Will the Daily Consumption of Commercially Available High-Protein Pasta and Cereal, in Comparison to Traditional Gluten-Free Pasta and Cereal, Favorably Impact Weight Loss and Satiety in Adults Adhering to Calorie Restricted Diets?

by

Andrew James

A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved July 2015 by the
Graduate Supervisory Committee:

Carol Johnston, Chair
Sandra Mayol-Kreiser
Christina Shepard

ARIZONA STATE UNIVERSITY

August 2015

ABSTRACT

Objective: This research examined the effectiveness of a weight loss diet incorporating high protein pasta and breakfast cereal products as compared to a weight loss diet using conventional versions of gluten-free pasta and breakfast cereal.

Design: In a 6-week parallel-arm food trial (representing the first phase of a 12-week cross-over trial), 26 overweight and obese (Mean BMI 43.1 ± 12.4 kg/m²) participants, free of related comorbidities, were randomly assigned to the Zone diet (~29% energy intake from protein) or a control diet (~9% energy from protein). Participants were included in the trial if they satisfied the criteria for elevated risk for metabolic syndrome (top half of the TG/HDL ratios of all who were tested). Participants were instructed to eat prepared meals (total of 7 cereal packets and 14 pasta meals weekly) that included patented food technologies for the Zone diet and commercially available gluten-free rice pasta and a conventional name brand boxed cereal for the control diet. Body composition was measured with a bioelectrical impedance scale at weeks 1, and 6. Food records and diet adherence were recorded daily by the participants.

Results: Both the Zone and control diets resulted in significant weight loss (-2.9 ± 3.1 kg vs. -2.7 ± 2.6 kg respectively) over time ($p = 0.03$) but not between groups ($p = 0.96$). Although not statistically significant, the Zone diet appears to have influenced more weight loss at trial weeks 3, 4, and 5 ($p = 0.46$) than the control diet. The change in FFM was significant ($p = 0.02$) between the Zone and control groups (1.4 ± 3.6 kg vs. -0.6 ± 1.5 kg respectively) at week-6. Study adherence did not differ significantly between diet groups ($p = 0.53$).

Conclusions: Energy-restricted diets are effective for short-term weight loss and high protein intake appears to promote protein sparing and preservation of FFM during weight loss. The macronutrient profile of the diet does not appear to influence calorie intake, but it does appear to influence the quality of weight loss. Other measures of body composition and overall health outcomes should be examined by future studies to appropriately identify the potential health effects between these diet types.

DEDICATION

I dedicate this thesis to my father, Mick James, whose untimely death related to complications from pancreatic cancer inspired me to become a Registered Dietitian to help others win their battles. I would also like to thank Dr. Pepper for giving me the extra pep I needed to finally finish this work.

ACKNOWLEDGMENTS

I would like to first thank my chair Dr. Carol Johnston for her generous time, support, and commitment to my academic endeavors and thesis completion. Over a period of 3 years and multiple thesis iterations, she has never faltered in her genuine attempts to see me succeed. Likewise, I extend the same appreciation to my committee members Tina Shepard, and Dr. Sandra Mayol-Kreiser. Both of these amazing women fought for me, struggled with me, and never wavered in their commitment to making me the best future Registered Dietitian I can be.

I want to acknowledge Dr. Jessica Knurick for allowing me to join her Zone food trial to complete my thesis. She worked incredibly hard to design and launch this study and I was incredibly fortunate and grateful to be a part of this magnificent experience. Additionally, I would like to thank the numerous student volunteers who helped produce all the meals for this trial to be successful. They spent countless hours in the metabolic kitchen to produce thousands of meals over a 3 month period which was no easy feat. Additionally, their help with the data entry and the numerous other roles were critical for my success. As with most studies at Arizona State, Ginger Hook played a major role in my success, from collecting and analyzing all the participant blood work to the fantastic moral support and encouragement. Anytime I felt lost in the lab, Ginger always seemed to be there to point me in the right direction. I am incredibly grateful to all the participants who decided it was a great idea to eat pasta every day for 12 weeks. There's no way I could manage that level of bravery and believe they should all be enshrined in the Guinness Book of World Records for their mental fortitude and iron stomachs.

Finally, I'd like to thank my mother for the incredible support during a time of transition in my life. She stood by my side when I decided to leave my career and go back to school. It was not an easy decision, but thanks to her support and positive outlook I get to experience the dream that so many people go their whole lives without achieving. I get to be a Registered Dietitian, and that is amazing.

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CHAPTER 1

INTRODUCTION

Although the rise of BMI-defined overweight and obesity in the United States (U.S.) over the past 35 years appears to be leveling off, nearly 70% of adults are currently overweight or obese. In 2010, more than a third (35.9%) of the country was categorized as obese, representing more than 78 million adults (1, 2). These statistics are staggering considering obese individuals spend more on healthcare than their normal-weight counterparts. In 2008, obese related healthcare expenses equaled $146 billion, including nearly 50% higher inpatient costs, more than a quarter (27%) increase in outpatient expenses, and 80% more spent on prescription drugs compared to normal-weight individuals (3). In addition to increased medical costs, obesity increases risk of all-cause mortality and morbidity from type 2 diabetes, hypertension, coronary heart disease, stroke, dyslipidemia and some types of cancers among a much longer list of associated diseases (4).

The prevalence of overweight and obesity within the U.S. changed little between 1960 and 1980 but began to rise steadily in the 80's and 90's and tapered off around 2003 to near current levels (1). The trend in the growing circumference of the population sparked a weight loss industry projected at $66 billion in the U.S. in 2013 including a vast array of new diets, services, and surgical procedures to manage the problem of ballooning proportions (5). With more than 100 million Americans initiating 4 to 5 weight loss diet attempts a year, the obesity management industry is projected to continue to grow near its historical average around 6% per year (5).

Creator of the Zone Diet, Dr. Barry Sears, argues that the obesity epidemic started around 1980 when government nutrition boards and scientific panels labeled a healthy diet as one low in the macronutrients fat and protein and high in carbohydrates (6). He believes that the country was part of an unsubstantiated social experiment created around these purported healthy recommendations and as a result, the population began to swell. This counterargument, as portrayed by the Zone Diet, is that a healthy diet contains a balanced macronutrient profile: moderate amounts of fat, carbohydrate, and protein (30, 40, and 30 percent energy respectively) (6).The Zone Diet has successfully marketed numerous food products with the intent to balance the macronutrient intake inline with its macronutrient recommendations. The proposed research will examine the weight loss efficacy of new Zone products, reengineered pasta and breakfast cereal products with balanced macronutrient profiles, as compared to conventional cereal and low gluten pasta.

Purpose of Study

The objective of this research is to identify the effectiveness of weight loss diets incorporating high protein pasta and breakfast cereal products as compared to weight loss diets using conventional versions of gluten-free pasta and breakfast cereal. The published literature suggests protein is more satiating than both carbohydrate and fat in both the short and long term (7). Additionally, high protein diets, irrespective of the protein source, appear to reduce total energy intake as a result of greater satiety (8, 9). However, a systematic review of randomized controlled trials (RCTs) of popular diets (Atkins, South Beach, Weight Watchers, and Zone) over a period of one year demonstrated they all achieved similarly modest weight loss regardless of macronutrient profiles (10). The

authors suggested the data reviewed from the RCTs were conflicting and the lack of true head-to-head comparisons resulted in the inability to identify any of the diets as being superior to the others.

Research Aim and Hypotheses

Based on reported mechanisms linking protein intake with satiation and weight loss (11), three hypotheses are proposed:

Hypothesis 1

The high protein (Zone pasta and cereal) diet will result in greater weight loss as compared to a diet incorporating gluten-free pasta and cereal.

Hypothesis 2

The high protein diet will support protein sparing resulting as indicated by a lower loss of lean mass.

Hypothesis 3

The increased satiety effect of protein will promote greater adherence to the diet with higher levels of protein.

Delimitations and Limitations

Volunteers for the study were directed to an online survey which was designed to address inclusion and exclusion criteria. Volunteers who met these criteria were eligible to participate in the study. Following strict eligibility criteria ensured intervening variables were held to a minimum when assessing the final results from the weight loss trial. Only apparently healthy individuals with no history of food allergies or dietary restrictions were eligible. Cigarette use, insulin use, and active disease states excluded individuals from participation. A total of 43 qualified participants were enrolled with the

target goal of completing the study with at least 25 participants, understanding expected attrition rates associated with feeding trials. All participants had their basal metabolic rate calculated (Mifflin-St. Jeor equation) and received a personalized calorie intake range with suggested additional food items to promote a 1-2 pound per week weight loss. Each participant received a total of 14 pasta meals and 7 cereal packets every week during the 12-week trial. The macronutrient profiles of the test foods varied by study group: macronutrient balanced or high carbohydrate/low protein.

The length of study presented challenges to the participants as 12 weeks represents an extensive time period to adhere to the trial and its goals. (Note: the research presented herein focuses on the data from the first 6 week phase of a 12 week crossover trial.) Participants were instructed to pick up their meals on a weekly basis which may have been challenging over the duration of the trial. Additionally, researchers have indicated food studies which are conducted over longer periods of time tend to result in gradually diminishing palatability ratings which could present adherence difficulties for participants eating the same foods every week regardless of protein content (12). It is believed a fair amount of attrition was to be expected with a trial of this duration based on the extent participants favor the food they receive. Participants self monitored their diet intake and behavior during the trial.

Definitions

Basal Metabolic Rate (BMR) – The minimal energy expenditure at rest (based on the Mifflin-St.Jeor equation).

Women: BMR = (10 x weight {kg}) + (6.25 x height {cm}) - (5 x age {yrs}) - 161

Men: BMR = (10 x weight {kg}) + (6.25 x height {cm}) - (5 x age {yrs}) + 5

Body Mass Index (BMI) – An index for comparing height to weight; calculated by dividing weight in kilograms by the square of height in meters; used to determine weight status.

Body Fat Percentage – Total percent of body fat compared to total weight. Measured with the Tanita scale via electrical impedance.

Lean Body Mass – Total body weight minus weight from body fat. Measured with the Tanita scale via electrical impedance.

Overweight – BMI range in adults between 25 kg/m^2 and 29.9 kg/m^2.

Obesity – BMI range in adults $\geq$ 30 kg/m^2.

Chapter 2

REVIEW OF LITERATURE

State of Obesity

Prevalence of Obesity. The National Center for Health Statistics stated nearly two-thirds of the population was classified as overweight (BMI 25-29.9 kg/m²) or obese (BMI ≥ 30 kg/m²) in 2004 but less than half of the population had the same classification in 1980 (13). According to data recorded from the National Health and Nutrition Examination Survey (NHANES), in 2008 the prevalence of obesity in the U.S. was recorded at 33.8% (78 million adults) of the population, representing more than 100% increase in obesity from the time the survey was conducted from 1976 to 1980 (15% obese) (14). Using BMI classifications for overweight and obese, little change in population weight status was recognized from 1960 through 1980 according to a comparison of the National Health Examination Survey (NHES I, 1960 – 1962) and NHANES (15). This trend changed dramatically after 1980, and by the time of the 1988-1994 NHANES was completed, an 8% increase in obesity was identified (16). A similar rise was again noted by the survey from 1999-2000 but this trend has leveled over the past decade (1).

As the population becomes more rotund, a greater share of the annual healthcare costs will need to be allocated to the management of overweight and obesity related health problems. These health problems are primarily the result of serious endocrine and metabolic changes that include type 2 diabetes, heart disease, and increased cancer risks. In addition to these ailments, obese individuals tend to have a lower quality of life, an increase in mortality and morbidity, and more functionally limiting restrictions (17). It is

difficult to project annual expenditures on the healthcare system as both direct and indirect costs are associated with this problem. Using data from the Medical Expenditure Panel Survey (MEPS), one group of researchers estimated that the cost to the U. S. was $147 billion dollars in 2008 which represented 9.1% of all healthcare expenditures that year (3). However, an earlier publication adjusted the MEPS data to factor in the weight of the respondents' biological relatives and found the impact of obesity on annual healthcare costs may have reached $190.2 billion (20.6% of total expenditure) by the year 2005 (18). Forecasting the prevalence of obesity through to the year 2030, a recent publication used regression modeling to project that the rate of obesity will increase to about 42% of the population, representing an estimated increase of about $550 billion in healthcare expenditures compared to 2010 levels (19). Others using more optimistic models suggest that obesity will plateau at 32% of the population by 2030 regardless of intervention strategies (20).

Dietary Guidelines. The contention from the Zone Diet is that the recent obesity epidemic stemmed from the primary dietary wisdom over the past 35 years which has espoused a low fat, low protein, and high carbohydrate diet as the proper ratio for a healthy and balanced nutritional approach (6). The argument in favor of a paradigm shift in our nutritional recommendations dictates that this dietary "wisdom" sparked a social experiment that is directly correlated to the rise in obesity recorded over this time period. Based upon the high carbohydrate model, the U.S. Department of Health and Human Services (HHS) and the U.S. Department of Agriculture (USDA) jointly launched the Dietary Guidelines for Americans (DGA) in 1980 and have published updates every 5 years (21). The macronutrient profile presented within these guidelines recommends that

45% to 65% of total calories comprise of carbohydrates, while 20% to 35% should come from fat, and the remaining 10% to 15% of calories from protein (22).

In 1997, the Dietary Reference Intakes (DRI) was founded with the recommendations from the Institute of Medicine and the National Academy of Sciences to establish quantitative estimates for dietary intake for healthy individuals. As part of the DRI, Acceptable Macronutrient Distribution Ranges (AMDRs) were created with a similar macronutrient profile to the DGA with one major difference. The range as a percent of energy for protein was increased up to 35% of total calories for adults (23). The increase in protein intake highlights a move from the traditional wisdom of the recent past but fails to leave behind the backbone of the high carbohydrate model. The trends in obesity began to level at the turn of the century, only a few years after the DRI had established new recommendations for a healthy diet (1). However, it is unlikely the higher protein recommendation had anything to do with the leveling obesity trend as Americans consume an average of 50% of their total calories from carbohydrates, 33% from fats, and about 15% from proteins which is consistent with both the DGA and DRI recommendations (24).

Standard American Diet. The rise in overweight and obesity in the U. S. coincides with the release of macronutrient recommendations from the government but is more likely connected to an increase in per capita caloric intake associated with the increase in food availability. The standard American diet (SAD) began to change when the availability of food increased substantially resulting in a significant increase in average caloric intake (24). According to the USDA's Economic Research Service (ERS), Americans consumed about 1,900 kcal per day in the 1950's and this intake level

rose to over 2,600 kcal per day by 2008 (25). The ERS noted that the bulk of this increase occurred from 1970 to 2000 which is within a similar timeframe coinciding with the rise in obesity. Grains (9.5 % increase), added fats (9% increase), and added sugars (4.7% increase) comprised the most significant increase in calories to the SAD during this time period which helps to explain why protein comprises only about 15% of the average diet (24). The abundance and accessibility to foods and beverages with added sugars and fats combined with a seemingly reduced availability of nutrient-dense options may offer a more plausible explanation for the sharp increase in weight gain over the past four decades (26).

