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IMPACT OF MEAL FREQUENCY ON APPETITE AND BLOOD MARKERS OF HEALTH IN OBESE WOMEN UTILIZING AN EQUI-HYPOCALORIC DIET DURING A BEHAVIORAL WEIGHT LOSS INTERVENTION

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BY

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DISSERTATION

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Impact of meal frequency on appetite and blood markers of health in obese women utilizing an equi-hypocaloric diet during a behavioral weight loss intervention

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ABSTRACT

Background: Obesity prevalence is a global pandemic and a public health concern. Beneficial weight loss strategies are needed for obesity management. Increased daily meal frequency (MF) is often associated with improvements in blood-markers of health and appetite control during weight loss; however, this has not been confirmed using portion-controlled meal products to reduce underreporting error.

Objective: The purpose of this study was to determine if increased MF can be a beneficial treatment strategy for weight loss in obese women by determining the changes of total ghrelin, blood glucose, insulin, lipid concentrations, and sensations of hunger and satiety due to an equi-hypocaloric diet intervention during a two meals per day sequence

(2 MF) versus a three meals and three snacks per day sequence (6 MF) using portion-controlled products.

Design: Eleven (N = 11) obese female subjects (BMI 39.1 ± 7.6 kg/m^2) ages 52 ± 7 years completed a six-week study using a randomized cross-over design. Subjects were randomized to either the 2 MF or 6 MF treatment condition for two-weeks, had a two-week washout of three meals and one snack per day, and then alternated treatment conditions.

Results: All subjects lost a similar percentage of body weight 6.6 ± 1.6% (6.2 ± 1.9 kg) which was not significantly different ($P > 0.05$) between the 2 MF and 6 MF conditions. Percent change analysis in fat-free mass (FFM) showed significant differences between conditions. Results showed 3.3 ± 2.6% decrease in FFM following the 2 MF condition, while following the 6 MF condition there was an average increase of 1.2 ± 1.7% in FFM ($P \leq 0.05$). There were no significant differences between conditions for fasting and area-under-the-curve (AUC) values of glucose, insulin, and total-ghrelin. Fasting high-density lipoprotein cholesterol (HDL-C) concentrations were significantly lower than baseline during the 6 MF condition (49 ± 10 vs. 53 ± 12 mg/dL, respectively) where the percent change in HDL-C during the 2 MF condition was significantly higher than in the 6 MF condition (1.3 ± 12.2% vs. 0.12 ± 10.3%). There was a significant decrease ($P = 0.006$) in glucose AUC from baseline to the six-week follow up. There were no significant differences found for fasting triglycerides, total cholesterol, or low-density lipoprotein cholesterol (LDL-C) between conditions. There was a significant difference from baseline to the six-week follow-up respectively for glucose AUC (13,122 ± 1,726

vs 12,296 ± 1,870 mg/dL·120 min) and hunger ratings (1,894 ± 887 vs 3,131 ± 1,563 mm·120 min, $P < 0.05$).

Conclusions For our sample increasing MF from two to six occasions per day did not promote greater improvements in blood-markers of health, appetite control, or total-ghrelin levels during a portion-controlled strict reduced-calorie diet intervention. Additionally, reduced MF may attenuate losses in HDL-C levels while increased MF may reduce losses in FFM during weight loss.

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SYMBOLS / ABBREVIATIONS

≥: greater than or equal to

>: greater than

≤: less than or equal to

<: less than

±: plus or minus

~: approximately

µg: microgram

µl: microliter

ml: milliliter

MF: meal frequency

mm: millimeters

ANOVA: analysis of variance

AUC: area under the curve

BIA: bioelectrical impedance analysis

BMI: body mass index

BW: body weight

%BF: body fat percentage

cm: centimeters

CVD: cardiovascular disease

ELISA: enzyme-linked immunosorbent assay

FFM: fat free mass

FM: fat mass

gm: gram

gm/d: grams per day

HDL-C: High-density lipoprotein cholesterol

LDL-C: Low-density lipoprotein cholesterol

RMR: resting metabolic rate

SD: standard deviation

VAS: visual analogue scale

VAS1: visual analogue scale question 1

VAS2: visual analogue scale question 2

VAS3: visual analogue scale question 3

VAS4: visual analogue scale question 4

CHAPTER I

Introduction

Obesity is a complex issue for clinical weight management professionals. Changing feeding behaviors by implementing increased dietary structure is becoming more commonly included in weight management strategies for patients undergoing supervised weight loss interventions. Some hypocaloric diets to induce body weight loss do result in unfavorable metabolic changes that can lead to increased ratings for hunger and cravings, increased circulating ghrelin, and suppressed postprandial ghrelin decline following meals (1).

Weight Loss and Meal Frequency

One fundamental principle that seems to be the most common component to current clinically supervised hypocaloric diets is the idea of structured daily meal frequency (MF). Most current dietary weight loss plans have substituted the classical eating pattern of three large meals per day to eating more frequently per day to spread out daily caloric intake (1, 2). Optimal MF has been hypothesized to have an effect on energy intake and thus body weight regulation due to its potential role in resting metabolic rate (RMR), hunger and satiety, and hormonal appetite regulation (1, 3). However, there appears to be no consistency within the literature in regard to MF and the optimal number of meals and/or snacks per day for weight management and obesity-related disease risk reduction. Nevertheless, most, if not all weight-loss programs advocate five or more small meals per day, providing claims that increased meal frequency can reduce self-reported scores of hunger and cravings, improve glucose and insulin control, and improve body composition (1, 2). Consuming an equi-hypocaloric diet of differing MF during

weight loss has been reported to have no statistically significant impact on the amount of weight lost (4-7). However, there has been evidence that increased meal frequency may improve metabolic markers of health, fuel utilization, appetite measures, as well as hunger and craving scores (1, 8-11). In the long-term increased MF may help with weight loss and weight maintenance.

Impact of Meal Frequency

Currently, limited research with obese (BMI $\geq$ 30 kg/m^2) subjects demonstrates the effects MF has on metabolic blood markers of health including serum glucose and insulin responses, serum lipid concentrations, total cholesterol, hormone levels (i.e. serum total ghrelin), and blood pressure (12, 13). One of the earliest studies showing the impact of MF on blood markers of health was done by Fabry et al. in 1964 (14). Along with results showing an inverse relationship of MF to body weight, Fabry and colleagues reported metabolic results showing that individuals who were currently consuming three or less meals per day had an increased prevalence of hyperinsulinemia and glucose intolerance compared to those consuming five or more meals per day (14). Additionally, two early studies by Gwinup and colleagues executed some initial descriptive investigations to study the effects of MF on serum glucose and lipids (15, 16). These studies demonstrated that 14 days of an equi-caloric diet of ten snacks versus three meals per day led to decreases in serum lipid and glucose concentrations (15, 16). It is important to note that these studies investigated normal-weight healthy adults.

Since these first investigations there have been limited studies investigating the beneficial responses of MF on serum lipid, glucose, and insulin concentrations using obese (7, 9, 10, 17) or normal weight adults (13, 18-20). Additionally, even fewer studies

have investigated the impact of MF on metabolic markers of health using obese subjects undergoing weight loss utilizing a hypocaloric diet (5, 7, 10). Surprisingly, most studies investigating metabolic markers of health relied on self-reported dietary intake (7, 9, 13, 18) where very few provided portion-controlled meals and/or meal replacements to control for measurement and portion control error (10, 17, 19). It has been shown that obese individuals significantly underreport energy intake, and obesity was found to be a significant predictor of underreporting energy intake (21, 22). One issue that may cause differing results among studies is that varied research methodologies have been used and limited research exists using strict dietary and calorie-controlled methods. More studies utilizing portion-controlled meals and/or meal replacements are needed to control for extraneous errors that typically occur with free-living dietary measures especially with participants who are undergoing weight loss.

According to a recent review of literature, the research-based evidence supporting the influence of MF on appetite regulation is inconclusive (12). It has been suggested that a "nibbling" or grazing pattern of eating (those who eat more frequently throughout the day) exerts various metabolic benefits (i.e increased resting metabolic rate (RMR) and/or food thermogenesis) when compared to those "gorging" (those eating two or less meals per day) (12). Research utilizing a metabolic or respiratory chamber to determine whole-body calorimetry during an isocaloric diet of varying MF showed no significant differences in acute changes in energy expenditure or food thermogenesis (6, 23, 24).

Although acute changes in energy expenditure or food thermogenesis has not been demonstrated during the short-term with varied MF, research investigating appetite and hunger responses shows promising results with increasing MF. Studies investigating

the effects of preprandial and postprandial hunger and satiety ratings report that hormones (i.e. total ghrelin) may play a direct role in daily caloric consumption (11, 20, 25). Using the visual analogue scale (VAS) for hunger, satiety and cravings, it has been demonstrated that VAS hunger scores can decrease and VAS satiety scores can increase with increased meal frequency (11, 20, 25). The VAS has been validated in previous appetite research to be an accurate measure for determining hunger, satiety, and/or food cravings at specific time periods (26).

Additionally, it has been proposed that gastric emptying may play a role in hunger and satiety ratings. Eating frequently can delay gastric emptying which will increase food exposure to the gut and produce necessary hormonal cascades (27). It is proposed that these hormonal responses will increase satiety and decrease hunger when the same caloric intake is spread across smaller more frequent meals (27).

Limitations in the Literature

Presently, there are few studies that have investigated differences in obese individuals undergoing weight loss while eating varied MF. Furthermore, the investigation of "gorging" (two meals per day) versus "nibbling" (three meals + three snacks per day) has yet to be explored in this population utilizing portion-controlled meal products. The "gorging" pattern of meal intake has been thought to enhance adipogenesis or fat storage and increase body weight; the weight gain is thought to be mainly attributed to the large fluctuations in fat storage and mobilization that occurs during gorging followed by prolonged periods of fasting (1, 28, 29).

The potent circulating orexogenic hormone ghrelin is the only known circulating hormone that increases hunger (30). Circulating ghrelin is known to stimulate

adipogenesis, increase hunger ratings, as well as display an endogenous secretory rhymicity that can be related closely to meal patterns (29). Diet-induced weight loss has been associated with a decrease in postprandial ghrelin suppression which could be the cause of the eventual regaining of weight lost (1). In examining current research comparing obese individuals and lean counterparts, obese subjects were found to have a negatively correlated relationship of ghrelin to body weight and percent body fat ($r = -0.50$, $r = -0.53$, respectively) (31). Additionally, according to Solomon et al. 2008 (32), there is a larger preprandial surge in ghrelin response with larger less frequent meals (two meals per day) versus smaller more frequent meals (12 snacks per day). This surge is thought to play a large role in disrupting metabolic balance by decreasing fat utilization and increasing fat storage. Although adiposity has been positively correlated with total ghrelin levels, the changes in total ghrelin in response to meal frequency during an equi-hypocaloric diet is not fully understood.

Problem Statement

Studies investigating the effect of varied MF on hunger/satiety, biochemical markers of appetite, and blood markers of health have shown conflicting results in obese subjects. Comparing these parameters using two meals per day versus three meals + three snacks per day has yet to be investigated in obese subjects through a cross-over design and portion-controlled meals. Thus, the current aim of this doctoral dissertation project is to assess the changes in appetite, blood lipid profiles, glucose and insulin metabolism, and sensations of hunger and satiety in obese women utilizing a commercially available weight loss program of varying meal frequencies as part of their clinically-supervised behavioral weight management program.

Purpose of Study

The purpose of the present study is to determine the effects of an equi-hypocaloric diet with varying meal frequency of "gorging" (two meals per day) versus "nibbling (three meals + three snacks per day) on total ghrelin, blood glucose, insulin, and lipid concentrations, and sensations of hunger and satiety during a six-week cross-over design.

Hypotheses

This study will test the following hypotheses:

1. There will be a significant difference ($P \leq 0.05$) in metabolic markers of health when subjects consume the two meals/day "gorging" sequence versus the three meal + three snacks/day "nibbling" sequence.
    a. It is hypothesized that fasting total cholesterol will decrease in response to the "nibbling" sequence following a test meal.
    b. It is hypothesized that fasting LDL cholesterol will decrease in response to the "nibbling" sequence following a test meal.
    c. It is hypothesized that fasting insulin AUC concentrations will decrease in response to the "nibbling" sequence following a test meal.
    d. It is hypothesized that fasting glucose AUC concentrations will decrease in response to the "nibbling" sequence following a test meal.

    Rationale: According to recent literature, LDL-C and total cholesterol as well as decreased fasting insulin and blood glucose responses all decrease with increased meal frequency (10, 13, 20). Additionally, early studies using healthy normal-weight subjects also reported that those who habitually consume two or less meals per day have an increased prevalence of hyperinsulinemia and glucose intolerance

compared to subjects consuming five or more meals per day (14); however, this has yet to be confirmed in obese subjects.

2. There will be a significant difference in VAS hunger and satiety ratings following the test meal for the allocated time intervals of 0, 30, 60, 90, and 120 minutes between the two test conditions.
    a. It is hypothesized that VAS hunger ratings AUC following a test meal will be significantly decreased following the two-week trial consuming the "nibbling" pattern versus "gorging" pattern.
    b. It is hypothesized that VAS satiety ratings AUC will be increased following the two-week trial consuming the "nibbling" pattern versus "gorging" pattern.

    Rationale: Bachman and Raynor (3) and Smeets et al. (11) reported that individuals with a higher frequency eating pattern of an isoenergetic diet gave lower hunger and increased satiety ratings as compared to those having a less frequent eating pattern. The increased MF versus the lower MF during an equi-hypocaloric diet is thought to delay gastric emptying and thus produce increased feelings of satiety (27).
3. There will be a significant difference in total ghrelin concentrations preprandially and postprandially in response to the test meal between the two test conditions; "gorging" and "nibbling".

    Rationale: According to Solomon et al., there is a larger preprandial surge in ghrelin response with larger, less frequent meals (two meals per day) compared to smaller more frequent meals (12 meals per day) (32). Reduced MF is thought to

play a role in disrupting metabolic balance by decreasing fat utilization and increasing fat storage (32). Furthermore, habitually reduced MF in obese subjects has been shown to have an effect on ghrelin concentrations. This eating pattern is associated with a negatively correlated relationship to body weight and percent body fat (31).

Scope of the Study

This study utilized an experimental randomized cross-over design. Testing occured under controlled medical supervision on location at Southwest Endocrinology Associates (SWENDO) as well as the University of New Mexico (UNM) Exercise Physiology Laboratory, both located in Albuquerque, NM. Medical and research staff on site include: physicians, nurses, exercise physiologists, dietitians, and trained research staff. Community-dwelling participants returned weekly to the clinic for follow-ups with the research team, attend education classes, and undergo measurements.

An *apriori* power analysis using G*Power Version 3.1.0 (Franz Faul, Universitat Kiel, Germany) indicated that a sample size of four to ten was needed to determine significance; 15 subjects were recruited. The study design was a two arm, two-week randomized cross-over design with a two-week washout (six-weeks total) where non-diabetic obese subjects (BMI ≥ 30 kg/m^2) were randomly assigned to an equi-hypocaloric diet of either the "gorging" meal pattern of two meals per day or the "nibbling" meal pattern of three meals + three snacks per day as recommended by Nutrisystem®. In accordance with SWENDO guidelines, dietary measures were standardized to restrict the dietary intake of all participants to 1200-1500 calories/day with a protein content of approximately 75g/day of protein depending upon body mass.

Subjects were given an identical test meal (~400 kcals) at baseline and prior to both conditions. Venous blood was sampled and total ghrelin, insulin, and glucose concentrations were analyzed. Subjects answered questions of hunger and satiety using a visual analogue scale (VAS) at time 0, 30, 60, 90, and 120 minutes following consumption. Subjects consumed their assigned portion-controlled meal pattern for two consecutive weeks. Following a two-week washout period of four eating occasions per day, they consumed the alternate MF pattern. Subjects returned for weekly measurements throughout the six-week period. Measurements included resting blood pressure, body weight, height, waist and hip circumferences, body composition (Tanita BC-418, bioelectrical impedance analyzer). They also attended weekly meetings at SWENDO and received necessary counseling. Following each two-week period, lipid profiles, glucose, insulin, and RMR were measured under fasting conditions.

Assumptions

The following assumptions were made for this study:

1. Subjects ingest meals and/or snacks as instructed and in accordance with treatment condition and record all intake with time of ingestion.
2. Food provided to the subject is eaten solely by the subject and is eaten in its entirety. If the subject did not consume the entire portion, subjects weighed the amount remaining and reported it on the food log.
3. Subject maintains her current physical activity regimen and documents all activity.
4. Subject follows all pre-test and pre-experimental guidelines as directed.
5. Subjects answer all questionnaires accurately and truthfully.

Limitations

1. Due to the nature of the study, we cannot observe all subjects during every eating occasion. Participants were free-living and adherence was maintained through self-report. It is a limitation that we are assuming the subjects will consume the allocated foods according to our instructions and that they will report this accurately.
2. Due to the nature of the study, we cannot observe all subjects as they write in their food logs. Accuracy and completeness of logs were not controlled in this free-living sample. We assume food logs are correct and were completed with detailed descriptions of food consumed and at what time of the day it was consumed.
3. Due to current resources and costs, a small sample size of approximately 15 women were recruited.
4. BIA (eight-electrode single frequency) was used to assess changes in body composition. The BIA will be used as an estimate of weekly body composition changes, if any. Total body water may be affected during weight loss which may be a factor that can influence BIA estimates.
5. Due to limited resources, the time course of measurements for insulin, glucose, lipids, and total ghrelin were measured at only five time intervals (0, 30, 60, 90, 120 minutes).

Significance of the Study

This project is significant because it is the first to investigate the effects of meal frequency on biochemical markers of appetite (total ghrelin) and health (glucose, insulin

and lipid concentrations) as well as hunger/satiety ratings in obese subjects utilizing portion-controlled products. Based on the results of the current study, this study can help recognize the affects of consuming two meals per day or six meals per day on certain blood-markers of health and appetite measures. With respect to the study population, this study aids in the understanding of the role altering MF has for obesity treatment.

Definition of Terms:

Acylated Ghrelin: The metabolically active form of ghrelin that has an acyl group attached. Acylated ghrelin is an endogenous ligand for the growth hormone secretagogue receptor (GHS-R1a) and stimulates feeding and growth hormone release.

Adipogenesis: is the process of cell differentiation by which preadipocytes become adipocytes.

Equi-Hypocaloric Diets: the comparison of two diets that provide equal macronutrient compositions and provides less caloric energy than is expended daily.

Free-Living Diet: diet consumed without restriction at the free-will of the individual.

Hypocaloric Diet: a diet that provides less caloric energy than is expended daily by the individual.

Isocaloric Diet: diet providing equal macronutrient compositions and caloric energy per day compared with another diet.

Meal: For this study, a meal will be ≥ 200 calories.

Preprandial: prior to consuming a meal.

Postprandial: after consuming a meal.

Thermogenesis: the process of heat production in organisms.

Snack: There are varied definitions in the literature, but for this study, a snack will be ≥100 - ≤ 200 calories.

Serum Total Ghrelin: the sum of acyl- and desacyl- ghrelin which together forms the circulating concentration of the 28 amino acid hunger-stimulating peptide hormone synthesized by gastric endocrine cells. Ghrelin is the only circulating hormone that stimulates senses of hunger.

References

1. Jakubowicz D, Froy O, Wainstein J, Boaz M. Meal timing and composition influence ghrelin levels, appetite scores and weight loss maintenance in overweight and obese adults. Steroids. 2012 Mar 10;77(4):323-31. PubMed PMID: 22178258. Epub 2011/12/20. eng.

2. Schwarz NA, Rigby BR, La Bounty P, Shelmadine B, Bowden RG. A review of weight control strategies and their effects on the regulation of hormonal balance. J Nutr Metab. 2011;2011:237932. PubMed PMID: 21822485. Epub 2011/08/09. eng.

3. Bachman JL, Raynor HA. Effects of manipulating eating frequency during a behavioral weight loss intervention: a pilot randomized controlled trial. Obesity (Silver Spring). 2012 May;20(5):985-92. PubMed PMID: 22173575. Epub 2011/12/17. eng.

4. Bortz WM, Wroldsen A, Issekutz B, Jr., Rodahl K. Weight loss and frequency of feeding. N Engl J Med. 1966 Feb 17;274(7):376-9. PubMed PMID: 5903121. Epub 1966/02/17. eng.

5. Finkelstein B, Fryer BA. Meal frequency and weight reduction of young women. Am J Clin Nutr. 1971 Apr;24(4):465-8. PubMed PMID: 5090633. Epub 1971/04/01. eng.

6. Garrow JS, Durrant M, Blaza S, Wilkins D, Royston P, Sunkin S. The effect of meal frequency and protein concentration on the composition of the weight lost by obese subjects. Br J Nutr. 1981 Jan;45(1):5-15. PubMed PMID: 7470437. Epub 1981/01/01. eng.

7. Poston WS, Haddock CK, Pinkston MM, Pace P, Karakoc ND, Reeves RS, et al. Weight loss with meal replacement and meal replacement plus snacks: a randomized trial. Int J Obes (Lond). 2005 Sep;29(9):1107-14. PubMed PMID: 15925955. Epub 2005/06/01. eng.

8. Cameron JD, Cyr MJ, Doucet E. Increased meal frequency does not promote greater weight loss in subjects who were prescribed an 8-week equi-energetic energy-restricted diet. Br J Nutr. 2010 Apr;103(8):1098-101. PubMed PMID: 19943985. Epub 2009/12/01. eng.

9. Farshchi HR, Taylor MA, Macdonald IA. Beneficial metabolic effects of regular meal frequency on dietary thermogenesis, insulin sensitivity, and fasting lipid profiles in healthy obese women. Am J Clin Nutr. 2005 Jan;81(1):16-24. PubMed PMID: 15640455. Epub 2005/01/11. eng.

10. Heden TD, Liu Y, Sims LJ, Whaley-Connell AT, Chockalingam A, Dellsperger KC, et al. Meal Frequency differentially alters postprandial triacylglycerol and insulin concentrations in obese women. Obesity (Silver Spring). 2012 Jun 22. PubMed PMID: 22836692. Epub 2012/07/28. Eng.

