a unit of whole-body radiation dose. For comparison purposes, the average person in the United States receives a radiation exposure of 300 mrem per year from natural background sources, such as from the sun, outer space, and from radioactive materials that are found naturally in the earth's air and soil. 13.5 mrem is less that you would receive from 17 days of natural background radiation. The radiation exposure is slight, and you have been informed of the risk, and you may choose not to complete the pQCT or DXA scans. If any of your scans cannot be read or used, a replacement scan will not be done to keep you from further exposure. If you are pregnant or think that you may be pregnant, you should not undergo either scan. In fact, a urinary pregnancy test will be done before any pQCT or DXA scan is taken. You will provide a small urine sample, and the investigator will use your urine sample to test if there is any human chorionic gonadotropin (hCG) in your urine. hCG is a hormone that is made by the body when a woman is pregnant. If the urinary test shows a positive results (pregnant), the investigator will tell you and remove you from the study. You will be referred to your obstetrician for care. Please be aware that if you have engaged in unprotected sex within the week before DXA and pQCT scans, there is a possibility that you could be pregnant without a positive pregnancy test result. If there is a possibility that you are pregnant, please inform an investigator immediately. All DXA and pQCT scans will be conducted by the investigator who is an International Society for Clinical Densitometry (ISCD) certified bone densitometry technologist.

The investigator is currently unaware of any specific risks associated with following any of the diets in this study. Please inform the investigator if you do not like yogurt or have yogurt intolerances or allergies. Also, investigators are unaware of any risks associated with participating in a daily walking program. If you feel shortness of breath or pain during walking, you should inform investigators immediately. If you experience any changes in your health during the study, you must tell the investigators.

4. Abnormal Test Results: Your blood pressure result will be shared with you by the registered nurse at each

test session. If your systolic (top number) blood pressure measurement is 140 mmHg or more and/or if your diastolic (bottom number) blood pressure measurement is 90 mmHg or more, you will be told to seek immediate medical attention from your physician, and the investigator will remove you from the study.

The blood samples will not be analyzed until the study has been completed. However, after all participants have completed their blood draws and bone density scans, we will have a follow-up meeting. At this meeting, you will be given your results. If there are any study results that suggest that medical attention is needed (for example, high blood cholesterol), you will be made aware of this within 30 days of analysis and recommended to contact your primary care physician for a follow-up examination.

5. Benefits to individual: You may benefit from participation in this research in several ways including: 1) determination of body composition by DXA; 2) assistance with a diet with supervision by a Registered Dietitian; 3) establishment of regular exercise regimen; 4) measurement of bone density; analysis of cholesterol level, blood sugar, insulin, and inflammatory markers; 5) blood pressure measurements; and 6) dietary intake analysis. You may or may not lose weight, but you will receive nutrition education by a Registered Dietitian. You will be provided with your results from every procedure at the completion of the study. Referral to appropriate health care professionals will be provided if necessary based on your results after completion of the study.

6. Benefits to society: This research will benefit society by demonstrating a new strategy for preserving lean tissue and bone mineral density in premenopausal women while they undergo safe weight loss.

7. Duration/time of the procedures and study: The study will consist of two informational meetings, five data collection sessions, weekly diet meetings, supervised walking sessions four times per week, bi-monthly weigh-in sessions and a follow-up meeting. The initial informational meetings will last no more than 60 minutes each, five data collection sessions that will last no more than 2 hours each, the three DXA scans sessions will last 30 minutes each, the weekly diet meetings will last 60 minutes each, daily walking sessions will last 30- or 40-minutes each, and the follow-up meeting will last no more than 60 minutes. The total time for this study is estimated at 100 hours over a 6-month period. You will also spend some time each day thinking about, preparing, and eating food.

8. Statement of confidentiality: *Your participation in this study is confidential. The data will be stored and secured at an investigator's office in a locked file cabinet. A three-digit code number will be assigned to you and used in place of your name. A master list of participants' code numbers will be kept in a separate locked file cabinet. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared. Only the investigators of this study or students of the Primary Investigator will be allowed access to any data. It is up to you to share your individual results with your Primary Health Care Provider, if you so choose.*

The following may review records related to this research: Penn State's Office for Research Protections, Penn State's Institutional Review Board, and the Office of Human Research Protections in the U.S. Dept. of Health and Human Services.