Mechanisms of Protein and Satiety

The increase in average caloric intake of the SAD may be a result of the addition of carbohydrates and fats into the diet without a similar increase in caloric intake from proteins. Overweight and obesity is a reflection of a diet that results in a positive energy balance or one where more calories are consumed than used in a given time period. Protein appears to have an increased satiety response when compared to carbohydrates and fats. The SAD may be a reflection of this effect as a reduction in the percentage of protein intake essentially inhibits its feeling of satiety for the other more readily accessible macronutrients. Increasing protein availability and intake into the diet should result in higher levels of satiety and help restore the energy balance equation. Several factors are associated with protein-induced satiety and its potential for weight loss. Protein increases energy expenditure through the thermic effect of food (TEF), and appears to increase hormones and metabolites that reduce hunger. Therefore, an increase

in protein intake may help the energy balance equation by both enhancing satiety (reducing intake) and increasing calorie output (27).

Diet-Induced Thermogenesis. Protein has the highest TEF for all the macronutrients and thus has the highest satiating efficiency of the three followed by carbohydrates and lastly by fats. The TEF is measured as a percentage increase in the BMR and is best explained by the metabolic responses to digestion, absorption, nutrient transport, and the metabolism and storage of nutrients (27). Protein was shown to have the highest effect (15% to 30%), followed by carbohydrates (5% to 10%) and fat (0% to 3%) (28). An increase in the TEF essentially dictates a greater calorie output and results in a positive effect on energy balance. This increase was exemplified by researchers who substituted carbohydrates (about 18% of energy) with either pork or soy protein, producing an average of 3% increase (pork = 492 kJ/d, 3.9%, $p = 0.05$; soy = 244 kJ/d, 1.9%, $p < 0.05$) in the 24 hour energy expenditure (29). The control meal was 28% composed of fat and 11% as protein, and the test meals were composed of 28% fat and 29% protein. In addition to the elevated energy output, they reported participants had a slightly lower energy intake with the higher protein diets despite using isoenergetic substitutions for carbohydrates. They noted participants had a difficult time finishing the meals likely due to the satiating effects of protein.

The protein-induced satiety was also registered in combination with the increased thermic effect of protein by researchers measuring participants in a respiration chamber (30). Using energy balanced meals, they compared the energy expenditures and satiety associated with high protein meal intervals (29% of total calories) and low protein meal intervals (9% of total calories). They noted that participants reported greater satiety and

fullness throughout the day on the high protein diet and had lowered feelings of hunger, appetite, and desire to eat despite being unable to note significance in the amount eaten between the two groups. They did find a significant difference between the diet-induced thermogenesis from the two groups (HP = 1295 ± 240 kJ/d, LP = 931 ± 315 kJ/d; $p < 0.01$), and noted a positive relationship ($r = 0.8$; $p < 0.01$) between the difference in the thermic effect of the diets with feelings of satiety. Crovetti, et al., noted a similar relationship between the TEF and satiety comparing a series of isoenergetic meals with different macronutrient profiles (31). High protein (68% of energy), high carbohydrate (69% of energy), and high fat (70% of energy) meals were offered to a group of 10 normal weight women and their energy expenditure was measured using indirect calorimetry. The high protein meal had a significantly higher TEF (261 ± 59 kJ; $p < 0.001$) than both carbohydrate (92 ± 67 kJ) and fat (97 ± 71 kJ). It also registered the highest satiety levels of the three macronutrients. A positive relationship between TEF and satiety for all three meal types ($r = 0.41$; $p = 0.025$) was noted.

Using an ad libitum approach instead of a controlled approach to meal intake, Raben et al., found that meals with similar energy densities but different macronutrient profiles did not elicit a change on appetite or energy intake (32). While they found a similar TEF as the previous researchers they did not find any relationship between energy expenditure and satiety. Comparing meals rich in protein (32% of energy), carbohydrate (65% of energy), and fat (65% of energy) on normal weight participants, they identified a 17% increase in TEF after the protein meal but found no significance between it and the other two meal types. They were surprised by the results but surmised the similar energy densities between the meals may explain the discrepancies between their results and

previous studies. In an interesting twist, the participants demonstrated a lower desire for sweet foods after the high carbohydrate meal compared to the high protein meal. The total food intake between the three groups was not significantly different however, suggesting that satiety and hunger may be less prone to change between the macronutrients when present with an ad libitum approach to eating. The results suggest that the macronutrient hierarchy in TEF may not coincide directly with the satiety hierarchy suggesting other mediating factors may be required to elucidate an increased satiated response to the thermic effect of protein.

Satiety Hormones. Other mechanisms involved with the increase in satiety following an increase in protein intake may help to explain the connection with the TEF. Hormonal changes related to protein intake and satiety may play an important role in how the energy balance equation is mediated within the body. Researchers demonstrated that satiety related to a high protein diet was mediated through an increase in Glucagon-like peptide-1 (GLP-1), an appetite suppressing hormone (33). GLP-1 inhibits the secretion of glucagon but also slows gut motility and gastrointestinal secretions which down regulates appetite, and increases satiety as a result (34). A total of 12 healthy women were on an isoenergetic diet of either a low protein (10% protein, 60% carbohydrate, and 30% fat) or a high protein (30% protein, 40% carbohydrate, and 30% fat) for a total of 4 days (33). On the last day both ghrelin, an appetite stimulating hormone, and GLP-1 were measured. They found GLP-1 concentrations were significantly higher in the high protein group and ghrelin concentrations were not significantly different between the two groups. They also found that the TEF and satiety were significantly higher while hunger was lower on the high protein diet. Despite the energy being essentially equivalent between

both meals, data from the respiration chamber revealed subjects were in energy balance on the high protein diet but actually registered a positive energy balance on the low protein diet.

Cholecystokinin (CCK), a peptide hormone which is also found in the gastrointestinal tract is another mechanism by which protein can stimulate an increase in satiety. Like GLP-1, CCK inhibits gastric emptying but is triggered by the digestion of proteins into amino acids, effectively reducing food intake as a result (34). Testing macronutrient effects on CCK and satiety, researchers enrolled a group of both lean and obese men to test a series of diets ranging in 4 different macronutrient profiles based upon protein levels (HP = 45%, AP = 35%, HF = 15%, and LP = 10%) (35). After eating a standardized breakfast, each group ate lunch from one of the 4 groups which were energy balanced. Hormones, hunger, and fullness were continuously monitored for a period of 3 hours and energy intake was measured at a buffet meal. The lean group ate significantly less from all but the LP (highest carbohydrate) test group. The obese individuals who received the HF or LP lunches registered the highest energy intake when presented with the buffet. The CCK response to both the HP and AP groups were sustained for the duration of time between the lunch and the buffet meals and were associated with the greatest levels of satiety.

Following a similar design to the previous researchers, Bowen et al., gave liquid preloads containing either whey (55g), casein (55g), lactose (56g), or glucose (56g) to a group of 19 overweight male participants following a buffet meal 3 hours later. Ghrelin, CCK, and satiety were all measured in 15 minute increments after the preload was ingested and the participant's energy intake was analyzed as a quotient of the buffet meal.

Energy intake was significantly higher (10 ± 3%; $p < 0.05$) after the glucose preload compared to the other three preloads and this result was predicted by the ghrelin levels ($p < 0.05$) measured at the 120 minute mark after preload ingestion. Higher CCK levels (71 ± 6%; $p < 0.05$) were measured for both the protein preloads after 90 minutes, and the levels at 180 minutes successfully predicted satiety for all 4 groups but had no significant link to energy intake. These results suggest that the suppression of ghrelin following the protein intake may be related to the satiety reported by participants and appeared to interact inversely with CCK levels (36). A recent animal study demonstrated that an infusion of CCK had an inhibitory effect on grelin resulting in a reduction in food intake (37).

Comparing satiety-related hormonal responses following whey and maltodextrin intake, a recent study measured GLP-1, CCK, peptide tyrosine-tyrosine (PYY), and pancreatic peptide's (PP) effect on satiety in a group of 18 normal-weight female participants (38). Upon ingestion of either an isoenergetic preload of protein or malodextrin (1333 kJ per 300ml versus 1370 kJ per 300ml respectively), the participants' hormone levels and measurements of appetite (hunger, fullness, desire to eat) were recorded at regular intervals prior to an ad libitum fried rice test meal. Similar to the previous reviewed literature, they confirmed both CCK (60 and 75 min post) and GLP-1 (90 min post) levels were significantly higher after consuming the protein preload. The investigators also noted a significant increase in PYY (90 and 120 min post) and PP (15 to 120 min post) compared to the maltodextrin intake. Despite registering increases in the satiety-related hormones, only PP had a significant negative relationship ($r = -0.21$; $P < 0.01$) with energy intake but had no effect on measurements for appetite. The subjects

with the highest plasma PP levels registered the least amount of food intake following the preload.

Amino Acids and Metabolites. In addition to testing satiety-related hormones, Chungchunlam et al., measured plasma amino acids and their relationship to energy intake and measurements of appetite (38). Following the whey protein preload, participants' plasma concentrations of total amino acid, urea, and ammonia were significantly higher within 15 minutes compared to the maltodextrin alternative ($p < 0.01$). Despite previous literature (39) suggesting a relationship between circulating amino acid concentrations and reduced energy intake, they did not observe relationships between plasma amino acids on total energy intake or any measurements of appetite. They did however, note a correlation between branched-chain amino acids (BCAA) and the rise in both GLP-1($r = 0.26$; $p < 0.01$) and PYY levels ($r = 0.48$; $p = 0.05$), suggesting a relationship between plasma amino acids and the release of satiety-related hormones. Additionally, a negative relationship ($r = -0.21$; $p < 0.01$) between urea levels and energy intake was noted suggesting a dose-dependent mechanism for satiety with protein intake.

Similar to whey protein, plasma amino acids from soy protein sources appeared to be correlated to satiety but not total energy intake. Veldhorst et al., gave 25 healthy participants energy-adjusted subject-specific breakfast meals categorized as either high protein (25% protein, 55% carbohydrate) or normal protein (10% protein, 55% carbohydrate) and measured plasma amino acids and satiety at regular intervals for 3 hours before measuring energy intake with an ad libitum lunch meal (40). Participants with the higher soy protein intake rated as being more satiated and less hungry which coincided with an increase in postprandial taurine concentrations (satiety: $r = 0.399$; $P <$

0.05; hunger: $r = 0.433$; $P < 0.05$). Like the whey protein study, feelings of satiety or reduced hunger showed no significant differences on energy intake following soy protein intake. The study also noted a similar increase in urea as Chungchunlam et al., described, but was unable to link urea to energy intake.

Plasma concentration of amino acids and their metabolites may impact satiety by initiating the release of satiety-related hormones but another group of researchers suggests amino acids stimulate vagal feedback from the gut to the satiety centers of the brain (41). Studying slides of mice hypothalamus and brainstems after a period of 2-weeks on either a high protein or high fat diet showed that the rats with higher protein intake had greater activation of the nucleus tractus solitarius (NTS). The NTS is a nerve bundle in the brain stem which receives both stretch receptors and chemoreceptors from the intestinal tract, and receives information from taste buds on the tongue. The NTS of mice on the high fat diet had lowered sensitivity to CCK and a lowered response to serum amino acids as a result. The investigators suggested that the feedback to the hypothalamus acted to suppress feelings of hunger while stimulation to the NTS affects satiety at a reflex-like level.

Bypassing the gastrointestinal tract, and thus any possibility for vagal feedback, altogether, researchers used parenteral nutrition to test plasma concentrations of amino acids on satiety and food intake (42). Six healthy participants, on three separate occasions received in random order an IV saline control, low-dose IVAA (125mg protein/kg/h), and a high-dose IVAA (250mg/kg/h) for a total of 360 minutes before being presented with a meal. Each subject rated measurements of appetite and satiety at 30-minute intervals and then their energy intake was recorded after the meal. As

expected, feelings of satiety was highest with the high-dose IVAA, followed by the low-dose IVAA, and finally by the saline solution ($p < 0.05$). However, feelings of hunger were not significantly different between the three groups and as a result the total amount of food consumed at the end of the trial was not significantly different. Similar to the other studies on amino acids effect on satiety, participants were significantly more satiated but total energy intake did not change as a result. The researchers also noted no significant correlations between plasma amino acid concentrations or CCK on satiety of any of the three groups, seemingly contradicting the findings of other studies measuring the same variables.

Protein-Induced Satiety and Energy Intake

Research into the mechanisms of protein-induced satiety demonstrated a number of factors on how protein intake is correlated with satiety, but many failed to define a significant link between those factors and an overall reduction in energy intake. Moving beyond the mechanisms of protein on satiety and focusing on the effects of the system as a whole may help to demonstrate a significant link between protein-induced satiety and a reduction in energy intake. Weigle et al., designed a food trial using subject-specific weight-maintaining prepared meals (30% protein, 20% fat, and 50% carbohydrate) to increase participants' protein intake from baseline levels (15% protein, 35% fat, and 50% carbohydrate) over a period of 2 weeks before transitioning them to an ad libitum diet using the same prepared meals for the following 12 weeks (43). After at least a week on the high protein isocaloric diet, participants reported an increase in fullness and a decrease in hunger which coincided with a reduction in caloric intake (494 ± 74 kcal/d) within 24 hours of starting the ad libitum portion of the trial. The participants maintained

a significant reduction in caloric intake (441 ± 63 kcal/d, $p < 0.001$) for the duration of the trial despite reported measures of appetite returning to baseline levels within a week of the transition. In an interesting twist, in this study, satiety regressed to the original mean while energy intake levels continued to be lower for a duration of approximately 12 weeks after switching to a high protein diet.

Similar results were noted by Latner et al., after measuring ad libitum intake at dinner by participants by comparing the satiating effects of three 450kcal liquid lunches composed of a mixture of proteins and carbohydrates (protein = 71.5% of energy, carbohydrate = 99% of energy, or 35.7% protein and 55.1% carbohydrate) (44). The participant group with the highest protein lunch ate 31% less (~300 kcal) calories than the carbohydrate group, and 20% (~90 kcal) less that the moderate protein group. Participants in the high carbohydrate group reported significantly higher hunger before dinner and reported greater satisfaction after eating than the other two groups. Using similarly disproportionate protein levels (81% versus 14% of energy) in their study, Johnson et al., showed participants reported higher satiety and ate a significantly smaller energy intake after the protein preload (45). However, they assessed sensory-specific satiety played a larger role than macronutrient composition on energy intake.