11. Smeets AJ, Westerterp-Plantenga MS. Acute effects on metabolism and appetite profile of one meal difference in the lower range of meal frequency. Br J Nutr. 2008 Jun;99(6):1316-21. PubMed PMID: 18053311. Epub 2007/12/07. eng.

12. La Bounty PM, Campbell BI, Wilson J, Galvan E, Berardi J, Kleiner SM, et al. International Society of Sports Nutrition position stand: Meal frequency. J Int Soc Sports Nutr. 2011;8:4. PubMed PMID: 21410984. Epub 2011/03/18. eng.

13. Titan SM, Bingham S, Welch A, Luben R, Oakes S, Day N, et al. Frequency of eating and concentrations of serum cholesterol in the Norfolk population of the European prospective investigation into cancer (EPIC-Norfolk): Cross sectional study. BMJ. 2001 Dec 1;323(7324):1286-8. PubMed PMID: 11731392. Epub 2001/12/04. eng.

14. Fabry P, Hejl Z, Fodor J, Braun T, Zvolankova K. The frequency of meals. Its relation to overweight, hypercholesterolaemia, and decreased glucose-tolerance. Lancet. 1964 Sep 19;2(7360):614-5. PubMed PMID: 14186149. Epub 1964/09/19. eng.

15. Gwinup G, Byron RC, Roush WH, Kruger FA, Hamwi GJ. Effect of Nibbling Versus Gorging on Serum Lipids in Man. Am J Clin Nutr. 1963 Oct;13:209-13. PubMed PMID: 14067443. Epub 1963/10/01. eng.

16. Gwinup G, Byron RC, Roush W, Kruger F, Hamwi GJ. Effect of nibbling versus gorging on glucose tolerance. Lancet. 1963 Jul 27;2(7300):165-7. PubMed PMID: 13951548. Epub 1963/07/27. eng.

17. Vander Wal JS, Waller SM, Klurfeld DM, McBurney MI, Cho S, Kapila M, et al. Effect of a post-dinner snack and partial meal replacement program on weight loss. Int J

Food Sci Nutr. 2006 Feb-Mar;57(1-2):97-106. PubMed PMID: 16849118. Epub 2006/07/20. eng.

18. Edelstein SL, Barrett-Connor EL, Wingard DL, Cohn BA. Increased meal frequency associated with decreased cholesterol concentrations; Rancho Bernardo, CA, 1984-1987. Am J Clin Nutr. 1992 Mar;55(3):664-9. PubMed PMID: 1550041. Epub 1992/03/01. eng.

19. Jenkins DJ, Wolever TM, Vuksan V, Brighenti F, Cunnane SC, Rao AV, et al. Nibbling versus gorging: metabolic advantages of increased meal frequency. N Engl J Med. 1989 Oct 5;321(14):929-34. PubMed PMID: 2674713. Epub 1989/10/05. eng.

20. Stote KS, Baer DJ, Spears K, Paul DR, Harris GK, Rumpler WV, et al. A controlled trial of reduced meal frequency without caloric restriction in healthy, normal-weight, middle-aged adults. Am J Clin Nutr. 2007 Apr;85(4):981-8. PubMed PMID: 17413096. Epub 2007/04/07. eng.

21. Bandini LG, Schoeller DA, Cyr HN, Dietz WH. Validity of reported energy intake in obese and nonobese adolescents. Am J Clin Nutr. 1990 Sep;52(3):421-5. PubMed PMID: 2393004. Epub 1990/09/01. eng.

22. Mendez MA, Wynter S, Wilks R, Forrester T. Under- and overreporting of energy is related to obesity, lifestyle factors and food group intakes in Jamaican adults. Public Health Nutr. 2004 Feb;7(1):9-19. PubMed PMID: 14972067. Epub 2004/02/20. eng.

23. Taylor MA, Garrow JS. Compared with nibbling, neither gorging nor a morning fast affect short-term energy balance in obese patients in a chamber calorimeter. Int J Obes Relat Metab Disord. 2001 Apr;25(4):519-28. PubMed PMID: 11319656. Epub 2001/04/25. eng.

24. Verboeket-van de Venne WP, Westerterp KR, Kester AD. Effect of the pattern of food intake on human energy metabolism. Br J Nutr. 1993 Jul;70(1):103-15. PubMed PMID: 8399092. Epub 1993/07/01. eng.

25. Odunsi ST, Vazquez-Roque MI, Camilleri M, Papathanasopoulos A, Clark MM, Wodrich L, et al. Effect of alginate on satiation, appetite, gastric function, and selected gut satiety hormones in overweight and obesity. Obesity (Silver Spring). 2010 Aug;18(8):1579-84. PubMed PMID: 19960001. Epub 2009/12/05. eng.

26. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. Int J Obes Relat Metab Disord. 2000 Jan;24(1):38-48. PubMed PMID: 10702749. Epub 2000/03/07. eng.

27. Jackson SJ, Leahy FE, Jebb SA, Prentice AM, Coward WA, Bluck LJ. Frequent feeding delays the gastric emptying of a subsequent meal. Appetite. 2007 Mar;48(2):199-205. PubMed PMID: 17081656. Epub 2006/11/04. eng.

28. Moran LJ, Noakes M, Clifton PM, Wittert GA, Le Roux CW, Ghatei MA, et al. Postprandial ghrelin, cholecystokinin, peptide YY, and appetite before and after weight loss in overweight women with and without polycystic ovary syndrome. Am J Clin Nutr. 2007 Dec;86(6):1603-10. PubMed PMID: 18065576. Epub 2007/12/11. eng.

29. Verbaeys I, Tolle V, Swennen Q, Zizzari P, Buyse J, Epelbaum J, et al. Scheduled feeding results in adipogenesis and increased acylated ghrelin. Am J Physiol Endocrinol Metab. 2011 Jun;300(6):E1103-11. PubMed PMID: 21427412. Epub 2011/03/24. eng.

30. Kanoski SE, Fortin SM, Ricks KM, Grill HJ. Ghrelin Signaling in the ventral hippocampus stimulates learned and motivational aspects of feeding via PI3K-Akt signaling. Biol Psychiatry. 2012 Aug 9. PubMed PMID: 22884970. Epub 2012/08/14. Eng.

31. Homaee HM, Moradi F, Azarbayjani MA, Peeri M. Relationships between acylated ghrelin with growth hormone, insulin resistance, lipid profile, and cardio respiratory function in lean and obese men. J Res Med Sci. 2011 Dec;16(12):1612-8. PubMed PMID: 22973371. Epub 2012/09/14. eng.

32. Solomon TP, Chambers ES, Jeukendrup AE, Toogood AA, Blannin AK. The effect of feeding frequency on insulin and ghrelin responses in human subjects. Br J Nutr. 2008 Oct;100(4):810-9. PubMed PMID: 18394217. Epub 2008/04/09. eng.

CHAPTER II

This chapter represents a review manuscript entitled “Potential role of meal frequency as a strategy for weight loss and health in overweight or obese adults” that is currently in review for publication in the journal, *Nutrition*. The manuscript is authored by Michelle Kulovitz, Len Kravitz, Christine Mermier, Ann Gibson, Carole Conn, Deborah Kolkmeyer, and Chad Kerksick. This manuscript follows the formatting and style guidelines of the journal. References cited are provided at the end of the manuscript. The referred table is following the cited references, which is the formatting guideline for the submitted journal, *Nutrition*.

Title: Potential role of meal frequency as a strategy for weight loss and health in overweight or obese adults

Running Title: Meal frequency and weight loss

By

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Abstract

Improved dietary strategies for weight loss are necessary to decrease metabolic disease risk in overweight or obese adults. Varying meal frequency, i.e., increasing or decreasing eating occasions beyond the traditional three meal per day pattern, has been thought to have an influence on body weight regulation, hunger control, and blood markers of health. It is common practice for weight management clinicians to recommend increasing meal frequency as a strategy for weight management and to improve metabolic parameters. However, limited research exists investigating the impact of meal frequency during controlled hypocaloric diet interventions. Furthermore, meal frequency literature often speculates efficacy of meal frequency treatments based on research using normal weight, overweight/obese, and/or the combination where much diversity exists within these various populations. In this review we will discuss that normal weight and overweight/obese populations as well as free-living versus investigator-controlled research trials should be studied independently. There is uncertainty in the literature when interpreting optimal meal frequency for obesity treatment because of these comparisons. Nevertheless, upon review of the scientific literature investigating overweight/obese adults during a hypocaloric dietary intervention, the relationship between meal frequency and body weight regulation, appetite, and health has not yet been determined. The objective of the present review is to survey the literature to assess if the alteration of meal frequency influences body weight regulation, hunger control, and/or blood markers of health in overweight or obese subjects undergoing a controlled hypocaloric diet to induce weight loss.

Keywords: obesity, meal frequency, eating frequency, eating occasions, weight loss, hypocaloric diet, appetite

Introduction

According to a recent release of Healthy People 2020, one primary public health concern in the United States is the prevalence of overweight and obese Americans (1). Currently, more than one-third of U.S. adults are obese with no indication that the prevalence is decreasing (2). It has been estimated that over 300,000 US adults (15.2% of all deaths) die each year due to obesity-related co-morbidities; of these factors, a poor diet and inadequate levels of physical activity hold great importance (3).

Presently, there are numerous dietary strategies that are believed to play a role in combating overeating and obesity (defined as body mass index, BMI $\geq$ 30 kg/m^2); however, the mechanisms are not well understood (4-6). For these reasons, evidence-based weight loss interventions are considered crucial for decreasing the prevalence of overweight and obese Americans.

Since the early 1960's, the idea of implementing increased dietary structure in regard to meal frequency (MF) has been debated. Currently weight management professionals recommend dietary weight loss plans that substitute the classical eating pattern (three large meals per day) with eating smaller meals more frequently throughout the day in order to spread out daily caloric intake (7, 8). Increased MF for weight management, body weight regulation, hunger control, and metabolic disease management is supported anecdotally, but this strategy lacks evidence in the associated scientific literature. Due to the potential impact of MF to manage hunger, satiety, regulation of appetite hormones, and lipemia, increasing MF has been hypothesized to impact energy intake and secondary to this, the favorable regulation of body weight (7, 9). Furthermore, it has been shown that weight loss maintainers tend to eat more frequently throughout the

day (3 meals and 2 snacks) than those who tend to regain weight lost, however the research is inconclusive during active weight loss (10). Thus it is the purpose of this review to investigate the role of MF as a dietary strategy for individuals undergoing active weight loss during controlled hypocaloric dietary interventions.

Observational research has demonstrated mixed results in free-living adults versus controlled research trials when investigating the relationship between MF and body weight. Some researchers contend that higher MF is related to a healthy weight (8, 11, 12). More specifically, those consuming a greater frequency of small meals throughout the day are more likely to have a normal BMI, healthy levels of certain markers of risk for diesease, (e.g. triglycerides, cholesterol, and glucose metabolism), and consequently, a reduced risk of developing or having a diagnosis of coronary heart disease (CHD) and/or other metabolic diseases such as obesity and type 2 diabetes (8, 11, 12). Conversely, others have reported that higher frequency of *ad libitum* eating may lead to increased weight gain and obesity because it presents increased opportunities to eat and overeat throughout the day (13-16).

Furthermore, research investigating the role of MF in disease regulation has shown variable results when investigating glucose and insulin levels and postprandial lipid profiles. Frequent meals have been proposed to reduce the occurrence of excess caloric consumption and provide better glucose control and reduced insulin secretion (17). Benefits of increasing MF on glucose control have been shown in overweight/obese (18) and in individuals who have impaired glucose tolerance (19, 20); however, research with normal weight or those who are normoglycemic subjects are mixed.

Increasing MF in overweight or obese subjects has shown: reduced glycemic load (20), delayed nutrient absorption (18), improved glucose and insulin metabolism (19-22), and improved hunger control (18, 22). However in healthy normal weight individuals and/or persons without impaired glucose metabolism, no significant differences were found in postprandial glucose regulation (23), in reducing the concentrations of lipid and/or hormones (24), or in hunger feelings (25).

Current Meal Patterns and Body Weight

One of the most notable limitations in the literature examining eating patterns associated with MF is the predominance of observational, cross-sectional studies. In this regard, several studies have reported an inverse relationship between eating frequency and body weight, body composition, or BMI in both normal weight and obese adults (26-28). According to a study investigating eating patterns and obesity prevalence in free-living US adults, eating four or more times per day was associated with a lower risk of obesity when compared to eating three or less times per day (28). However, those who habitually skipped breakfast were 1.35 times more likely to be obese than those who always had breakfast (28). Research analyzing eating patterns of US adults showed that obese individuals eating *ad libitum* are more likely to skip breakfast, "gorge" during mid-morning or lunch, and then "gorge" again for dinner with no snacks or meals in between (28). Observational studies investigating eating patterns of weight-stable and weight-gaining individuals showed that weight-gaining individuals eat an average of 1645 kJ/d more than weight-stable individuals; this difference is attributed to increased carbohydrate and fat consumption through larger portion sizes (29). These studies provide evidence for the need of more controlled feeding studies. Findings from Pearcey

and de Castro 2002 suggest that without holding total caloric intake constant the usefulness of MF and meal timing data can be limited (29).

Current Limitations in the Literature

Several limitations exist within the MF research to date. There is a lack of standardized terminology; eating occasions are described with multiple terms such as meal frequency, eating frequency, and feeding frequency. Furthermore, there are differences in definitions of caloric requirements for a meal or snack (11, 23, 30). Additionally, due to the differences in responses seen with the healthy normal-weight versus overweight/obese adults, comparisons should be made between similar populations rather than grouping them together. Grouping different populations may produce differing responses to MF, lipid and glucose metabolism, hormonal appetite regulation, or sensations of hunger/satiety (21, 22, 24, 29). Differences also occur, in part, due to varied research methodologies; limited research exists using strict dietary and/or controlled methods. Although it is important to understand the behaviors of free-living adults, controlled trials should not be compared to free-living observational studies to investigate plausible strategies to be used by clinical professionals for weight management. Moreover, due to the common error seen with individuals underreporting caloric intake, there are other potential differences that can occur when drawing conclusions about optimal MF when using self-reported dietary intake versus portion-controlled products (31).

Therefore, the goal of this review is to clarify the impact of different MF on body composition and weight management, lipid and glucose metabolism, hunger and satiety, and hormonal appetite regulation in overweight/obese adults based on scientific evidence

from randomized controlled hypocaloric diet trials. Previous review papers investigating the impact of varied MF on these parameters have not studied the impact of MF with only overweight/obese adults undergoing a hypocaloric diet intervention to induce weight loss (14, 32-34). Since outcome measures can vary between normal weight and overweight/obese subjects, the results of interventions with overweight/obese during a hypocaloric diet trial will be discussed in order to discern possible treatment mechanisms for obesity management. This paper will investigate randomized controlled research trials using reduced-calorie controlled dietary interventions that include altering MF. We also will assess whether there is evidence to suggest that increasing MF during a reduced-calorie diet may be a treatment strategy for reducing obesity. Additionally, we will highlight areas in need of further research.

Literature Search Criteria

The scientific literature was reviewed and studies were incorporated in this review if they included healthy overweight or obese (BMI $\geq$ 25 kg/m^2) adults (males and/or females ages 18-66 years) who were consuming a hypocaloric diet during a controlled feeding study. Keywords included in the search were a combination of "obese", "hypocaloric", "reduced calorie", "meal", "eating", "frequency", "occasion", and "timing". The following sources were used: MedLine, PubMed, Proquest, Cochrane Database, and Google Scholar. Studies published between 1970 and 2012 were included if they were written in the English language and incorporated a randomized study design with a hypocaloric dietary intervention in an overweight or obese population that investigated one or more of the following outcome measures: changes in body weight or composition; blood markers of health including glucose, insulin, or lipid concentrations;

hunger or satiety responses via appetite hormone measurements; or estimates of hunger sensation or satiety following a test meal. A summary of studies yielded by the search criteria and distribution of terms provided is presented in Table 1.

Body Composition

A number of studies using both animal and human subjects have reported that MF can affect body weight and composition (26-28, 30). Fabry and colleagues (1964) were among the first researchers to discover an inverse relationship associated with observed habitual MF and body weight in normal-weight humans (27). This research is thought to be largely responsible for the idea that meal timing may play a role in appetite regulation and body composition. Since that time, there has been much debate in the literature regarding the role of MF for the treatment of obesity as it relates to body weight and/or composition. For example, some recent cross-sectional studies have reported no relationship between MF and BMI or body fatness (9, 25, 35) while a 2007 study reported opposing results indicating a positive relationship between MF and obesity (30).

Eight experimental studies investigating overweight/obese individuals during a hypocaloric weight loss intervention designed to induce body weight or composition changes met the search criteria. Limited evidence demonstrating a relationship between weight loss in the overweight/obese population and MF exists. Of these studies, the most recent was conducted by Bachman and Raynor in 2012 (9). This controlled trial randomized participants (age: 51±9.9 years, BMI: 35.5± 4.8 kg/m^2, 57.8% female) into either a "gorging" three meals/day eating approach (n=25) or a "grazing" approach where they were instructed to eat at least 100 kcals every 2-3 hours and to distribute calories as they wished (n=26) (9). Assessments for weight loss and average hunger ratings were

done at 0, 3, and 6 months. No significant differences were found in energy intake or BMI between groups at any time point (9). It is important to note that subjects were told to maintain a non-individualized diet of either 1,200 kcals/day (body weight ≤ 200lbs) or 1,500 kcals/day (body weight >200 lbs) for the 6-month study duration. Adherence was based on self-reported 3-day food records (9).

Another study using a controlled experimental design was conducted by Cameron et al. in 2010 (25). They examined 16 obese (BMI 37.1±4.5 kg/m^2) subjects during an eight-week equi-energetic hypocaloric diet. These subjects consumed either three meals + three snacks per day or three meals per day with no snacks. Loses of body weight, fat-mass, and lean mass were similar between groups (25). Cameron and colleagues (25) did prescribe an individualized hypocaloric diet where a 700 kcal/day deficit was individualized to study participants.

These findings are similar to a number of previously published studies (36-41), in which no significant differences were reported in body weight changes in overweight/obese subjects using three versus six eating occasions (36, 40, 41), four versus five eating occasions (37), or two versus three or five eating occasions (39). Only two of these studies used partial meal replacements and/or portioned products to alleviate some measurement error (37, 38). Other studies provided meal plans and relied on self-reported intake for adherence. In this respect, Vander Wal and colleagues (38) used Kashi® products for breakfast, mid-morning snack, and lunch with a balanced meal for dinner, while Poston et al. (38) provided Slimfast® products to be eaten a minimum of two times/day. Although both used some portion controlled products, no significant differences were found in the amount of weight lost after eight (37) or 24 weeks (38).

Currently, there are many MF patterns that have been tested with respect to the number of meals/snacks administered as well as the duration of intervention. However, the research shows no significant findings were evident for changes in body composition with varied MF. At this time, it appears MF does not have a significant role in body composition changes or weight reduction during controlled feeding trials with overweight/obese adults. The lack of significant differences in weight loss or changes in body fat findings with varied MF may suggest that the differing patterns of MF and methods of measurement may or may not play a role in the results. The variable methodologies used in these studies makes it difficult to develop a conclusion with respect to the relationship between MF and body composition changes. Furthermore, based on the knowledge that underreporting of calories consumed is common in both obese and non-obese individuals, findings from studies using self-reported dietary intake must be interpreted with the caution due to the probability that underreporting has occurred (31).

Markers of Health

According to the Centers for Disease Control *Summary of Health Statistics*, 2010, 34% of Americans 18 years and older are overweight and 28% are obese, while over 25% had been diagnosed with coronary heart disease (CHD). Type 2 diabetes (T2DM), dyslipidemia, hypertension, and physical inactivity are some of the leading risk factors for developing CHD in the United States (42). There is a paucity of research on MF's effect on metabolic markers of health inclusive of glucose and insulin metabolism as well as blood lipid concentrations during a hypocaloric diet for overweight/obese subjects.

There are several studies investigating the changes in postprandial insulin and glucose concentrations in normal weight (17, 23, 43, 44) and/or non-hypocaloric diet interventions (44, 45). After the review of studies meeting our search criteria, only seven studies were found that investigated one or more of the following: glucose concentrations, insulin responses, and/or blood lipid changes in overweight or obese populations.

Insulin and Glucose Metabolism

Mechanisms for improved glucose regulation in overweight/obese individuals or those with impaired glucose tolerance indicate that increased MF can decrease the postprandial surge of glucose and thus decrease the amount of insulin released in response (38). Furthermore, small sporadic meals are also thought to aid in T2DM management. They are thought to provide more stable blood sugar levels by increasing glucose uptake and disposal by the muscles as fuel as well as providing a possible suppression of free fatty acid (FFA) release from the adipose tissue (14, 21). Although there is very limited research on glucose and insulin responses and MF in overweight/obese subjects, a controlled study was conducted by Bertelsen et al. (19). T2DM patients (BMI 32.2±1.3 kg/m^2) were studied to investigate postprandial glucose and insulin responses. Researchers investigated the differences between two isocaloric conditions in which two bolus feedings and six intermittent feedings were analyzed on separate occasions for differences in glucose and insulin responses (19). They found that the two meal approach induced an 84% greater maximum amplitude in glucose concentrations as well as increased circulating FFA when compared to the six meal

approach (19). Their preliminary findings were indicative of poor metabolic control that can be ameliorated with higher MF in this population.