9. Right to ask questions: Please contact Dr. Shelly Nickols-Richardson (Primary Investigator) at 814-863-2920 with questions, complaints or concerns about the research. You can also call this number if you feel this study has harmed you. If you have any questions, concerns, problems about your rights as a research participant or would like to offer input, please contact Penn State University's Office for Research Protections (ORP) at (814) 865-1775. The ORP cannot answer questions about research procedures. All questions about research procedures can only be answered by the research personnel.

10. Payment for participation: You will receive $45 for scheduling and attending the physician clearance appointment. You will receive $10 per hour for each of the four, 2-hour test sessions. Lastly, if you complete the study you will receive $50. Therefore, if you participate in all the measurement sessions, you will receive $175 for the whole study. Total payments within one calendar year that exceed $600 will require the University to report these payments to the IRB annually. This may require you to claim the compensation that you receive for participation in this study as taxable income. A gym membership will be set-up for you. You will not be paid directly for this gym membership, but you will not have to pay for the gym membership.

11. Voluntary participation: Your decision to be in this research is voluntary. You can stop at any time. You do not have to answer any questions you do not want to answer. Refusal to take part in or withdrawing from this study will involve no penalty or loss of benefits you would receive otherwise. There may be reasons for which the investigators find that you should discontinue the study.

12. Event of injury: Medical care is available in the event of injury resulting from research but neither financial compensation nor free medical treatment is provided. You are not waiving any rights that you may have against the University for injury resulting from negligence of the University or investigators.

You must be between the ages of 20 to 45 years to take part in this research study. If you agree to take part in this research study and with the information outlined above, please sign your name and indicate the date below.

You will be given a copy of this signed and dated consent form for your records.

_____ _____

Participant Signature Date

_____ _____

Person Obtaining Consent Date

MEDICAL HISTORY FORM / BONE LAB / Pennsylvania State University

Title of Project: Yoplait Light Can Be Included in a Healthy Lifestyle Plan that Facilitates Short- and Long-Term Weight and Fat Mass Losses and Maintenance of Weight Loss and Bone Health in Premenopausal Women

---

Date: _____

Name (please print): _____

Name of physician (please print): _____

Date of Birth: _____ Age (years): _____ Gender: FEMALE MALE Ethnicity: _____

Medical History

Please indicate any current or previous conditions or problems you have experienced or have been told by a physician that you have had:

	Yes	**No**
Heart disease or any heart problems:	_____	_____
Respiratory disease or breathing problems:	_____	_____
Circulation problems:	_____	_____
Kidney disease or problems:	_____	_____
Urinary problems:	_____	_____
Reproductive problems:	_____	_____
Muscle problems:	_____	_____
Skeletal problems including osteoporosis or osteopenia:	_____	_____
Fainting or dizziness, especially with exertion:	_____	_____
Neurological problems/disorders:	_____	_____
High blood pressure or low blood pressure:	_____	_____
High blood cholesterol:	_____	_____
Diabetes mellitus:	_____	_____
Thyroid problems:	_____	_____
Eating disorders (bulimia, anorexia):	_____	_____
Crohn's disease:	_____	_____
Allergies:	_____	_____
Insomnia:	_____	_____
Other (Please list): _____	_____	_____

If "yes" to any of the above please indicate the date, explain, and describe:

_____

_____

Please list any hospitalizations/operations/recent illnesses (Type/Date):_____

_____

_____

Medications

Please indicate any current medications that you are taking on a daily or weekly basis:

	Yes	No
Steroids (such as Prednisone):	_____	_____
Thyroid medications (such as Synthroid):	_____	_____
Bisphosphonates (such as Fosamax):	_____	_____
Anticonvulsants (such as Dilantin):	_____	_____
Glucocorticoids (such as Dexamethasone):	_____	_____
Medications for Weight Loss (such as Meridia):	_____	_____
Other bone medications (such as Miacalcin):	_____	_____

Please list any nutritional supplements, herbal products, or other medications, (prescription and over-the-counter) you are currently taking on a daily or weekly basis and the doses per day: _____

_____

Family Health History

Has anyone in your family (blood relatives only) been diagnosed or treated for any of the following?

