ABSTRACT

Acute Effects of Caloric Intake and Macronutrient Type on Body Weight, Body Composition, Resting Energy Expenditure, and Total Metabolic Rate

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Committee Chairperson: Richard B. Kreider, Ph.D.

Obesity is a growing epidemic with no consensus regarding solutions. Thirty healthy overweight and obese female subjects (41.9 ± 12 years, 166 cm ± 7 cm, a 100.5 kg ± 19.6 kg, 46.0% ± 4.0% body fat, and BMI 37.4 ± 6.9) participated in the study. A 14-day dietary intervention examined acute effects of energy balance and macronutrient type on dietary intake, REE, body weight, body composition, and thyroid panel. Significant changes occurred in weight loss over time (p < 0.001); body fat mass over time (p < 0.001) and time x diet (p = 0.02); body fat percentage over time (p < 0.002) and time x diet (p = 0.023); REE over time (p = 0.03), and thyroid panel. Body weight continued to decrease during positive energy balance, yet metabolism rebounded. Results suggest energy balance has a greater overall effect on REE and weight loss than macronutrient type.
Acute Effects of Caloric Intake and Macronutrient Type on Body Weight, Body Composition, Resting Energy Expenditure, and Total Metabolic Rate

by

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A Thesis

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CHAPTER ONE

Introduction

The prevalence of obesity in the United States and other countries around the world has grown to epidemic proportions despite increased public awareness and efforts to control weight (Mokdad et al., 2001). However, there remains no scientific consensus on the causes (i.e. diet, genetics, environment, emotional distress/disturbances, etc.) of the rising prevalence of obesity, and even less consensus regarding optimal methods for weight loss and prevention of weight regain (Flegal et al., 2002). In fact, little research exists regarding how metabolic rate is affected during dieting and the role macronutrients play. With little scientific conclusion on obesity, several non-scientific dietary strategies for weight loss have become popular with consumers. These dietary strategies stem from conventional dietary theories revolving around energy balance regardless of macronutrient breakdown to newer strategies involving the manipulation of the macronutrient content of the diet.

Energy balance is defined as the difference between metabolizable energy intake and total energy expenditure (Schutz, 1995). The basic idea is if caloric intake is less than energy output, then negative energy balance will occur. Negative energy balance over time will stimulate the human body to access stored fat for energy, thus decreasing weight by using fat stores. Conventional weight loss and weight maintenance strategies have focused on energy balance.

Total energy expenditure consists of three main components: basal metabolism or resting energy expenditure (REE), thermic effect of food (TEF), and physical activity
The three components of total energy expenditure actually decrease in response to an energy deficit and weight loss (Schoeller & Buchholz, 2005). Therefore, body weight and body composition both influence energy expenditure (Schutz, 1995). Total energy expenditure decreases with weight loss because basal metabolic rate decreases with the loss of lean tissue mass (Schutz, 1995). This phenomenon could therefore limit or slow weight loss. The loss of lean tissue mass is inevitable during weight loss and typically accounts for approximately 25% of the tissue lost (Ballor & Poehlman, 1994). On the other hand, when caloric intake exceeds energy expenditure, the result is a positive energy balance and weight gain (McArdle, 2001).

With increases in weight, there is an increase in basal metabolic rate due to the growth of lean tissue mass to support the increase in fat mass (Schutz, 1995).

Changes in dietary macronutrient content may also profoundly influence metabolism (Harber et al., 2005). Many mainstream diets have focused on dietary protein (i.e. high protein diets or high protein/high fat diets). Proponents of diets with an increase in the ratio of protein to carbohydrates suggest the diet may increase the thermic effect of food (TEF), thus affecting total energy expenditure (Luscombe et al., 2002). On the other hand, carbohydrates, arguably, have an influential role as well. It has been proposed that a reduction in carbohydrate availability may mediate the metabolic response to fasting, which suggests that low carbohydrate availability may be a stronger metabolic regulator than a negative energy balance (Klein & Wolfe, 1992). Shifting the body to a fasting mode could be detrimental to REE and weight loss efforts.

Carbohydrates are also known for their protein-sparing effect under most normal conditions (Whitney & Rady-Rolfes, 2004).
The present study is needed to further examine the acute effects of caloric intake and macronutrient type on body weight, body composition, resting energy expenditure, and blood markers of metabolic rate. Additional research is needed to identify dietary strategies for the management of obesity. This study will be helpful in determining how long people can optimally restrict dietary intake before REE and hormonal regulation of metabolic rate is impacted. The data will also help to determine the amount of calories and length of time needed to promote increases in REE.

**Statement of the Problem**

Does caloric intake and macronutrient intake influence body weight, body composition, and resting energy expenditure in sedentary overweight females?

**Purposes**

1. To determine the effects of macronutrient type (high protein vs. high carbohydrate) on metabolic rate, weight loss, and body composition during the negative energy balance phase of the diet.