However, research results investigating the changes in insulin and/or glucose responses to differing MF in healthy overweight/obese subjects are mixed; some found no significant differences (36, 38, 40), while others found significant differences between feeding patterns (18). Poston et al. (38) investigated if meal replacements with snacks would have an impact on blood glucose and insulin responses in obese men and women who were already "snackers" versus "non-snackers". The researchers found no significant differences between those consuming three larger meals consisting of meal replacements and no snacks as compared to those consuming meal replacements plus snacks three times per day (38). The caloric prescription for this study required participants to maintain a daily diet of 1200 kcals for women and 1500 kcals for men. It is important to note that the researchers relied on self-reported dietary intake by study participants, a condition in which underreporting of energy intake is known to occur (31). Similar findings were shown in a study by Forslund et al. 2008 (36), who studied 140 obese adults eating on three versus six occasions per day during a one-year intervention. Individualized baseline caloric intake was assessed to adjust diets so that intake was reduced by approximately 30% to induce weight loss. Fasting glucose and insulin concentrations were assessed at baseline and one-year later with no significant differences being found between MF groups (36). Potential shortcomings of this study relates to the lack of postprandial glucose or insulin responses as well as the use of self-reported food records and telephone interviews to determine compliance with caloric intake and allocated MF. Only fasting levels of glucose and insulin at baseline and

following the one-year intervention were used for assessment. Using similar methods and a 60-day intervention, Finkelstein and Fryer, (40) also reported no significant changes in fasting glucose concentrations from baseline to the follow-up in healthy young obese women.

Heden et al. (18) recruited eight healthy obese women (BMI 34.5±1.3 kg/m^2) for assignment into one of two isocaloric 12-hour treatment conditions of three eating occasions and six eating occasions. The changes in postprandial insulin concentrations were studied following both interventions. The insulin incremental area under the curve (AUC) was significantly higher during the three-meal condition when compared to the six-meal condition when subjects were followed for 12 hours. Insulin is thought to be indirectly related to the formation of fatty acids. Since insulin secretion upregulates enzymes known to be involved in cholesterol synthesis and enhance lipogenesis, increased insulin levels may promote the progression of CVD (11, 23).

Blood Lipids

Elevated levels of plasma triglycerides, LDL cholesterol, and total cholesterol combined with decreased concentrations of plasma HDL cholesterol are lipid profile markers associated with increased risk for coronary artery disease (46). Cross-sectional evaluations with the general population have found that both total cholesterol and LDL cholesterol are decreased with habitual increases in MF (47, 48). As with other areas, more research is needed to fully elucidate any relationship between MF and lipid profile changes, particularly in specialized in-need populations such as overweight/obese adults undergoing a reduced-calorie diet (11).

Significant associations investigating MF and blood lipid concentrations were found in two studies meeting search criteria. In a randomized controlled trial (RCT) on MF by Forslund et al. (36) researchers found no significant differences between conditions for fasting levels of total cholesterol, LDL cholesterol, and triglycerides; the exception was HDL cholesterol. Using self-reported intake, those consuming three meals/day for one year were found to have significantly higher HDL cholesterol values than did those consuming three meals plus three snacks each day (36). Additionally, Heden et al. (18) found a significantly lower AUC value for triglycerides during the three daily eating occasions as compared to the six-eating occasion condition in obese subjects. Researchers argue that one potential mechanism for these findings could be the greater postprandial insulin concentrations during the three-meal condition (see Postprandial Lipemia section) (18).

Additionally, four other studies have also reported no significant differences in blood lipids due to varied MF in overweight or obese adults during a reduced-calorie diet. Vander Wal et al. (37) reported that an eight-week meal replacement program plus one late night snack did not significantly improve total cholesterol, LDL cholesterol, triglycerides, or HDL cholesterol values in obese adults. It appears that the intervention may not have been robust enough to see improvements in these parameters because only one extra eating occasion was added to the subject's typical regimen. However, Finkelsein and Fryer, (40) Antoine et al. (41) and Poston et al. (38) reported similar findings. Collectively, no significant differences were identified relative to total cholesterol, LDL cholesterol, triglycerides, or HDL cholesterol following a 60-day RCT

with eight obese females, a two-week RCT with ten obese females, or a 24-week RCT with 100 obese adults, respectively.

Postprandial Lipemia

Research using obese individuals following a hypocaloric intervention (18) and interventions designed to promote weight maintenance (23) have shown significant increases in postprandial insulin concentrations during a three-meal condition versus either six or 17 meals, respectively. Heden et al. (18) speculated that a possible mechanism for significantly lower triglyceride AUC for the three-meal condition is mediated by postprandial insulin responses. The increased insulin response is thought to lead to a decreased surge in triglycerides. Since insulin stimulates adipose tissue lipoprotein lipase activity this will function to hydrolyze triglycerides to non-esterified fatty acids and glycerol (18, 49). The non-esterified fatty acids are taken up by adipocytes and are then re-esterified into a triglyceride droplet to be stored (49). This lower triglyceride AUC is said to occur approximately one hour after the fall of the free fatty acids (49). Consequently, the increased insulin concentrations that occur because of an increase postprandial glucose surge during lower MF, may be increasing postprandial triglyceride clearance and its storage following meals (18).

Overall, evidence showing changes in glucose and insulin responses and blood lipids in overweight/obese adults during a reduced calorie diet are limited. Some of the differences in study findings are thought to be because of differing MF, sample sizes, and/or study conditions (18). More controlled research designed to increase adherence and compliance to dietary interventions is needed. Currently, there is not enough evidence in the literature to support or refute the impact of MF during a hypocaloric diet

being beneficial for glucose and/or insulin control as well as blood lipid changes during a weight loss program.

Appetite

Hypocaloric diets often leave individuals feeling hungry. This increases their risk of relapse and decreases compliance (38). However, a recent study has investigated an approach to reduce feelings of hunger by increasing MF and spreading out daily caloric intake (7). The effect of MF on hunger and satiety during a hypocaloric diet in overweight/obese individuals is less understood than with normal weight individuals.

Hunger and Satiety Ratings

There are papers which discuss MF and its effects on gastric sensations of "emptiness" or "fullness" prior to or following a meal (50), however only two studies were found using a controlled reduced-calorie diet of altered MF in overweight or obese subjects (9, 25). Eating more frequently has been thought to reduce hunger leading to a reduced overall energy intake (7, 9). Bachman and Raynor (9) showed that participants in the "grazing" MF condition had a significant reduction in hunger ratings, while those "gorging" had no significant changes in hunger throughout the duration of their study (9). These authors further suggest that consuming a reduced calorie diet to induce weight loss is better tolerated by obese patients when MF is increased (9). Conversely, no significant differences were shown by Cameron and colleagues (25) for the desire to eat, hunger, fullness, or prospective food consumption.

It has been suggested that the use of increased MF as a dietary strategy in free-living adults is related to the amount of food consumed at subsequent meals. Using a small (N=7) sample size of obese men, Speechly et al. (51) investigated the effects of

eating 1/3 their daily energy needs (~1000 kcals) as either a single meal or as five equal portions on subsequent *ad libitum* food intake. Although there were no significant differences between hunger ratings, those consuming the single preload ate 27% more (or ~358 kcals) during the *ad libitum* meal than those consuming the same meal in five portions (51). This study suggests that the increased MF can be a successful dietary strategy for reducing total daily caloric intake with some dietary portion and meal timing control. Future research merits investigation of this approach during a reduced calorie intervention for weight loss. To date it is unknown if these same results can be a benefit when increasing MF in order to keep calories consumed less than expended.

Appetite Stimulating Hormones and Hypothalamic Control.

The majority of the research investigating the hormonal responses of weight management and weight reduction interventions has studied the responses of two main hormones known to stimulate appetite: ghrelin and peptide YY (PYY) (8, 50). Ghrelin is a hormone produced primarily in the gastrointestinal tract where the majority of the hormone is secreted from the stomach to trigger the hypothalamus to sense feelings of hunger (50). PYY is a gut hormone that belongs to the pancreatic polypeptide family. It is released in response to meals, and its reduction stimulates hunger cascades (52). Some researchers suggest that increasing MF may have a direct effect on gastric stretch, hormones, and emptying that contribute to hunger and satiety during weight loss (51). Solomon and colleagues (53), suggest that a larger preprandial surge in ghrelin occurs with larger less frequent meals which is influenced by the increased gastric stretch at meal times. This could cause hormonal influences that increase initiation of meals that can lead to more between-meal snacking behavior and a higher daily caloric intake (53,

54). Additionally, the postprandial release of ghrelin is somewhat mediated by the release of insulin; ghrelin and insulin have an inverse relationship in healthy normal weight adults (53, 55).

Cameron et al. (25), conducted the only controlled feeding study found to investigate ghrelin and PYY in obese (BMI 37.1±4.5 kg/m^2) individuals undergoing a controlled hypocaloric feeding study for eight weeks. They reported no significant differences in feelings of hunger, fullness, or ghrelin and PYY between groups consuming an equi-hypocaloric diet in either three or six meals per day (25).

Although there is no unanimous agreement in the literature, it does appear that eating more frequently during a hypocaloric diet intervention can reduce hunger and increase satiety ratings compared to eating less frequently; however, the research using controlled feeding studies in overweight/obese adults is limited. Increased MF may lead to reduced consumption at subsequent meals and/or overall daily caloric intake (53). In conjunction with a hypocaloric diet plan, the impact of increasing MF and its ability to reduce hunger stimulating hormones and feelings of hunger offers an interesting preliminary mechanistic basis to warrant further implementation into practice. Regardless, more research is needed before we have the evidence to put this into practice.

Conclusions

While there is widespread anecdotal evidence, the amount of controlled research that has examined the impact of differing MF strategies is limited. Although more studies have been published recently, the availability of research in more specific populations of interest such as the overweight and obese is still lacking. Currently the literature lacks consistency in terms of the number of meals/snacks administered, as well as the duration

of interventions. Additional research is still needed to more fully understand the effects of MF on body weight and composition, markers of health, hunger and satiety ratings, and hormonal changes particularly in obese samples. Finally, most of the literature reviewed uses self-reported dietary intake for adherence to prescribed MF or caloric intake.

Future Direction

Interesting areas of exploration yet to be studied are changes in body composition, glucose, insulin, lipemia, and appetite measures in obese individuals utilizing pre-packaged portion-controlled meal replacements for all food products consumed. This approach could be advantageous in order to minimize the underreporting error (a nearly ubiquitous problem with dietary interventions research), while also minimizing the logistical burden of eating more often. Although more evidence is becoming available, the literature surrounding the impact of meal frequency is still sparse. This area of research needs to be explored because of the associated findings that can be applied to clinical situations as well as the common desire to lose or better manage weight. From a macro perspective this may be a small step toward attaining the Healthy People 2020 goals of decreasing the current obesity prevalence and decreasing obesity-related co-morbidities in the United States and across the globe.

References

1. Healthy People. Healthy People, 2020. In: Services USDoAaUSDoHaH, editor. Washinton DC2010.

2. Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity in the United States, 2009-2010. NCHS Data Brief. 2012 Jan(82):1-8. PubMed PMID: 22617494. Epub 2012/05/24. eng.

3. Mokdad AH, Marks JS, Stroup DF, Gerberding JL. Actual causes of death in the United States, 2000. JAMA. 2004 Mar 10;291(10):1238-45. PubMed PMID: 15010446. Epub 2004/03/11. eng.

4. Hedley AA, Ogden CL, Johnson CL, Carroll MD, Curtin LR, Flegal KM. Prevalence of overweight and obesity among US children, adolescents, and adults, 1999-2002. JAMA. 2004 Jun 16;291(23):2847-50. PubMed PMID: 15199035. Epub 2004/06/17. eng.

5. Song YM, Lee K, Sung J, Yang YJ. Changes in eating behaviors and body weight in Koreans: the Healthy Twin Study. Nutrition. 2013 Jan;29(1):66-70. PubMed PMID: 22858202. Epub 2012/08/04. eng.

6. de Castro JM. The Award for Nutrition and Metabolism. In search of the structure of a function: the eating behavior of free-living humans. Nutrition. 2007 Apr;23(4):374-7. PubMed PMID: 17331703. Epub 2007/03/03. eng.

7. Jakubowicz D, Froy O, Wainstein J, Boaz M. Meal timing and composition influence ghrelin levels, appetite scores and weight loss maintenance in overweight and obese adults. Steroids. 2012 Mar 10;77(4):323-31. PubMed PMID: 22178258. Epub 2011/12/20. eng.

8. Schwarz NA, Rigby BR, La Bounty P, Shelmadine B, Bowden RG. A review of weight control strategies and their effects on the regulation of hormonal balance. J Nutr Metab. 2011;2011:237932. PubMed PMID: 21822485. Epub 2011/08/09. eng.

9. Bachman JL, Raynor HA. Effects of manipulating eating frequency during a behavioral weight loss intervention: a pilot randomized controlled trial. Obesity (Silver Spring). 2012 May;20(5):985-92. PubMed PMID: 22173575. Epub 2011/12/17. eng.

10. Bachman JL, Phelan S, Wing RR, Raynor HA. Eating frequency is higher in weight loss maintainers and normal-weight individuals than in overweight individuals. J Am Diet Assoc. 2011 Nov;111(11):1730-4. PubMed PMID: 22027056. Epub 2011/10/27. eng.

11. Bhutani S, Varady KA. Nibbling versus feasting: which meal pattern is better for heart disease prevention? Nutr Rev. 2009 Oct;67(10):591-8. PubMed PMID: 19785690. Epub 2009/09/30. eng.

12. Edelstein SL, Barrett-Connor EL, Wingard DL, Cohn BA. Increased meal frequency associated with decreased cholesterol concentrations; Rancho Bernardo, CA, 1984-1987. Am J Clin Nutr. 1992 Mar;55(3):664-9. PubMed PMID: 1550041. Epub 1992/03/01. eng.

13. Hartmann C, Siegrist M, van der Horst K. Snack frequency: associations with healthy and unhealthy food choices. Public Health Nutr. 2012 Aug 16:1-10. PubMed PMID: 22894987. Epub 2012/08/17. Eng.

14. Palmer MA, Capra S, Baines SK. Association between eating frequency, weight, and health. Nutr Rev. 2009 Jul;67(7):379-90. PubMed PMID: 19566598. Epub 2009/07/02. eng.

15. Berteus Forslund H, Lindroos AK, Sjostrom L, Lissner L. Meal patterns and obesity in Swedish women-a simple instrument describing usual meal types, frequency and temporal distribution. Eur J Clin Nutr. 2002 Aug;56(8):740-7. PubMed PMID: 12122550. Epub 2002/07/18. eng.

16. Drummond S, Crombie N, Kirk T. A critique of the effects of snacking on body weight status. Eur J Clin Nutr. 1996 Dec;50(12):779-83. PubMed PMID: 8968697. Epub 1996/12/01. eng.

17. Gwinup G. Insulin resistance and antagonism and their clinical significance. W V Med J. 1963 Jul;59:190-2. PubMed PMID: 13951547. Epub 1963/07/01. eng.

18. Heden TD, Liu Y, Sims LJ, Whaley-Connell AT, Chockalingam A, Dellsperger KC, et al. Meal Frequency Differentially Alters Postprandial Triacylglycerol and Insulin Concentrations in Obese Women. Obesity (Silver Spring). 2012 Jun 22. PubMed PMID: 22836692. Epub 2012/07/28. Eng.

19. Bertelsen J, Christiansen C, Thomsen C, Poulsen PL, Vestergaard S, Steinov A, et al. Effect of meal frequency on blood glucose, insulin, and free fatty acids in NIDDM subjects. Diabetes Care. 1993 Jan;16(1):4-7. PubMed PMID: 8422824. Epub 1993/01/01. eng.

20. Jenkins DJ, Wolever TM, Ocana AM, Vuksan V, Cunnane SC, Jenkins M, et al. Metabolic effects of reducing rate of glucose ingestion by single bolus versus continuous sipping. Diabetes. 1990 Jul;39(7):775-81. PubMed PMID: 2191884. Epub 1990/07/01. eng.

21. Wolever TM. Metabolic effects of continuous feeding. Metabolism. 1990 Sep;39(9):947-51. PubMed PMID: 2202886. Epub 1990/09/01. eng.

22. Leidy HJ, Armstrong CL, Tang M, Mattes RD, Campbell WW. The influence of higher protein intake and greater eating frequency on appetite control in overweight and obese men. Obesity (Silver Spring). 2010 Sep;18(9):1725-32. PubMed PMID: 20339363. Epub 2010/03/27. eng.

23. Jenkins DJ, Wolever TM, Vuksan V, Brighenti F, Cunnane SC, Rao AV, et al. Nibbling versus gorging: metabolic advantages of increased meal frequency. N Engl J Med. 1989 Oct 5;321(14):929-34. PubMed PMID: 2674713. Epub 1989/10/05. eng.

24. Murphy MC, Chapman C, Lovegrove JA, Isherwood SG, Morgan LM, Wright JW, et al. Meal frequency; does it determine postprandial lipaemia? Eur J Clin Nutr. 1996 Aug;50(8):491-7. PubMed PMID: 8863008. Epub 1996/08/01. eng.

25. Cameron JD, Cyr MJ, Doucet E. Increased meal frequency does not promote greater weight loss in subjects who were prescribed an 8-week equi-energetic energy-restricted diet. Br J Nutr. 2010 Apr;103(8):1098-101. PubMed PMID: 19943985. Epub 2009/12/01. eng.

26. Duval K, Strychar I, Cyr MJ, Prud'homme D, Rabasa-Lhoret R, Doucet E. Physical activity is a confounding factor of the relation between eating frequency and body composition. Am J Clin Nutr. 2008 Nov;88(5):1200-5. PubMed PMID: 18996853. Epub 2008/11/11. eng.

27. Fabry P, Hejl Z, Fodor J, Braun T, Zvolankova K. The Frequency of Meals. Its Relation to Overweight, Hypercholesterolaemia, and Decreased Glucose-Tolerance. Lancet. 1964 Sep 19;2(7360):614-5. PubMed PMID: 14186149. Epub 1964/09/19. eng.

28. Ma Y, Bertone ER, Stanek EJ, 3rd, Reed GW, Hebert JR, Cohen NL, et al. Association between eating patterns and obesity in a free-living US adult population. Am J Epidemiol. 2003 Jul 1;158(1):85-92. PubMed PMID: 12835290. Epub 2003/07/02. eng.

29. Pearcey SM, de Castro JM. Food intake and meal patterns of weight-stable and weight-gaining persons. Am J Clin Nutr. 2002 Jul;76(1):107-12. PubMed PMID: 12081823. Epub 2002/06/26. eng.

30. Yannakoulia M, Melistas L, Solomou E, Yiannakouris N. Association of eating frequency with body fatness in pre- and postmenopausal women. Obesity (Silver Spring). 2007 Jan;15(1):100-6. PubMed PMID: 17228037. Epub 2007/01/18. eng.

31. Bandini LG, Schoeller DA, Cyr HN, Dietz WH. Validity of reported energy intake in obese and nonobese adolescents. Am J Clin Nutr. 1990 Sep;52(3):421-5. PubMed PMID: 2393004. Epub 1990/09/01. eng.

32. La Bounty PM, Campbell BI, Wilson J, Galvan E, Berardi J, Kleiner SM, et al. International Society of Sports Nutrition position stand: meal frequency. J Int Soc Sports Nutr. 2011;8:4. PubMed PMID: 21410984. Epub 2011/03/18. eng.

33. Leidy HJ, Campbell WW. The effect of eating frequency on appetite control and food intake: brief synopsis of controlled feeding studies. J Nutr. 2011 Jan;141(1):154-7. PubMed PMID: 21123467. Epub 2010/12/03. eng.

34. Kirk TR. Role of dietary carbohydrate and frequent eating in body-weight control. Proc Nutr Soc. 2000 Aug;59(3):349-58. PubMed PMID: 10997651. Epub 2000/09/21. eng.

35. Mills JP, Perry CD, Reicks M. Eating frequency is associated with energy intake but not obesity in midlife women. Obesity (Silver Spring). 2011 Mar;19(3):552-9. PubMed PMID: 20966909. Epub 2010/10/23. eng.

36. Berteus Forslund H. Should snacks be recommended in obesity treatment? A 1-year randomized clinical trial. Eur J Clin Nutr. 2008;62(1):1-10.

37. Vander Wal JS, Waller SM, Klurfeld DM, McBurney MI, Cho S, Kapila M, et al. Effect of a post-dinner snack and partial meal replacement program on weight loss. Int J Food Sci Nutr. 2006 Feb-Mar;57(1-2):97-106. PubMed PMID: 16849118. Epub 2006/07/20. eng.

38. Poston WS, Haddock CK, Pinkston MM, Pace P, Karakoc ND, Reeves RS, et al. Weight loss with meal replacement and meal replacement plus snacks: a randomized trial. Int J Obes (Lond). 2005 Sep;29(9):1107-14. PubMed PMID: 15925955. Epub 2005/06/01. eng.

39. Verboeket-van de Venne WP, Westerterp KR, Kester AD. Effect of the pattern of food intake on human energy metabolism. Br J Nutr. 1993 Jul;70(1):103-15. PubMed PMID: 8399092. Epub 1993/07/01. eng.

40. Finkelstein B, Fryer BA. Meal frequency and weight reduction of young women. Am J Clin Nutr. 1971 Apr;24(4):465-8. PubMed PMID: 5090633. Epub 1971/04/01. eng.

41. Antoine JM, Rohr R, Gagey MJ, Bleyer RE, Debry G. Feeding frequency and nitrogen balance in weight-reducing obese women. Hum Nutr Clin Nutr. 1984 Jan;38(1):31-8. PubMed PMID: 6693294. Epub 1984/01/01. eng.

42. Schiller JS, Lucas JW, Ward BW, Peregoy JA. Summary health statistics for U.S. adults: National Health Interview Survey, 2010. Vital Health Stat 10. 2012 Jan(252):1-207. PubMed PMID: 22834228. Epub 2012/07/28. eng.

43. Gwinup G, Byron RC, Roush WH, Kruger FA, Hamwi GJ. Effect of Nibbling Versus Gorging on Serum Lipids in Man. Am J Clin Nutr. 1963 Oct;13:209-13. PubMed PMID: 14067443. Epub 1963/10/01. eng.

44. Stote KS, Baer DJ, Spears K, Paul DR, Harris GK, Rumpler WV, et al. A controlled trial of reduced meal frequency without caloric restriction in healthy, normal-weight, middle-aged adults. Am J Clin Nutr. 2007 Apr;85(4):981-8. PubMed PMID: 17413096. Epub 2007/04/07. eng.