	Yes	No	Relationship	Age
Heart attack	_____	_____	_____	_____
Heart disease	_____	_____	_____	_____
High blood pressure	_____	_____	_____	_____
Stroke	_____	_____	_____	_____
Kidney disease	_____	_____	_____	_____
Diabetes	_____	_____	_____	_____
Crohn's disease	_____	_____	_____	_____
Thyroid disorders	_____	_____	_____	_____
Osteoporosis	_____	_____	_____	_____
Osteopenia	_____	_____	_____	_____

Have you broken any bone(s)? Yes _____ No ____

If "yes," please list bone(s) and age(s) at time of break: _____

_____

Health Habits

Do you add salt to your food? Yes _____ No _____

Are you on any special type of diet? Yes _____ No _____

If "yes," please describe:

_____

_____

_

Do you drink caffeinated beverages? Yes _____ No _____ If "yes," how many cups per day? _____
(such as sodas, colas, coffee, tea, lattes, hot chocolate, "energy" drinks)

Do you drink alcoholic beverages? Yes _____ No _____ If "yes," how many cups per day? _____

What is the average number of alcoholic drinks that you consume on the weekend? _____

Did you use tobacco products in the past (more than 12 months ago)? Yes _____ No _____

Do you currently use tobacco products? Yes _____ No _____ If "yes," what type of tobacco products do you use, how frequently do you use them, and what number do you use per day?

_____

_____

_

Work Schedule and Patterns

Do you engage in night-time work? YES NO

If yes, please explain: _____

_____

Exercise Habits

Do you engage in regular exercise? Yes _____ No _____

If "yes" please list:

Activity	**Frequency (times per week)**	**Duration (minutes)**
_____	_____	_____
_____	_____	_____
_____	_____	_____
_____	_____	_____

Do you ever feel faint, short of breath, or chest discomfort with exertion? Yes _____ No _____

If "yes," please explain:

_____

_____

_

Are there any orthopedic limitations you have that may restrict your ability to exercise? Yes ___ No ___

If "yes" please explain: _____

_____

Questions Related to Reproductive Function

Do you use an oral contraceptive? Yes _____ No _____

If "yes" what brand and dose of oral contraceptive? _____

If "yes," for how long have you used this oral contraceptive? _____

If "yes," do you use oral contraceptives due to menstrual cycle irregularities? Yes ____ No ____

Have you undergone a hysterectomy and/or ovariectomy? Yes _____ No _____

If "yes," when? _____

If "yes," do you use hormone replacement therapy? Yes _____ No _____

If "yes," what brand and dose of estrogen or hormone replacement therapy do you use? _____

_____

If "yes," for how long have you used this estrogen or hormone replacement therapy?

_____

When was the first day of your last menses? _____

Have you had any abnormal menses or absence of menses in the last 12 months? Yes _____ No _____

If "yes", describe: _____

_____

Are you pregnant or do you think that you may be pregnant? YES NO

Are you attempting to become pregnant? YES NO

How many menstrual cycles do you have per year?

a) 12 to 14 per year
b) 9 to 11 per year
c) 6 to 8 per year
d) 3 to 5 per year
e) < 3 per year

Do you have children? YES NO If "yes" how many children do you have? _____

Are you currently breastfeeding? YES NO

Weight History

What is your current weight? _____

How much did you weigh six months ago? _____

How much did you weigh one year ago? _____

During the last 2 years, how many times have you lost 5 pounds?

NEVER ONCE TWICE THREE OR MORE

During the last 2 years, how many times have you gained 5 pounds?

NEVER ONCE TWICE THREE OR MORE

Do you desire to lose weight? YES NO

Have you ever undergone surgery to lose weight (Gastric Bypass or Gastric Banding)? YES NO

What is your height? _____

Body mass index: _____ (kg/m^2) (Please leave this line blank; an investigator will calculate.)

Yogurt Product Consumption

Do you consume yogurt products? Yes _____ No _____

If "yes," how often do you consume yogurt or yogurt products and how much?

a) three or more yogurt servings (3 cups) per day
b) one or two yogurt servings per day
c) two or three yogurt servings per week
d) three or less servings per month
e) do not consume yogurt
f) If "other" frequency, please describe: _____

Do you have a yogurt allergy or intolerance? Yes _____ No _____

Do you avoid yogurt products? Yes _____ No _____

If "yes" to either of these questions, please explain: _____

_____

I confirm that the above information is correct.