The reduced sensory appeal of the high levels of protein in the preload was one factor for the reduction in calorie intake, highlighting that the reduction in calories reported was at least partly a coefficient of participant dissatisfaction (45). However, MacKenzie-Shalders et al., demonstrated diminishing returns on satiety and energy intake as protein intake increases (46). Testing a whey protein supplement (20g, 40g, 60g, or 80g protein) on 10 male athletes, they found all men reported significantly lower ratings

of hunger (50% to65%, $p < 0.05$) for the duration of the 3 hours between taking the supplement and eating an ad libitum meal. Regardless of their appetite sensations, each participant's energy intake from the meal was not significantly different from one another, representing about 30% of their estimated energy requirement. An increase above 20 grams of whey protein presented no beneficial impact in lowering food intake or increasing satiety despite nearly a 1000 kJ increase in energy consumed in the highest protein supplement compared with the lowest protein supplement. Another group of researchers determined that whey protein doses above 10g had a significant impact on reducing food intake, but comparatively no increase in benefit was detected between 10g, 20g, 30g, and 40g conditions (47).

Protein and Weight Loss

Higher amounts of protein intake above DGA levels have been demonstrated to have a positive relationship on the increase of satiety and other measures of appetite. The suppression of appetite in response to protein has a negative relationship with energy intake and that relationship may be dose-dependent. A number of studies and trials have been conducted to determine if protein-induced satiety and its relation to energy intake would result in reduction in total body weight. Evaluating studies focusing on total weight loss, body composition, and the safety of high-protein diets are necessary to understand the efficacy of increasing daily intake of protein.

Short Term Weight Loss. Soenen et al., created an energy-restricted 2 x 2 factorial weight loss trial (132 participants) over a period of 3 months (energy intake = 33% of energy requirements) followed by a 9 month period designed for weight maintenance (energy intake = 67% of energy requirements). The macronutrient

composition (protein/carbohydrate/fat) and labels of the 4 diets is as follows: HPLC – 20/25/55, HPNC – 20/50/30, NPLC – 10/25/65, and NPNC – 10/50/40. After 3 months, the both HP groups lost significant amount of weight (-14.1 ± 4kg; $p < 0.001$) and fat mass (-11.9 ± 1.7kg; $p < 0.001$) independent of carbohydrate intake. The same held true for the NP groups in regards to both weight loss (-11.5 ± 4kg; $p < 0.001$) and fat mass (-9.3 ± 0.7kg; $p < 0.00s$). No significantly relevant difference was noted between the change in weight loss and fat mass between the 2 protein groups. A similar trend was noted at the 12 month mark with both the HP and NP groups maintaining significant weight loss independent of carbohydrate intake. The authors of the study concluded that weight loss and weight maintenance were dependent on the high protein aspect of the diet but not the low carbohydrate component independent of fat content (48). Unfortunately, they did not measure satiety or any measure of appetite between the 4 test groups to determine participant comfort during the trial.

Johnston et al. both confirmed the successful impact of a high-protein diet for weight loss independent of the carbohydrate component and an increase of reported satiety and satisfaction by the participants after a pair of food trials over a period of 6 weeks. Maintaining the same protein content (~30% of energy) between both test groups, they compared the results of a ketogenic low-carbohydrate (KLC) test diet (~5% energy carbohydrate) with a nonketogenic low-carbohydrate (NLC) diet (~40% energy carbohydrate) to determine the role carbohydrates play in a high-protein dietary approach (49). After 6 weeks, participants in both groups had similar success with weight loss (KLC = 6.3 ± 0.6 kg, NLC = 7.2 ± 0.8 kg) but a diet-specific weight loss advantage was not noted. Participants in both groups reported an overall improvement in hunger ratings

(No particular feeling to satisfied) but no significance on this measure of satiety was reported between the two test meals. In a separate weight loss food trial, they compared an isocaloric high-protein, low-fat (HPLF) diet (protein = 30% of energy, fat < 30%) with a high-carbohydrate, low-fat (HCLF) diet (carbohydrate = 60% of energy, fat < 30%) to determine successful outcomes (50). After the 6-week intervention, participants in both groups were equally successful in reducing both body weight (HPLF = -5.7 ± 0.6%, HCLF = -5.9 ± 0.5%) and fat mass (HPLF = -8.9 ± 2.2%, HCLF = -10.6 ± 1.4%) with no significance reported between the two test groups. Participants in the HPLF group reported greater diet satisfaction during the first 4 weeks of the trial but the data did not reach the significance threshold.

Analyzing perceived hunger and weight loss when comparing low-carbohydrate, high-protein (LCHP) diets with high-carbohydrate, low-fat diets (HCLF), Nickols-Richardson et al., created a 6 week food trial to determine if either diet was statistically superior (51). Overweight premenopausal women were recruited for the study received instructions on calorie restricted diets (1,500 – 1,700 kcal/d) and were separated into two groups including either LCHP (carbohydrates < 40g/d, protein was unrestricted) or HCLF (60% energy from carbohydrate, 15% protein, and 25% fat) diets. At the end of the trial, the women on the LCHP diet lost significantly ($p < 0.05$) more weight (5.7%) compared to the HCLF diet (3.3%), but both groups had a significant ($p < 0.0001$) reduction in BMI which did not differ between the two groups. In addition, participants in the LCHP diet reported a significant score reduction ($p < 0.03$) in perceptions of hunger after the trial (3.2 ± 2.4) compared to their baseline scores (6.3 ± 4.1) whereas the HCLF did not. However, they noted the estimated average daily energy intake was not significantly

different between the two groups despite the differences reported in perceptions of hunger. Perceptions of hunger, independent from energy intake, is recognized as an important factor which impacts body weight regulation, body composition, and success with weight loss goals, suggesting the reduction in hunger felt by the LCHP diet plays an important role in healthy outcomes of a weight loss intervention (52).

Over a 12-week period following a structured plan, hypo-caloric diets composed with a high-protein (HP = 27% energy from protein, 44% carbohydrate, 29% fat) component was compared with a standard-protein (SP = 16% energy from protein, 57% carbohydrate, 27% fat) diet to determine outcomes on total weight loss, total fat loss, and reductions in abdominal fat (53). At the end of the trial both weight loss (HP = 7.82 ± 0.37 kg; SP = 7.65 ± 0.39 kg) and total fat loss (HP = 6.8 ± 4.3 kg; SP = 6.4 ± 4.7 kg) between the two groups were not significant but effective nonetheless. However, participants in the HP group with the highest level (>1.54 mmol/L at baseline) of triglycerides (TAG) lost significantly more weight ($p = 0.01$), total fat ($p = 0.007$), and abdominal fat ($p = 0.005$) compared to the SP group with similarly elevated TAG levels. The results suggest high-protein diets may be particularly effective for individuals with elevated TAG levels, a common condition within obese populations, when compared to other weight loss regimens.

Testing high-protein intake within morbidly obese populations may highlight the importance between the relationship of extremely elevated TAG levels and the effectiveness of short-term high-protein diets. Triffoni-Melo et al., separated a group of 19 class III morbidly obese women into two experimental groups receiving a 1,200 kcal diet for a total of 8 days (54). The control group received a diet with an energy intake

macronutrient profile containing 171g (54%) carbohydrate, 74g (23%) protein, and 32g (23%) of fat while the experimental group received 45g (15%) carbohydrate, 105g (35%) protein, and 67g (50%) fat. At the end of the trial, the experimental group realized a significantly ($p = 0.01$) larger weight loss (4.4 kg) when compared to the control group (2.6 kg). In addition to the lost weight, the TAG levels in the experimental group from day 1 to day 8 was statistically lower within the group (-44.3, $p < 0.05$) when compared to the control group which did not see any significant change during this time. However, no significance was noted between the two groups relevant to changes in TAG. This may be related to the fact that TAG levels at baseline approached significant differences ($p = 0.09$) between the experimental and control groups (161.2 ± 70.0 mg/dl vs. 124.6 ± 46.7 respectively).

A number of studies have shown that high-protein diets are at least as effective as other weight loss strategies and increased protein intake in terms of short-term dieting may be more beneficial in certain populations. In addition to short-term weight loss, high-protein diets may also prove to be more beneficial when looking at weight loss maintenance. Following a 6 week period of energy restricted dieting, a group of 48 participants were placed on a low-fat diet for a period of 12 weeks and received either a maltodextrin (HC), casein (HP-C), or whey (HP-W) supplement (2 x 25g/d) for the duration of the trial. The participants taking either of the HP supplements had significantly better weight maintenance (-2.3kg, $p = 0.04$) and maintained lower fat mass (-2.2kg, $p = 0.02$) when compared to the HC group. No statistical advantages were noted between the whey and casein supplement regarding weight maintenance and biomarkers from all three supplements were not significantly different (55).

Long-term Weight Loss. Dalle Grave et al., conducted a randomized trial over a period of 52 weeks to determine weight loss on energy-restricted high-protein versus high-carbohydrate diets within a morbidly obese population (56). After 3 weeks of inpatient treatment, a total of 88 participants continued the remaining 48 weeks of their assigned weight loss treatment (1200 kcal/d for women, 1500 kcal/d for men) in an outpatient setting. The participants were randomly assigned into either a high-protein group (HPD = 34% energy from protein, 20% fat, 46% carbohydrate) or a high-carbohydrate group (HCD = 17% energy from protein, 20% fat, 64% carbohydrate). After 1 year, the HPD group lost an average of 15% body weight and the HCD lost about 13.3% but no significant difference was noted between the two groups. Interestingly, the attrition rates were statistically similar between groups which might be explained by the possibility that the outpatient nature of the trial reinforced participant adherence by providing a form of social support and structure. The unintentional compliance reinforcement may explain the similar level of weight loss experienced. Unfortunately no measures of body composition were recorded during the trial to allow for comparisons between the two groups.

Over a period of 6 months, Skov et al., compared weight loss and body composition between the administration of both high-carbohydrate (HC) and high-protein (HP) diets in overweight and obese participants (57). The HP diet energy composition consisted of 25% protein and 45% carbohydrate while the HC diet contained 12% protein and 58% carbohydrate with fat being held constant at 30% between the two diet plans. After 3 months, the HP group lost a significantly ($p < 0.02$) greater amount of average weight (-7.5 kg) compared to the HC group (-5.0 kg) and the weight loss was composed

of significantly more fat respectively (-5.8 kg vs. -3.8 kg, $p < 0.02$). Additionally, at the 3 month mark, more participants from the HP group had lost over 5 kg body weight compared to the HC group (79% vs. 52%, $p < 0.05$). A similar trend was reported between the two groups at the end of the 6 month trial. The HP group averaged more weight loss than the HC group (8.7 kg vs. 5.0 kg; $p = 0.0002$) and a greater percentage of weight loss was from fat (7.6 kg vs. 4.3 kg; $p < 0.0001$). More participants lost over 10 kg on the HP diet than the HC diet as well (35% vs. 9%; $p < 0.001$). Overall, a higher percentage of participants on the HP diet realized clinically relevant weight loss and this was exemplified by comparison in the reduction of intra-abdominal adipose tissue between the two groups (HP = -33 cm^2, HC = -16.8cm^2; $p < 0.0001$). The researchers credit the increase in weight loss of the HP group over the HC group to the satiating effect of protein as participants in the HP group reported significantly lower energy intake than the HC group (2 MJ lower intake; $p <0.001$) which is consistent with prior research (58).

Weight loss on a high-protein diet has been shown to be quite effective over a period of up to 6 months, but once the time frame is extended up to 12 months the results appear to be less clear. Foster et al, enrolled 63 obese participants into a one year food trial comparing the effects of weight loss from either a low-carbohydrate, high-protein (HP) diet or a conventional low-calorie, high-carbohydrate (HC) diet (59). Similar to Skov et al., they found that participants in the HP group lost significantly more weight than the HC group at both the 3-month (-6.8 ± 5.0% vs. -2.7 ± 3.7%; $p =0.001$) and 6-month mark (-7.0 ± 6.5% vs. -3.2 ± 5.6%; $p = 0.02$). After 12 months however, no significant difference was found between the two groups (-4.4 ± 6.7 vs. -2.5 ± 6.3%; p

=0.26). Due to the hands off approach by the researchers, attrition rates were high in both groups which may help to explain the failure to find significant differences between the two groups at the end of the trial. A total of 85% of participants completed 6 months of the trial but only 59% completed the full 12 months. Measures for body composition were not recorded so no comparison between the clinical significance of the weight loss could be assessed.

Wycherley et al., designed a year long food trial to determine the long-term effects a high-protein diet had on not only weight loss, but body composition (60). A total of 120 obese men were put on a low-fat (25% total energy), energy-restricted isocaloric diet composed of either high protein (HP = 35% energy protein and 40% carbohydrate) or high carbohydrates (HC = 17% protein, 58% carbohydrate). Of the 68 participants who finished the trial, all of them had statistically similar reductions in total weight and fat mass. The HP group did however have a significant reduction in percent body fat mass at week 12 ($-5.0 \pm 3.3\%$ vs -3.1 ± 3.7; $p < 0.01$) compared to the HC group as it related to a significant reduction in percent loss of total body fat free mass (FFM) at week 52 (-2.6 ± 3.7 vs. -3.8 ± 4.7; $p < 0.05$). The reduced loss of the metabolically active FFM in the HP group is important to long-term weight loss maintenance as diets below protein requirements (based upon nitrogen balance) increase the risk of body weight regain (62). Even though the HP group did not lose more weight over the duration of the trial, the weight loss is clinically more significant.

Evaluating weight maintenance over a period of 6 months, Larsen et al., enrolled more than 900 participants who had recently lost at least 8% of their total weight loss into a food trial to determine the effects of protein and glycemic load on body composition

(62). In a 2-by-2 factorial design, they essentially created 4 diets including high-protein, high-glycemic index (HPHG), low-protein, high glycemic index (LPHG), low-protein, high glycemic index (LPHG), and low-protein, low glycemic index (LPLG). The high-protein diets equated to a total energy value of 25% of calories from protein and the low-protein diet had a total of 13%. No restrictions were placed on energy intake, but they targeted at least a difference of 15 glycemic index points between the high and low diets. Attrition rates in the HP groups and the LG groups were significantly lower than those of the LPHG group (26.4% and 25.6% vs. 37.4%; $p = 0.02$ and $p = 0.01$). In addition to the highest attrition rate in the study, the LPHG group was the only one of the four to gain a significant amount of weight back by the end of the trial (1.67 kg {0.48 to 2.87}, $p = 0.05$). The HP groups, regardless of glycemic index, gained less weight (0.95 kg, $p = 0.05$) back compared with the LP groups. No other significant data regarding body composition was reported. In conclusion, they suggested that high-protein diets with lower glycemic indexes may prove to be an ideal for weight loss maintenance.

Safety and efficacy.

Evaluating the efficacy and safety of high-protein diets, Krebs et al., divided participants into a high-protein, low-carbohydrate (HPLC = 2-2.5g/kg of protein/body weight) group or a low fat LF = 30% of energy) group for a period of 13 weeks where they here heavily monitored for safety (63). Both test groups resulted in significant weight loss for both groups but not between groups. The HPLC actually resulted in a slight but significant average loss of FFM ($p = 0.05$) where the LF diet did not, contradicting a number of other trials focused on high-protein diets. At the end of the trial DEXA measurements were taken and neither group had significant changes in bone

mineral density. Both diet regimens resulted in improvements in several biomarkers including significant reductions in both total and LDL cholesterol levels. Triglyceride levels were significantly ($p = 0.0003$) reduced (approximately 3 times more) in the HPLC group compared with the LF diet (between groups, $p = 0.03$). Both fasting glucose and 2-hour glucose did not differ between the two diets. Participants on the HPLC diet showed no adverse affects to the diet and biochemical indices like BUN, liver function tests, creatinine, and other serum chemistries came back WNL. The only significant issues reported with the high-protein diet compared with the low-fat diet during the trial was complaints of headache ($p = 0.04$). The researchers were a little surprised by the reduction of FFM within the HPLC group, but they concluded that the diet was both effective and safe for weight loss in obese populations.