2. To examine the acute effects of reducing and increasing caloric intake on REE, hormonal markers of metabolic rate, body water, and body composition in overweight sedentary women.

**Hypotheses**

H₀: There will be no difference in changes in body weight over time.

H₁: There will be no difference between types of diets and changes in body weight.

H₂: There will be no difference in changes in body composition over time.

H₃: There will be no difference between types of diet and changes in body composition.

H₄: There will be no difference in changes in resting energy expenditure over time.
H₅: There will be no difference between types of diet and changes in resting energy expenditure.

H₆: There will be no difference in changes in blood markers of metabolic rate over time (thyroid stimulation hormone, thyroxine, triiodothyronine, free thyroxine index).

H₇: There will be no difference between types of diet and changes in blood markers of metabolic rate (thyroid stimulation hormone, thyroxine, triiodothyronine, free thyroxine index).

Delimitations

The study was conducted within the following parameters:

1. Thirty (30) sedentary, overweight females (ages 18-65, BMI > 27) participated in the study.

2. Subjects were recruited from the general public by radio and newspaper ads.

3. All familiarization and testing sessions were conducted in the Exercise & Sport Nutrition Laboratory (ESNL) in the Department of Health, Human Performance & Recreation at Baylor University.

4. A battery of assessments were conducted pre-dietary intervention and throughout the course of the intervention to determine the changes in variables being examined.

5. Each participant was randomly assigned to one of two diet protocols to follow for the duration of the study.

Limitations

1. Participants were responsible for purchasing and preparing food according to the diet plan.

2. The short duration of the diet protocol may have limited the changes due to dietary influence.

Assumptions

1. Subjects fasted for 12 hours prior to each testing session.

2. Subjects maintained consistent levels of physical activity.
3. Subjects adhered to the dietary protocols over the course of the study.

4. Subjects reported any adverse events to lab staff.

**Definition of Terms**

- **Basal Metabolic Rate (BMR)** - the rate of energy use for metabolism under specified conditions; after a 12-hour fast and restful sleep, without any physical activity or emotional excitement, and in a comfortable setting.

- **BUN** - blood urea nitrogen; waste product in the blood from the breakdown of protein. The kidneys filter blood and remove urea.

- **Creatinine** - waste product from protein in the diet and from the muscles of the body that is removed from the body by the kidneys.

- **Fat Mass** - the weight of the human body consisting of fat tissue.

- **Free Thyroxine Index** - a mathematical calculation used to correct the estimated total thyroxine (T4) for the amount of thyroxine-binding globulin present.

- **Higher Carbohydrate Diet** - lower in protein content relative to the high protein diet; diet contains 60 grams of protein per day. Based on a 200 lb body weight, the higher carbohydrate diet provided ~0.7g/kg/day.

- **Higher Protein Diet** - higher in protein content relative to the high carbohydrate diet; diet contains 115 grams of protein per day. Based on a 200 lb body weight, the higher protein diet provided ~1.3g/kg/day.

- **Lean Body Mass** - also known as fat free mass; the weight of the human body minus the fat content.

- **Resting Energy Expenditure** - similar to the BMR, a measure of the energy use of a person at rest in a comfortable setting, but with less stringent criteria for recent food intake and physical activity.

- **Thermogenesis** - the generation of heat; used in physiology and nutrition as an index of how much energy the body is expending. Thermogenesis of the human body includes basal metabolism, physical activity, and the thermic effect of food.

- **Thyroid Stimulating Hormone** - also known as TSH. A hormone produced by the pituitary gland at the base of the brain in response to signals from the hypothalamus gland in the brain. Thyroid stimulating hormone (TSH) promotes the growth of the thyroid gland in the neck and stimulates it to produce more thyroid hormones.
• Thyroxine - abbreviated T4. A hormone made by the thyroid gland that has four iodine molecules attached to its molecular structure. T4 and other thyroid hormones help regulate growth and control the rate of chemical reactions (metabolism) in the body.

• Triiodothyronine - also known as T3, liothyronine; a hormone made by the thyroid gland. It has three iodine molecules attached to its molecular structure, and affects almost every process in the body, including body temperature, growth, and heart rate.

• Triiodothyronine Uptake - also known as T3U.
CHAPTER TWO

Review of Literature

*Obesity Epidemic*

Obesity is a chronic, relapsing, stigmatized, neuro-chemical disease that continues to dramatically increase in prevalence (Bray & Champagne, 2005; Schoeller & Buchholz, 2005). Increases have been observed in all age, sex, and racial groups (Schoeller & Buchholz, 2005; Snitker et al., 1997). The rapid increase in the prevalence of obesity in the United States began around 1980 when the Dietary Guidelines emphasized reducing dietary fat and replacing energy with carbohydrate (Schoeller & Buchholz, 2005).