_____ _____

Print Name Signature Date

Screening Form Review for Exclusion/Inclusion Criteria
ID#_____
Date_____
Title of Project: Yoplait Light Can Be Included in a Healthy Lifestyle Plan that Facilitates Short- and Long-Term Weight and Fat Mass Losses and Maintenance of Weight Loss and Bone Health in Premenopausal Women

1.	Age: 20 to 45 years	YES	NO
2.	Female	YES	NO
3.	Absence of		
	Impaired renal function	YES	NO
	Diabetes	YES	NO
	Thyroid disorders	YES	NO
	Crohn's disease	YES	NO
	Osteopenia or Osteoporosis	YES	NO
4.	Absence of		
	Steroid use	YES	NO
	Thyroid hormones	YES	NO
	Bisphosphonates	YES	NO
	Anticonvulsants	YES	NO
	Glucocorticoids	YES	NO
	Weight loss medications	YES	NO
5.	Caffeinated beverage intake (≤16 oz/d)	YES	NO
6.	Alcohol intake (≤1drink/d)	YES	NO
7.	Absence of cigarette smoking	YES	NO
8.	Moderate physical activity (≤2 hrs/wk)	YES	NO
9.	Eumenorrhea (9-14 cycles/yr)	YES	NO
10.	Oral contraceptive use (<2yrs)	YES	NO
11.	Absence of hysterectomy w/o HRT	YES	NO
12.	Absence of ovariectomy w/o HRT	YES	NO
13.	Absence of gastric bypass surgery	YES	NO
14.	Absence of pregnancy/attempting to become pregnant	YES	NO
15.	Stable body weight in last 6 months (≤5 lb change)	YES	NO
16.	Desire to lose weight	YES	NO
17.	Body mass index (25.0-35.0)	YES	NO
18.	Absence of yogurt allergy/intolerance/aversion/ high consumption (≥24 oz/d)	YES	NO
19.	Zung Scale score (<50)	YES	NO

All answers must be YES

Title of Project: Yoplait Light Can Be Included in a Healthy Lifestyle Plan that Facilitates Short- and Long-Term Weight and Fat Mass Losses and Maintenance of Weight Loss and Bone Health in Premenopausal Women

Health Care Provider Clearance Form

Dear Physician/Gynecologist/Physician's Assistant/Registered Nurse/Nurse Practitioner:
This patient would like to participate in a study titled, "Yoplait Light Can Be Included in a Healthy Lifestyle Plan that Facilitates Short- and Long-Term Weight and Fat Mass Losses and Maintenance of Weight Loss and Bone Health in Premenopausal Women," conducted by Shelly Nickols-Richardson, PhD, RD, of the Nutritional Sciences Department at The Pennsylvania State University. The purpose of this study is to determine effects of following a weight-loss diet with exercise on weight, blood glucose level, markers of inflammation, bone turnover, mineral metabolism, cholesterol and blood pressure, resting heart rate and overall bone mineral density in overweight and obese women ages 20 to 45 years during a 24-week intervention period. An energy-restricted diet with two different levels of protein designed to induce weight loss will be assigned to each woman. Please indicate below that this patient has completed the enclosed Medical History Form in a manner that is consistent with the medical records of this patient. If this patient participates in this study, she will discontinue use of vitamin and mineral supplements for the 24-week study period. The patient will undergo a series of fasting blood draws at 6-week intervals over 24 weeks and bone densitometry scans at three intervals.

Results of Health Care Provider Screening: Please make certain that all questions on this form are completed in a manner consistent with the medical records of this patient. If unusual problems are present or not disclosed that may affect the candidate's safety or eligibility for the study, note this/these finding(s) below and submit to the investigator.

By signing this form, you agree that this woman is free of osteoporosis, osteopenia, impaired renal function, abnormal lipid metabolism, diagnosed cardiovascular or metabolic (e.g., diabetes) disease, including hypertension, can participate in moderate physical activity such as walking, has not undergone weight loss surgery, and is premenopausal and in good physical health to participate in an assigned dietary intervention.

THIS CANDIDATE QUALIFIES FOR PARTICIPATION IN THE STUDY, SUBJECT TO FINAL VERIFICATION BY THE INVESTIGATOR. Yes: ___ No: ___.

If "No," please explain reasons for which individual is not eligible to participate in this study.