Individuals eating ad libitum with short-term high-protein diets have been shown to lose an average of about 1 kg per week (64). Over the course of 12 months, high-protein diets resulted in less clear results compared to high-carbohydrate diets. Weight loss associated with protein usually results in a reduction in FFM and an increase in the maintenance of lean mass as part of a calorie-restricted diet, related to the composition of protein in the diet (65). A recent meta- regression examined high-protein intake as part of a short-term diet approach indicating that 1.05 g protein/kg of actual body weight resulted in protein sparing nearly 0.6 kg over a period of 6 weeks and 1.2 kg over a period of 12 weeks (66). The efficacy of weight loss with high-protein diets appears to be greater with short-term diets but more studies need to be completed to determine whether or not high-protein diets are warranted for long-term weight loss goals.

While high-protein diets are efficacious for weight loss, the DGA recommends lower amounts as part of a healthy diet citing worries about bone and kidney health with prolonged elevated protein intake. The recommendations of the DGA highlight safety concerns but the question is whether or not these concerns are warranted. Protein plays a vital role in bone collagen synthesis but increased protein intake has been linked to the promotion of increased excretion of calcium in the urine (67). The increase in calcium excretion has been linked to the argument that high-protein intake is linked to a negative impact on bone health and osteoporosis. The American Journal of Clinical Nutrition reported that dietary protein in fact seems to play a positive role in bone health and stated that at the very least protein in not detrimental (68). A related systematic review on dietary protein and bone health reported a beneficial influence of protein supplementation on bone mineral density (as protein intake increases calcium absorption from the diet), but noted the significant positive effects came from meat protein and not vegetable or dairy proteins (69). While urinary calcium may increase as a result of protein intake, it does not appear to be relevant to bone health suggesting high-protein diets are safe regarding calcium in the body.

The focus on urinary calcium excretion brings attention to another concern circulating around high protein diets; the effect on renal function and health. Frank et al., stated high-protein intake can lead to glomerular hyperfiltration, proteinuria, diuresis, and increased risk for nephrolithiasis under certain conditions and can exacerbate chronic kidney disease (70). High protein diets, especially considering short-term durations, raise questions about whether increased protein intake within healthy populations has any negative impact on normal kidney function. A review of both epidemiologic and

experimental data on the safety of protein and kidney health concluded little evidence exists to suggest high-protein diets are dangerous or have adverse effects in individuals with normal renal function (71). A study focusing on trained athletes suggests protein intake of up to 2.8 g/kg of body weight showed no negative effects on kidney health. (72). However, a separate study has suggested that intakes higher than this amount could significantly increase risk for developing both kidney and uric acid stones (73). None of the weight loss diets reviewed had intakes anywhere close to these levels suggesting high-protein diets for weight loss are safe in relation to kidney health without the presence of established renal diseases.

Despite promising research promoting the safety and efficacy of high-protein diets, Hernandez-Alonso et al., found that chronic high-protein intake is associated with increased body weight and total death risk (74). They analyzed the PREDIMED trial focusing on dietary protein levels reported on food-frequency questionnaires. They found that as protein intake increased as percentage of energy, the risk for weight gain (HR: 1.9; $p = 0.05$) rose significantly when it replace carbohydrates in the diet but not fat. Additionally, they found that increased protein intake was also linked to greater risk for all-cause death when it was substitute for both carbohydrates (HR: 1.59; $p = 0.05$) and fat (HR: 1.66; $p = 0.05$). The study reports the results were related to only long-term high-protein intake and was only associated with animal protein. The increase mortality risk may be associated with another study as Sandhu et al., found that an increased intake of all meat or red meat of at least 100 g/d was associated with a 12-17% increase risk of colorectal cancer (75). None of the weight loss studies reviewed sustained protein intake long enough to be significantly implicated in the risk for increased mortality.

Studies Related to the Zone Diet

High-protein, lower-carbohydrate diets have been shown to be both effective and safe for weight loss but studies focusing on popular diets with similar macronutrient profiles are scarce. Most people who make a diet attempt are going to choose a name brand diet emphasizing the need to address the health effects of popular diets. Dansinger et al., compared 4 popular diets representing different macronutrient profiles to determine the effectiveness on weight loss (76). Participants were assigned diets including Atkins (restricted carbohydrate), Zone (Balanced macronutrient profile), Weight Watchers (restricted calories), or Ornish (restricted fat) for a period of 12 months. They were instructed to maintain strict adherence to the diets for 2 months before being permitted to maintain the diet ad libitum for the remainder of the trial. Weight loss between the diet groups was not found to be significant but the amount of weight loss was correlated to the reported adherence levels ($r = 0.60$; $p < 0.001$) essentially meaning the participants who followed the diets lost the most weight. A total of 53% of participants finished the trial on the Atkins diet while 65% completed with both the Zone and Weight Watchers, and only 50% completed the stricter Ornish diet. Despite the difference in completion rates, no significance was noted between the four groups. However, participants finishing in the top ranking for adherence, independent from diet type, lost an average of 7% of their initial body weight suggesting diet compliance was an important factor in this study. All groups realized a mean reduction in total caloric intake for the duration of the trial but no diet was significantly better that the other.

Following a very similar study design, Gardner et al., was able to find a significant advantage of one diet over the other three (77). They randomly assigned 311

overweight and obese premenopausal women into one of four diets to determine outcomes on weight loss: Atkins, Zone, Ornish, and LEARN. Similar to the previous study, the participants were instructed to put in maximum effort for the first 2 months and then were on their own for the remaining 10 months of the trial. At the end of the trial they noted the participants in the Atkins group had significantly greater amount of weight loss (Atkins = -4.7 ± 1.6 kg, Zone = - 1.6 ± 1.2 kg; $p = 0.05$) when compared to the Zone group but not the other two diets. In fact, the other 3 diets all resulted in weight loss but not significant between the groups. The Atkins faired significantly better than all the diets at months 2 and 6 of the trial ($p = 0.05$ for both). Dietary retention for all 4 groups was similar but diet adherence was not measured or statistically analyzed by the researchers. The previous study comparing a similar spectrum of diets controlled for participant adherence, noting the weight loss was significantly correlated to the level of adherence. Considering both studies followed a nearly identical design, the significance of the weight loss may be skewed towards adherence levels compared to diet effectiveness. Clearly more research needs to be completed to determine the health effects of these popular diets.

Chapter 3

METHODS

Participants

The primary inclusions for the study were healthy adults between the ages of 20 and 75 with a BMI between 25 and 42kg/m². Participants were screened for chronic conditions, food allergies and diet restrictions, and advised on the use of pasta and cereal as the primary portion of their diet through the duration of the trial. Exclusion criteria included the use of insulin and cigarettes, plans to change diet and/or physical activity levels during the duration of the trial, and/ or weight change greater than +/- 10 pounds within three months of the study. In addition to these factors, pregnant or lactating women or women who have given birth within the prior six months were excluded from the study. Prescription medication use was permitted so long as the use had been consistent for at least three months and would remain consistent for the duration of the trial. Participants were required to have access to a refrigerator which was necessary to safely store the test foods.

Participants were recruited through the Arizona State University community via electronic messaging, posted flyers, and word of mouth. All interested individuals were instructed to complete the online eligibility survey to determine if they qualified for the study. Those individuals who were eligible to partake in the study were invited to the nutrition laboratory at the ASU downtown campus to discuss the food trial in detail. Upon signing the informed consent for the study (Appendix B), the participants completed a health history questionnaire (Appendix C) and set up a time to collect a fasting blood sample to determine the ratio of triglyceride to cholesterol levels

(TG/HDL). Recruiting ceased once a total of 45 participants met the inclusion criteria and were enrolled into the study.

Study Design

Before the study began, the 45 participants were instructed on how to follow a weight loss diet while incorporating the trial foods (Appendix D). They completed a 3-day diet record at the start of the trial and recorded their diet daily during the 12 week trial in a spiral notebook. During their initial visit, the participants scheduled a weekly time and date to receive their prepared meals for the duration of the study. The 7-day rotating menu consisted of breakfast cereal, a pasta meal for lunch (fusilli-based) and for dinner (orzo-based). These meals were prepared, portioned, and frozen weekly in the Arizona State University metabolic kitchen. A total of 7 cereal packets and 14 pasta meals were prepared weekly for each participant. The test meals included pasta and cereal developed using patented food technologies from the Zone Diet, Inc. The control dishes were produced using commercially available gluten-free rice pasta and a conventional name brand boxed cereal.

The ingredients and nutritional information are shown in **Table 1** for both the experimental and control meals. The average macronutrient breakdown for calories, percent energy intake, and weight provided by the test meals are shown in **Table 2**. The participants were provided with food packages each week to be consumed the following week for the duration of the 12-week trial. Following a blinded crossover design, participants received the respective test and control meals for each six week segment of the trial. As the test meals only provided an average of 950 kcal/d, all participants received diet counseling on foods to consume in addition to the test foods to keep to a

calorie total that would permit modest weight loss weekly (approximately 1 pound per week: see Appendix D). Participants received information pertaining to their specific caloric intake needs along with recommended foods to consume to meet those needs in addition to the test meals. Participants were able to eat additional foods ad libitum and received no further instruction on food intake during the trial.

The participants' height was measured at the beginning of the trial and their weight was measured weekly when they received their food packages using a spring scale (Health o meter Doctor's scale, Neosha, MO). Waist and hip circumferences and body composition using bioelectrical impedance technology (Tanita scale, Arlington Heights, IL) and DEXA (at weeks 6 and 12 only) were taken along with a fasting venous blood sample at trial weeks 0, 6, and 12. (Apart from the calculation of the TG/HDL ratio which was used as an inclusion criteria, blood analyses were not further analyzed for this report. Also, data are only reported for the first 6 week phase of the 12-week trial.) Participants were instructed to avoid moderate to intensive exercise at least a day prior to taking these measurements at weeks 0, 6, and 12 to improve accuracy. In addition to these measures, participants recorded their adherence and compliance to their respective diet on a calendar. These records included whether or not they ate each of the three food items per day and were the only measure of adherence reported. Additional foods were reported in their spiral notebook as part of their food diary but were used solely to calculate energy intake and were not used to measure adherence. No tool to measure satiety was implemented during the trial. Energy intake was calculated by scoring the most complete daily diet record each week to estimate the intake for the entire week

during the first half of the study. Physical activity questionnaires (Appendix E) were completed at 3-week intervals to assess any influence on the weight loss program.

Table 1. Nutrition Facts for Test Meals

Test Pasta and Cereal

Zone PastaRX - Orzo		Zone PastaRX - Fusilli		Zone Cereal	
Serving Size: 1 Cup (60g)		Serving Size: 1 Cup (60g)		Serving Size: 1 Cup (60g)	
	Amount Per Serving		Amount Per Serving		Amount Per Serving
Calories	240	**Calories**	240	**Calories**	240
Fat	4g	**Fat**	4g	**Fat**	10.5g
Carbohydrate	28g	**Carbohydrate**	28g	**Carbohydrate**	24g
Protein	20g	**Protein**	20g	**Protein**	21g

Control Pasta and Cereal

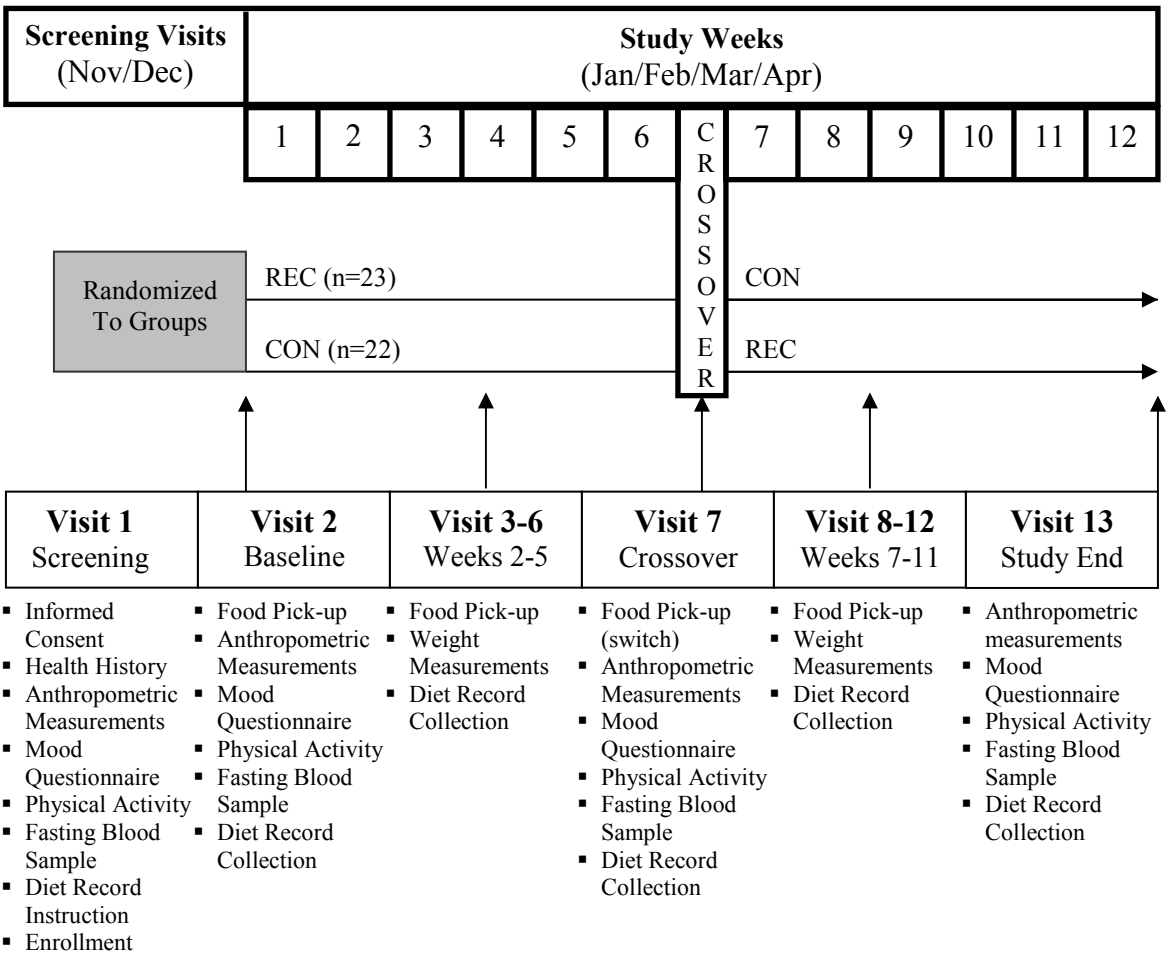
Gluten Free- Orzo		Gluten Free - Fusilli		Control Cereal	
Serving Size: 2 oz (58g)		Serving Size: 2 oz (58g)		Serving Size: 1 Cup (60g)	
	Amount Per Serving		Amount Per Serving		Amount Per Serving
Calories	200	**Calories**	200	**Calories**	260
Fat	1.5g	**Fat**	1.5g	**Fat**	5g
Carbohydrate	44g	**Carbohydrate**	44g	**Carbohydrate**	52g
Protein	3g	**Protein**	3g	**Protein**	4g