Obesity increases health risk and the cost of health care (Bray & Champagne, 2005). Overweight and obese individuals have greater amounts of body fat and increased risk of several chronic health issues including: diabetes mellitus, gall bladder disease, cardiovascular disease, hypertension, osteoarthritis, and several types of cancer (Bray & Champagne, 2005; Layman et al., 2003). Overweight and obese individuals also battle prejudice among social and economic situations and score lower on many scales that assess quality of life (Bray & Champagne, 2005). However, several of these risks and issues can be reversed by weight loss (Bray & Champagne, 2005).

Despite increased public awareness and efforts to control body weight, there remains no scientific consensus on dietary and other causes of the increasing obesity prevalence (Eisenstein et al., 2002). There also remains no scientific consensus of optimal methods for weight loss and prevention of weight regain (Eisenstein et al., 2002).
Energy Balance

Total energy expenditure (TEE) consists of basal metabolic rate (BMR), the thermic effect of food (TEF), and physical activity. BMR is the metabolic cost of processes such as the maintenance of trans-membrane ion gradients and resting cardiopulmonary activity and accounts for approximately 60% of TEE (Leibel et al., 1995). TEF includes the energy expended during digestion, transport, and deposition of nutrients and accounts for approximately 10% of TEE (Leibel et al., 1995). Physical activity involves the number of calories burned during activity and accounts for approximately 30% of TEE (Leibel et al., 1995). Eating fewer calories and burning more, typically through exercise, will result in a negative energy balance. When negative energy balance occurs over time, the body must access stored body fat for energy. This string of events leads to weight loss. Regardless of macronutrients, negative energy balance does stimulate weight loss (Layman et al., 2003).

A number of studies have shown that 24-hour energy expenditure decreases during energy restriction (Whitehead et al., 1996). In fact, all three components of TEE decrease in response to energy deficit and weight loss (Schoeller & Buchholz, 2005). Therefore, body weight and body composition both influence energy expenditure (Schutz, 1995). In studies varying in size and duration of negative energy balance, the magnitude of the decrease in 24-hour energy expenditure has been found to be 10-20% of the initial 24-hour energy expenditure (Whitehead et al., 1996). Leibel et al. (1995) found a decrease of approximately 15% in 24-hour energy expenditure, corrected for body composition, in obese and non-obese men and women who had lost 10% of their initial body weight. TEE decreases with weight loss due to the decrease in metabolic rate.
associated with the loss of lean tissue (Schutz, 1995). The loss of lean tissue is inevitable during weight loss and accounts for approximately 25% of the tissue lost (Ballor & Poehlman, 1994). Decreases in 24-hour energy expenditure are undesirable for individuals trying to lose weight since it will reduce the energy deficit. The decreased 24-hour energy expenditure then leads to a decrease in the rate of weight loss over time (Whitehead et al., 1996).

*Macronutrient Influence*

One of the debates surrounding the obesity epidemic is the optimal balance of macronutrients for adult health (Layman et al., 2003). Given the lack of scientific unity on obesity cause and treatments, several weight loss prescriptions, both sound and unsound, have become popular in mainstream culture (Eisenstein et al., 2002). Replacing fat with carbohydrate was the focus of weight loss strategies in the 1980s, but recent diets suggest replacing carbohydrates with protein (Eisenstein et al., 2002; Layman et al., 2003). Supporters of the macronutrient influence emphasize that energy balance does not explain: 1) why men and women distribute fat differently; 2) how fat distribution changes with age; 3) why weight loss stops after a period of dieting (Bray & Champagne, 2005).

Research reports that high carbohydrate diets reduce oxidation of body fat, increase blood triglycerides, and reduce satiety (Layman et al., 2003). The purported benefits of a higher protein intake include weight loss, decreases in hunger, and prevention, reduction, and possibly even resolution of several chronic diseases (Eisenstein et al., 2002). Luscombe et al. (2002) theorizes that an increase in the ratio of protein to carbohydrates may increase the TEF, thus affecting total energy expenditure.
If an increase in energy expenditure can occur from eating a high protein diet, yet hunger stays the same or decreases, then body weight would decrease (Eisenstein et al., 2002).

Layman et al. (2003) discovered beneficial effects on body composition and blood lipids when protein was substituted for carbohydrates. The body must maintain glucose homeostasis. Under conditions of lower carbohydrate (<200g/day), the body relies on hepatic production to maintain blood glucose levels. Gluconeogenesis in the liver may use dietary amino acids for carbon substrates (Layman et al., 2003). When the glucose homeostasis hypothesis was applied, Layman et al. (2003) found that obese women consuming a diet with a CHO/protein ratio <1.5 for 10 weeks minimized fasting and postprandial changes in blood glucose and enhanced insulin sensitivity. The same study indicated that the higher protein diet was more effective in improving body composition. The changes in the ratio of fat loss/lean loss suggested the higher protein diet improved utilization of body fat while maintaining lean body mass (Layman et al., 2003).