_____
_____
_____
_____

Please sign to indicate that the above information is correct:

_____ _____

Print Name Signature Date

You may contact the Primary Investigator at:
Shelly Nickols-Richardson
smn13@psu.edu or (814-863-2920)
The Pennsylvania State University

(For investigator use and review, do not fill out, thank you)
Subject is: ELIGIBLE _____ or INELIGIBLE _____
Reviewer Signature_____ Date _____

APPENDIX B

DIET-RELATED MATERIALS

Daily Meal Plan

Jumpstart Phase

YL Group (1,200 kcals/day)[a]

Breakfast:	1	starch (select from list)[b]
	1	fruit (participant choice)
	1	YL
Lunch:	1	starch (select from list)[b]
	1	fruit (participant choice)
	1	YL
Snack:	2	vegetable (participant choice)
	1	non-fat dairy (participant choice)
Dinner:	2	non-fat dairy (participant choice)
	2	vegetable (participant choice)
	6	lean meat (participant choice)
	1	fat

[a] **Participants may consume 1 additional fruit and 1 additional vegetable as snack choices at mid-morning or mid-evening if needed.**

[b] **Starch list: 1 cup plain Cheerios, 1 cup Multi-Grain Cheerios, 1 cup Rice Chex, 1 Nature Valley Crunchy granola bar (choice of flavors)**

Daily Meal Plan

Jumpstart Phase

EXG Group 1: (1,500 kcals/day)

**All foods are participant's choice*

Breakfast:	1	starch
	1	fruit
	1	non-fat dairy
Lunch:	2	starch
	1	fruit
	1	non-fat dairy
	2	vegetable
	1	medium-fat meat
	2	fat
Snack:	1	starch
	1	fruit
Dinner:	2	starch
	1	fruit
	1	non-fat dairy
	2	vegetable
	2	medium-fat meat
	2	fat

Daily Meal Plan

Jumpstart Phase

EXG Group 2: (1,700 kcals/day)

**All foods are participant's choice*

Breakfast:	2	starch
	1	fruit
	1	non-fat dairy
Lunch:	2	starch
	1	fruit
	1	non-fat dairy
	2	vegetable
	1	medium-fat meat
	2	fat
Snack:	1	starch
	1	fruit
	1	vegetable
Dinner:	3	starch
	1	non-fat dairy
	3	vegetable
	2	medium-fat meat
	3	fat

Daily Meal Plan

Weight Loss Phase

YL Group 1: (1,500 kcals/day)

Breakfast:	1	starch (select from list)[a]
	1	fruit
	1	YL
Lunch:	1	starch
	1	fruit
	1	non-fat dairy
	1	vegetable
	3	lean meat
	1	fat
Snack:	1	fruit
	1	YL
	1	vegetable
	3	lean meat
Dinner:	1	non-fat dairy
	2	vegetable
	3	medium-fat meat
	2	fat

[a] **Starch list: 1 cup plain Cheerios, 1 cup Multi-Grain Cheerios, 1 cup Rice Chex, 1 Nature Valley Crunchy granola bar (choice of flavors)**

Daily Meal Plan

Weight Loss Phase

YL Group 2: (1,700 kcals/day)

Breakfast:	1	starch (select from list)[a]
	1	fruit
	1	YL
Lunch:	1	starch
	1	fruit
	1	non-fat dairy
	2	vegetable
	3	lean meat
	1	fat
Snack:	1	fruit
	1	YL
	3	lean meat
Dinner:	1	starch
	2	non-fat dairy
	2	vegetable
	3	medium-fat meat
	3	fat

[a] **Starch list: 1 cup plain Cheerios, 1 cup Multi-Grain Cheerios, 1 cup Rice Chex, 1 Nature Valley Crunchy granola bar (choice of flavors)**

Daily Meal Plan

Weight Loss Phase

EXG Group 1: (1,500 kcals/day)

*All foods are participant's choice

Breakfast:	1	starch
	1	fruit
	1	non-fat dairy
Lunch:	2	starch
	1	fruit
	1	non-fat dairy
	2	vegetable
	1	medium-fat meat
	2	fat
Snack:	1	starch
	1	fruit
Dinner:	2	starch
	1	fruit
	1	non-fat dairy
	2	vegetable
	2	medium-fat meat
	2	fat

Daily Meal Plan

Weight Loss Phase

EXG Group 2: (1,700 kcals/day)

*All foods are participant's choice

Breakfast:	2	starch
	1	fruit
	1	non-fat dairy
Lunch:	2	starch
	1	fruit
	1	non-fat dairy
	2	vegetable
	1	medium-fat meat
	2	fat
Snack:	1	starch
	1	fruit
	1	vegetable
Dinner:	3	starch
	1	non-fat dairy
	3	vegetable
	2	medium-fat meat
	3	fat