45. Smeets AJ, Westerterp-Plantenga MS. Acute effects on metabolism and appetite profile of one meal difference in the lower range of meal frequency. Br J Nutr. 2008 Jun;99(6):1316-21. PubMed PMID: 18053311. Epub 2007/12/07. eng.

46. Robinson JG, Wang S, Smith BJ, Jacobson TA. Meta-analysis of the relationship between non-high-density lipoprotein cholesterol reduction and coronary heart disease risk. J Am Coll Cardiol. 2009 Jan 27;53(4):316-22. PubMed PMID: 19161879. Epub 2009/01/24. eng.

47. Titan SM, Bingham S, Welch A, Luben R, Oakes S, Day N, et al. Frequency of eating and concentrations of serum cholesterol in the Norfolk population of the European prospective investigation into cancer (EPIC-Norfolk): cross sectional study. BMJ. 2001 Dec 1;323(7324):1286-8. PubMed PMID: 11731392. Epub 2001/12/04. eng.

48. McGrath SA, Gibney MJ. The effects of altered frequency of eating on plasma lipids in free-living healthy males on normal self-selected diets. Eur J Clin Nutr. 1994 Jun;48(6):402-7. PubMed PMID: 7925222. Epub 1994/06/01. eng.

49. Sadur CN, Eckel RH. Insulin stimulation of adipose tissue lipoprotein lipase. Use of the euglycemic clamp technique. J Clin Invest. 1982 May;69(5):1119-25. PubMed PMID: 7040473. Epub 1982/05/01. eng.

50. Odunsi ST, Vazquez-Roque MI, Camilleri M, Papathanasopoulos A, Clark MM, Wodrich L, et al. Effect of alginate on satiation, appetite, gastric function, and selected gut satiety hormones in overweight and obesity. Obesity (Silver Spring). 2010 Aug;18(8):1579-84. PubMed PMID: 19960001. Epub 2009/12/05. eng.

51. Speechly DP, Rogers GG, Buffenstein R. Acute appetite reduction associated with an increased frequency of eating in obese males. Int J Obes Relat Metab Disord. 1999 Nov;23(11):1151-9. PubMed PMID: 10578205. Epub 1999/12/01. eng.

52. Batterham RL, Heffron H, Kapoor S, Chivers JE, Chandarana K, Herzog H, et al. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. Cell Metab. 2006 Sep;4(3):223-33. PubMed PMID: 16950139. Epub 2006/09/05. eng.

53. Solomon TP, Chambers ES, Jeukendrup AE, Toogood AA, Blannin AK. The effect of feeding frequency on insulin and ghrelin responses in human subjects. Br J Nutr. 2008 Oct;100(4):810-9. PubMed PMID: 18394217. Epub 2008/04/09. eng.

54. Kanoski SE, Fortin SM, Ricks KM, Grill HJ. Ghrelin Signaling in the Ventral Hippocampus Stimulates Learned and Motivational Aspects of Feeding via PI3K-Akt Signaling. Biol Psychiatry. 2012 Aug 9. PubMed PMID: 22884970. Epub 2012/08/14. Eng.

55. Flanagan DE, Evans ML, Monsod TP, Rife F, Heptulla RA, Tamborlane WV, et al. The influence of insulin on circulating ghrelin. Am J Physiol Endocrinol Metab. 2003 Feb;284(2):E313-6. PubMed PMID: 12531744. Epub 2003/01/18. eng.

Table 1. Summary of weight-loss studies meeting search criteria.

Study (year)	Population	Methodology	Measurements	Significant Findings
Bachman and Raynor (2012)[7]	51±9.9 years 35.5± 1.3 kg/m^2 57.8% female	6-month RCT of (1200-1500 kcals/day). Randomized to either the three-meal group (3 meals/day) (n=25) or the grazing group (at least 100kcals every 2-3 hours) (n=26)	3-day diet records, VAS, physical activity questionnaire, and body composition (BIA) at 0, 3 & 6 months.	Grazing reported significant reductions in VAS hunger from 0 to 6 months.
Heden et al. (2012)[16]	39± 3 years BMI 34.5± 4.8 kg/m^2 8 Female	RCT 2-days; one 3 meal the other 6 meal feedings MR (1500 kcals/day) Subjects monitored for 12-hours.	Body composition (BodPod), waist & hip circumferences, fasting blood glucose and cholesterol, LDL, HDL, LDL, triglycerides at 0 and every 30 min for 12-hours.	Insulin iAUC significantly higher during the 3 meal condition. iAUC for triglycerides significantly lower for 3 meal condition.
Cameron et al. (2010)[23]	18-55 years, 37.1±4.5 kg/m^2 8 Female, 8 Male	8-week RCT (of -2931 kJ or –700 kcals /day). Randomized to either the high MF (3 meals +3 snacks/day) or low-MF (3 meals/day)	RMR, body composition (DEXA), VAS, Total Ghrelin, Peptide YY, at 0 & 8-weeks	No significant findings
Berteus Forslund et al. (2008)[34]	18-60 years 38.3±5.3 kg/m^2 104 Female, 36 Male	52-week RCT (individualized caloric restriction) Randomized to either the 3 meals +3 snacks/day group or the 3 meals/day group.	Dietary compliance monitored by phone interviews. Body weight, waist and hip circumferences, blood pressure, fasting glucose, insulin, cholesterol, LDL, HDL, and triglycerides at 0 & 52-weeks.	HDL had inverse relationship to the number of snacks eaten per day.
Vander Wal et al. (2006)[35]	18-65 years Mean BMI 38 kg/m^2 61 Female, 19 Male	8-week RCT Partial MR program (Kashi ®) n= 29 to 'post-dinner snack' (3 meals + 1 snack) and n=32 to 'no snack' (3 meals/day) group	Dietary compliance via questionnaire and empty packages. Body composition (BodPod), waist circumference, triglycerides, cholesterol, HDL, LDL, at 0 & 8-weeks	No significant findings
Poston et al. (2005)[36]	35-55 years BMI 25-30 kg/m^2 96-84% Female	24-week RCT partial MR program (SlimFast ®) (1200 kcals women, 1500 kcals men). 'Snackers' vs 'non-snackers' randomized to 3 MR only or 3 MR + 3 snacks	Dietary compliance via self-reported intake. Body weight, blood pressure, fasting glucose, insulin, cholesterol, HDL, LDL, VLDL at 0, 12, & 24-weeks	No significant findings
Bertelsen et al. (1993)[17]	64±2 years 32.2± 1.3 kg/m^2 NIDDM Patients 4 Female, 8 Male	2-days one of 2 meals another 6 meal feedings (811 kcals/day). Subjects monitored for 8-hours blood drawn every 30 min.	Glucose, insulin, & FFA at 0 & every 30 min for 8 hours.	2 meals produced an 84% greater max amplitude in glucose concentrations and increased FFA
Antonie et al. (1984)[39]	16-59 years BMI 31.8 kg/m^2 10 Female	14-day crossover (1200 kcals/day) randomized to 3 meals or 6 meals per day	All food except fruits/veggies provided to subjects. Nitrogen balance, fasting cholesterol and triglycerides at 0 and 14-days.	Nitrogen losses lower with higher MF.
Finkelstein and Fryer (1971)[38]	20-22 years BMI 27-33 8 Female	60-day RCT (1,700 kcals or 1,400 kcals/day) n=4 randomized to 6 meals/day n=4 randomized to 3 meals/day	Meals served in metabolic suite for adherence. Body weight, 6-day nitrogen balance, fasting glucose, total serum lipids, and cholesterol at 0 and 60-days	No significant findings

Abbreviations: BIA= bioelectrical impendence, BodPod= air displacement plethysmography, DEXA=dual-energy-X-ray absorptiometry, FFA= Free-fatty acids, MF= Meal Frequency, MR=Meal replacement, NIDDM=non-insulin dependent diabetes mellitus, RCT= Randomized controlled trial, VAS= Visual analogue scale

CHAPTER III

RESEARCH MANUSCRIPT

This chapter presents a research manuscript entitled “Meal frequency shows mixed results in blood-markers of health and appetite in obese women during an equi-hypocaloric portion-controlled weight loss intervention”. This manuscript will be submitted to the *Journal of the Academy of Nutrition and Dietetics*. It is authored by Michelle Kulovitz, Jason Beam, James McCormick, Len Kravitz, Christine Mermier, Carole Conn, Ann Gibson, Robert Ferraro, and Chad Kerksick. The manuscript follows the formatting and style guidelines of the journal and provides the figures and tables following the cited references as requested by the target journal. References are provided at the end of the chapter.

Title: Meal frequency shows mixed results in blood-markers of health and appetite in obese women during an equi-hypocaloric portion-controlled weight loss intervention

Running Title: Meal frequency and equi-hypocaloric diet intervention

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ABSTRACT

Background: Increased meal frequency (MF) may be associated with improvements in blood-markers of health and appetite during weight loss; however this claim has not been investigated using portion-controlled products with obese women.

Objective: To determine if consuming a portion-controlled equi-hypocaloric diet as a two meals per day (2 MF) or three meals plus three snacks per day sequence (6 MF) can be a successful strategy for obese women to improve weight loss, total ghrelin, glucose, insulin, lipid concentrations, and appetite regulation.

Design: Eleven (N = 11) obese female subjects (BMI 39.1 ± 7.6 kg/m^2) were randomized to either the 2 MF or 6 MF treatment condition for two weeks, completed a two-week washout, and alternated treatment conditions for the remaining two weeks.

Results: Body mass was successfully lost ($P < 0.05$) under both feeding regimens (2 MF: 2.8 ± 1.5 vs. 6 MF: 1.9 ± 1.5 kg). There were no significant differences found between conditions for fasting and AUC values of glucose, insulin, and total ghrelin. On average FFM decreased by 3.3 ± 2.6% following the 2 MF condition and an average increase of 1.2 ± 1.7% in FFM following the 6 MF condition ($P \leq 0.05$). Fasting HDL-C percent increased during the 2 MF condition was significantly greater than the 6 MF condition (1.3 ± 12.2% vs. 0.12 ± 10.3%) ($P \leq 0.05$). In a similar fashion between conditions, there was a significant decrease in glucose AUC (2 MF:13,122 ± 1726 vs. 6 MF:12,296 ± 1,870 mg/dL·120 min, $P \leq 0.05$) and increased hunger ratings (2 MF: 1,894 ± 887 vs. 6 MF: 3,131 ± 1,563 mm·120 min) independent of MF.

Conclusions: Increased MF does not promote greater improvements in blood-markers of health, appetite control, or total-ghrelin levels during a portion-controlled reduced-calorie

diet intervention in obese women. While reduced MF may attenuate losses of HDL-C, increased MF may reduce FFM loss during weight loss. Based on our results, we cannot support an altered MF during an equi-hypocaloric diet as superior to a reduced-calorie diet alone.

Keywords: ghrelin, obesity, hunger, appetite, reduced-calorie diet, eating frequency

INTRODUCTION

Obesity is a multi-factorial issue in clinical weight management. Changing eating behaviors by implementing increased dietary structure is becoming more commonly included in weight management strategies for patients undergoing supervised weight loss interventions. Some hypocaloric diets designed to induce body weight loss do, however, result in unfavorable metabolic changes that can lead to increased ratings of hunger and cravings, increased levels of key adipokines such as circulating ghrelin, as well as increased postprandial ghrelin concentrations following meals[1].

One fundamental principle that seems to be the most common component to clinically supervised hypocaloric dietary interventions is the idea of structured daily meal frequency (MF), where consuming meals at regular time intervals is thought to be associated with greater weight loss success[2]. Increased MF has been hypothesized to have an effect on energy intake and body weight regulation due to its potential role in resting metabolic rate (RMR), hunger and satiety, and appetite hormones (i.e. total ghrelin) [1, 3].

Currently, there is limited research with obese (BMI $\geq$ 30 kg/m^2) subjects demonstrating any impact of MF on metabolic blood-based markers of health including glucose and insulin responses, serum lipid concentrations, and levels of specific appetite hormones (i.e. serum total ghrelin). Abnormal levels of circulating low-density

lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), total cholesterol, triglycerides (TG), and insulin levels are acknowledged as factors that may increase cardiovascular disease (CVD) risk[4]. Presently, findings on the impact of altering MF on serum lipid, glucose, and insulin concentrations using obese [4-7] or normal weight adults [8-11] is inconclusive. Moreover, few studies have investigated the impact of MF and blood-based markers of health using obese subjects undergoing a clinically supervised weight loss trial [5, 6, 12]. It has been noted that many different people, but in particular obese individuals, significantly underreport their energy intake. Furthermore obesity was found to be a significant predictor of underreporting energy intake [13, 14]. Secondary to these known outcomes, it is surprising that most MF studies investigating changes in various metabolic markers of health have relied on self-reported dietary intake [4, 6, 8, 11]. On the contrary, very few studies have provided portion-controlled meals and/or meal replacements to control for measurement and portion control error [5, 7, 9].

Human appetite regulation in response to weight changes involves multiple complex biochemical regulatory pathways within the body that result in many factors that influence human hunger and satiety sensations[15]. According to a recent review, the evidence supporting the influence of MF on appetite regulation is inconclusive [16]. It has been suggested that a "nibbling" or grazing pattern of eating (eating more frequently throughout the day) may exert various favorable metabolic benefits (i.e increased resting metabolic rate, and/or improved markers of health) when compared to "gorging" (eating $\leq$ 2 meals per day) [16]. However, consistency within the literature is lacking in regard to MF and the optimal number of meals and/or snacks per day for weight management.

Consistent reports in the literature indicate that consuming an equi-hypocaloric diet of differing MF during weight loss has no statistically significant impact on the amount of weight that is lost following 60 days to eight-weeks of active weight loss [12, 17-19]. However, some evidence exists to suggest that increased MF may improve both blood-based markers of health and fuel utilization, indicators of appetite, as well as hunger ratings[4]. In consideration of these findings, it remains possible that altering MF may help with weight loss and weight maintenance in overweight or obese individuals [1, 4, 5, 19, 20].

To date, no research study has investigated changes in blood-markers of health, hunger and appetite responses of obese women during a strict calorie- and portion-controlled hypocaloric diet examining the impact of two meals per day versus six meals per day to induce weight loss. The use of portion-controlled products may assist in compliance with a reduced-calorie diet because they do not require the subject to weigh or measure food items. This may alleviate some of the underreporting error that is commonly associated with food recall accuracy by obese individuals. Thus, the primary purpose of the present study was to determine the changes in total-ghrelin, blood glucose, insulin, lipid concentrations, and sensations of hunger due to an equi-hypocaloric diet intervention with varying meal frequency in obese women during a "gorging" (two meals per day sequence, 2 MF) versus "nibbling" (three meals and three snacks per day, 6 MF) sequence. We hypothesized that the extent of change in postprandial blood-markers of health would be more favorable and sensations of hunger would be less following the "nibbling" sequence.

MATERIALS AND METHODS

Subjects

Fifteen non-diabetic obese women aged 35-60 years with a BMI ≥ 30 kg/m^2 were recruited for this study (Table 1). Eleven subjects completed the study. Two subjects were withdrawn due to non-compliance; one subject withdrew due to family issues, and the other withdrew due to time constraints. The Human Research Review Committee (HRRC) of the University of New Mexico approved this study prior to the commencement of testing. All subjects were tested at a local weight management clinic: Southwest Endocrinology Associates Weight Management Center located in Albuquerque, NM.

The inclusion criteria for all study participants required that they were: non-diabetic, currently following a sedentary to moderately-active lifestyle, weight stable or gaining (≤ 3kg over the last 6 months), eating ≤ four meals and/or snacks per day, able to travel to the clinic for weekly meetings, able to travel biweekly to the clinic in the morning for blood draws, and able to have a venous catheter placed for blood collection.

Individuals were excluded if they were: using medications (i.e diuretics, some glucosamides, etc) or dietary supplements (i.e. ephedra, thermogenics, capsaicin, caffeine, or any herbal or botanical purported to increase weight loss) that could affect body composition, have a pacemaker, were a current a smoker or nicotine user, experienced a loss in body weight >3 kg over the last six months, or experienced any dramatic change in physical activity patterns within the six months prior to the onset of the study. Other exclusion criteria included being previously diagnosed or treated for: diabetes, abnormal thyroid conditions, liver or kidney diseases, gastrointestinal problems that could affect dietary adherence or absorption, cancer or other wasting disorders, were pregnant, lactating, or were planning to become pregnant during study duration.

Moreover, any participant who was unwilling to comply with any aspect of the study protocol (i.e. travel to the testing site weekly, attend group or individual intervention meetings, assessments, complete a daily detailed food diary, or properly store food provided for the study duration) was excluded. Lastly, any individual who presented with or reported an allergy to soy, peanut, latex, had a gluten intolerance or sensitivity or had an active eating disorder was excluded.

Eligible subjects who were currently prescribed and/or taking anti-hypertensive or lipid-lowering medications were asked to maintain all medications as prescribed. All subjects were asked to maintain their usual physical activity levels, document all physical activity, and to refrain from drinking alcoholic during the study.

Participants were required to sign an approved informed consent form prior to their participation. Subjects completed a preliminary screening, which included: health history, meal frequency, and physical activity questionnaires. Participants completing the preliminary screening who met all eligibility requirements, attended the orientation meeting, completed baseline assessments were randomized into one of two treatment conditions.

Design

This research study utilized a randomized, cross-over, repeated measures quasi-experimental design which consisted of four testing sessions conducted at similar times in the morning. Additionally, participants were required to meet weekly at the clinic for measurements and behavioral education sessions.

Compliance and Retention

A group orientation and familiarization meeting for all study participants was mandatory for inclusion in the study. Subjects were taught how to log food intake and time of food intake, how to log physical activity, and how to use their food scale. If a subject did not consume all the food provided they were asked to weigh the food left over and document it in their food log. Additionally, subjects received weekly reminders of the importance of adherence, accuracy, and truthfulness of dietary intake and physical activity reporting, regardless if they strayed from the prescribed meal plan or their normal physical activity pattern at any time point during the study.

Meal frequency intervention

Subjects were randomly assigned to an equi-hypocaloric diet of either the 2 MF meal pattern or the 6 MF meal pattern. The 2 MF pattern participants were instructed to consume allocated meals every 5-6 hours while awake; while the 6 MF pattern participants were instructed to consume allocated meals/snacks every 2-3 hours while awake. Meals provided to subjects were defined as an eating occasion with a caloric intake between 200 and 300 kcals, while a snack was defined as an eating occasion between 100 and 200 kcals. During the washout phase participants were instructed to consume the allocated meals and snack every 3-4 hours. Throughout all treatment conditions food products were identical as subjects received the identical assortment of pre-packaged food products for the study duration. Dietary measures were standardized ensuring the dietary intake of all participants was approximately 1200 calories/day with ~75g/day of protein. Daily subject dietary macronutrients averaged 52% carbohydrates, 27% protein, and 21% fat. Subjects consumed their designated meal pattern eating only

Nutrisystem® meal products which were supplied to the subject. Participants also supplemented this diet with a limited selection of Nutrisystem® approved fresh fruits and vegetables in specific quantities. Fresh items added accounted for only approximately 187 kcals/day, 10% of daily caloric intake of the subjects' daily total of ~1200 kcals per day. The subjects consumed identical food products throughout the study. Subjects were randomized into one of the two MF patterns for two weeks (Phase I). Following a two-week period of three Nutrisystem® meals + one snack per day (Washout), the subjects consumed the alternate pattern for two weeks (Phase II).

Protocol for experimental trial visits

Participants reported to the clinic in the morning after a 12- hour overnight fast with water *ad libitum* and having completed a dietary and food recall log. Subjects voided their bladder and bowels prior to measurements. The measurements included: resting metabolic rate, body weight (BW), height, waist and hip circumferences, and body composition.

Subsequently, they consumed a test meal (400 kcals, 49% carbohydrates, 25% protein, 26% fat) at baseline, following Phase I, prior to and following Phase II. This test meal was chosen because it was of similar macronutrient content as the daily macronutrient composition of the prescribed meal plan and it contained the average caloric intake of meals for both the 2 MF and 6 MF conditions (similar to the meals consumed during the washout condition). Following each two-week period, lipid profiles, glucose/insulin metabolism, and RMR were measured under fasting conditions.

Participants also engaged in weekly group/individual meetings that utilized identical educational content and behavior modification strategies important for weight loss and weight maintenance. This also provided participants the opportunity to ask questions and discuss concerns.

Experimental procedures

Anthropometric measurements

Anthropometric measurements taken by trained research personnel include: waist and hip circumferences, height, and weight. Barefoot standing height was measured to the nearest one cm using a standard wall stadiometer (SECA Model #2161814009). Body weight was measured to the nearest 0.1 kg using a calibrated body weight scale (UWE Model# UFM-L-300). Waist circumference was measured over light clothing to the nearest 0.5 cm superior to the suprailiac crest, and hip circumference was measured to the nearest 0.5 cm at the largest circumference of the hips and buttocks. For body composition estimation, subjects were asked to follow all recommended pre-test guidelines for bioelectrical impedance analysis per manufacturer instructions prior to each measurement. The Tanita-BC-418 eight-electrode bioelectrical impedance analysis device (Tanita Corporation, Arlington Heights, IL, USA) was used to assess average changes in body composition in relation to changes in percent body fat (%BF), fat-free mass (FFM), and fat-mass (FM). This device has been validated for use in adult populations (BMI: 16-41 kg/m^2) for body composition analysis [21].

Resting metabolic rate (RMR)

RMR was tested using the BodyGem ® (Microlife, Clearwater, FL) in accordance with manufacturer instructions. BodyGem® has been validated against a metabolic cart in healthy normal weight individuals and was found to be more accurate than using Harris-Benedict prediction equations for RMR [22]. Participants were seated in a quiet room and had not participated in any strenuous exercise for at least 24-hours prior to measurement. RMR was measured prior to beginning and upon completing each two-week MF condition (baseline, week two, week four, and week six).