Substituting dietary protein for carbohydrate in an energy restricted diet maintained levels of thyroid hormones and reduced the insulin response to a test meal; both endocrine events are consistent with higher rates of lipolysis (Layman et al., 2003). The changes in body composition associated with the higher protein diet may be related to the targeting of body fat or sparing of muscle proteins, or both (Layman et al., 2003).

Whitehead et al. (1996) completed an acute dietary intervention lasting 7 days and varying macronutrients during energy restriction. The results indicated that maintaining protein intake during energy restriction could reduce the decrease in sleeping metabolic rate (SMR) and 24 hour energy expenditure. Overall, there was a decrease in 24 hour energy expenditure of 3% on the high protein diet, 6% on the high carbohydrate diet, and
7% on the high fat diet (Whitehead et al., 1996). When expressed per kilogram of body weight, this translated into a 0.4% decrease on the high protein diet; 3.5% on the high carbohydrate diet; and 4.8% on the high fat diet (Whitehead et al., 1996). The differences were not large, but could play a considerable role during weight loss over an extended period of time. Whitehead et al. (1996) related the smaller decrease in 24 hour energy expenditure to the greater postprandial increase in energy expenditure associated with protein compared to iso-energetic amount of carbohydrates or fat. Another possible explanation includes the proportion of metabolically active tissue lost was less on the high protein diet and that protein intake influences levels of hormones, such as thyroid hormones (Whitehead et al., 1996).

**Thyroid Hormones and Metabolism**

The thyroid gland makes thyroid hormone. Thyroid hormone helps: 1) the body use energy, 2) the body to stay warm, 3) organs to function and work together ("Thyroid function tests", 2005). Thyroxine (T4) is the major thyroid hormone secreted. T4 is converted to triiodothyronine (T3), which is the active form. The amount of T4 produced by the thyroid gland is controlled by thyroid stimulating hormone (TSH). It is well known that thyroid hormones affect BMR (Handbook of obesity: Etiology and pathophysiology, 2004). For example, hyperthyroid patients have an increased BMR, which is dependent upon the T3 plasma concentration (Handbook of obesity: Etiology and pathophysiology, 2004). However, there is no general scientific agreement regarding the mechanisms in which thyroid hormones stimulate heat production (Handbook of obesity: Etiology and pathophysiology, 2004). A few theories exist regarding how thyroid hormones may affect thermogenesis. Thyroid hormones may increase the Na, K-
ATPase activity in various tissues and may stimulate the rate of protein turnover \((\text{Handbook of obesity: Etiology and pathophysiology}, 2004)\). Thyroid hormones may also potentiate the effects of the sympathetic nervous system in a permissive manner to allow sympathetic activity to accelerate and generate heat \((\text{Handbook of obesity: Etiology and pathophysiology}, 2004)\).

Recent research has discovered an interesting relationship between thyroid function and energy expenditure regarding weight loss. BMR is positively related to fat free mass (FFM), body fat (BF), and total T3 (Stenlof et al., 1993). BMR is negatively related to free T4 (Stenlof et al., 1993). A positive correlation exists between the ratio T3/T4 and energy expenditure (Buemann et al., 1998). T4 provokes an important stimulation of energy expenditure. Serum concentrations of T3 are reduced in humans during caloric restriction and increase during refeeding (Spaulding et al., 1976). The rapid decline in T3 during starvation varies from 1 to 4 days and may depend on the carbohydrate content of the pre-fasting diet (Jung et al., 1980). Interestingly, the decrease in T3 is less in obese women than obese men (Jung et al., 1980). Carbohydrate deprivation decreased plasma T3 levels and increased protein catabolism. Starvation decreased plasma T3 levels, resting energy expenditure, and nitrogen excretion (Bisschop et al., 2001). Elevated TSH in obese women is significantly reduced by diet induced weight loss (Kok et al., 2005). The decrease in TSH and free T3 may blunt energy expenditure in response to long-term caloric restriction; thus, lowering TEE and frustrating weight loss efforts (Kok et al., 2005). High protein, low energy diets may not prevent the changes in thyroid hormone levels, which suggests that high protein diets create the same hormonal profiles as seen in energy restriction (Barrows & Snook, 1987).
CHAPTER THREE

Methods

Subjects

Thirty healthy untrained, overweight and obese female subjects between the ages 18 to 65 participated in this study. Subjects were 41.9 ± 12 years, 166 cm ± 7 cm, a 100.5 kg ± 19.6 kg, 46.0% ± 4.0% body fat, and had a BMI of 37.4 ± 6.9. Overweight was defined as having a Body Mass Index (BMI) greater than 27; overweight and obese individuals were allowed to participate. Subjects were excluded from participation if they had any metabolic disorder including known electrolyte abnormalities; heart disease, arrhythmias, diabetes, or thyroid disease; uncontrolled hypertension, hepatorenal, autoimmune, or neurological disease, and/or if they had taken weight loss dietary supplements (e.g., thermogenics, etc.) or medications within three months prior to the start of the study. The only exception was if the prospective subject had a medical condition or history that the subject’s personal physician felt was controlled and therefore was not a limitation for them to participate. Eligible subjects were informed of the requirements of the study and signed informed consent statements in compliance with the Human Subjects Guidelines of Baylor University and the American College of Sports Medicine.