Table 2. Prepared Food Profiles: Average total calories, percent energy intake, and weight of macronutrients provided by Zone and control diet test meals.[1]

	Zone Diet: Orzo Pasta (364 kcal provided)			Control Diet: Orzo Pasta (324 kcal provided)	
	Energy Intake (%)	Weight (g)		Energy Intake (kcal)	Weight (g)
Protein	26	24.7		8	10.5
Fat	28	11.5		25	9.4
Carbohydrate	43	39.1		68	52.7
Fiber	-	6.0		-	5.2
Total Energy	98	-		101	-
	Zone Diet: Fusilli Pasta (358 kcal provided)			Control Diet: Fusilli Pasta (324 kcal provided)	
Protein	28	23.9		13	6.9
Fat	29	11.4		26	8.9
Carbohydrate	44	39.2		65	55.2
Fiber	-	4.5		-	3.5
Total Energy	100	-		104	-
	Zone Diet: Cereal (240 kcal provided)			Control Diet: Cereal (260 kcal provided)	
Protein	35	21		6	4
Fat	39	10.5		19	5
Carbohydrate	40	24		80	52
Fiber	-	5		-	2
Total Energy	114	-		105	-
	Zone Diet: Totals[2] (962 kcal provided)			Control Diet: Totals[3] (908 kcal provided)	
Protein	29	69.5		9	21.4
Fat	31	33.4		22	23.3
Carbohydrate	43	102.4		66	159.9
Fiber	-	15.5		-	10.7
Total Energy	103	-		98	-

[1]Data in table represent averages provided by the recipes in appendix F.
[2]Zone diet recommended macronutrient profile percentages are 30/40/30 (protein/carbohydrate/fat)
[3]DGA recommended macronutrient profile percentages are 10-15/45-65/20-35 (protein/carbohydrate/fat)

Study Timeline



Variables

All anthropometric measurements were taken using the same equipment calibrated prior to the start of the feeding trial. All weight measurements were gathered at roughly the same time every week for each participant and recorded in a coded chart which was uploaded into a spreadsheet at the conclusion of the study. The average of three waist and hip circumference measurements were recorded at the respective timeframes within the study and then recorded by the same individual to reduce variance within each measure. Diet records and calendars were used to determine compliance with

the prepared meals and calculate additional calories provided by other foods consumed during the trial. Fasting venous blood samples were collected by the same research nurse at the nutrition laboratories at the Arizona State University downtown Phoenix campus and analyzed to calculate the participant's TG and HDL levels.

The combination of weight, body composition, and waist and hip circumferences provided a detailed summary of fat loss or gain throughout the duration of the 12-week study. For the primary hypotheses to hold true the expectation would be for the test group to realize a reduction in total body weight with a greater percentage of loss from fat and not lean body tissues. Determining the composition of any weight lost provided evidence on the effectiveness of the feeding trial at lowering body weight. A fasting blood sample was employed to qualify participants for the study based upon elevated triglyceride and cholesterol levels. An elevated TG/HDL ratio is strongly related to overweight status and is regarded as a reliable indicator for metabolic syndrome and heart health. Enrollment into the study required an elevated TG/HDL ratio, however, blood data was not presented for this report. Finally, the use of physical activity questionnaires at scheduled intervals helped to identify possible influences within the study outcome.

Laboratory Analysis

Blood indices were measured from intravenous blood samples drawn after a 12-hour fast. The blood draws were initially used to measure TG and cholesterol levels and calculate a TG/HDL ratio to identify exclusion criteria and set baseline measures. Inclusion factors considered for the study included TG/HDL levels within the top half of the ratios of all who were tested. Fasting venous blood draws were collected at weeks 0, 6, and 12 were used to calculate the change from baseline to the end of each six week

crossover period. The same laboratory researcher took every blood sample and calculated the TG/HDL ratio to reduce potential for bias. A standard lipid panel was utilized to calculate TG and cholesterol levels and the TG/HDL was calculated based upon the results.

Statistical Analysis

Statistical calculations were completed using SPSS Statistical Analysis software version 19. All variables were controlled for gender to account for the gender differences in body mass and composition. Data were normally distributed and analyzed by independent t-test and univariate analysis of variance. Data is reported as means ± standard error (SE) unless indicated differently. Variables were considered significantly different at $p \leq 0.05$. Participant energy intake was calculated by using the best daily diet record entry each week as an estimate for the entire week. Food Processor SQL was used to calculate calorie intake from food records. Weekly activity was quantified ($kcal \cdot kg^{-1} \cdot wk^{-1}$) from survey data measuring duration and frequency of energy expenditure sessions per week.

Chapter 4

DATA & RESULTS

A total of 41 people were enrolled to participate in the food trial and following stratification by BMI, age, and gender they were randomly assigned into either the Zone group (n = 21) or the control group (n = 20). Prior to the start of the trial, one individual withdrew for personal reasons (control group). Of the 40 remaining participants that initiated the trial, 18 withdrew before week-6 due to schedule conflicts, dissatisfaction with the test meals, or other personal reasons. A total of 11 participants in each of the test groups finished the 6-week parallel-arm trial and were assessed for data analysis. One participant in the Zone group was not measured for FFM at baseline. In addition, 6 participants in the Zone group and 5 participants in the control group did not turn in food records at the end of the trial and were not assessed as part of change in energy intake.

Although participants were stratified by gender, age, and BMI prior to randomization to the two groups, attrition resulted in unequal gender balance in the final samples at week 6 (Table 3). However, when controlling for gender, no significant differences were identified between group baseline characteristics indicating they were effectively randomized (Table 3). The initial 3-day food record suggested that the Zone group (1718.6 ± 638.0) and control group (1719.9 ± 594.3) were eating a similar quantity of calories on average ($p = 0.66$) despite a relative but insignificant difference ($p = 0.23$) in calculated BMR suggesting an expression of gender variance. Baseline measurements for body composition were similarly distributed as FFM ($p = 0.58$) and body fat percentages ($p = 0.54$) did not differ significantly between groups. As a weight loss study, change in body composition may be the most important comparative measure to

follow. The Zone group had an average of 37.9 ± 10.4% of their weight composed of adipose and 62.9 ± 12.1 kg was FFM. The percent body fat in the control group was 40.0 ± 7.9% with 50.8 ± 12.3 kg of FFM.

Table 3. Participant Statistics at Baseline[1,2]

	Zone (n = 11)	Control (n = 11)	P-Value[3]
Gender (M/F)	5 / 6	1 / 10	0.06
Age (years)	40.6 ± 12.7	45.6 ± 12.0	0.58
Height (cm)	175.3 ± 8.0	167.3 ± 8.2	0.22
Weight (kg)	100.9 ± 16.1	86.1 ± 23.1	0.46
BMI (kg/m^2)	32.9 ± 5.2	30.8 ± 7.6	0.78
Waist Circ. (cm)	107.4 ± 12.9	94.9 ± 17.3	0.20
Body Fat (%)	37.9 ± 10.4	40.0 ± 7.9	0.54
FFM (kg)[4]	62.9 ± 12.1	50.8 ± 12.3	0.58
Activity ($kcal \cdot kg^{-1} \cdot wk^{-1}$)	53.7 ± 32.2	41.2 ± 25.5	0.63
Energy Intake – (kcal)[5]	1718.6 ± 638.0	1719.9 ± 594.3	0.66
Calculated BMR (kcal)	1815.9 ± 240.4	1532.2 ± 304.4	0.23

[1]Zone – Zone pasta and Zone cereal group; Control – Gluten free pasta and control cereal group.
[2]Values reported as means ± Standard Deviation
[3] Data normally distributed, analysis by univariate ANOVA controlling for gender.
[4]Zone: n = 10; Control: n = 11
[5]Zone: n = 7; Control: n = 8

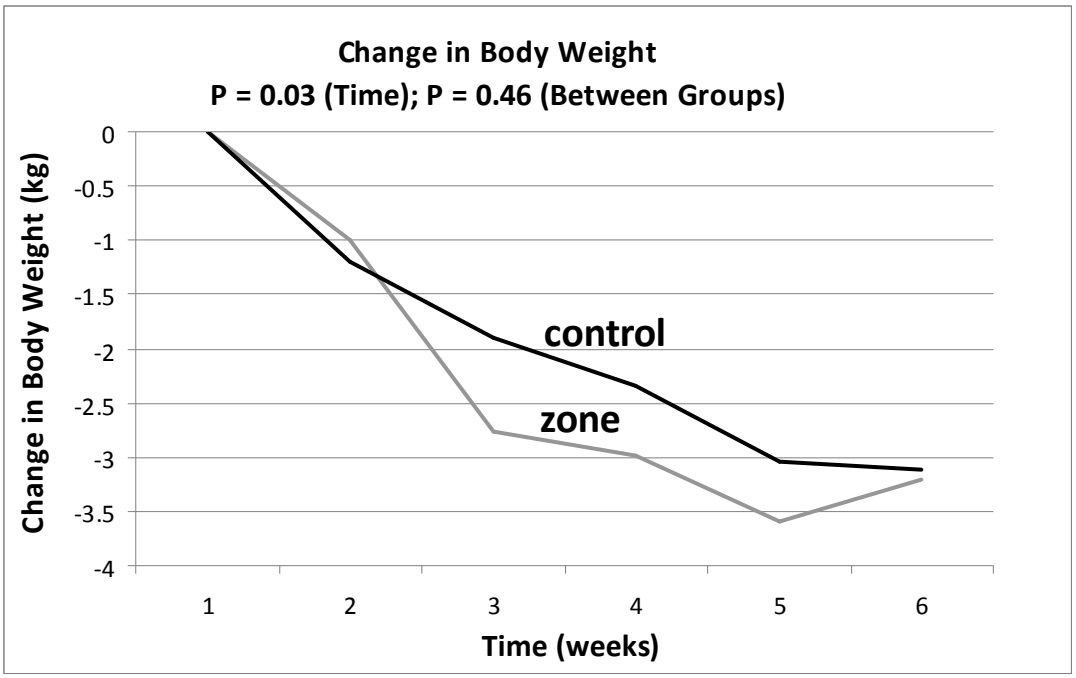
At the completion of week 6, an independent t-test revealed that participants in both groups lost a significant amount of weight over time ($p = 0.03$) but no significant differences were realized between groups ($p = 0.96$; Table 4). Participants lost an average of 2.9 ± 3.1 kg with the Zone diet and 2.7 ± 2.6 kg with the control diet. However, when the data are presented graphically it appears that the Zone diet may have

influenced weight loss at trial weeks 3, 4, and 5 (Figure 1). Following the trend line, the Zone diet produced a greater weight loss after the second week (-2.8 kg vs. -1.2 kg) and continued to be below the control line until week 5 (-3.6 kg vs. -3.0 kg). Although not significant, a greater disparity between groups appears when plotting the weight loss data for female participants only (Figure 2). The females in the Zone group lost more weight after week 2 (-3.1 kg vs. -1.8) and the trend continued to be below the control line to the end of the trial (-4.3 kg vs. -2.9 kg). It should be noted that the weekly weight measurements were taken in the afternoon (at food pick-up) using the Health o meter Doctor's scale, Neosha, MO, while the baseline and final weight measurements were recorded in a fasted state utilizing the Tanita scale, Arlington Heights, IL.

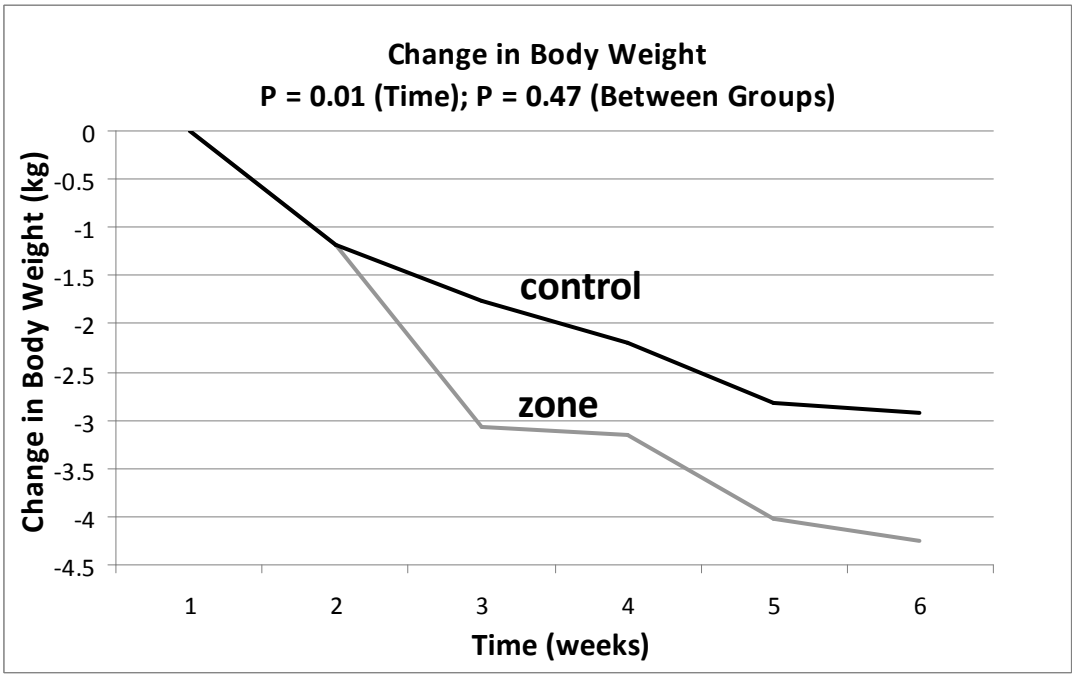
Table 4. Week 6 Values and Change from Baseline[1,2]			
	Zone (n = 11)	Control (n = 11)	P-value[3]
Weight at Week 6 (kg)	98.1 ± 15.7	83.4 ± 21.0	
Weight Change	-2.9 ± 3.1	-2.7 ± 2.6	0.96
FFM at Week 6 (kg)	64.4 ± 11.8	50.3 ± 11.5	
FFM Change	1.4 ± 3.6	-0.55 ± 1.5	0.02
Body Fat at Week 6 (%)	35.5 ± 9.2	38.8 ± 7.5	
Change in Body Fat	-2.9 ± 4.9	-1.2 ± 1.8	0.16
Energy Intake at Week 6 (kcal) [4]	1025.0 ± 555.0	941.7 ± 565.6	
Energy Intake Change	-328.6 ± 752.0	-229.2 ± 757.0	0.59
Activity Level at Week 3 (kcal· kg^{-1}· wk^{-1})	45.6 ± 32.6	43.8 ± 51.2	0.88
Activity Level at Week 6 (kcal· kg^{-1}· wk^{-1})	43.6 ± 27.3	40.1 ± 21.7	0.63

[1]Zone – Zone pasta and Zone cereal group; Control – Gluten free pasta and control cereal group.
[2]Values reported as means ± Standard Deviation
[3]Data normally distributed, analysis by univariate ANOVA controlling for gender.
[4]Zone: n = 5; Control: n = 6

Figure 1. Change in body weight for Zone and Control groups between baseline and week-6 of the food trial. Data based on afternoon weekly weights on Health o meter Doctor's scale, Neosha, MO.