Blood sampling

Venous blood samples were collected using a 22-gauge Teflon catheter inserted into an antecubital arm or dorsal hand vein of the participant. A baseline blood sample (~15 mL) was collected into sterilized vacutainer serum separator tubes. Following consumption of the test meal, a blood sample was collected every 30 minutes over a two-hour period (0, 30, 60, 90, 120 minutes). The samples were first allowed to clot and then were centrifuged (3500 rpm) at 25°C for 15 minutes. All serum samples were then stored in separate tubes at -80°C until analysis to minimize freeze-thaw cycles of the samples. Identical procedures were followed at each time interval. The catheter was kept patent by flushing with 1.0-2.0 mL of saline after every blood draw.

Processing of blood samples

Serum samples were sent to a local private laboratory (Albuquerque, NM) for determination of glucose and lipid panels (total-cholesterol, LDL-C, HDL-C, TG). Serum total-ghrelin (includes both acyl and des-acyl ghrelin) samples were analyzed in duplicate in house using an enzyme immunoassay kit (EZGRT-89K; EMD Millipore, St. Charles, MO). Serum insulin concentrations were analyzed in duplicate in house using an ELISA

kit (EIA-2935; DRG International, Inc. Springfield, New Jersey, USA). ELISA procedures were performed using a Bio-Rad iMark microplate reader (168-1130EDU, Bio-Rad,) at a wavelength of 450 nm. Standard curves were produced using a 4-parameter logistic equation and Microplate Manager software (version 6.0) from Bio-Rad (Hercules, CA). High and low controls were calculated for total-ghrelin which yielded an average coefficient of variation of 5.1% for the high control and 13.94% for the low control. Control values for each insulin plate yielded an average coefficient of variation of 12.8%.

Visual analogue scales

The visual analogue scales (VAS) were 100 mm in length; the scale and questions have been validated for sensations reproducibility in test meal studies[15]. Subjective satiety and hunger were assessed via four VAS questions at the same time intervals when blood was collected and analyzed (0, 30, 60, 90, 120 minutes following test meal consumption). Subjective measures assessed by the VAS included: hunger or feelings of "emptiness" (VAS1), satiety (VAS2), "fullness" (VAS3), and the "urge to eat right now"(VAS4).

Statistics

An *apriori* power analysis was performed to determine the number of subjects that would be needed to detect significant differences. A sample size of 10 was determined by a power estimation via G*Power Version 3.1.0 (Franz Faul, Universitat Kiel, Germany) utilizing statistical methods of ANOVA with repeated measures including within-subjects interactions.

All data are presented as means ± SD, and the data was analyzed using SPSS (version 19.0, Chicago, IL). Postprandial blood measurements following the test meal conditions were compared using the area under the curve (AUC), techniques as calculated in Prism (GraphPad Software, La Jolla, CA) using the trapezoidal method [23]. Data for anthropometrics, RMR, as well as fasting and AUC measures for blood markers were analyzed using a one-way (four conditions) repeated measures ANOVA to identify significant within-subjects main effects. Pairwise comparisons using paired-samples t-tests were conducted for any significant main effects with a Tukeys HSD post-hoc correction. Blood marker and VAS values during the two-hour trials were analyzed using a two-way (four conditions by five time points) repeated measures ANOVA to indentify if there any significant within-subjects main effects and intervention effects. Pairwise comparisons using paired samples t-tests were conducted for any significant main effects with a Tukeys HSD post-hoc correction. In order to observe changes between the 2 MF and 6 MF conditions, percent change data was calculated based on changes occurring due the two-week treatment condition. A value of $P \leq 0.05$ was set to determine statistical significance.

RESULTS

Anthropometric and metabolic measurements

Average subject characteristics and anthropometircs at baseline and at the six-week follow up visit are shown in Table 1. There was a significant main effect for BW, BMI, waist and hip circumferences, FM, FFM, and percent body fat (%BF), observed after the six week intervention ($P \leq 0.05$) (Table 1). Pairwise comparisons indicated that

BW, BMI, waist and hip circumferences, FM, FFM, %BF, were significantly lower after the 2 MF, washout, and 6 MF when compared to baseline (Table 2). On average there was a decrease of 3.3 ± 2.6% in FFM following the 2 MF condition, and an average increase of 1.2 ± 1.7% in FFM following the 6 MF condition ($P \leq 0.05$) (Figure 1). In addition, FFM was found to be significantly higher ($P \leq 0.05$) after the 6 MF condition (51.1 ± 8.7 kg) when compared to the washout (50.6 ± 8.9 kg). There was no significant main effect for condition or time for RMR from baseline to all other measured time points ($P > 0.05$).

<<<Insert Table 1 about here>>>

<<<Insert Figure 1 about here>>>

Serum insulin and glucose

Although, fasting concentrations of glucose and insulin decreased from baseline to the six-week follow-up, these differences were not significant. No significant main main effects of time were found between fasting concentrations of glucose, insulin, or insulin AUC. Glucose AUC showed a significant decrease ($P = 0.006$) from baseline to the six-week follow up (Table 3). Additionally, as expected, there was a statistically significant main effect of time for glucose AUC between conditions ($P = 0.009$). Pairwise comparisons indicated that glucose concentrations at 60 post test meal were significantly ($P = 0.022$) lower at the six-week follow-up than at baseline (Figure 2).

<<<Insert Figure 2 about here>>>

Serum lipid panel

No significant main effects of time were reported for fasting triglycerides, total cholesterol, or LDL-C between conditions; fasting HDL-C levels, however, were found

to be different across conditions ($P \leq 0.05$). Pairwise comparisons indicated that there were significant differences between baseline and all other time points measured for HDL-C ($P \leq 0.05$). In addition, percent changes in HDL-C were found to be significantly greater ($P \leq 0.05$) during the 2 MF condition ($1.3 \pm 12.2\%$) when compared to the 6 MF ($0.12 \pm 10.3\%$).

Serum total-ghrelin responses

Fasting total-ghrelin values were found to be higher following the 6 MF condition compared to the 2 MF condition; however this change was not statistically significant (Figure 3). Although the results were not significant, fasting baseline levels of total-ghrelin at all other measured time points were increased ($P > 0.05$). Additionally, there were no significant main effects for fasting total-ghrelin and total-ghrelin AUC between conditions or at the six-week follow up.

<<<Insert Figure 3 about here>>>

Appetite Measures

Changes in subjective hunger (VAS1), satiety (VAS2), fullness (VAS3), and the urge to eat (VAS4) showed significant increases when compared to baseline and following consumption of the test meal values independent of condition ($P \leq 0.05$). However, there was a significant main effect for VAS1 AUC ($P = 0.042$) between conditions. Pairwise comparisons indicated that VAS1 AUC was significantly higher ($P \leq 0.05$) during the 2 MF, washout, 6 MF, and at the six-week follow-up conditions when compared to baseline.

DISCUSSION

The aim of the present study was to determine the effects of a portion-controlled equi-hypocaloric diet of 2 MF and 6 MF on total-ghrelin, glucose, insulin, and lipid concentrations. Additionally, we investigated changes in sensations of hunger and satiety in obese women.

The primary finding of this study was that a 2 MF sequence may result in a greater percent elevation in HDL-C concentrations when compared to the 6 MF sequence. Alternatively, FFM changes were limited under 6 MF when compared the 2 MF sequence. In addition, fasting total-ghrelin, insulin, glucose, total-cholesterol, LDL-C, and triglyceride concentrations did not differ significantly between two-weeks of consuming two MF conditions.

A similar percentage of body weight was lost following the 2 MF (2.7 ± 1.4%) and 6 MF (2.0 ± 1.5%) conditions. Subjects had an average percent body weight loss of 6.6 ± 1.6% (6.2 ± 1.9 kg) following the six-week trial. MF did impact the amount of FFM lost, but not the amount of FM lost between trials. There was a significant difference in FFM changes between the 2 MF and 6 MF treatment conditions whereby subjects experienced a 3.3 ± 2.6% average decrease following the 2 MF condition and a 1.2 ± 1.7% average increase following the 6 MF condition ($P \leq 0.05$) (Figure 1). Although urinary nitrogen was not measured during this study, a possible explanation is that subjects had greater nitrogen losses during the 2 MF condition versus the 6 MF condition. Even though dietary protein intake was held constant between trials, there have been reports that reduced MF can promote greater protein breakdown and perhaps greater amounts of weight lost as FFM due to the increased lengths of time between feedings[18, 24]. During the 2 MF condition, subjects would typically go without eating for five to six

hours between main meals and 15-18 hours between their evening meal and the first meal of the following day. Additionally, the consumption of dietary protein throughout the day at six eating occasions versus two eating occasions may have a sparing effect on FFM in non-dieting scenarios as well as during weight loss; however, this should be confirmed with additional controlled research[25, 26].

In conjunction with the present findings, other studies have reported favorable changes in cholesterol components that mirror decreases in body mass[27, 28], a change which may reduce the risk of CVD[4, 29]. When exercise is held constant, weight loss is associated with a reduction in HDL-C which may negatively impact the known beneficial properties of HDL-C with respect to atherosclerotic progression[27]. Results of the present study indicate that obese women consuming the 2 MF condition may be able to preserve greater HDL-C concentrations during weight loss as compared to consuming the same food and caloric intake in the 6 MF condition (Table 3). In accordance with previous findings in healthy normal weight females[30], current results indicate that consuming a 2 MF sequence results in a significantly greater percent change in HDL-C ($P \leq 0.05$) when compared to the change seen during the 6 MF sequence ($1.3 \pm 12.2\%$ vs $0.12 \pm 10.3\%$, respectively). Previous evidence also indicates that obese subjects consuming a hypocaloric diet with reduced meal frequency (three meals per day) may significantly decrease triglyceride concentrations when compared to six meals[5]; similar changes were also reported for total-cholesterol, insulin, and glucose concentrations in lean normal-weight healthy men[9, 31] and normal-weight men and women[10] without calorie restriction. Findings from the present study do not support these outcomes. Non-significant reductions from baseline were seen for triglyceride, insulin, and glucose concentrations

with both feeding regimens (Table 3); no significant differences were revealed between the two feeding frequencies. Our findings may suggest that when a dietary intervention is carried beyond a single dose response, as a part of a portion-controlled weight loss program, meal frequency may no longer operate as an intervening force, but more work is needed to further explore this suggestion.

Non-significant decreases in fasting insulin (9.3%) and insulin AUC (7.7%) were found with 6 MF in comparison to 2 MF. These findings are consistent with previous research that also indicated no statistically significant differences occurred between MF conditions for insulin in healthy normal-weight men[32], healthy normal-weight women [30], and overweight men and women [6, 33]. In examining these data, high variability in individual responses reduced the ability to detect significance, but this does not eliminate the possibility that our findings may wield some level of practical or clinical significance. For example, in a study investigating increased MF on glucose and insulin concentrations in subjects with Type II diabetes mellitus, there was a significantly greater amplitude in glucose concentrations following the two meal condition versus the six meal condition[34]. These results were similar to our findings; we observed reduced insulin concentrations as well as a 0.56% increase and a 3.3% decrease in glucose AUC following the 2 MF and 6 MF conditions, respectively (Table 3). Although these results were not statistically significant between the 2 MF and 6 MF conditions, we did see an overall significant decrease ($P \leq 0.05$) in glucose AUC from baseline to the six-week follow-up (Table 3) indicating that weight loss, independent of meal frequency may be enough to significantly reduce glucose concentrations postprandially.

As anticipated, appetite measures using AUC calculations for the four VAS questions resulted in significantly increased perceived hunger rating (VAS1) AUC from baseline to all other measure time points ($P \leq 0.05$). This indicates subjects were more hungry due to the reduced calorie diet. Interestingly, there were no significant differences in hunger ratings between the 2 MF and the 6 MF conditions. These results were similar to previous findings within healthy normal-weight subjects where increasing MF from three to six meals per day significantly increased hunger[35-37]. These results may indicate that when caloric intake is held constant within a controlled feeding study using portioned products, obese subjects may rate hunger to be higher independent of MF. Studies have found that obese individuals have an increased likelihood of underreporting of energy intake, especially with snack items[4, 35]. Since previous research investigating hunger ratings based on test-meal responses in obese subjects used self-reported energy intake for adherence, it may be possible findings showing increase satiety rating may be due to underreporting errors resulting in increased caloric consumption.[36] Some literature has also shown that increased MF can have a negative effect in body weight regulation because it increases the opportunities to eat and subsequently lead to more underreporting of energy intake.[10, 35] If, in fact, the use of portion-controlled products in the present study increased compliance and decreased associated underreporting error; this may indicate that total daily caloric intake may be independent of MF for hunger control in clinical practice.

Circulating ghrelin is known to increase hunger ratings and display an endogenous secretory rhymicity that can be related closely with meal patterns [38]. Our results indicated that both total ghrelin fasting concentrations and total ghrelin AUC were

found to be similar to baseline at all measured time points. Similar to our findings, diet-induced weight loss has been associated with a decrease in postprandial ghrelin suppression[1]. We hypothesized that total ghrelin AUC concentrations would be lower following the 6 MF when compared to the 2 MF; however, total ghrelin AUC following the 2 MF condition was found to be lower than the 6 MF condition. Furthermore, fasting total ghrelin concentrations and AUC were 18.5% and 22.5% higher, respectively, following the 6 MF condition compared to 9.8% and 3.2% higher, respectively, following the 2 MF condition; this pattern is similar to previous findings in obese adults[39]. Total fasting ghrelin concentrations were found to be significantly correlated to fasting ratings of hunger (VAS1) at baseline ($r = 0.650$, $P = 0.03$) and following the 2 MF condition ($r = 0.641$, $P = 0.034$), but not following the 6 MF condition. Previous evidence exists that caloric restriction alone is known to increase ghrelin levels and to be correlated with associated hunger responses in obese men[40].

Findings of the current study should be interpreted within the context of the study limitations. Four of the original fifteen women withdrew or were dropped from the study. This confounded the already high individual variability in our data and subsequently reduced our statistical power. It does suggest, however, that changes in these parameters are not acute, and may require an adaptation to MF patterns. Furthermore, our two-week dietary manipulation in treatment conditions may not have been robust enough to elicit changes in glucose, insulin, or total ghrelin concentrations to a degree that produced statistical significance.

CONCLUSIONS

This is the first study to investigate changes in blood-markers of health and appetite regulation using all portion-controlled products during a meal frequency intervention. According to the present results, increasing MF to eating six occasions per day did not promote greater improvements in blood-markers of health, appetite control, or total ghrelin levels during a portion-controlled strict reduced-calorie diet intervention in these obese women. Within the context of this study design, reducing MF to two eating occasions per day may attenuate losses in the beneficial function of HDL-C during weight loss when compared to eating on six occasions per day. Conversely, consuming a reduced-calorie 2 MF diet may contribute to greater losses in FFM, while a 6 MF approach may aid in preservation of FFM. Current evidence does not support increased MF as a successful strategy to aid in reducing subjective hunger or appetite control when strict dietary measures are taken to reduce underreporting error in obese women. Future research is needed with longer intervention periods of two versus six meals per day using portion-controlled products.

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References

1. Jakubowicz D, Froy O, Wainstein J, Boaz M. Meal timing and composition influence ghrelin levels, appetite scores and weight loss maintenance in overweight and obese adults. *Steroids.* Mar 10 2012;77(4):323-331.

2. Kong A, Beresford SA, Alfano CM, et al. Self-monitoring and eating-related behaviors are associated with 12-month weight loss in postmenopausal overweight-to-obese women. *J Acad Nutr Diet.* Sep 2012;112(9):1428-1435.

3. Bachman JL, Raynor HA. Effects of manipulating eating frequency during a behavioral weight loss intervention: a pilot randomized controlled trial. *Obesity (Silver Spring).* May 2012;20(5):985-992.

4. Farshchi HR, Taylor MA, Macdonald IA. Beneficial metabolic effects of regular meal frequency on dietary thermogenesis, insulin sensitivity, and fasting lipid profiles in healthy obese women. *Am J Clin Nutr.* Jan 2005;81(1):16-24.

5. Heden TD, Liu Y, Sims LJ, et al. Meal frequency differentially alters postprandial triacylglycerol and insulin concentrations in obese women. *Obesity (Silver Spring).* Jun 22 2012.

6. Poston WS, Haddock CK, Pinkston MM, et al. Weight loss with meal replacement and meal replacement plus snacks: a randomized trial. *Int J Obes (Lond).* Sep 2005;29(9):1107-1114.

7. Vander Wal JS, Waller SM, Klurfeld DM, et al. Effect of a post-dinner snack and partial meal replacement program on weight loss. *Int J Food Sci Nutr.* Feb-Mar 2006;57(1-2):97-106.

8. Edelstein SL, Barrett-Connor EL, Wingard DL, Cohn BA. Increased meal frequency associated with decreased cholesterol concentrations; Rancho Bernardo, CA, 1984-1987. *Am J Clin Nutr.* Mar 1992;55(3):664-669.

9. Jenkins DJ, Wolever TM, Vuksan V, et al. Nibbling versus gorging: metabolic advantages of increased meal frequency. *N Engl J Med.* Oct 5 1989;321(14):929-934.

10. Stote KS, Baer DJ, Spears K, et al. A controlled trial of reduced meal frequency without caloric restriction in healthy, normal-weight, middle-aged adults. *Am J Clin Nutr.* Apr 2007;85(4):981-988.

11. Titan SM, Bingham S, Welch A, et al. Frequency of eating and concentrations of serum cholesterol in the Norfolk population of the European prospective investigation into cancer (EPIC-Norfolk): cross sectional study. *BMJ.* Dec 1 2001;323(7324):1286-1288.

12. Finkelstein B, Fryer BA. Meal frequency and weight reduction of young women. *Am J Clin Nutr.* Apr 1971;24(4):465-468.

13. Bandini LG, Schoeller DA, Cyr HN, Dietz WH. Validity of reported energy intake in obese and nonobese adolescents. *Am J Clin Nutr.* Sep 1990;52(3):421-425.

14. Mendez MA, Wynter S, Wilks R, Forrester T. Under- and overreporting of energy is related to obesity, lifestyle factors and food group intakes in Jamaican adults. *Public Health Nutr.* Feb 2004;7(1):9-19.

15. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes Relat Metab Disord.* Jan 2000;24(1):38-48.

16. La Bounty PM, Campbell BI, Wilson J, et al. International Society of Sports Nutrition position stand: meal frequency. *J Int Soc Sports Nutr.* 2011;8:4.

17. Bortz WM, Wroldsen A, Issekutz B, Jr., Rodahl K. Weight loss and frequency of feeding. *N Engl J Med.* Feb 17 1966;274(7):376-379.

18. Garrow JS, Durrant M, Blaza S, Wilkins D, Royston P, Sunkin S. The effect of meal frequency and protein concentration on the composition of the weight lost by obese subjects. *Br J Nutr.* Jan 1981;45(1):5-15.

19. Cameron JD, Cyr MJ, Doucet E. Increased meal frequency does not promote greater weight loss in subjects who were prescribed an 8-week equi-energetic energy-restricted diet. *Br J Nutr.* Apr 2010;103(8):1098-1101.

20. Smeets AJ, Westerterp-Plantenga MS. Acute effects on metabolism and appetite profile of one meal difference in the lower range of meal frequency. *Br J Nutr.* Jun 2008;99(6):1316-1321.

21. Jebb SA, Cole TJ, Doman D, Murgatroyd PR, Prentice AM. Evaluation of the novel Tanita body-fat analyser to measure body composition by comparison with a four-compartment model. *Br J Nutr.* Feb 2000;83(2):115-122.

22. Melanson EL, Coelho LB, Tran ZV, Haugen HA, Kearney JT, Hill JO. Validation of the BodyGem hand-held calorimeter. *Int J Obes Relat Metab Disord.* Nov 2004;28(11):1479-1484.

23. Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology.* Oct 2003;28(7):916-931.

24. Arnal M, Obled C, Attaix D, Patureau-Mirand P, Bonin D. Dietary control of protein turnover. *Diabete Metab.* Nov-Dec 1987;13(6):630-642.

25. Areta JL, Burke LM, Ross ML, et al. Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *J Physiol.* May 1 2013;591(Pt 9):2319-2331.

26. Westerterp-Plantenga MS, Lemmens SG, Westerterp KR. Dietary protein - its role in satiety, energetics, weight loss and health. *Br J Nutr.* Aug 2012;108 Suppl 2:S105-112.

27. Aicher BO, Haser EK, Freeman LA, et al. Diet-induced weight loss in overweight or obese women and changes in high-density lipoprotein levels and function. *Obesity (Silver Spring).* Oct 2012;20(10):2057-2062.

28. Brinton EA, Eisenberg S, Breslow JL. A low-fat diet decreases high density lipoprotein (HDL) cholesterol levels by decreasing HDL apolipoprotein transport rates. *J Clin Invest.* Jan 1990;85(1):144-151.

29. Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation.* Jan 1989;79(1):8-15.

30. Murphy MC, Chapman C, Lovegrove JA, et al. Meal frequency; does it determine postprandial lipaemia? *Eur J Clin Nutr.* Aug 1996;50(8):491-497.

31. Munsters MJ, Saris WH. Effects of meal frequency on metabolic profiles and substrate partitioning in lean healthy males. *PLoS One.* 2012;7(6):e38632.

32. McGrath SA, Gibney MJ. The effects of altered frequency of eating on plasma lipids in free-living healthy males on normal self-selected diets. *Eur J Clin Nutr.* Jun 1994;48(6):402-407.

33. Leidy HJ, Armstrong CL, Tang M, Mattes RD, Campbell WW. The influence of higher protein intake and greater eating frequency on appetite control in overweight and obese men. *Obesity (Silver Spring).* Sep 2010;18(9):1725-1732.

34. Bertelsen J, Christiansen C, Thomsen C, et al. Effect of meal frequency on blood glucose, insulin, and free fatty acids in NIDDM subjects. *Diabetes Care.* Jan 1993;16(1):4-7.

35. Goris AH, Westerterp-Plantenga MS, Westerterp KR. Undereating and underrecording of habitual food intake in obese men: selective underreporting of fat intake. *Am J Clin Nutr.* Jan 2000;71(1):130-134.

36. Moran LJ, Noakes M, Clifton PM, et al. Postprandial ghrelin, cholecystokinin, peptide YY, and appetite before and after weight loss in overweight women with and without polycystic ovary syndrome. *Am J Clin Nutr.* Dec 2007;86(6):1603-1610.