Study Site

All familiarization and testing assessments were conducted in the Exercise & Sport Nutrition Laboratory (ESNL) in the Department of Healthy, Human Performance, and Recreation at Baylor University.
Study Design

The study included baseline testing followed by fourteen days of dietary intervention. Table 1 shows the research design and time course for assessments. Participants came to the ESNL for testing eleven times over the fourteen day dietary intervention. The independent variable was dietary intake. Dependent variables included: estimated dietary energy intake; REE, body weight, body water, body composition, hip and waist anthropometric measurements; and fasting clinical blood profiles (substrates, electrolytes, muscle and liver enzymes, red cells, white cells, and thyroid panel).

Table 1

Overview of Research Design and Testing Schedule

<table>
<thead>
<tr>
<th>Familiarization and Entry</th>
<th>Baseline Day 0</th>
<th>Days 1, 2, 3, 4, 7, 8, 9, 10, 11, 14</th>
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<tr>
<td>Phone Interview</td>
<td>Body Mass</td>
<td>Body Mass</td>
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<tr>
<td></td>
<td>Body Water</td>
<td>Body Water</td>
</tr>
<tr>
<td>Familiarization Session</td>
<td>DEXA Body Composition</td>
<td>DEXA Body Composition</td>
</tr>
<tr>
<td></td>
<td>Fasting Blood Collection</td>
<td>Fasting Blood Collection</td>
</tr>
<tr>
<td>Assessment of medical</td>
<td>Resting BP/ECG</td>
<td>Resting BP/ECG</td>
</tr>
<tr>
<td>history to determine</td>
<td>Resting Energy Expenditure</td>
<td>Resting Energy Expenditure</td>
</tr>
<tr>
<td>qualifications to</td>
<td>Anthropometric Measurements</td>
<td>Anthropometric Measurements</td>
</tr>
<tr>
<td>participate in study</td>
<td>Subjects matched according to FFM and age for random assignment into diet regimen.</td>
<td></td>
</tr>
</tbody>
</table>

Entry and Medical Screening Session

Subjects were recruited by radio and newspaper ads in Waco, Texas, and surrounding communities. The ads briefly described the study, outline qualifications, and instructed participants to call the ESNL. Subjects expressing interest in participating in
this study were interviewed on the phone to determine whether they appeared to qualify to participate in this study. Subjects believed to meet eligibility criteria were then invited to attend an entry/familiarization session. Any subject who did not meet entry criteria was required to obtain medical clearance from their personal physician prior to participating in baseline assessments.

**Familiarization Session**

Subjects eligible to participate in the study were familiarized to the study protocol via a verbal and written explanation outlining the study design. During this session, subjects signed Informed Consent Statements and completed personal and medical histories. Copies of all forms used over the course of this study can be found in the appendices. This familiarization session also described the dietary program and familiarized the subjects to the tests to be performed. Subjects were then given an appointment time to perform baseline assessments.

**Baseline Testing**

Following the familiarization session, the subjects recorded all food intake on dietary record forms for four days (4-d). Subjects were instructed to refrain from exercise for 48 hours and fast for 8-hours prior to baseline testing. Subjects reported to the ESNL for clinical assessments. Once reporting to the lab, subjects were weighed, had total body water determined by bioelectrical impedance (BIA), and had body composition determined using a Hologic Discovery W Dual Energy X-ray Absorptiometer (DEXA). Subjects then had REE, blood pressure, and resting heart rate, and hip and waist measurements determined using standard procedures. Subjects donated approximately 20 ml of fasting blood using venipuncture techniques of an antecubital vein in the forearm
according to standard procedures. Blood samples were analyzed in the Exercise & Biochemical Nutrition lab for standard clinical chemistry profiles (glucose, total protein, blood urea nitrogen, creatinine, BUN/creatinine ratio, uric acid, AST, ALT, CK, LDH, GGT, albumin, globulin, sodium, chloride, calcium, carbon dioxide, total bilirubin, alkaline phosphatase, triglycerides, cholesterol, HDL, LDL) and whole blood cell counts (including hemoglobin, hematocrit, red blood cell counts, MCV, MCH, MCHC, RDW, white blood cell counts, neutrophils, lymphocytes, monocytes, eosinophils, baosophils). In addition, serum samples were assayed for a thyroid panel including triiodothyronine (T3), thyroxine (T4), and thyroid stimulating hormone (TSH).