Firgure 2. Change in female body weight for Zone and Control groups between baseline and week-6 of the food trial. Data based on afternoon weekly weights on Health o meter Doctor's scale, Neosha, MO.



Analyzing change in body composition from baseline, the change in FFM was significant ($p = 0.02$) between the two groups at week-6 (Table 4). A univariate analysis was conducted to control for gender. The Zone group had a mean increase in FFM of 1.4 ± 3.6 kg while the control group saw a mean decrease in FFM equal to -0.6 ± 1.5 kg. Physical activity and exercise did not appear to effect change in FFM over the course of the study as the activity level between groups remained similar (week 3; $p = 0.88$; week 6; $p = 0.63$). The significance reported in FFM was not related to the change in body fat percentages as neither group saw a statistical advantage with this measure of body composition ($p = 0.16$). Both groups registered a reduction in body fat: -2.9 ± 4.9% and -1.2 ± 1.8% for the Zone and control group respectively (ns; $p = 0.32$).

Energy intake was less at week 6 in both groups compared to baseline levels and was calculated through data analysis of a series of 3-day records. Participants on the Zone diet reported a mean difference of -329 ± 752 kcal while the control diet reported consuming 229 ± 757 less calories daily ($p = 0.59$ for interaction; $p = 0.12$ for time effect). Essentially, both groups maintained a similar caloric intake over the duration of the study regardless of the macronutrient composition of the meals they ate (Table 3). However, only about half the participants completed the 3-day records (Zone: $n = 5$, Control: $n = 6$) which limits the statistical interpretation of these data. In addition to the 3-day record, participants kept daily records on how well they adhered to the diet interventions (Table 5). Participants were instructed to track the intake of the 3 meals they received every day and adherence was calculated by the total meals they ate for the duration of the study. The control group reported an average of 81.1 ± 6.9% adherence to

the prepared meals and the zone group reported 83.4 ± 12.5%, but the differences between groups were insignificant (p = 0.53).

Table 5. Adherence Based on Percent Food Items Consumed (Target = 3x/day)[1,2]			
	Zone (n = 8)	Control (n = 10)	P-value[3]
Adherence (%)	83.4 ± 12.5	81.1 ± 6.9	0.53
[1]Zone – Zone pasta and Zone cereal group; Control – Gluten free pasta and control cereal group. [2]Values reported as means ± Standard Deviation [3]Data normally distributed, analysis by independent t-test controlling for gender.			

Chapter 5

DISCUSSION

This randomized, parallel-arm, food trial demonstrated that consuming an energy-restricted diet higher in protein content improved FFM outcomes more than a high carbohydrate, isocaloric diet, but both diets resulted in significant body weight reductions over time. Both diet groups lost similar weight after 6 weeks (~ 3% body mass), however the Zone group gained FFM during this time while the percent contribution of FFM was about 20% of the weight loss experienced in the control group. The change in FFM between the two groups was not related to their body fat percentages as both group experienced similar results. Despite a difference of about 2 kg in FFM between them, both groups retained a statistically equal amount of fat mass.

The FFM sparing effect of the Zone group was anticipated as other research has reported comparable findings with a similar high protein profile (30% of energy intake). Over a 12 week energy-restricted weight loss intervention, Leidy et al. observed that their high protein group lost a similar amount of weight (~9 kg) compared with the normal protein group (18% protein) but had only about a 1.5 kg loss of FFM compared to 2.8 kg loss respectively (78). Our study compared groups with a larger difference in protein intake (29% vs. 9% energy) which may explain why the Zone group had a much larger change in FFM between the two groups. Similarly, a meta-analysis focused on high protein hypocaloric diets reported that increased protein intake was associated with the sparing of FFM similar to levels provided in the Zone group (66). They found no significance between diet type and weight loss or body fat percentages which is consistent with our results.

Kreiger et al. concluded in their meta-analysis that the change in body composition related to high protein intake was independent of energy intake which is also consistent with our findings (66). Both the Zone and control groups reduced their caloric intake by similar amounts and were essentially consuming the same amount of energy through the duration of the trial. In addition, both group's activity levels remained statistically similar suggesting the change in FFM was independent of both energy intake and energy expenditures. As a result the increase in protein intake seems to be responsible for mitigating FFM loss under energy-restricted conditions. FFM is strongly associated with BMR as it accounts for roughly 60-70% of total daily energy expenditure (79). The implication is that a diet high in protein could have significant benefit for sustained weight loss as more FFM is spared over time. Conversely, losing FFM over time can make weight loss more difficult and prevent the realization of the beneficial effects of reducing body fat on comorbidities. The lack of long-term studies focused on the benefits of FFM preservation make it difficult to define any clinical significance as it relates to weight loss (60).

No statistically significant differences were observed between the two diets for change related to body fat percentage ($p = 0.16$) despite the superior change in FFM experienced by the Zone group compared to the control. Results from other studies have contradicted these finding as they reported body fat percentage decreased significantly with high-protein diets compared to their high-carbohydrate counterparts (80, 81). Layman et al. found that a high-protein diet had a significantly ($p < 0.05$) greater ratio of fat loss to lean mass compared with the high-carbohydrate group over a 10 week period (80). Our treatment period of only 6 weeks may have been too short for changes in body

fat to reach significant levels. However, Clifton et al. may offer another explanation for why changes in body fat were significant in some studies but not in ours (53). They found that body fat percentages were significantly different between high-protein and high-carbohydrate diets only when the participants had high TAG levels at baseline (>1.54 mmol/L). Participants with lower levels did not see any change between the two treatment groups. The significant change in FFM independent of loss in body fat percent may be more of a reflection of the participant TAG levels than the timeframe of the treatment period.

As a weight loss trial, the design of our weight loss plan was successful as both treatment groups lost a significant amount of weight over time ($p = 0.03$). These results are not uncommon when compared to related research studies. However, studies on short-term high-protein diets like ours were more likely to see significant differences between groups where long-term studies were more likely to see similar results. Triffoni-Melo et al. noted a significant difference between treatment groups after only 8 days (54). Other researchers found significant reductions in body weight over a 6 week diet intervention (51). Skov et al. found that increased protein intake resulted in greater weight loss at 3 months and to a lesser extent at 6 months (57). The statistical weight loss advantage continued to support high protein diets up to a period of 3 months but weaned at the 12 month mark (48). High-protein weight loss studies focusing on 6 months or greater tend to demonstrate similar results to ours. In fact when diet periods were extended to 52 weeks, a number of studies reported significant weight loss over time but not between treatment groups (59, 60, 76). Despite our findings, it appears high-

protein diets are more efficacious for weight loss under short-term dieting conditions and are at least as effective for weight loss as other diet approaches in the long-term.

Although not statistically significant, the graphical representation of weight loss between our treatment groups appears to highlight the Zone diet as being more effective than the high-carbohydrate control. The trend at weeks 2, 3, and 5 shows that the zone group had lost more weight than the control group and appeared to be more effective as a result. However, by week 6 the Zone group appeared to have gained enough weight back to comparable measures as the control. The gender differences between test groups led us to analyze the trend line between females to determine what effect sex may have impacted on the chart effect. Once this was completed, the trend seemed to improve to a greater extent for the Zone group suggesting a possible relation between the Zone diet and weight loss. A possible explanation for the weight gain by week 6 could be participant fatigue for the engineered carbohydrate product offered in the Zone treatment group. The pasta product has a different texture and mouthfeel to the control pasta which may have resulted in reduced participant adherence. By the final week it is possible participants may have begun to eat foods not in compliance with the recommendations made to them prior to the study. While both groups had similar adherence scores at the end of the trial, these scores were not analyzed for week-by-week effect.

The increased satiety related to protein intake, as reported by previous research, was believed to increase adherence to the Zone diet group on account of its increased protein content (27). Pesta et al noted diets higher in protein intake resulted in increased feeling of satiety which resulted in a decrease in energy intake. In addition, Weigle et al noted feelings of fullness increased while hunger decreased in participants eating a high

protein diet (43). The increased protein content of the Zone diet was thought to improve adherence to an isocaloric diet as participants would have more feelings of fullness and less feelings of hunger (desire to eat) despite eating less food. Adherence was measured in this study by how often participants ate the test meals and not how well they managed their diet as a whole. With both groups instructed to eat their test meals, using them as an indicator of adherence may have failed to address whether or not the satiety effects of protein improved participant adherence. The similarity in adherence between the two diets might suggest that participants were following instructions and not influenced by the diet type as a result. The fact both participant groups maintained similar levels of energy intake through the duration of the trial suggests the protein content between the diets had little effect on overall food intake.

Limitations. This food trial had a number of limitations to consider. As with most food trials our study was impacted by high attrition rates. Only 54% of the initial enrollment finished the duration of the trial as 19 participants dropped out for a variety of reasons. The attrition rates were 6% higher than we had anticipated which may limit the generalizability of our findings as a result. However, the dropout rate was comparable to other similar weight loss trials (50, 60, 82). Participant adherence to the treatment and diet recommendations are subjective and not entirely accurate. We attempted to maintain simple guidelines to follow and tracked their diets in order to mitigate participant bias but we have no means to measure the effectiveness of our efforts. Similarly, diet records are often not reliable and highly volatile as a measure of energy intake. We attempted to improve the accuracy of diet records by evaluating the most complete diet record each week and then projecting that evaluation as a representation for the entire week. The

length of the trial presented adherence and compliance challenges for the participants resulting in issues with reliability after the crossover. Without an appropriate wash-out period, the first phase of the trial may have presented carry-over effects which could not be sufficiently ruled out as an explanation for the differences noted between the treatments after the crossover. As a result, only the first 6 weeks were used for data analysis.

Our initial and final weight measures were recorded in a fasted state on a Tanita scale while the weekly weights were measured in the afternoon on a Health o meter Doctor's scale. The variation between how body weight was tracked may have impacted the significance of our results. In addition, the Tanita scale was used to determine FFM and percent body fat which is not as valid of a measure as a DEXA Scan would have provided. Similarly, the way in which BMR was measured may have impacted the significance of our results as well. The Mifflin-St. Jeor calculation provided a rough estimate of BMR, but the use of a respiration chamber or an indirect calorimeter would have provided a much more accurate measure.

This study may very well be the first of its kind to truly blind participants from diet type by masking the sources of protein. Both the Zone and the control groups received cereal and pasta dishes and had no way to determine one type was higher protein than the other. In other similar food trials, participants know they are eating more protein as they are instructed to eat more protein rich food sources like meat and dairy. In other Zone trials, participants are instructed to eat a diet high in meat and vegetables while typically high carbohydrate food sources like pasta and cereal are omitted. Moreover, in many published trials, participants were given the diet book to follow (e.g., the Atkins

book or the Zone book); hence, participants clearly knew which diet they were following. In addition, while participants did receive structured food packages, the bulk of their diet was ad libitum increasing generalizability to the population as a whole. Prior to enrollment, participants were screened for an extensive list of exclusion criteria to mitigate intervening variables from expressing influence into our data. The inclusion criteria into the study ensured participants were statistically similar between both groups which promote validation for our results.

Conclusions. The parallel-arm food trial suggests that energy-restricted diets are effective for short-term weight loss. Additionally, the results implicate high-protein diets as possibly having a protective effect on body composition as FFM was spared during the trial. Conversely, FFM was lost with a high-carbohydrate diet. The macronutrient profile of the diet does not appear to influence calorie intake, but it does appear to influence the quality of weight loss. Other measures of body composition and overall health outcomes need to be investigate further by future studies to appropriately identify the potential health effects between these diet types. More research is needed to determine effective techniques to increase participant adherence to the diets and to reduce attrition rates common with food trials.

In summary, hypothesis 1 was rejected as both participant groups lost a significant amount of weight over time but no significance was found between the two groups. Hypothesis 2 was supported as the experiment group resulted in a net gain in FFM which was significantly different than the control group which resulted in a net loss in FFM. Hypothesis 3 was rejected as both participant groups had similar energy intake

and reported compliance to their respective diets indicating the satiating effect of protein had no impact on adherence during this weight loss trial.

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APPENDIX A

IRB APPROVAL

ASU Knowledge Enterprise Development

APPROVAL FULL BOARD

Carol Johnston
SNHP - Nutrition
602/827-2265
CAROL.JOHNSTON@asu.edu

Dear Carol Johnston:

On 9/17/2014 the ASU IRB reviewed the following protocol:

Type of Review:	Initial Study
Title:	The Effects of Re-Engineered Carbohydrates on Metabolic Syndrome
Investigator:	Carol Johnston
IRB ID:	STUDY00001550
Funding:	Name: Inflammation Research Foundation;
Grant Title:	
Grant ID:	
Documents Reviewed:	• consent, Category: Consent Form; • JUNE2014Proposal%284%29 (1).pdf, Category: Grant application; • protocol, Category: IRB Protocol; • physical activity questionnaire, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • mood questionnaire, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • health history questionnaire, Category: Measures (Survey questions/Interview questions /interview guides/focus group questions); • Adherence calendar, Category: Participant materials (specific directions for them); • recruitment ad, Category: Recruitment Materials; • online survey, Category: Screening forms;

The IRB approved the protocol from 9/17/2014 to 9/16/2015 inclusive. Before 9/16/2015, you are to submit a completed "FORM: Continuing Review (HRP-212)" and required attachments to request continuing approval or closure.

If continuing review approval is not granted before the expiration date of 9/16/2015 approval of this protocol expires on that date. When consent is appropriate, you must use final, watermarked versions available under the "Documents" tab in ERA-IRB.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103).

Sincerely,

IRB Administrator

APPENDIX B

INFORMED CONSENT

CONSENT FORM
Effects of Re-Engineered Carbohydrate Meal Plans on Body Weight and Other Health Parameters

INTRODUCTION

The purposes of this form are to provide you (as a prospective research study participant) information that may affect your decision as to whether or not to participate in this research and to record the consent of those who agree to be involved in the study.

RESEARCHERS

Dr. Carol Johnston, Professor in the School of Nutrition and Health Promotion, and Jessica Knurick, a doctoral student, have invited your participation in a research study that will last 12 weeks.