37. Ohkawara K, Cornier MA, Kohrt WM, Melanson EL. Effects of increased meal frequency on fat oxidation and perceived hunger. *Obesity (Silver Spring).* Feb 2013;21(2):336-343.

38. Verbaeys I, Tolle V, Swennen Q, et al. Scheduled feeding results in adipogenesis and increased acylated ghrelin. *Am J Physiol Endocrinol Metab.* Jun 2011;300(6):E1103-1111.

39. Stock S, Leichner P, Wong AC, et al. Ghrelin, peptide YY, glucose-dependent insulinotropic polypeptide, and hunger responses to a mixed meal in anorexic, obese, and control female adolescents. *J Clin Endocrinol Metab.* Apr 2005;90(4):2161-2168.

40. Brennan IM, Seimon RV, Luscombe-Marsh ND, Otto B, Horowitz M, Feinle-Bisset C. Effects of acute dietary restriction on gut motor, hormone and energy intake responses to duodenal fat in obese men. *Int J Obes (Lond).* Mar 2011;35(3):448-456.

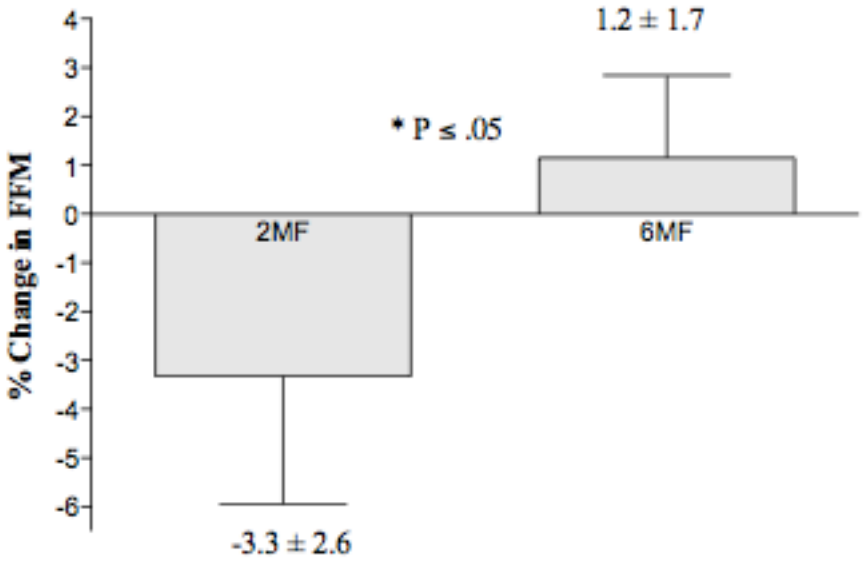


Figure 1. Percent change in FFM between the two-meal frequency (2 MF) and six-meal frequency (6 MF). When subjects consumed the 2 MF pattern for two-weeks, their percent change in fat-free mass (FFM) produced a significantly greater decrease in FFM loss during the 2 MF condition compared to an increase during the 6 MF condition ($P \leq 0.05$).

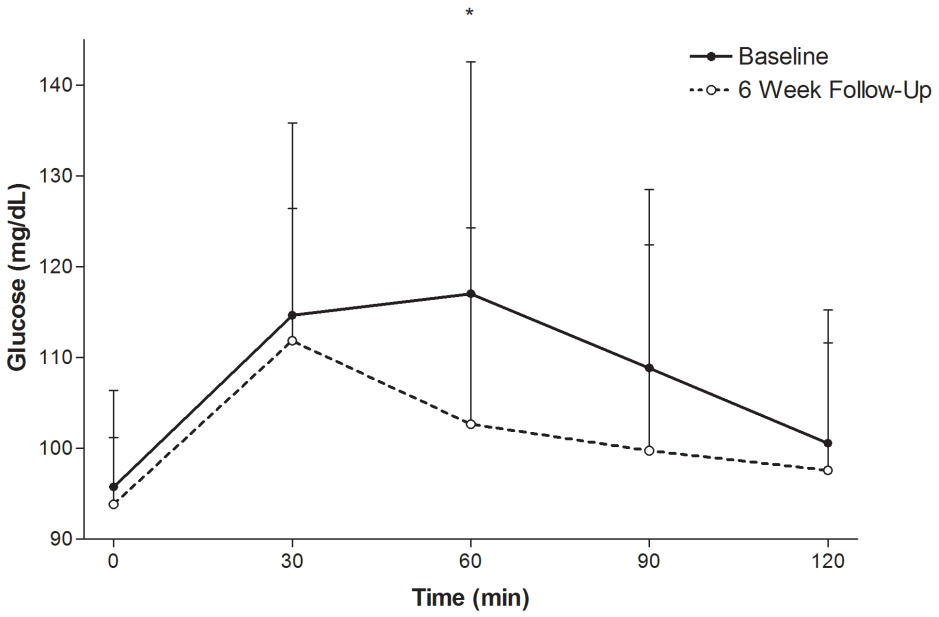
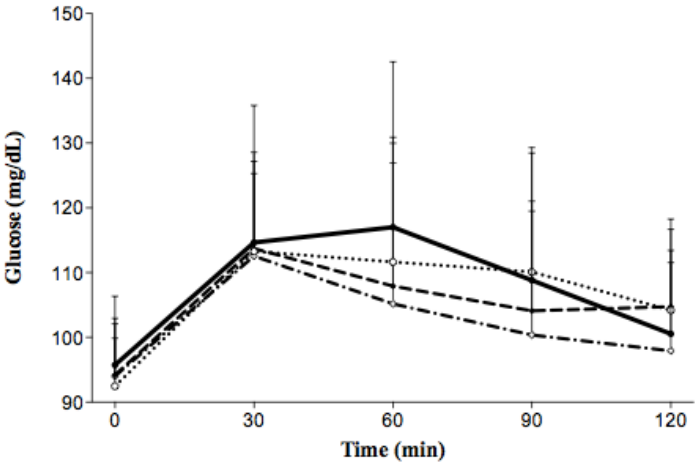


Figure. 2 There was a significant main effect for time for glucose between baseline to the six-week follow-up (P = 0.009).
*Significantly decreased at time 60 versus baseline (P = 0.022)

a)



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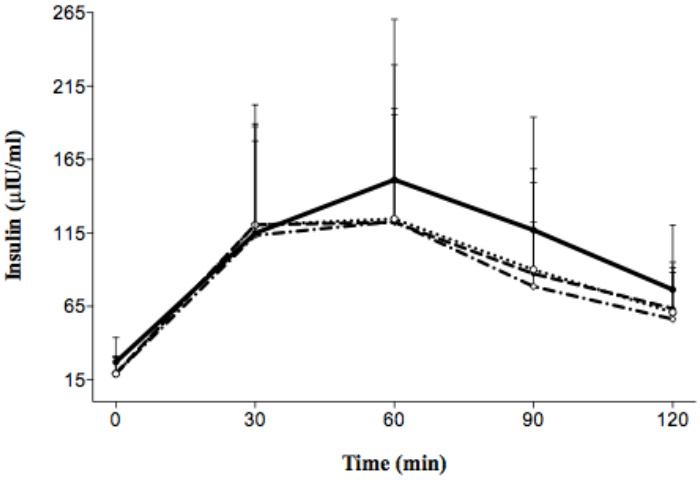


c)

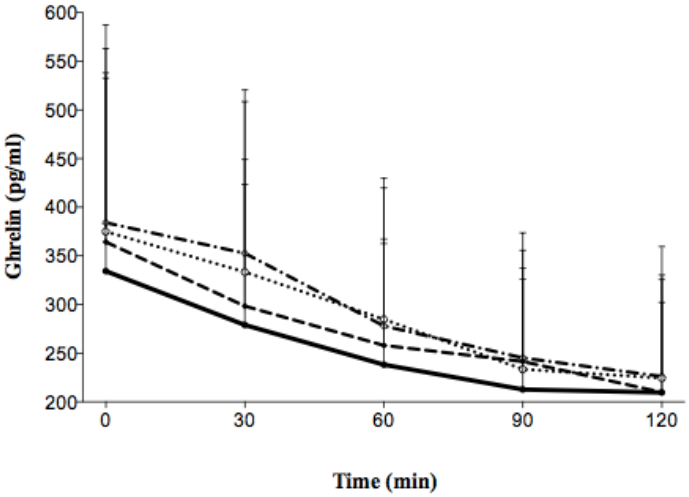


Figure 3. a) glucose, b) insulin, and c) total-ghrelin; responses to all conditions. There were no significant differences in average fasting values or AUC between conditions; a)P = 0.197 b)P = 0.211 c) P = 0.059

Baseline 2MF Washout 6MF

Table 1. Characteristics of Participants		
	Baseline	6-Week Follow-up
Age (yrs)	52 ± 7	52 ± 7
Height (m)	1.61 ± 0.07	1.61 ± 0.07
Weight (kg)	101.7 ± 22.6	95.5 ± 21.7*
BMI (kg/m^2)	39.1 ± 7.6	36.7 ± 7.2*
FM (kg)	48.4 ± 13.6	43.9 ± 13.5*
FFM (kg)	52. 7 ± 9.0	51.1 ± 8.8*
WC (cm)	120. 6 ± 14.0	112.8 ± 15.3*
HC (cm)	129.1 ± 15. 4	120.6 ± 13.4*
WHR	0.94 ± 0.07	0.93 ± 0.06
%BF	46.8 ± 3.9	45.1 ± 4.6*
Caloric intake (kcals/day)	2207 ± 422	1200 ± 110*
RMR (kcals/day)	1637 ± 464	1579 ± 358

BMI: body mass index, *FM*: fat-mass, *FFM*: fat-free mass, *WC*: waist circumference, *HC*: hip circumference, *WHR*: waist-to-hip ratio, *%BF*: percent body fat, *RMR*: resting metabolic rate.
N=11,*$P \leq 0.05$ vs baseline

Table 2.Anthropometric and RMR Results Summary

	Treatment			
	Baseline	2 MF	Washout	6 MF
Weight (kg)	101.7 ± 22.6	97.2 ± 22.6*	96.6 ± 22.2*	96.4 ± 21.4*
FM (kg)	48.1 ± 13.9	45.7 ± 14.5*	45.1 ± 13.8*	44.6 ± 13.4*
FFM(kg)	52.7 ± 9.0	50.9 ± 8.9*	50.6 ± 8.9*	51.1 ± 8.7*†
RMR (kcals/day)	1637 ± 464	1621 ± 347	1685 ± 450	1627 ± 415

2 MF: 2 meal frequency pattern, *6 MF*: 6 meal frequency pattern, *Washou*t: 4 meal frequency pattern, *FM*: fat-mass, *FFM*: fat-free mass, *RMR*: resting metabolic rate
*P < 0.05 vs. baseline, †P ≤ 0.05 vs. washout

Table 3. Results Summary

Fasting and AUC values for insulin, glucose, total-ghrelin, and VAS during 2-h trial following two-week intervention of altered meal frequency.

	Treatment				
	Baseline	2 MF	Washout	6 MF	6-Week Follow-Up
Fasting Insulin (μIU/L)[ae]	27 ± 17	19 ± 12	19 ± 11	19 ± 11	20.2 ± 12.0
Fasting Glucose (md/dL)[a]	96 ± 11	92 ± 10	94 ± 9	94 ± 6	94 ± 7
Fasting Total-Ghrelin (pg/ml)[a]	334 ± 204	375 ± 188	364 ± 169	353 ± 168	397 ± 205
Insulin AUC (μIU/L·120 min)[ae]	12881 ± 7747	11254 ± 7418	11150 ± 5810	10577 ± 5908	11381± 7206
Glucose AUC (mg/dL·120 min)[a]	13122 ± 1726	13000 ± 1663	12755 ± 1494	12421 ± 1773	12296 ± 1870*
Total-Ghrelin AUC (pg/ml·120 min)[a]	30586 ± 16545	34545 ± 17081	32553 ± 13335	35436 ± 17574	34958 ± 18111
Fasting Triglycerides (mg/dL)[bc]	118 ± 40	114 ± 40	130 ± 57	115 ± 47	113 ± 45
Fasting Total-Cholesterol (mg/dL)[bd]	190 ± 34	181 ± 44	181 ± 34	176 ± 40	177 ± 40
Fasting HDL-Cholesterol (mg/dL)[bd]	53 ± 12	52 ± 12	49 ± 11*†	49 ± 10*	49 ± 9
Fasting LDL-Cholesterol (mg/dL)[bd]	113 ± 28	106 ± 40	107 ± 32	104 ± 35	105 ± 36
VAS 1 AUC (mm·120 min)[a]	1894 ± 887	3106 ± 1740*	2981 ± 1159*	2673 ± 1458*	3131 ± 1563*
VAS 2 AUC (mm·120 min)[a]	11254 ± 7418	7506 ± 2083	7640 ± 1469	7582 ± 1460	7370 ± 1957
VAS 3 AUC (mm·120 min)[a]	7390 ± 2808	7064 ± 2225	7668 ± 1906	7826 ± 1945	7167 ± 2331
VAS 4 AUC (mm·120 min)[a]	4002 ± 2254	3727 ± 1941	4023 ± 1964	3649 ± 1986	3873 ± 1980

2 MF: 2 meal frequency pattern, *6 MF*: 6 meal frequency pattern, *Washou*t: 4 meal frequency pattern,
VAS: visual analogue scale questions

[a]N=11

[b]N=10, One subject was not included due to missing data

[c]To convert Triglyceride mg/dL to mmol/L, multiply mg/dL by 0.0113.

[d]To convert total- cholesterol, HDL-cholesterol, LDL-cholesterol l mg/dL to mmol/L, multiply mg/dL by 0.026.
[e]To convert insulin µIU/L to pmol/L multiply µIU/L by 6.0.
[f]To convert glucose mg/dL to mmol/L, multiply mg/dL by 0.0555.
[g]To convert total-ghrelin pg/ml to pmol/L, multiply mg/ml by 0.296.

*$P<.05$ vs baseline
†$P \leq .05$ vs 2 MF

CHAPTER IV

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

SUMMARY

The review manuscript entitled "Potential role of meal frequency as a strategy for weight loss and health in overweight or obese adults" summarized the current literature as it relates to meal frequency during weight loss and its function in obesity treatment. Due to the common belief that increasing meal frequency may have an impact on body weight and other factors, its role in obesity management was in need of review ground in scientific evidence. The objective of this review was to highlight only original research using a reduced-calorie diet for weight loss in overweight or obese subjects utilizing varied meal frequencies as a strategy for improving one or more of the following: weight loss, metabolic markers of health (including fasting glucose, insulin, lipid concentrations), and/or appetite responses. It is believed this comprehensive review can help provide some clarity within the literature regarding meal frequency as a potential treatment therapy to aid in weight loss, improve metabolic markers of health, and help with appetite control in overweight or obese adults. The findings of this research review discussed that normal weight versus overweight/obese populations as well as free-living versus calorie-controlled research trials should be studied independently. Currently, because of these mixed comparisons, there is uncertainty in the literature when interpreting optimal meal frequency for obesity treatment. This review focused on the discussion of scientific literature using overweight/obese adults during a hypocaloric

dietary intervention, the relationship between meal frequency and body weight regulation, appetite, and health.

The research manuscript entitled "Meal frequency shows mixed results in blood-markers of health and appetite in obese women during an equi-hypocaloric weight loss intervention" provides evidence that altered meal frequency of either 2 MF versus 6 MF does not significantly alter fasting or area under the curve values for: insulin, glucose, total-ghrelin, or subjective hunger or satiety ratings in obese women serving as their own controls. Some mixed beneficial results were found after using portion-controlled products to aid in improving dietary compliance and reducing underreporting error. While reduced MF may attenuate losses of HDL-C, increased MF may reduce loss of FFM during weight loss. Based on these results, no conclusions can be made to indicate that altering MF during an equi-hypocaloric diet exerts any additional impact beyond those of a reduced-calorie diet.

CONCLUSIONS

This research adds significant findings to the scientific literature on meal frequency because it was the first of its kind to use portion-controlled products during a meal frequency trial for obese women. Three significant findings have been identified. First, increasing MF to six eating occasions per day does not promote greater improvements in blood-markers of health, appetite control, or total-ghrelin levels during a portion-controlled strict reduced-calorie diet intervention. Secondly, within the context of this study design, reducing MF to two eating occasions per day may attenuate losses in the beneficial function of HDL-C during weight loss when compared to eating six

occasions per day. Lastly, consuming a reduced-calorie diet as 2 MF may contribute to greater losses in FFM, while a 6 MF may aid in preservation of FFM.

Current evidence does not support that increased MF is a successful strategy to aid in reducing subjective hunger or appetite control when strict dietary measures are taken to reduce underreporting error in obese women. Future research is needed with longer intervention periods of two versus six meals per day using portion-controlled products.

RECOMMENDATIONS

This research may have been improved if researchers had access to subjects' medical charts for blood markers such as fasting lipid, glucose, and insulin concentrations prior to beginning the trial. It is possible that the large variability seen in many variables was inherent in the sample and was present even before the intervention. Within the constraints of the study design, it was not possible to obtain baseline measurements for subjects until all of the subjects had completed the trial. By analyzing the data as it was collected, we would have been able to observe individual variability from the start. Additionally, this information would have provided valuable information to the researchers ensuring the subjects' self-reported medical history was accurate could have alerted us to individuals with high baseline variability.

We have three recommendations for future studies. First, the impact of meal frequency using portion-controlled products during a reduced-calorie diet for a longer duration than two weeks for each pattern should be investigated. Due to the testimonials from subjects regarding the duration of consuming only the portion-controlled products,

it may be advantageous to split subjects into two groups (2 MF and 6 MF) for the entire six-week duration compared to a cross-over design. Secondly, an investigation of the impact of meal frequency on the observed blood-markers of health and appetite along with a moderate-intensity and/or high-intensity exercise regimen would enrich the literature. Limited research exists investigating appetite responses in obese women following moderate and/or high-intensity aerobic exercise. It would be advantageous to investigate the impact of a reduced-calorie diet for weight loss using portion-controlled products in obese women in conjunction with moderate and/or high-intensity exercise for changes in the observed blood-markers of health and appetite. (3) Third, examining body composition and metabolic changes in obese women following a resistance training and/or combination resistance + aerobic training program while undergoing a reduced-calorie diet for weight loss using portion-controlled entrees would provide additional insight into the influence of exercise modality on the variables of interest.

APPENDICES

A. HIPAA Form

B. Informed Consent

C. Data and Safety Monitoring Plan

D. Flyer

E. Health History Questionnaire

F. Visual Analogue Scale Questions

G. Subject Weekly Questionnaire

H. Supplemental Figure 1

I. Supplemental Figure 2

APPENDIX A.

UNIVERSITY OF NEW MEXICO HEALTH SCIENCES CENTER

HIPAA[1] AUTHORIZATION TO USE AND DISCLOSE

PROTECTED HEALTH INFORMATION FOR RESEARCH PURPOSES

Title of Study: Impact of meal frequency on appetite and blood markers of health in obese women utilizing an equi-hypocaloric diet during a behavioral weight loss intervention

Principal Investigators: **Dr. Len Kravitz, Ph.D. & Dr. Carole Conn, Ph.D. RD, LD, FACSM**
UNMHSC Department: **Heath, Exercise, and Sports Sciences; Individual, Family, and Community Education**
Mailing Address: **MSC 04-1610, Johnson Center, University of New Mexico, Albuquerque, NM 87131**

Co-Investigators: **Michelle Kulovitz, M.S., Jason Beam, M.S., Ailish White, M.S., Christine Mermier, Ph.D., Ann Gibson, Ph.D, Micah Zuhl, Ph.D., Roy Salgado, M.S., James Jeremy McCormick**

Sponsor: N/A

1. **What is the purpose of this form?** You have been asked to take part in a research study. The consent form for this study describes your participation, and that information still applies. This extra form is required by the federal Health Insurance Portability and Accountability Act (HIPAA). The purpose of this form is to get your permission (authorization) to use health information about you that is created by or used in connection with this research.

2. **What if I don't want my personal health information (PHI) to be used in this research study?** You do not have to give this permission. Your decision not to sign this form will not change your ability to get health care outside of this research study. However, if you do not sign, then you will not be allowed to participate in the study.

3. **What PHI am I allowing to be used for this research?** The information that may be used includes: a) Blood glucose, insulin, and lipid concentrations
   b) Blood acylated-ghrelin concentrations
   c) Height, weight, resting blood pressure, and waist and hip circumferences
   d) Resting energy expenditure
   e) Subjective responses to visual analogue scale questions

4. **Where will researchers go to find my PHI?** We will ask you to fill out a questionnaire about your health and your current eating patters. We may ask to see your personal information in records at hospitals, clinics or doctor's offices where you

[1] HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of health information.

may have received care in the past, including but not limited to facilities in the UNM health care system.

5. **Where will researchers go to find my PHI?** We may ask to see your personal information in records at hospitals, clinics or doctor's offices where you may have received care in the past, including but not limited to facilities in the UNM health care system.

6. **Who will be allowed to use my information for this research and why?** The researchers named above and their staff will be allowed to see and use your health information for this research study. It may be used to check on your progress during the study, or analyze it along with information from other study participants. Sometimes research information is shared with collaborators or other institutions. Your records may also be reviewed by representatives of the research sponsor or funding agency, the Food and Drug Administration (FDA) to check for quality, safety or effectiveness, or the Human Research Review Committee (HRRC) for the purposes of oversight and subject safety and compliance with human research regulations.

7. **Will my information be used in any other way?** Your information used under this permission may be subject to re-disclosure outside of the research study and be no longer protected under certain circumstances such as required reporting of abuse or neglect, required reporting for law enforcement purposes, and for health oversight activities and public health purposes.

8. **What if I change my mind after I give this permission?** You can change your mind and withdraw this permission at any time by sending a written notice to the Principal Investigator at the mailing address listed at the top of this form to inform the researcher of your decision. If you withdraw this permission, the researcher may only use and share your information that has already been collected for this study. No additional health information about you will be collected by or given to the researcher for the purposes of this study.