Randomization and Dietary Intervention

Thirty subjects were randomized into one of the following groups:

1. Higher Carbohydrate Diet (HC Diet)
   1,000 kcals/day for 7-days
   [115 g/d (46%) carbohydrate, 60 g/d (24%) protein, 33 g/d (30%) fat]
   2,600 kcals/day for 7-days
   [355 g/d (55%) carbohydrate, 100 g/d (15%) protein, 85 g/d (30%) fat]

2. Higher Protein Diet (HP Diet)
   1,000 kcals/day for 7-days
   [60 g/d (24%) carbohydrate, 115 g/d (46%) protein, 33 g/d (30%) fat]
   2,600 kcals/day for 7-days
   [355 g/d (55%) carbohydrate, 100 g/d (15%) protein, 85 g/d (30%) fat]

Subjects consumed 1,000 kcals/day for seven days consisting of either a higher carbohydrate or higher protein diet (in percentage but not relative terms). Once completed, the subject consumed a high carbohydrate diet (55% carbohydrate, 15% protein, and 30% fat) for seven days consisting of 2,600 kcals/day. Diets were prepared by a registered dietitian and consisted of less than 60 g/day of carbohydrate or more than 115 g/day of protein. Each participant received a diet booklet containing menus for the
14-day intervention. Each page in the booklet contained the menu for one day and provided a checklist for participants to fill out as they ate over the course of the day. Substitutions of equal calorie and nutrient content were listed for most menu items to add variety over the 14-day intervention. Diet logs were analyzed from the participant diet booklets using ESHA Food Processor Software to insure compliance.

**Assessment Schedule**

The study included baseline testing followed by a 14-day dietary intervention as described in Table 1. Participants came to the ESNL for testing eleven times over the fourteen day dietary intervention. Subjects returned to the lab following a 12-hour a fast on days 1, 2, 3, 4, 7, 8, 9, 10, 11, and 14 of the study. Subjects were weighed; had total body water and DEXA body composition measurements determined; had REE, resting heart rate and blood pressure measurements obtained; and donated approximately 20 ml of venous blood. These tests helped determine the acute effects of changes in caloric and macronutrient intake on metabolism and body composition.

**Medical Monitoring**

Interested subjects were invited to familiarization sessions. During this time, subjects signed consent forms and completed medical history information. Subjects then underwent a general exam to determine whether the subject met entry criteria to participate in the study. A trained researcher evaluated the medical and training history questionnaires to determine whether the subject met entry criteria and could therefore participate in the study. Trained, non-physician exercise specialists certified in CPR supervised subjects undergoing exercise assessments. A telephone and an automated electronic defibrillator were located in the laboratory in case of any emergencies and
there were no less than two researchers working with each subject during testing sessions. In the event of any unlikely emergency one researcher would check for vital signs and began any necessary interventions while the other researcher contacted Baylor’s campus police, which is standard university policy. Instructions for emergencies were posted above the phone in the ESNL in the event that any other research investigators were available for assistance. Subjects were informed to report any unexpected problems or adverse events they encountered during the course of the study to Richard B. Kreider, PhD, EPC or Chris Rasmussen, MS, MX, EPC, CSCS. If clinically significant side effects were reported, the subjects was referred to discuss the problem with the research nurse (currently Melyn Galbreath, FNP) or Lori Greenwood, PhD, ATC who is an Associate Professor of Athletic Training at Baylor University. If deemed necessary, Dr. Greenwood referred the subject to Ron Wilson, MD for medical follow-up. Dr. Wilson is one of the Sports Medicine physicians for Baylor University and is an adjunct Professor in the Department of HHPR. He agreed to provide medical support and consultation for this study and to our lab. If necessary, Dr. Wilson evaluated the complaint and made a recommendation whether any medical treatment was needed and/or whether the subject could continue in the study. If Dr. Wilson felt medical follow-up was necessary, the subject was referred to obtain medical treatment from their personal physician. This was a similar referral/medical follow-up system that Baylor athletes currently have in place with the exception that subjects in this study were not provided medical care. New findings and/or medical referrals of unexpected problems and/or adverse events were documented, placed in the subjects research file, and reported to the Baylor IRB committee.
Methods

*Dietary inventories.* Subjects recorded all food and fluid intake on dietary record forms for four days prior to baseline testing. The record included three week days and one weekend day of food consumption. Dietary intake was assessed using the Food Processor III Nutrition Software.