STUDY PURPOSE

The purpose of this research is to evaluate the effect of re-engineered carbohydrate (REC) meal plans on body weight and biomarkers of health including blood cholesterol, blood glucose, and satiety. Dr. Barry Sears, creator of the Zone Diet, has developed a new line of Zone 1-2-3 foods using his patented 'Molecular Baking' to balance the protein:carbohydrate ratio of commonly 'high carbohydrate' foods. The REC used in this trial is a pasta product. Dr. Sear's Inflammation Foundation is funding this trial.

DESCRIPTION OF RESEARCH STUDY

You have informed us that you are healthy, 20-75 y of age, and not a smoker and that you do not have any food allergies or diet restrictions. If you decide to participate in this study, you will join a study examining the effect of a new pasta product (designed to have more protein and less carbohydrate) on body weight and biomarkers of health including blood cholesterol and glucose. To be eligible for the diet program, your TG/HDL ratio will need to be >3.5 for men and >2.5 for women. We will test this for you. You will be asked to consume the foods provided to you during the 12 week study, but you may add vegetables and other foods to the meals as desired. Meal plans will be individualized to meet your specific calorie needs; you will receive the new pasta for 6 weeks and the traditional pasta for 6 weeks (however, the order of pasta is randomized and neither you or the study coordinator will know which pasta you are consuming during the study. You will be asked to record diet adherence daily and not to change your typical physical activity pattern or consume dietary supplements during the study. A health history questionnaire will be completed at the initial visit and a short mood survey will be completed on 6 occasions during the study. The study involves 13-16 visits (at weekly intervals) to the test site at the ASU Nutrition Labs on the Phoenix Downtown Campus. These visits will take ~1/2 hour and include picking up weekly food allotments. You will be provided with a cooler tote on wheels to easily take the food to your car. Fasting blood samples will be collected at 4 of these visits. A trained phlebotomist or registered nurse will draw all blood samples; each sample of blood is about 2 tablespoons.

At week 6 and week 12, body composition (relative amounts of fat and lean tissue) will be determined by using an FDA‐approved bone density measurement machine. The procedure is called Dual‐energy X‐ray Absorptiometry (DEXA). You will be asked to lie face up on an open, padded table for 7 minutes while the scanner arm of the DEXA machine passes over the entire body. You can wear regular clothing but any metal must be removed. You will be exposed to a small amount of radiation (1‐4 microSieverts) that is within an acceptable range per the FDA. For comparison, you would be exposed to approximately 80 microSieverts on a transatlantic airline flight of 8 hours, 50 microSieverts living in Denver, Colorado, at an elevation of 5,000 feet for approximately 4 weeks, or 30 to 40 microSieverts during a typical chest x‐ray. (For test accuracy, you will be asked about test procedures using barium/isotopes in the recent past and be scheduled for visit 1 with an adequate lapse of time.)

If you say YES, then your participation will last for 12 weeks at the Arizona State University Downtown Phoenix Campus. Approximately 45 subjects will be recruited for this study.

RISKS

Potential risks of participating in this study involve general risks associated with giving a blood sample such as bruising or irritation at the site of venipuncture and faintness. Meal plans are composed of commercial breakfast items (cereals/bars/shakes) and the lunch and dinner meals will be prepared in the ASU Nutrition Program's metabolic kitchens and frozen in appropriate portion sizes. Trained staff will prepare the meals under the direction of a Chef, and these staff will hold food handlers certificates from Maricopa County. Meals will be provided in coolers so the food will be preserved during the drive home.

Anytime you are exposed to radiation there is potential risk. If there is ANY chance of being pregnant then you should not undergo the DEXA scanning. Females will take a urine pregnancy test prior to the DEXA scan. A certified X‐ray technician will complete all DEXA scans. As with any research, there is some possibility that you may be subject to risks that have not yet been identified.

BENEFITS

There is a possibility that you will lose some weight during this trial. In addition, your participation in the research may contribute to knowledge regarding the efficacy of a new food product for weight loss and improved health biomarkers.

NEW INFORMATION

If the researchers find new information during the study that would reasonably change your decision about participating, then they will provide this information to you.

CONFIDENTIALITY

All information obtained in this study is strictly confidential unless disclosure is required by law. The results of this research study may be used in reports, presentations, and

publications, but the researchers will not identify you. In order to maintain confidentiality of your records, Dr. Carol Johnston will assign you a participant number. Your name will not appear on any records aside from this consent form. This form will be kept in Dr. Carol Johnston's locked office to maintain your confidentiality.

WITHDRAWAL PRIVILEGE

It is OK for you to say no. Even if you say yes now, you are free to say no later, and withdraw from the study at any time. Your decision will not affect your relationship with Arizona State University or otherwise cause a loss of benefits to which you might otherwise be entitled.

COSTS AND PAYMENTS

The researchers want your decision about participating in the study to be absolutely voluntary. Yet they recognize that your participation may pose some costs related to time and travel. Participants will receive cash incentives during the trial: $15, $25, $35, $45, and $55 at study weeks 0 (start of trial), 3, 6, 9, and 12. [total = $175]

COMPENSATION FOR ILLNESS AND INJURY

If you agree to participate in the study, then your consent does not waive any of your legal rights. However, no funds have been set aside to compensate you in the event of injury.

VOLUNTARY CONSENT

Any questions you have concerning the research study or your participation in the study, before or after your consent, will be answered by Carol Johnston, carol.johnston@asu.edu [or (602)827-2265] and Jessica Knurick, jknurick@asu.edu.

If you have questions about your rights as a subject/participant in this research, or if you feel you have been placed at risk; you can contact the Chair of the Human Subjects Institutional Review Board, through the ASU Office of Research Integrity and Assurance, at 480-965 6788. This form explains the nature, demands, benefits and any risk of the project. By signing this form you agree knowingly to assume any risks involved. Remember, your participation is voluntary. You may choose not to participate or to withdraw your consent and discontinue participation at any time without penalty or loss of benefit. In signing this consent form, you are not waiving any legal claims, rights, or remedies. A copy of this consent form will be offered to you.

Your signature below indicates that you consent to participate in the above study.

_____ _____

Subject's Signature Printed Name Date

_____ _____

email phone contact number

INVESTIGATOR'S STATEMENT

"I certify that I have explained to the above individual the nature and purpose, the potential benefits and possible risks associated with participation in this research study, have answered any questions that have been raised, and have witnessed the above signature. These elements of Informed Consent conform to the Assurance given by Arizona State University to the Office for Human Research Protections to protect the rights of human subjects. I have provided (offered) the subject/participant a copy of this signed consent document."

Signature of Investigator_____ Date_____

APPENDIX C

HEALTH HISTORY QUESTIONAIRRE

Health History Questionnaire

ID#_____

Age:_____

Gender (please circle): Female Male

Smoker (please circle): Yes No

To be completed by investigator

Height:_____ft. _____in.

Weight:_____lbs.

Waist:_____in.

1. Are you taking any medications regularly? (including aspirin, steroids, birth control, etc.) Y N

   If yes, please list the medication, when you started taking it, and how often the medication is taken:

_____

_____

2. Do you currently take supplements? (vitamins, minerals, herbs, etc.) Y N

   If yes, what supplements and how often?

_____

_____

3. Has a doctor ever told you that you have any of the following conditions?

Heart disease?	Y N	Thyroid problems?	Y N
Kidney disease?	Y N	Cancer?	Y N
Liver disease?	Y N	High blood pressure?	Y N
Type 2 Diabetes?	Y N		

Other chronic conditions? (please specify): _____

Food Allergy? Y N (if yes, what type?)_____

4. If female, are you pregnant or planning on becoming pregnant in the next 16 weeks? Y N

   Are you currently breast-feeding? Y N

5. Have you lost or gained >10 pounds in the last 3 months? Y N

   If yes, please explain: _____

6. Have you ever fainted at a blood draw? Y N

   Are you willing to participate in a blood draw? Y N

   Have you donated blood in the past 8 weeks? Y N

TURN OVER

7. Will you be willing to consume the diet plan (all food provided) for 12 weeks? Y N

Are you able to pick up food packets weekly during the trial? Y N

Do you have a refrigerator and freezer at your residence to store food? Y N

8. You may lose up to 6 pounds or more during the study. Is this ok with you? Y N

9. Would you be able to restrict supplement use for 12 weeks? Y N

10. Do you follow a specific diet? (weight loss/gain, vegetarian, low-fat, etc.) Y N

11. Will you have a problem fasting overnight (10-12 hr) prior to the blood draw? Y N

12. Will you be able to maintain (e.g., not change) your typical physical activity pattern during the trial? Y N

13. Over a 7 day period, how many times do you engage in any regular activity long enough to work up a sweat (e.g., heart beats rapidly)? _____

How many times per week do you exercise moderately?_____

14. Please circle the total time you spend in each category for an average week.

Light activities such as: slow walking, golf, easy swimming, gardening, etc.

Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Moderate activities such as: moderate walking, cycling, swimming, weight lifting, etc.

Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

Vigorous activities such as: fast walking, jogging, cycling, heavy/intense weight lifting, etc.

Hours per week: 0 1 2 3 4 5 6 7 8 9 10+

15. Please describe any other medical conditions or situations that may affect you ability to participate in a research trial (i.e., pregnancy, infections, travel, vacations, deadlines, etc.).

APPENDIX D

FOOD LIST

FOOD LIST – select a variety of foods

Vegetables contain **25 calories** and 5 grams of carbohydrate. One serving equals:

½ C	Cooked vegetables (carrots, broccoli, zucchini, cabbage, etc.)
1 C	Raw vegetables or salad greens
½ C	Vegetable juice

If you're hungry, eat more fresh or steamed vegetables.

Fat-Free and Very Low-Fat Milk contain **90 calories** per serving. One serving equals:

1 C	Milk, fat-free or 1% fat
¾ C	Yogurt, plain nonfat or low-fat
1 C	Yogurt, artificially sweetened

Very Lean Protein choices have **35 calories** and 1 gram of fat per serving. One serving equals:

1 oz	Turkey breast or chicken breast, skin removed
1 oz	Fish fillet (flounder, sole, scrod, cod, etc.)
1 oz	Canned tuna in water
1 oz	Shellfish (clams, lobster, scallop, shrimp)
¾ C	Cottage cheese, nonfat or low-fat
2	Egg whites
¼ C	Egg substitute
1 oz	Fat-free cheese
½ C	Beans, cooked (black beans, kidney, chick peas or lentils): count as 1 starch/bread and 1 very lean protein

Fruits contain 15 grams of carbohydrate and **60 calories**. One serving equals:

1 small	Apple, banana, orange, nectarine
1 med.	Fresh peach
1	Kiwi
½	Grapefruit

Lean Protein choices have **55 calories** and 2–3 grams of fat per serving. One serving equals:

1 oz	Chicken or turkey —dark meat, skin removed
1 oz	Salmon, swordfish, herring
1 oz	Lean beef (flank steak, London broil, tenderloin, roast beef)*
1 oz	Veal or lamb, roast or lean chop*
1 oz	Pork, tenderloin or fresh ham*
1 oz	Low-fat cheese (with 3 g or less of fat per ounce)
1 oz	Low-fat luncheon meats (with 3 g or less of fat per ounce)
¼ C	4.5% cottage cheese
2 med.	Sardines

*** Limit to 1–2 times per week**

Medium-Fat Proteins have **75 calories** and 5 grams of fat per serving. One serving equals:

1 oz	Beef (any prime cut), corned beef, ground beef**
1 oz	Pork chop
1	Whole egg (medium)**
1 oz	Mozzarella cheese
¼ C	Ricotta cheese
4 oz	Tofu (note this is a heart healthy choice)

**** Choose these very infrequently**

Starches contain 15 grams of carbohydrate and **80 calories** per serving. One serving equals:

1 slice	Bread (white, pumpernickel, whole wheat, rye)
2 slices	Reduced-calorie or "lite" bread
¼ (1 oz)	Bagel (varies)
½	English muffin
½	Hamburger bun
¾ C	Cold cereal
⅓ C	Rice, brown or white, cooked
⅓ C	Barley or couscous, cooked
⅓ C	Legumes (dried beans, peas or lentils), cooked
½ C	Pasta, cooked
½ C	Bulgar, cooked
½ C	Corn, sweet potato, or green peas
3 oz	Baked sweet or white potato
¾ oz	Pretzels
3 C	Popcorn, hot air popped or microwave (80% light)

Fats contain **45 calories** and 5 grams of fat per serving. One serving equals:

1 tsp	Oil (vegetable, corn, canola, olive, etc.)
1 tsp	Butter or stick margarine
1 tsp	Mayonnaise
1 Tbsp	Reduced-fat margarine or mayonnaise
1 Tbsp	Salad dressing
1 Tbsp	Cream cheese
2 Tbsp	Lite cream cheese
1/8th	Avocado
8 large	Black olives
10 large	Stuffed green olives
1 slice	Bacon

Zone Study

Here is a food list with calories for planning your daily food intake [***in addition to the study foods***] based on your assigned calorie group

500 calories

800 calories

1100 calories

1400 calories

1700 calories

Note for candies etc, use about 2 starches + 2 fats (but will depend on choice and amount)

Ice cream, frozen yogurt, cakes, candy, chips, snack bars, etc.

APPENDIX E

PHYSICAL ACTIVITY QUESTIONAIRRE

ID# _____ Study week/Date of visit _____

Please circle **the number of times** you did the following kinds of exercises **for more than 15 minutes** last week.

- **Mild exercise** (minimal effort):

Easy walking, golf, gardening, bowling, yoga, fishing, horseshoes, archery, etc.

Times per week: 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14+

- **Moderate exercise** (not exhausting):

Fast walking, easy bicycling, tennis, easy swimming, badminton, dancing, volleyball,
baseball, etc.

Times per week: 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14+

- **Strenuous exercise activities** (heart beats rapidly):

Running, jogging, hocky, football, soccer, squash, basketball, cross country skiing,
judo, roller skating, vigorous swimming, vigorous long distance bicycling, etc.

Times per week: 0 1 2 3 4 5 6 7 8 9 10 11 12 13 14+

APPENDIX F

PREPARED MEAL RECIPES

Orzo Recipes

"Rice" and Tomatoes	
Yield: 1 serving Total time: 15 minutes	
Ingredients:	
1/2 cup	Zone orzo
1 tsp	dehydrated onion flakes
1/4 tsp	chili powder
1/4 tsp	oregano
1/4 tsp	cumin
1/4 tsp	garlic powder
1/4 tsp	cayenne pepper - (optional)
1/4 cup	canned tomatoes - diced with sauce or fresh or tomato sauce
1/2 tsp	lime juice - fresh squeezed
	salt - to taste
1 1/2 tsp	extra virgin olive oil
Instructions: Prepare Zone orzo according to package directions. Drain and put back in sauce pan. Stir in remaining ingredients and enjoy.	