9. **What are the privacy protections for my PHI used in this research study?** HIPAA regulations apply to personal health information in the records of health care providers and other groups that share such information. There are some differences in how these regulations apply to research, as opposed to regular health care. One difference is that you may not be able to look at your own records that relate to this research study. These records may include your medical record, which you may not be able to look at until the study is over. The HIPAA privacy protections may no longer apply once your PHI has been shared with others who may be involved in this research.

10. **How long does this permission allow my PHI to be used?** If you decide to be in this research study, your permission to access and use your health information in this study may not expire, unless you revoke or cancel it. Otherwise, we will use your information as long as it is needed for the duration of the study.

I am the research participant or the personal representative authorized to act on behalf of the participant. By signing this form, I am giving permission for my personal health

information to be used in research as described above. I will be given a copy of this authorization form after I have signed it.

_____	_____	_____
Name of Research Subject	Signature of Subject/Legal Representative	Date

_____

Describe authority of legal representative

_____	_____	_____
Name of Person Obtaining Authorization	Signature	Date

APPENDIX B.

The University of New Mexico Health Sciences Center
Consent to Participate in Research

Impact of meal frequency on appetite and blood markers of health in obese women utilizing an equi-hypocaloric diet during a behavioral weight loss intervention

3/20/2013

Introduction

You are being asked to participate in a research study that is being done by Dr. Len Kravitz and Dr. Carole Conn who are the Principal Investigators as well as Michelle Kulovitz, M.S., and their associates from the Department of Health, Exercise, and Sports Sciences at the University of New Mexico. This research is studying the effect of the number of eating occasions per day on weight loss, blood markers of health, and appetite control.

Ingesting meals with the same amount of calories at different time periods throughout the day has been shown to have an effect on body weight regulation, metabolic disease management, and hunger. There is limited research with obese women undergoing a controlled weight loss trial with varied meal frequency. Our study will look at a reduced-calorie diet using Nutrisystems ® portion-controlled meal entrees that will be consumed during both two occasions per day and six occasions per day for two weeks each. Our research team is questioning whether that the number of eating occasions per day may have an effect on the amount of weight lost, blood glucose, insulin, lipids, and/or appetite measures, and perhaps on how hungry you feel after eating.

You are being asked to participate in this study because you are a non-diabetic woman between the ages of 35-60, with a body mass index (BMI) ≥ 30, who on average consumes meals or snacks on four occasions throughout the day, is sedentary to moderately active, weight stable (≤ 3 kg or 6.5 pounds over the last 6 months), and does not have any diagnosed uncontrolled cardiovascular, pulmonary, or metabolic diseases. Approximately 20 people will take part in this study conducted by the University of New Mexico.

This form will explain the research study, and will also explain the possible risks as well as the possible benefits to you. We encourage you to talk with your family and friends before you decide to take part in this research study. If you have any questions, please ask one of the study investigators.

What will happen if I decide to participate?

If you agree to participate, the following things will happen:

Overview

1. You will be asked to visit Southwest Endocrinology Associates (SWENDO) or the UNM Exercise Physiology Lab in the Johnson Center on the University of New Mexico main campus eleven times over the course of 6-7 weeks.
2. During your first visit, you will attend a group orientation meeting where all aspects of the research study will be explained in detail and provide an opportunity for you to ask questions.
3. Throughout the 6-7 week trial you will be asked to visit either SWENDO or the UNM Exercise Physiology Lab on four separate occasions separated by 2 weeks for a test meal in the morning where you will have your blood drawn periodically.
4. Additionally, throughout the 6-7 week trial you will be asked to visit SWENDO weekly for group or individual meetings and weigh-ins.

First Visit

1. During your first visit, you will sign this consent form, a HIPAA form, and fill out a health history and meal frequency questionnaire. Additionally, only your signed consent form will be added to your SWENDO medical chart. Additionally, your SWENDO physician will be aware of your study participation.
2. After filling out paperwork, you will attend the participant orientation meeting where you will be shown and introduced to the responsibilities that will be asked of you throughout this study. This meeting will cover:

    - How to log food
    - How to log daily physical activity
    - Food scale use. If you do not consume all food provided or planned you will be asked to weigh left over food and document in food log.
    - Importance of adherence, accuracy, and truthfulness with intake.

3. We will give you a food and physical activity log so that you can keep track of your food and physical activity three days before your second visit to SWENDO and throughout the study.
4. We will explain to you how to complete this log, and we will also schedule the date and time for your second visit before you leave.
5. We will also give you a sheet with pre-test guidelines that you should follow before each test meal trial.

Second, Third, Fourth, and Fifth Two-Week Visits

1. Upon arrival to your second through fifth two-week appointments to either SWENDO or the UNM Exercise Physiology Lab, you will void your bladder and bowel and change into lightweight clothing.

2. We will measure your height, weight, and blood pressure
3. Body composition will be estimated using a device where you stand barefoot on a platform while holding handles (bioelectrical impedance). This will take approximately two minutes.
4. The calories you burn at rest (resting energy expenditure) will be measured using a hand-held device that you breathe into. This will take a maximum of 15 minutes. This measurement is in addition to the typical testing done by the SWENDO program.
5. We will prep either your right or left arm or hand for a needle stick.
6. We will insert a flexible needle into your arm or hand that will remain throughout the rest of your visit. This will allow us to take your blood without having to stick you multiple times.
7. We will draw 16 ml (~1 Tbsp) of blood. We will then insert 1 ml (1/5 tsp) of saline into the catheter to keep it from getting clotted by blood.
8. We will set up your test meal and you will be asked to consume this test meal within 15 minutes.
9. Thirty minutes after consumption of your test meal, we will draw 1 ml (1/5 tsp) of blood and discard it. We will then draw 16 ml (~1 Tbsp) of blood for later analysis of blood sugar and insulin concentrations, lipids, and acylated-ghrelin (a hormone responsible for hunger). We will also ask you how you are feeling on a Visual Analogue Scale on your current hunger, satiety, and desire to eat.
10. You will then sit and rest for 2 hours while we draw 16 ml (~1 Tbsp) of blood every 30 minutes for 120 minutes. For each blood draw, we will draw 1 ml (1/5 tsp) of blood and discard it. We will then draw 16 ml (~1 Tbsp) of blood for later analysis of blood sugar and insulin concentrations. We will clear out the catheter with 1 ml (1/5 tsp) saline to keep the catheter from clotting. We will also ask you how you are feeling on a Visual Analogue Scale on your current hunger, satiety, and desire to eat every 30 minutes.
11. After the 2 hours, we will take the catheter out of your arm.
12. We will schedule the date and time of your next two-week visit and your weekly visit.
13. We will ask you to consume all Nutrisystems ® food products that we will provide to you during your weekly clinic visits.
14. You will be asked to follow the meal frequency pattern that you were randomly assigned to consume for two weeks.
15. All food and beverages you consume will be asked to be recorded in your food diary along with the time you ate the food or beverage.
16. You will repeat the above procedures when you come to SWENDO or the lab for your third, fourth, and fifth appointments scheduled every two weeks.
17. These appointments will last four hours or less each.
18. We will collect a total of 85 ml (5.7 Tbsp) each visit for a total of 340 ml (23 Tbsp) over the 6-week study.
19. Results of all of your blood tests will be explained to you after the conclusion of the study. If during the study, any of your lab values (glucose, lipids, insulin, or acylated-ghrelin) are outside the clinical range of normal, your

SWENDO overseeing physician will be contacted for a follow-up of any necessary blood parameters.

Weekly Meeting Visits

20. You will be asked to attend weekly group or individual one-on-one meetings at SWENDO that will cover different topics that relate to healthy weight loss and behaviors that will help you during the study and after the study.
21. During these weekly visits we will measure your height, weight, blood pressure, and re-test your body composition analysis.
22. These meetings will be 1.5 hours or less each.

How long will I be in this study?

Participation in this study will take no more than a total of 20 hours over a period of 6-7 weeks.

What are the phases for the varied meal frequency protocol?

You will be randomly assigned to a diet that is reduced calorie diet of approximately 1200 calories per day to induce weight loss. You will be randomized to either 2 large meals per day or 3 meals + 3 snacks per day. The 2 meal pattern will instruct you to consume provided meals every 5-6 hours (while you are awake), while the three meal pattern will instruct you to consume provided meals/snacks every 3-4 hours (while you are awake) daily. Food products will be identical and dietary measures will be standardized ensuring your dietary intake will be ~1200 calories/day with ~75g/day of protein. Your nutrients will average 52% carbohydrates, 27% protein, and 22% fat which has been shown to provide adequate calories and nutrients during weight loss and is currently consumed by thousands of Nutisystems ® customers. You will consume your randomly selected meal pattern eating only Nutrisystems ® meal products provided to you and Nutrisystems ® directed grocery items for the entire study duration. You will consume your randomized meal frequency pattern for two weeks (Phase I), have a two-week washout period of 3 meals + 1 snack per day (Washout), and then consume the alternate pattern for two weeks (Phase II). All procedures for consumption and storage of food products will be covered during your first visit and/or orientation meeting. All Nutrisystems ® food products will be provided to you each week during your weekly clinic visits.

What are the risks or side effects of being in this study?

Changing Meal Pattern Risks

- Some people may feel inconvenienced to eat on a strict schedule. There is also a potential risk of discomfort/hunger as a result of eating a reduced calorie diet. You will be asked to consume all provided foods for the day during two meals and three meals and three snacks for two weeks each.

There will be a two-week washout period where you will be asked to consume the same diet with Nutrisystems ® food products as three meals and one snack per day. Additionally, some may find it inconvenient to record all food/beverages and exercise in a journal.

Blood Drawing Risks

- Risks of blood drawing include fainting (<0.01% or <1 out of 10,000 people), lightheadedness (<0.01% or <1 out of 10,000 people), bruising at the site of needle puncture (~2% or ~2 out of 100 people), and localized infection (<0.01% or <1 out of 10,000 people).

Weight Loss Risks

- Although it is expected that you will lose some body weight, there is a possibility that you may not lose any weight during this study.

Loss of Privacy Risks

- There are risks of stress, emotional distress, inconvenience and possible loss of privacy and confidentiality associated with participating in a research study.

For more information about risks and side effects, ask the investigator.

What are the benefits to being in this study?

There are some direct benefits to you as well as societal and public health benefits of this research study. Since this study is investigating methods to induce healthy weight loss in obese women as it relates to body weight reduction, changes in body composition, changes in appetite hormones, lipids, and hunger ratings, it can provide benefits directly to you about changes in your body during a reduced calorie diet as well as potential benefits in hunger/satiety due to two eating occasions versus six eating occasions of the same daily caloric intake.

Since this study will include a behavioral education program, the knowledge gained by the group and individual support classes, one-on-one couseling, and knowledge of portion control will be information that can help you in weight management.

This study may elicit potential public health benefits to society, including advancement of knowledge for obesity therapies that include treatment strategies for obesity, related metabolic diseases, and appetite responses to a reduced calorie diet. Additionally, results of this resarch may be used in clinical practice by physicians, nurses, and registered dietitians to aid in weight management.

What other choices do I have if I do not want to be in this study?

Your participation is voluntary, and if you decide to not be in the study then you will not be contacted again. Additional choices are available to you without participation in this research study. There are many choices for obesity treatment for women that include commercial or medically supervised diet and/or exercise programs that may or many not utilize physicians, registered dietitians, or registered nurses for consultations. You are encouraged to consult your primary care physician for advice on participation in this research study and/or to obtain further information on other weight management choices that may be right for you.

How will my information be kept confidential?

We will take measures to protect the security of all your personal information, but we cannot guarantee confidentiality of all study data.

Information contained in your study records is used by study staff. The University of New Mexico Health Sciences Center Human Research Review Committee (HRRC) that oversees human subject research, and the Food and Drug Administration and/or other entities may be permitted to access your records. There may be times when we are required by law to share your information. However, your name will not be used in any published reports about this study.

Your information will be stored in a locked cabinet in the UNM Exercise Physiology Lab and/or at SWENDO where only study personnel will have access to it. In addition, you will be given a study number that will be used for data collection and analysis. Your information will be destroyed after the completion of data analysis. Your name will not be used in any published reports about this study. Additionally, your name or other identifying information will not be given to Nutrisystems ® for solicitation of products or for any other reason. You will not be contacted by Nutrisystems ® at any point during the research study.

What are the costs of taking part in this study?

There are no costs to you for study related material. However, the cost for the regular SWENDO health care will be up to $180 for the 6-week duration. Additionally, there are costs of fresh produce, dairy, and lean protein from the grocery store, and your time.

What will happen if I am injured or become sick because I took part in this study?

If you are injured or become sick as a result of this study, UNMHSC will provide you with emergency treatment, at your cost.

No commitment is made by the University of New Mexico Health Sciences Center (UNMHSC) or Southwest Endocrinology Associates (SWENDO) to provide free medical care or money for injuries to participants in this study.

In the event that you have an injury or illness that is caused by your participation in this study, reimbursement for all related costs of care will be sought from your insurer, managed care plan, or other benefits program. If you do not have insurance, you may be responsible for these costs. You will also be responsible for any associated co-payments or deductibles required by your insurance.

It is important for you to tell the investigator immediately if you have been injured or become sick because of taking part in this study. If you have any questions about these issues, or believe that you have been treated carelessly in the study, please contact the Human Research Review Committee (HRRC) at the University of New Mexico Health Sciences Center, Albuquerque, New Mexico 87131, (505) 272-1129 for more information.

Will I be paid for taking part in this study?

You will not be paid for your time during this study. However the use of Nutrisystems ® portion-controlled entrees and products will be provided to you free of charge. You will receive free food products plus free shipping to be picked up at SWENDO for the duration of the study after your orientation session.

How will I know if you learn something new that may change my mind about participating?

You will be informed of any significant new findings that become available during the course of the study, such as changes in the risks or benefits resulting from participating in the research or new alternatives to participation that might change your mind about participating.

Can I stop being in the study once I begin?

Your participation in this study is completely voluntary. You have the right to choose not to participate or to withdraw your participation at any point in this study without affecting your future health care or other services to which you are entitled. The Nutrisystems ® food products will not be sent to you if you withdraw from the study.

Whom can I call with questions or complaints about this study?

If you have any questions, concerns or complaints at any time about the research study, Dr. Carole Conn, Dr. Len Kravitz or Ms. Michelle Kulovitz, or his/her associates will be glad to answer them at (505) 277-2658.

If you need to contact someone after business hours or on weekends, please call (714)-809-8528 and ask for Michelle Kulovitz.

If you would like to speak with someone other than the research team, you may call the UNMHSC HRRC at (505) 272-1129.

Whom can I call with questions about my rights as a research participant?

If you have questions regarding your rights as a research participant, you may call the UNMHSC HRRC at (505) 272-1129. The HRRC is a group of people from UNM and the community who provide independent oversight of safety and ethical issues related to research involving human participants. For more information, you may also access the HRRC website at http://hsc.unm.edu/som/research/hrrc/.

CONSENT

You are making a decision whether to participate (or to have your child participate) in this study. Your signature below indicates that you/your child read the information provided (or the information was read to you/your child). By signing this consent form, you are not waiving any of your (your child's) legal rights as a research participant.

I have had an opportunity to ask questions and all questions have been answered to my satisfaction. By signing this consent form, I agree to participate (or let my child participate) in this study. A copy of this consent form will be provided to you.

_____ _____ _____

Name of Adult Subject (print) Signature of Adult Subject Date

INVESTIGATOR SIGNATURE

I have explained the research to the participant and answered all of his/her questions. I believe that he/she understands the information described in this consent form and freely consents to participate.

_____

Name of Investigator/ Research Team Member (type or print)

_____ _____

(Signature of Investigator/ Research Team Member) Date

APPENDIX C.

UNM HEALTH SCIENCES CENTER
Office of Research

Data and Safety Monitoring Plan (DSMP)
Attachment 6

NOTE: For NMCCA studies, please complete Section 1 and attach the NMCCA DSMP short form.

Check the proposed level of risk and provide justification for this determination:

☐ **Minimal Risk:**
The probability and magnitude of harm or discomfort anticipated in the research are not greater in and of themselves than those ordinarily encountered in daily life or during the performance of routine physicals or psychological examinations or tests.
Justify: _____

☒ **Moderate Risk:**
Risks are more than minimal but less than high risk as defined below.
Justify: Subjects will be expected to follow a different meal frequency pattern than they are typically used to. Subjects will be asked to consume the food provided to them at time speciifcations allocated to their group randomization. Subjects will consume a hypocaloric diet of approximately 1200 kcals per day of commerically available portion controlled meals by Nutrisystems ®. Thousands of individuals take part in this type of program. However, the placement of an indwelling venous catheter for blood draws would be considered to incure slightly more risk than a routine physical.

☐ **High Risk:**
Interventions associated with risk of serious adverse events at high or uncertain frequency; studies in populations associated with very high risk of serious adverse clinical events based on underlying disease or in whom assessment of treatment associated adverse events may be difficult.
Justify: _____

APPENDIX D.

RESEARCH STUDY

Women Needed for UNM Weight Loss and Appetite Study!
HRPO #13-097

What is the study about?

The effect of meal frequency on body weight, metabolic disease management, and appetite regulation during a behavioral weight management program.

Who Can Volunteer?

35-60 year old non-diabetic females with a Body Mass Index (BMI) ≥ 30, weight stable for 6 months or more (≤ 6.6 lbs change), who want to lose weight!

Where will it take place?

Southwest Endocrinology Associates and/or UNM Exercise Physiology Lab

Will I get paid for participating?

No, however you will receive FREE Nutrisystems® meal entrees for the study duration as well as results from your testing.

Who Can I Contact for More Information?

Michelle Kulovitz, mkulovi@unm.edu, 714-809-8528

UNM Weight Loss Study
Michelle Kulovitz
714-809-8528
mkulovi@unm.edu

APPENDIX E.

HEALTH HISTORY & MEAL FREQUENCY QUESTIONNAIRE

HRPO # 13-097

Subject Number________________________ Date__/__/__

Phone (H or cell)________________ Age___ Gender___ Ethnicity______

Email____

Emergency contact (name, phone #)_______________________________________

♦♦

MEDICAL HISTORY

Physical injuries:____

Limitations____

Have you ever had any of the following cardiovascular problems? Please check all that apply.

Heart attack/Myocardial Infarction___ Heart surgery ___ Valve problems ____
Chest pain or pressure ___ Swollen ankles ___ Dizziness ____
Arrhythmias/Palpitations ___ Heart murmur ___ Shortness of breath ____
Congestive heart failure ___

Have you ever had any of the following? Please check all that apply.

Hepatitis/HIV ____ Depression ____ Cancer (specifytype) _____
Rheumatic fever _____ High blood pressure______ Thyroid problems ______
Kidney/liver disease ____ Gastrointestinal Disorders ____ Total cholesterol >200 mg/dl ____
Diabetes (specify type)____ Asthma____ HDL cholesterol <35 mg/dl ____
Emphysema ____ Stroke____ LDL cholesterol >135 mg/dl____
Pacemaker ____ Triglycerides>150 mg/dl____

If you checked YES to one of the above please explain your current treatment:

____

____

Do immediate blood relatives (biological parents & siblings **only**) have any of the conditions listed above? If yes, list the problem, and family member age at diagnosis.

____

Do you currently have any other medical conditions not listed?
Details____

____

Indicate level of your overall health. Excellent ____ Good ____ Fair ____ Poor_____

Are you taking any medications, vitamins or dietary supplements now? Y N

If yes, what are they?____

Do you have allergies to any medications or supplements? If yes, what are they?

____

Are you allergic to latex? Y N

Have you experienced a change in body weight ≥ 3 kg (6.6 lbs) within the last 6 months? Y N

If yes, elaborate.____

Have you ever been diagnosed with an eating disorder? Y N

Are you pregnant or planning to become pregnant in the next 3 months? Y N

What is your current menopausal status?

☐Eumenorrheic ☐Amenorrheic ☐Premenopausal ☐Menopausal

♦♦

LIFESTYLE FACTORS

Do you currently smoke? Y N Have you ever used tobacco/smoked? Y N

If yes: type ________________

How long?______ Quantity____/day Years since quitting_______________

How often do you drink the following?

Caffeinated: coffee or tea _________oz/wk Soft drinks _________oz/wk

♦♦

EXERCISE

These questions will ask you about the time you spent being physically active in the last **7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

<u>In the last 7 days:</u>

Did you perform any exercise? Y N

If NO, is this typical for you? Y N Please explain____

If YES, please answer the following:

What did you do for exercise? ____

How often? _______________days/ week

How long did you typically perform your exercise? ___________________ minutes/day

Rate the intensity of your exercise: ☐Light ☐Moderate ☐Vigorous

Is the exercise this past week typical for you? Y N

If NO, Explain: ____

Have you changed your exercise regimen dramatically within the last 6 months? Y N

If YES, How? ____

♦♦♦

<u>MEAL FREQUENCY/FOOD INTAKE</u>

These questions will ask you about the amount of meals and snacks you had in the last **7 days**. Please answer each question to the best of your knowledge. If there was a disruption this past week to your typical eating pattern please explain.

<u>In the last 7 days:</u>

On average how many meals (≥200 calories) do you try to have in one day? ___________

How many days/week do you eat this way?_______________

If variable, please explain____

On average how many snacks (100 to < 200 calories) do you have in one day? _________

How many days/week do you eat this way?_______________

If variable, please explain____

On average how many times a day would you say you eat something ?_____

Do you have any nut/soy allergies? Y N

Do you have any food allergies? Y N If YES, Please Explain_____

Are there any food items that you absolutely will/cannot not eat? Y N If YES, Please Explain_____

Do you have freezer/refrigerator space to accommodate food to be provided to you? Y N

APPENDIX F.

How hungry do you feel?

I am not hungry at all ——— I have never been more hungry

How satisfied do you feel?

I am completely empty ——— I cannot eat another bite

How full do you feel?

Not at all full ——— Totally full

How much do you think you can eat?

Nothing at all ——— A lot

APPENDIX G.