*Body composition assessments.* Subjects underwent body composition tests in the ESNL. Prior to each assessment, height was measured using standard anthropometry and total body weight was measured using a calibrated electronic scale with a precision of +/- 0.02 kg. Total body water was then estimated using a Xitron 4200 Bioelectrical Impedance Analyzer (*San Diego, CA*) which measures bio-resistance of water and body tissues based on a minute low energy, high frequency current (500 micro-amps at a frequency of 50 kHz) transmitted through the body. This analyzer is commercially available and has been used in the health care/fitness industry as a means to assess body composition and body water for over 20 years. The use of this device has been approved by the Food and Drug Administration (FDA) to assess total body water and the current to be used has been deemed safe (NIH, 1996). This was measured through four electrodes placed on the body: one electrode was placed on the posterior surface of the right wrist, in between the radial and ulna styloid processes (wrist bones), another electrode was placed on the posterior surface of the right hand at the distal base of the second metacarpal; the third electrode was placed on the anterior surface of the right foot at the distal end of the first metatarsal; and the fourth electrode was placed on the anterior surface of the right foot at the base of the tibia and fibula. Subjects laid on a table in the supine position and electrodes were connected to the analyzer. After the subject was
connected, age, gender, weight, height, and activity level were entered into the unit by the technician. After the unit had measured the resistance, which takes approximately 30 seconds, the unit then calculated total body water and body water percent. Body composition/bone density measurements was then determined using a calibrated Hologic 4500W dual-energy x-ray absorptiometry (DEXA) by qualified personnel with limited x-ray technology training under the supervision of Richard B. Kreider, PhD, MX. The DEXA body composition test involved having the subject lie down on their back in a standardized position in a pair of shorts/t-shirt or a gown. A low dose of radiation then scanned their entire body for approximately six (6) minutes. The DEXA segmented regions of the body (right arm, left arm, trunk, right leg, and left leg) into three compartments for determination of fat, soft tissue (muscle), and bone mass. Radiation exposure from DEXA for the whole body scan was approximately 1.5mR per scan. This was similar to the amount of natural background radiation a person would receive in one month while living in Texas. The maximal permissible x-ray dose for non-occupational exposure is 500 mR per year. Total radiation dose was approximately 15mR for the entire study. Since women of child bearing age served as subjects in this study, each subject completed a questionnaire related to their menstrual cycle timing, sexual activity, use of birth control pills, and desire to become pregnant. DEXA tests were performed within 14-days of the onset of their period in menstruating women of child bearing age who did not use oral contraceptives according to NCRP and ARP radiology standards in order to reduce the possibility of exposure of an unknown fetus to radiation.

**Resting heart rate and blood pressure.** Heart rate was determined by palpitation of the radial artery using standard procedures (ACSM, 2000). Blood pressure was
assessed in the supine position after resting for 5-min using a mercurial sphygmomanometer using standard procedures (ACSM, 2000).

**Blood samples.** Subjects fasted overnight for twelve (12) hours and then donated approximately 4 teaspoons of fasting venous blood (20 milliliters). Blood samples were obtained using standard phlebotomy procedures using standard sterile venipuncture of an antecubital vein by laboratory technicians trained in phlebotomy in compliance with guidelines established by the Texas Department of Health and Human Services. The phlebotomists and lab technicians wore personal protective clothing (gloves, lab coats, etc.) when handling blood samples. Subjects were seated in a phlebotomy chair. Their arm was cleaned with a sterile alcohol wipe and sterile gauze. A standard rubber tourniquet was then placed on the brachium. An antecubital vein was palpated and then a 23 gauge sterile needle attached to a plastic vacutainer holder was inserted into the vein using standard procedures. Three serum separation vacutainer tubes (red tops) and one EDTA vacutainer tubes (purple top) were inserted into the vacutainer holder for blood collection in succession using multiple sample phlebotomy techniques. Once samples were obtained, the vacutainer holder and needle were removed. The needle was discarded as hazardous waste in a plastic sharps container. The site of the blood draw was then cleaned with a sterile alcohol wipe and gauze and a sterile Band-Aid were placed on the site. The blood collection tubes were labeled and placed in a test tube rack. Laboratory technicians (who had received blood borne pathogen training and wore personal protective clothing) centrifuged the serum samples, transferred serum into labeled serum storage containers, and prepared samples for shipment or storage into a refrigerator or freezer for subsequent analysis. Serum and whole blood samples were
analyzed in the Exercise Biochemical and Nutrition Laboratory (EBNL) at Baylor University for assay of a standard clinical chemistry profiles (glucose, total protein, blood urea nitrogen, creatinine, BUN/creatinine ratio, uric acid, AST, ALT, CK, LDH, GGT, albumin, globulin, sodium, chloride, calcium, carbon dioxide, total bilirubin, alkaline phosphatase, triglycerides, cholesterol, HDL, LDL) and whole blood cell counts (including hemoglobin, hematocrit, red blood cell counts, MCV, MCH, MCHC, RDW, white blood cell counts, neutrophils, lymphocytes, monocytes, eosinophils, baosphils) in order to evaluate markers of catabolism and clinical safety of the dietary protocols. Serum microcentrifuge samples were assayed for thyroid panel including triiodothyronine (T3), thyroxine (T4), and thyroid stimulating hormone (TSH) in the EBNL at Baylor University. Analysis of these blood parameters determined the effects of the dietary interventions on general markers of clinical health status and metabolism.