Daily Meal Plan

Maintenance Phase

YL Group 1: (1,500 kcals/day)

Breakfast:	1	starch
	1	fruit
	1	non-fat dairy[a]
Lunch:	1	starch
	1	fruit
	1	non-fat dairy[a]
	1	vegetable
	3	lean meat
	1	fat
Snack:	1	fruit
	1	non-fat dairy[a]
	1	vegetable
	3	lean meat
Dinner:	1	non-fat dairy
	2	vegetable
	3	medium-fat meat
	2	fat

[a] 2 of these must be YL

Daily Meal Plan

Maintenance Phase

YL Group 2: (1,700 kcals/day)

Breakfast:	1	starch
	1	fruit
	1	non-fat dairy[a]
Lunch:	1	starch
	1	fruit
	1	non-fat dairy[a]
	2	vegetable
	3	lean meat
	2	fat
Snack: 1	fruit	
	1	non-fat dairy[a]
	3	lean meat
Dinner:	1	starch
	2	non-fat dairy
	2	vegetable
	3	medium-fat meat
	2	fat

[a] 2 of these must be YL

Daily Meal Plan

Maintenance Phase

EXG Group 1: (1,500 kcals/day)

*All foods are participant's choice

Breakfast:	1	starch
	1	fruit
	1	non-fat dairy
Lunch:	2	starch
	1	fruit
	1	non-fat dairy
	2	vegetable
	1	medium-fat meat
	2	fat
Snack:	1	starch
	1	fruit
Dinner:	2	starch
	1	fruit
	1	non-fat dairy
	2	vegetable
	2	medium-fat meat
	2	fat

Daily Meal Plan

Maintenance Phase

EXG Group 2: (1,700 kcals/day)

*All foods are participant's choice

Breakfast:	2	starch
	1	fruit
	1	non-fat dairy
Lunch:	2	starch
	1	fruit
	1	non-fat dairy
	2	vegetable
	1	medium-fat meat
	2	fat
Snack:	1	starch
	1	fruit
	1	vegetable
Dinner:	3	starch
	1	non-fat dairy
	3	vegetable
	2	medium-fat meat
	3	fat

VITA

JULIE D. SHLISKY, PhD
Jds533@psu.edu
702-497-1789
625 Walnut Street, State College, PA 16801

EDUCATION

PhD in Nutritional Science, June 2009-August 2012
The Pennsylvania State University, University Park, PA, 3.8 GPA
Dissertation Topic: A lifestyle intervention promoting weight loss with moderate or standard protein and non-fat dairy intake and daily walking: impact on body composition, metabolic parameters and bone health in premenopausal women with overweight and obesity

BA in Biological Sciences, concentrating in Molecular Biology, May 2006
Goucher College, Baltimore Maryland, 3.4 GPA

RESEARCH AND RELEVANT EXPERIENCE

Graduate Research Assistant. The Pennsylvania State University, Department of Nutritional Sciences. University Park, PA. June 2009-Present.

- Performed all anthropometric measurements throughout 6-month weight-loss intervention in premenopausal women.
- Processed and analyzed participant blood samples for biochemical measurement.
- Developed daily walking protocols.
- Assisted in development of weekly nutrition education classes.
- Led nutrition education sessions for study participants several times a week.

DuPont Laboratory Scientist. DuPont Chemical Solutions Enterprise, Clean and Disinfect Business. Wilmington, Delaware. June 2006-August 2008.

- Assisted in development of novel combinations of currently registered products to increase efficacy against viral pathogens.
- Developed rapid screening methodology to determine disinfectant performance.
- Performed standardized microbiological testing on disinfectant formulations for DuPont Animal and Human Health businesses.

Honors Independent Research "Characterization of behavior of prokaryotic *E. coli* RNA polymerase during elongation through regions of bent DNA caused by polyadenylated sequences using altered conditions." Dr. Judith Levin, Goucher College, Baltimore, Maryland. 2005–2006

Supplemental Instruction Instructor Biology 220 Genetics. Goucher College, Baltimore, Maryland. 2004- 2006.

CERTIFICATIONS

Certified Bone Densitometry Technologist (CBDT), International Society for Clinical Densitometry. December 2009–December 2012.

- Certified in the operation and analysis of bone imaging technologies such as Dual X-ray absorptiometry (DXA) and peripheral quantitative computed tomography (pQCT).