Broccoli Orzo	
Yield: 1 serving Total time: 15 minutes	
Ingredients:	
1/2 cup	Zone orzo
1 cup	frozen broccoli
1 tbsp	sundried tomatoes
1/4 tsp	garlic powder
1/4 tsp	turmeric
	salt & pepper
1 tbsp	fresh squeezed lemon juice
1 1/2 tsps	extra virgin olive oil
Instructions: Cook Zone orzo as directed with broccoli. Drain. Reserve 1/2 cup of liquid and set aside. Add orzo back to pan. Stir in remaining ingredients and heat to desired temperature. Add some reserved pasta water it you want a little "sauce".	

Three Bean Salad	
Yield: 1 serving Total time: 15 minutes	
Ingredients:	
1/2 cup	Zone orzo
1/2 cup	green beans - raw chopped (or cooked and cooled with orzo)
1 tbsp	kidney beans canned - rinsed/drained
1 tbsp	garbanzo beans canned - rinsed/drained
1 tbsp	parsley - chopped
1 1/2 tsps	extra virgin olive oil
1 tbsp	lemon juice - to taste
	salt and pepper - to taste
Instructions: Cook Zone orzo (add green beans near the end) as directed and let cool. Meanwhile, combine remaining ingredients in a good sized bowl and season to taste. Stir in orzo.	

White Bean Orzo	
Yield: 1 serving Total time: 20 minutes	
Ingredients:	
1/2 cup	Zone orzo
1 1/2 tsps	olive oil
1 clove	garlic
1	plum tomato - chopped
2 tbsps	cannellini beans, Bush's - rinsed/drained
	basil - to taste
	oregano - to taste
1 tbsp	parsley - chopped
1 1/2 tsps	grated Parmesan
	salt and pepper - to taste
Instructions: Cook Zone orzo as directed. Reserve 1/2 cup of liquid and drain. Meanwhile, heat olive oil and garlic in a medium saucepan and season. Add chopped tomato, beans and dried herbs. Add some of the reserved pasta liquid and stir to desired sauce consistency. Pour over pasta and season with Parmesan, salt and pepper.	

Orange and Dill Orzo	Awesome Pasta Salad
Yield: 1 serving Total time: 15 minutes	Yield: 1 serving Total time: 20 minutes
Ingredients: 1/2 cup Zone orzo 3/4 cup vegetable broth 2 tbsps fresh-squeezed orange juice - (use a good juice orange) and some zest 1 tbsp finely minced fresh dill - (or 1 teaspoon dried dill) 2 tsps pistachio nuts - minced 1 tsp extra virgin olive oil 1/2 cups broccoli slaw Dole - raw	Ingredients: 1/2 cup Zone orzo 7 cherry tomatoes - halved 1/2 oz low-fat provolone cheese - cubed 1/2 green bell pepper - chopped 1 tbsp black olives - drained, sliced 1 tbsp pimentos - drained, chopped 1 tsp extra virgin olive oil 1 tbsps fresh-squeezed lemon juice - to taste garlic powder - to taste Italian seasoning - to taste salt and pepper - to taste water.
Instructions: Heat oil in a small saucepan over medium heat; add onion. Cook, stirring frequently, until tender. Add a tablespoon of the broth as needed. Meanwhile bring broth to a boil. Add Zone orzo and stir until broth is almost all absorbed, about 5 minutes. Set aside. Grate the zest from the orange and then squeeze out all the juice. Fluff the Zone orzo thoroughly with a fork and add both the zest and juice, along with the dill. Mix well. Cover tightly (remove comma) and set aside until serving time. Without removing the cover, heat just before serving in a microwave or a regular oven at 325°F. Serve hot over the broccoli slaw, sprinkled lightly with pistachios and drizzled with extra virgin olive oil.	Instructions: Prepare Zone orzo according to package directions. Drain and rinse in cold water to stop cooking; drain again. Set aside to cool. Meanwhile whisk the extra virgin olive oil, lemon juice, garlic powder, Italian seasoning, salt and pepper together in a small bowl. (If you like a lot of dressing add 1 tablespoon water and more lemon juice.) In a large bowl combine pasta with tomatoes, cheese, green pepper, olives, and pimentos. Pour in salad dressing and toss to coat.

Strawberry Almond Breakfast Pudding	Spicy Kale Orzo
Yield: 1 serving Total time: 10 minutes	Yield: 1 serving Total time: 10 minutes
Ingredients: 1/2 cup Zone orzo 2 tsps almond butter 2 tbsps 0%-Fat Greek yogurt sprinkle pumpkin pie spice 1/2 cup strawberries - quartered	Ingredients: 1/2 cup Zone orzo 3/4 cup beef broth 1 cup kale - chopped 1 clove garlic - crushed 1/4 tsp turmeric 1 1/2 tsps extra virgin olive oil
Instructions: Prepare orzo according to package directions. Drain. Stir in the almond butter and yogurt. Top with pumpkin pie spice and strawberries.	Instructions: Bring broth to a boil. Add Zone orzo and kale. Stir until broth is almost all absorbed, about 4 minutes. Cover and let rest for 4 minutes. Stir in garlic, turmeric and extra virgin olive oil.

Cold Sesame Slaw	Vegetable Orzo
Yield: 1 serving Total time: 10 minutes	Yield: 1 serving Total time: 15 minutes
Ingredients: 1/2 cup Zone orzo 1/2 cup broccoli slaw Dole 1/4 cup red bell pepper - sliced 1 tbsp water chestnuts - canned, chopped 1/4 tangerine - cut in half across the segments. Separate that in 1/2 Squeeze the remainder for juice 1 tbsp fresh-squeezed tangerine juice - (squeeze the remainder) 1 tsp sesame seeds 2 tbsps rice wine vinegar 1 tsp sesame oil	Ingredients: 1/2 cup Zone orzo 3/4 cup chicken broth 1 tsp olive oil 1 clove garlic - pressed 3 hearts artichoke hearts canned - quartered 3 tbsp roasted red pepper 1/4 cup tomatoes - diced 1 tbsp black olives - sliced 2 tsps dried parsley
Instructions: Prepare Zone orzo according to package directions. Drain and rinse in cold water to stop cooking. Drain and rinse again. Set aside to cool. Meanwhile, mix broccoli slaw with pepper slices, water chestnuts, tangerine pieces (only 1/4 the tangerine), tangerine juice, sesame seeds, rice wine vinegar, and sesame oil.	Instructions: Bring the Zone orzo and chicken broth to a boil, stirring occasionally. Lower heat to medium and cook until broth is absorbed, stirring all the while, about 6 minutes. Heat olive oil in skillet. Add garlic, artichoke hearts, roasted red peppers, tomatoes, black olives, parsley and orzo. Heat until warmed through. Transfer to a serving bowl and enjoy!

Fusilli Recipes

Fusilli with Spinach	
Yield: 1 serving Total time: 20 minutes	
Ingredients:	
1 cup	Zone fusilli
1 1/2 tsps	olive oil
1/4 cup	onion - chopped
1/2 clove	garlic - pressed
5 oz	baby spinach
1 tbsp	fresh squeezed lemon juice
1 tsp	fresh dill or 1/3 dried
	salt and pepper - to taste
Instructions: Cook Zone fusilli as directed. Reserve 1/2 cup of liquid and drain. Meanwhile, heat olive oil and add onion. Sauté for a few minutes. Add garlic and sauté until wilted. Add baby spinach to wilt for about 30 seconds. Toss into fusilli and moisten with reserved liquid (comma out) to desired consistency, adding slowly (you may not want to use all). Sprinkle with herbs, salt and pepper.	

Fusilli with Spinach and Tomato	
Yield: 1 serving Total time: 20 minutes	
Ingredients:	
1 cup	Zone fusilli
2/3 cup	tomato sauce
1 1/2 tsps	olive oil
2 1/2 cups	baby spinach
1 tsp	grated Parmesan
	oregano - to taste
	salt and pepper - to taste
Instructions: Cook Zone fusilli as directed. Reserve 1/2 cup of liquid and drain. Meanwhile, heat olive oil and add onion. Sauté for a few minutes. Add garlic and sauté until wilted. Add baby spinach to wilt for about 30 seconds Toss into fusilli and moisten with reserved liquid, to desired consistency, adding slowly (you may not want to use all). Sprinkle with cheese, oregano, salt and pepper.	

Fusilli with Broccoli	
Yield: 1 serving Total time: 20 minutes	
Ingredients:	
3/4 cup	Zone fusilli
1 1/2 tsps	olive oil
1/2 cup	onion - chopped
1/2 clove	garlic - pressed
1/2 cup	chicken broth
1 cup	broccoli – florets (whole or chopped)
	herbs - to taste (such as thyme, sage, oregano or tarragon)
	salt and pepper - to taste
Instructions: Cook Zone fusilli as directed. Reserve 1/2 cup of liquid and drain. Meanwhile, heat olive oil and add onion. Sauté for a few minutes, add garlic and sauté until wilted. Add broccoli and cover for 5 minutes. Toss into fusilli and moisten with reserved liquid, to desired consistency, adding slowly (you may not want to use all). Sprinkle with herbs, salt and pepper.	

Fusilli with Red Clam Sauce	
Yield: 1 serving Total time: 15 minutes	
Ingredients:	
3/4 cup	Zone fusilli
1 tsp	olive oil
1/2 cup	tomato sauce
1/4 cup	Chicken of the Sea minced clams
2 tbsps	lemon juice
2 tbsps	fresh parsley - chopped
1/4 tsp	thyme - ground
1 tbsp	grated Parmesan
	salt and pepper - to taste
Instructions: Cook Zone fusilli as directed. Drain and toss with olive oil. Meanwhile, heat sauce in a medium saucepan. Add clams, lemon juice, parsley and thyme. Simmer for 5 minutes. Serve over hot fusilli seasoned with grated Parmesan, salt and pepper.	

Apple-Walnut Pasta with Chicken Sausage	
Yield: 1 serving Total time: 15 minutes	
2/3 cup	Zone fusilli
1/2 tsp	olive oil
2 tbsp	dry white cooking wine
1 slice	onion
1/2 link	Al fresco sweet apple chicken sausage - sliced
1/4 medium	apple - diced
3/4 tsp	walnuts - crushed, toasted if you want
1 cup	baby spinach leaves
Instructions: Cook Zone pasta as directed. Drain. Reserve 1/2 cup of liquid and set aside. Meanwhile, heat oil and wine in nonstick skillet. Sauté onion until translucent, about 3-4 minutes. Add cut sausage and cook until heated through, about 2 minutes. Stir in diced apples, walnuts, spinach and reserved pasta. Add some of the reserved water if needed. Cook for an additional 2 minutes until all ingredients are warm and spinach is wilted.	

Fusiili Fagiole	
Yield: 1 serving Total time: 20 minutes	
Ingredients:	
1 cup	Zone fusilli
1 1/2 tsps	olive oil
1 clove	garlic
2	plum tomatos - chopped
	basil - to taste
	oregano - to taste
1 tbsp	parsley - chopped
2 tbsps	cannellini beans, Bush's - rinsed/drained
1 1/2 tsps	grated Parmesan
	salt and pepper - to taste
Instructions: Cook Zone fusilli as directed. Reserve 1/2 cup of liquid and drain. Meanwhile, heat olive oil and garlic in a medium saucepan and season. Add chopped tomato, beans and dried herbs. Add some of the reserved pasta liquid and stir to desired sauce consistency. Pour over pasta and season with Parmesan, salt and pepper.	

Mediterranean Salad	
Yield: 1 serving Total time: 15 minutes	
Ingredients:	
1 cup	Zone fusilli
4	cherry tomatoes - halved
1/2 cup	cucumber - chopped
2	olives - chopped
1/2 oz	feta cheese - crumpled
1/2 tsp	extra virgin olive oil
2 tbsps	fresh-squeezed lemon juice -
juice	of 1 lemon
	oregano - to taste
	black pepper
Instructions: Cook Zone pasta as directed and let cool. Meanwhile, cut the tomato and cucumber into chunks. Add to cooled pasta along with remaining ingredients.	

Spinach Basil Pesto Pasta w/ Tomato Salad	
Yield: 1 serving Total time: 20 minutes	
Ingredients:	
3/4 cup	Zone fusilli
2 cups	baby spinach
1/4 cup	roasted red pepper
1/4 cup	basil leaves - packed
1 clove	garlic - peeled and cut in half
1 1/2 tbsps	water - more if needed
1/2 tbsp	extra virgin olive oil
Side Salad:	
2	tomatoes - cut into 6 slices
1/2 oz	Polly-o fat free mozzarella
6 leaves	basil
Instructions: Cook Zone fusilli as directed. Drain and set aside. Meanwhile, place the spinach, basil, garlic, water and olive oil into a food processor or blender. Blend until smooth and toss with pasta. Serve immediately with cheese and basil on top of tomato slices.	

American Chop Suey with Salad		Baked Fusilli with Zucchini	
Yield: 1 serving Total time: 20 minutes		Yield: 1 serving Total time: 30 minutes	
Ingredients:		Ingredients:	
2/3 cup	Zone fusilli	3/4 tsp	olive oil
1 tsp	olive oil	1/2 med	zucchini - cut into small cubes
1/2	celery stalk - chopped	1/2 cup	green beans - trimmed and cut diagonally in half
3 tbsps	onion - diced		
1 clove	garlic - minced	1/2 (14 oz) can	canned tomatoes - diced
3 tbsps	red bell pepper - diced	3/4 cup	Zone fusilli
1 1/2 oz	extra-lean ground turkey breast	1/2 cup	tomato sauce
	cooking spray	2 tbsps	water
1/2 (14.5 oz) can	canned tomatoes - diced	1/4 tsp	Italian seasoning
1/4 tsp	crushed red pepper flakes	1 slice	Canadian bacon - chopped to bite-sized pieces
1/4 tsp	fresh chopped basil		
	salt and pepper - to taste		salt and pepper - to taste
1 tbsp	fresh-squeezed lemon juice	1 1/2 tbsps	mozzarella cheese, shredded, part skim milk
1 tsp	extra virgin olive oil	1 tsp	grated Parmesan
1/2 cup	lettuce		cooking spray
1/4	tomato	1/2 tsp	extra virgin olive oil
1/4	cucumber		
Instructions: Cook Zone fusilli 3-4 minutes. Drain and set aside. Meanwhile, heat oil to medium/high in a skillet. Add celery and onion. Cook for a few minutes first and then add the garlic and peppers. Remove from pan, spray with cooking spray and sauté turkey until no longer pink. Return veggie mixture to pan along with par-cooked fusilli. Top with canned tomatoes and crushed red pepper, stir well, and cover and simmer for about 8 minutes. Top with fresh basil just before serving. Make a small side salad.		Instructions: Preheat oven to 375°F. Heat oil in skillet over medium-high heat. Add zucchini and green beans. Cover and cook, stirring occasionally for 5 minutes. Stir in tomatoes (with juice), tomato sauce, water, Italian seasoning, Canadian bacon, salt and pepper. Cover and simmer 4 minutes. Meanwhile, Prepare Zone fusilli according to package directions, (reduce cooking time by 5 minutes). Drain and stir in with vegetables. Transfer into small baking dish coated with cooking spray. Sprinkle with cheeses. Cover and bake 10 minutes or until bubbly. Uncover and bake 5-10 minutes longer.	