Weekly Data Collection Sheet- Participant Survey
HRPO#13-097

Participant #:_____
Date: _____
Trial #/Week #:_____

1. Did you experience any discomfort this week with regards to the meal plan? Y / N
If so, Please
explain_____

2. Overall, how would you rate your hunger this week?
(not hungry at all) 1 2 3 4 5 6 7 8 9 10 (so hungry I couldn't bare it)

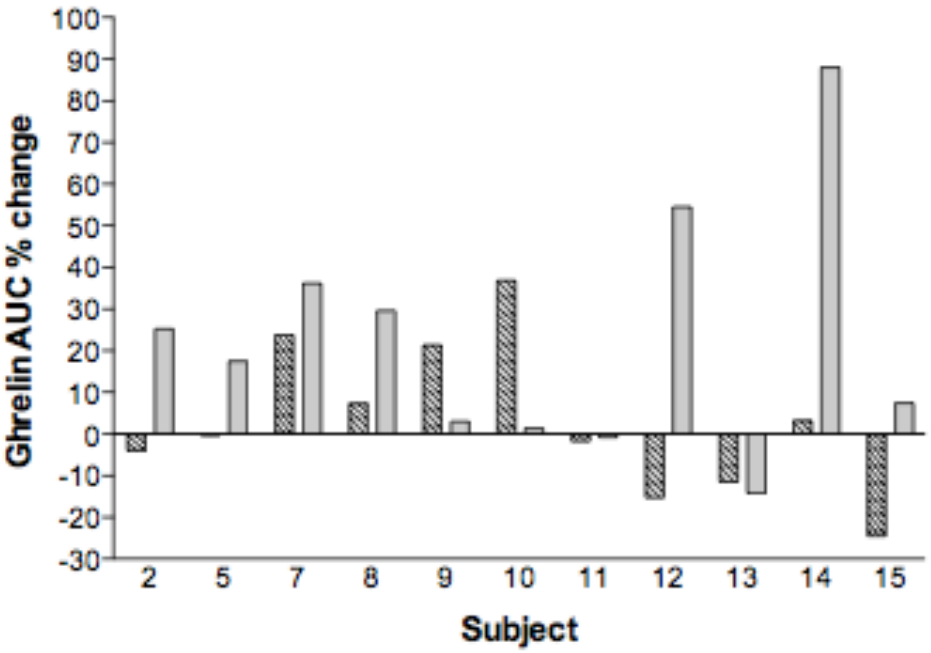
3. How would you rate your adherence to the prescribed meal plan this week?
(didn't follow it at all) 1 2 3 4 5 6 7 8 9 10 (I ate right on schedule every day)

4. Did you consume any foods that were not NutriSystems ® or NutriSystems ® prescribed grocery items? Y / N
If so, Please
explain_____

5. Do you have any comments or concerns that you would like to express to the researchers? Y / N
If so, Please
explain_____
_____
_____

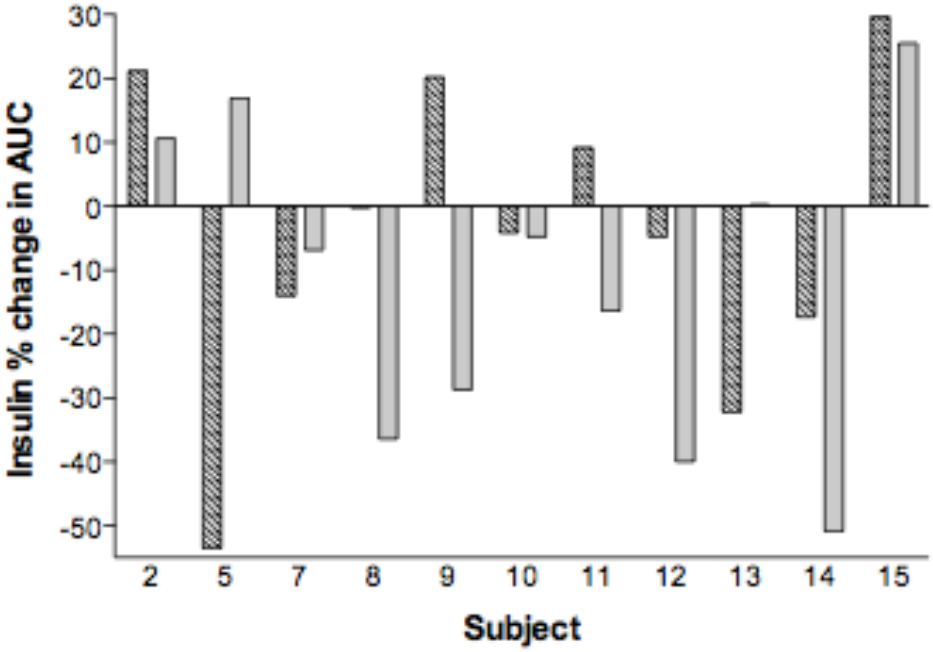
Thank you so much!!
Your participation and adherence is vital to the advancement of research!!!

APPENDIX H.



Supplemental Figure 1. Individual subject results for percent change total ghrelin AUC. There were no significant differences for total-ghrelin AUC percent change. ($P > 0.05$). Dark grey bars = 2 MF, light shaded bars = 6 MF

APPENDIX I.



Supplemental Figure 2 Individual subject results for percent change for insulin AUC. There were no significant differences for Insulin AUC percent change. ($P > 0.05$) Dark grey bars = 2 MF, light shaded bars = 6 MF

REFERENCES

Aicher BO, Haser EK, Freeman LA, et al. Diet-induced weight loss in overweight or obese women and changes in high-density lipoprotein levels and function. *Obesity (Silver Spring).* Oct 2012;20(10):2057-2062.

Antoine JM, Rohr R, Gagey MJ, Bleyer RE, Debry G. Feeding frequency and nitrogen balance in weight-reducing obese women. Hum Nutr Clin Nutr. 1984 Jan;38(1):31-8. PubMed PMID: 6693294. Epub 1984/01/01. eng.

Areta JL, Burke LM, Ross ML, et al. Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *J Physiol.* May 1 2013;591(Pt 9):2319-2331.

Arnal M, Obled C, Attaix D, Patureau-Mirand P, Bonin D. Dietary control of protein turnover. *Diabete Metab.* Nov-Dec 1987;13(6):630-642.

Bachman JL, Raynor HA. Effects of manipulating eating frequency during a behavioral weight loss intervention: a pilot randomized controlled trial. *Obesity (Silver Spring).* May 2012;20(5):985-992.

Bandini LG, Schoeller DA, Cyr HN, Dietz WH. Validity of reported energy intake in obese and nonobese adolescents. *Am J Clin Nutr.* Sep 1990;52(3):421-425.

Batterham RL, Heffron H, Kapoor S, Chivers JE, Chandarana K, Herzog H, et al. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. *Cell Metab*. 2006 Sep;4(3):223-33. PubMed PMID: 16950139. Epub 2006/09/05. eng.

Bertelsen J, Christiansen C, Thomsen C, et al. Effect of meal frequency on blood glucose, insulin, and free fatty acids in NIDDM subjects. *Diabetes Care.* Jan 1993;16(1):4-7.

Berteus Forslund H, Lindroos AK, Sjostrom L, Lissner L. Meal patterns and obesity in Swedish women-a simple instrument describing usual meal types, frequency and temporal distribution. Eur J Clin Nutr. 2002 Aug;56(8):740-7. PubMed PMID: 12122550. Epub 2002/07/18. eng.

Berteus Forslund H. Should snacks be recommended in obesity treatment? A 1-year randomized clinical trial. *Eur J Clin Nutr.* 2008;62(1):1-10.

Bhutani S, Varady KA. Nibbling versus feasting: which meal pattern is better for heart disease prevention? *Nutr Rev.* 2009 Oct;67(10):591-8. PubMed PMID: 19785690. Epub 2009/09/30. eng.

Bortz WM, Wroldsen A, Issekutz B, Jr., Rodahl K. Weight loss and frequency of feeding. *N Engl J Med.* Feb 17 1966;274(7):376-379.

Brennan IM, Seimon RV, Luscombe-Marsh ND, Otto B, Horowitz M, Feinle-Bisset C. Effects of acute dietary restriction on gut motor, hormone and energy intake responses to duodenal fat in obese men. *Int J Obes (Lond).* Mar 2011;35(3):448-456.

Brinton EA, Eisenberg S, Breslow JL. A low-fat diet decreases high density lipoprotein (HDL) cholesterol levels by decreasing HDL apolipoprotein transport rates. *J Clin Invest.* Jan 1990;85(1):144-151.

Cameron JD, Cyr MJ, Doucet E. Increased meal frequency does not promote greater weight loss in subjects who were prescribed an 8-week equi-energetic energy-restricted diet. *Br J Nutr.* Apr 2010;103(8):1098-1101.

de Castro JM. The Award for Nutrition and Metabolism. In search of the structure of a function: the eating behavior of free-living humans. *Nutrition*. 2007 Apr;23(4):374-7.

Drummond S, Crombie N, Kirk T. A critique of the effects of snacking on body weight status. *Eur J Clin Nutr.* 1996 Dec;50(12):779-83. PubMed PMID: 8968697.

Duval K, Strychar I, Cyr MJ, Prud'homme D, Rabasa-Lhoret R, Doucet E. Physical activity is a confounding factor of the relation between eating frequency and body composition. *Am J Clin Nutr*. 2008 Nov;88(5):1200-5.

Edelstein SL, Barrett-Connor EL, Wingard DL, Cohn BA. Increased meal frequency associated with decreased cholesterol concentrations; Rancho Bernardo, CA, 1984-1987. *Am J Clin Nutr.* Mar 1992;55(3):664-669.

Edelstein SL, Barrett-Connor EL, Wingard DL, Cohn BA. Increased meal frequency associated with decreased cholesterol concentrations; Rancho Bernardo, CA, 1984-1987. Am J Clin Nutr. 1992 Mar;55(3):664-9.

Fabry P, Hejl Z, Fodor J, Braun T, Zvolankova K. The frequency of meals. Its relation to overweight, hypercholesterolaemia, and decreased glucose-tolerance. *Lancet.* 1964 Sep 19;2(7360):614-5.

Farshchi HR, Taylor MA, Macdonald IA. Beneficial metabolic effects of regular meal frequency on dietary thermogenesis, insulin sensitivity, and fasting lipid profiles in healthy obese women. *Am J Clin Nutr.* Jan 2005;81(1):16-24.

Finkelstein B, Fryer BA. Meal frequency and weight reduction of young women. *Am J Clin Nutr.* Apr 1971;24(4):465-468.

Flanagan DE, Evans ML, Monsod TP, Rife F, Heptulla RA, Tamborlane WV, et al. The influence of insulin on circulating ghrelin. *Am J Physiol Endocrinol Metab.* 2003 Feb;284(2):E313-6.

Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes Relat Metab Disord.* Jan 2000;24(1):38-48.

Garrow JS, Durrant M, Blaza S, Wilkins D, Royston P, Sunkin S. The effect of meal frequency and protein concentration on the composition of the weight lost by obese subjects. *Br J Nutr.* Jan 1981;45(1):5-15.

Gordon DJ, Probstfield JL, Garrison RJ, et al. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. *Circulation.* Jan 1989;79(1):8-15.

Goris AH, Westerterp-Plantenga MS, Westerterp KR. Undereating and underrecording of habitual food intake in obese men: selective underreporting of fat intake. *Am J Clin Nutr.* Jan 2000;71(1):130-134.

Gwinup G, Byron RC, Roush W, Kruger F, Hamwi GJ. Effect of nibbling versus gorging on glucose tolerance. Lancet. 1963 Jul 27;2(7300):165-7. PubMed PMID: 13951548. Epub 1963/07/27. eng.

Gwinup G. Insulin resistance and antagonism and their clinical significance. *W V Med J.* 1963 Jul;59:190-2. PubMed PMID: 13951547. Epub 1963/07/01. eng.

Hartmann C, Siegrist M, van der Horst K. Snack frequency: associations with healthy and unhealthy food choices. *Public Health Nutr.* 2012 Aug 16:1-10.

Healthy People. Healthy People, 2020. In: Services USDoAaUSDoHaH, editor. Washinton DC2010.

Heden TD, Liu Y, Sims LJ, et al. Meal Frequency Differentially Alters Postprandial Triacylglycerol and Insulin Concentrations in Obese Women. *Obesity (Silver Spring).* Jun 22 2012.

Homaee HM, Moradi F, Azarbayjani MA, Peeri M. Relationships between acylated ghrelin with growth hormone, insulin resistance, lipid profile, and cardio respiratory function in lean and obese men. *J Res Med Sci*. 2011 Dec;16(12):1612-8.

Jackson SJ, Leahy FE, Jebb SA, Prentice AM, Coward WA, Bluck LJ. Frequent feeding delays the gastric emptying of a subsequent meal. Appetite. 2007 Mar;48(2):199-205.

Jakubowicz D, Froy O, Wainstein J, Boaz M. Meal timing and composition influence ghrelin levels, appetite scores and weight loss maintenance in overweight and obese adults. *Steroids.* Mar 10 2012;77(4):323-331.

Jebb SA, Cole TJ, Doman D, Murgatroyd PR, Prentice AM. Evaluation of the novel Tanita body-fat analyser to measure body composition by comparison with a four-compartment model. *Br J Nutr.* Feb 2000;83(2):115-122.

Jenkins DJ, Wolever TM, Ocana AM, Vuksan V, Cunnane SC, Jenkins M, et al. Metabolic effects of reducing rate of glucose ingestion by single bolus versus continuous sipping. Diabetes. 1990 Jul;39(7):775-81. PubMed PMID: 2191884. Epub 1990/07/01. eng.

Jenkins DJ, Wolever TM, Vuksan V, et al. Nibbling versus gorging: metabolic advantages of increased meal frequency. *N Engl J Med.* Oct 5 1989;321(14):929-934.

Kanoski SE, Fortin SM, Ricks KM, Grill HJ. Ghrelin Signaling in the Ventral Hippocampus Stimulates Learned and Motivational Aspects of Feeding via PI3K-Akt Signaling. *Biol Psychiatry*. 2012 Aug 9.

Kirk TR. Role of dietary carbohydrate and frequent eating in body-weight control. *Proc Nutr Soc.* 2000 Aug;59(3):349-58.

Kong A, Beresford SA, Alfano CM, et al. Self-monitoring and eating-related behaviors are associated with 12-month weight loss in postmenopausal overweight-to-obese women. *J Acad Nutr Diet.* Sep 2012;112(9):1428-1435.

La Bounty PM, Campbell BI, Wilson J, et al. International Society of Sports Nutrition position stand: meal frequency. *J Int Soc Sports Nutr.* 2011;8:4.

Leidy HJ, Armstrong CL, Tang M, Mattes RD, Campbell WW. The influence of higher protein intake and greater eating frequency on appetite control in overweight and obese men. *Obesity (Silver Spring).* Sep 2010;18(9):1725-1732.

Leidy HJ, Campbell WW. The effect of eating frequency on appetite control and food intake: brief synopsis of controlled feeding studies. *J Nutr*. 2011 Jan;141(1):154-7.

Ma Y, Bertone ER, Stanek EJ, 3rd, Reed GW, Hebert JR, Cohen NL, et al. Association between eating patterns and obesity in a free-living US adult population. *Am J Epidemiol.* 2003 Jul 1;158(1):85-92.

McGrath SA, Gibney MJ. The effects of altered frequency of eating on plasma lipids in free-living healthy males on normal self-selected diets. *Eur J Clin Nutr.* Jun 1994;48(6):402-407.

Melanson EL, Coelho LB, Tran ZV, Haugen HA, Kearney JT, Hill JO. Validation of the BodyGem hand-held calorimeter. *Int J Obes Relat Metab Disord.* Nov 2004;28(11):1479-1484.

Mendez MA, Wynter S, Wilks R, Forrester T. Under- and overreporting of energy is related to obesity, lifestyle factors and food group intakes in Jamaican adults. *Public Health Nutr.* Feb 2004;7(1):9-19.

Mills JP, Perry CD, Reicks M. Eating frequency is associated with energy intake but not obesity in midlife women. *Obesity (Silver Spring).* 2011 Mar;19(3):552-9.

Mokdad AH, Marks JS, Stroup DF, Gerberding JL. Actual causes of death in the United States, 2000. *JAMA*. 2004 Mar 10;291(10):1238-45.

Moran LJ, Noakes M, Clifton PM, et al. Postprandial ghrelin, cholecystokinin, peptide YY, and appetite before and after weight loss in overweight women with and without polycystic ovary syndrome. *Am J Clin Nutr.* Dec 2007;86(6):1603-1610.

Munsters MJ, Saris WH. Effects of meal frequency on metabolic profiles and substrate partitioning in lean healthy males. *PLoS One.* 2012;7(6):e38632.

Murphy MC, Chapman C, Lovegrove JA, et al. Meal frequency; does it determine postprandial lipaemia? *Eur J Clin Nutr.* Aug 1996;50(8):491-497.

Odunsi ST, Vazquez-Roque MI, Camilleri M, Papathanasopoulos A, Clark MM, Wodrich L, et al. Effect of alginate on satiation, appetite, gastric function, and selected gut satiety hormones in overweight and obesity. *Obesity (Silver Spring).* 2010 Aug;18(8):1579-84.

Ogden CL, Carroll MD, Kit BK, Flegal KM. Prevalence of obesity in the United States, 2009-2010. *NCHS Data Brief*. 2012 Jan(82):1-8.

Ohkawara K, Cornier MA, Kohrt WM, Melanson EL. Effects of increased meal frequency on fat oxidation and perceived hunger. *Obesity (Silver Spring).* Feb 2013;21(2):336-343.

Palmer MA, Capra S, Baines SK. Association between eating frequency, weight, and health. *Nutr Rev*. 2009 Jul;67(7):379-90.

Pearcey SM, de Castro JM. Food intake and meal patterns of weight-stable and weight-gaining persons. *Am J Clin Nutr.* 2002 Jul;76(1):107-12.

Poston WS, Haddock CK, Pinkston MM, et al. Weight loss with meal replacement and meal replacement plus snacks: a randomized trial. *Int J Obes (Lond).* Sep 2005;29(9):1107-1114.

Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology.* Oct 2003;28(7):916-931.

Robinson JG, Wang S, Smith BJ, Jacobson TA. Meta-analysis of the relationship between non-high-density lipoprotein cholesterol reduction and coronary heart disease risk. *J Am Coll Cardiol*. 2009 Jan 27;53(4):316-22.

Sadur CN, Eckel RH. Insulin stimulation of adipose tissue lipoprotein lipase. Use of the euglycemic clamp technique. *J Clin Invest*. 1982 May;69(5):1119-25

Schiller JS, Lucas JW, Ward BW, Peregoy JA. Summary health statistics for U.S. adults: National Health Interview Survey, 2010. *Vital Health Stat 10*. 2012 Jan(252):1-207.

Schwarz NA, Rigby BR, La Bounty P, Shelmadine B, Bowden RG. A review of weight control strategies and their effects on the regulation of hormonal balance. *J Nutr Metab*. 2011;2011:237932.

Schwarz NA, Rigby BR, La Bounty P, Shelmadine B, Bowden RG. A review of weight control strategies and their effects on the regulation of hormonal balance. *J Nutr Metab*. 2011;2011:237932.

Smeets AJ, Westerterp-Plantenga MS. Acute effects on metabolism and appetite profile of one meal difference in the lower range of meal frequency. *Br J Nutr.* Jun 2008;99(6):1316-1321.

Solomon TP, Chambers ES, Jeukendrup AE, Toogood AA, Blannin AK. The effect of feeding frequency on insulin and ghrelin responses in human subjects. *Br J Nutr*. 2008 Oct;100(4):810-9.

Song YM, Lee K, Sung J, Yang YJ. Changes in eating behaviors and body weight in Koreans: the Healthy Twin Study. *Nutrition.* 2013 Jan;29(1):66-70. PubMed PMID: 22858202. Epub 2012/08/04. eng.

Speechly DP, Rogers GG, Buffenstein R. Acute appetite reduction associated with an increased frequency of eating in obese males. *Int J Obes Relat Metab Disord*. 1999 Nov;23(11):1151-9.

Stock S, Leichner P, Wong AC, et al. Ghrelin, peptide YY, glucose-dependent insulinotropic polypeptide, and hunger responses to a mixed meal in anorexic, obese, and control female adolescents. *J Clin Endocrinol Metab.* Apr 2005;90(4):2161-2168.

Stote KS, Baer DJ, Spears K, et al. A controlled trial of reduced meal frequency without caloric restriction in healthy, normal-weight, middle-aged adults. *Am J Clin Nutr.* Apr 2007;85(4):981-988.

Taylor MA, Garrow JS. Compared with nibbling, neither gorging nor a morning fast affect short-term energy balance in obese patients in a chamber calorimeter. *Int J Obes Relat Metab Disord.* 2001 Apr;25(4):519-28.

Titan SM, Bingham S, Welch A, et al. Frequency of eating and concentrations of serum cholesterol in the Norfolk population of the European prospective investigation into cancer (EPIC-Norfolk): cross sectional study. *BMJ.* Dec 1 2001;323(7324):1286-1288.

Vander Wal JS, Waller SM, Klurfeld DM, et al. Effect of a post-dinner snack and partial meal replacement program on weight loss. *Int J Food Sci Nutr.* Feb-Mar 2006;57(1-2):97-106.

Verbaeys I, Tolle V, Swennen Q, et al. Scheduled feeding results in adipogenesis and increased acylated ghrelin. *Am J Physiol Endocrinol Metab.* Jun 2011;300(6):E1103-1111.

Verboeket-van de Venne WP, Westerterp KR, Kester AD. Effect of the pattern of food intake on human energy metabolism. *Br J Nutr.* 1993 Jul;70(1):103-15.

Westerterp-Plantenga MS, Lemmens SG, Westerterp KR. Dietary protein - its role in satiety, energetics, weight loss and health. *Br J Nutr.* Aug 2012;108 Suppl 2:S105-112.

Wolever TM. Metabolic effects of continuous feeding. *Metabolism.* 1990 Sep;39(9):947-51.

Yannakoulia M, Melistas L, Solomou E, Yiannakouris N. Association of eating frequency with body fatness in pre- and postmenopausal women. *Obesity (Silver Spring).* 2007 Jan;15(1):100-6.