*Resting energy expenditure assessment.* Resting energy expenditure assessments were made according to standard protocols using the Parvo Medics TrueMax 2400 Metabolic Measurement System (Sandy, UT). Subjects laid down on an exam table and had a light blanket placed over them to keep warm. A transparent metabolic canopy was then placed over the subject’s neck and head so that metabolic measurements could be obtained. The subject laid motionless, but awake for 20-minutes. Metabolic measurements were then obtained to determine resting oxygen uptake and energy expenditure.
Data Analysis

Separate 2 x 11 (Group x Test) factorial analysis of variance (ANOVAs) were run with repeated measures on Test for each criterion variable. Data was considered statistically significant when the probability of type I error was 0.05 or less. If a significant group, treatment and/or interaction alpha level was observed, least significant differences (LSD) post-hoc analyses were performed to determine where significance was obtained. Data for thyroid hormones and REE were correlated using Pearson product-moment correlation coefficient.
CHAPTER FOUR

Results

Thirty healthy women signed informed consent statements and participated in this study. Of these, all 30 subjects completed the study. Subjects were 41.9 ± 12 years, 166 cm ± 7 cm, a 100.5 kg ± 19.6 kg, 46.0% ± 4.0% body fat, and had a BMI of 37.4 ± 6.9. All volunteers completed the protocol and the diets were well-tolerated.

Diet Compliance

Diet compliance was monitored via dietary records. Participants completed 4-day diet inventories prior to starting the study protocol. Table 2 provides the means ± SD of dietary intake at baseline; values include overall caloric intake as well as a breakdown of the macronutrients: carbohydrate, protein, and fat. Diet inventories revealed that prior to the study participants consumed on average 2,239 ± 598 calories a day. Baseline diet consumption of the participants was determined to ensure they were consuming more calories than the negative energy balance phase of the study protocol (i.e. 1,000 kcals/day during days 1-7 of the study).

Table 2

Average Baseline Caloric Intake

<table>
<thead>
<tr>
<th></th>
<th>Minimum</th>
<th>Maximum</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caloric intake (kcals/day)</td>
<td>1,306</td>
<td>3,568</td>
<td>2,239 ± 598</td>
</tr>
<tr>
<td>Carbohydrate intake (grams/day)</td>
<td>123</td>
<td>758</td>
<td>295 ± 130</td>
</tr>
<tr>
<td>Protein Intake (grams/day)</td>
<td>42</td>
<td>138</td>
<td>78 ± 24</td>
</tr>
<tr>
<td>Fat Intake (grams/day)</td>
<td>45</td>
<td>206</td>
<td>98 ± 35</td>
</tr>
</tbody>
</table>
Table 3 summarizes the prescribed and the actual diet consumed by the participants over the course of the study. Significant differences (p = 0.04) were found between the higher carbohydrate and higher protein diets during days 1-7, which was expected based on the respective macronutrient prescriptions; however, in addition to the macronutrient differences, on average, the HC diet group consumed 100 more calories a day than the HP diet group during days 1-7. This 100 calorie difference was considered significant (p=0.04) and may have affected overall results. No significant differences in calorie intake occurred during days 8-14 of the study, and overall caloric intake over the 14 day period was not significantly different.

Table 3

Comparison of Prescribed versus Actual Caloric Intake

<table>
<thead>
<tr>
<th>Phase</th>
<th>Prescribed Diet</th>
<th>Actual Intake Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days 1-7 Negative energy balance</td>
<td>1,000 kcal HC Diet</td>
<td>1,112 ± 127&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>1,000 kcal HP Diet</td>
<td>1,009 ± 133&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Days 8-14 Positive energy balance</td>
<td>2,600 kcal HC Diet</td>
<td>2,016 ± 266</td>
</tr>
<tr>
<td></td>
<td>2,600 kcal HP Diet</td>
<td>2,026 ± 354</td>
</tr>
<tr>
<td>Overall average for entire 14 day study</td>
<td>HC Diet</td>
<td>1,564 ± 180&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>HP Diet</td>
<td>1,518 ± 231&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> = Significant differences between caloric intake (p = 0.04)

<sup>b</sup> = No significance between overall average calories for the 14-day period

Despite a 1,000 calorie prescribed diet, the HC group consumed an average of 1,112 calories a day. This was significantly different than the HP diet during days 1-7. However, the overall average for days 1-14 was not significantly different.
Table 4 indicates the means ± SD intake of carbohydrates, protein, and fat throughout the study. Significant differences were seen between carbohydrate (p < 0.001), and protein intake (p < 0.001) during days 1-7. Again, differences were expected based on the prescribed macronutrient intake. Carbohydrate and protein intake were not significantly different during days 8-14, which was expected based on the prescribed macronutrient intake. Fat intake was similar between the two diet groups throughout the study.

Table 4

<table>
<thead>
<tr>
<th>Macronutrient Intake (g)</th>
<th>Phase</th>
<th>Prescribed Diet</th>
<th>CHO Mean ± SD</th>
<th>PRO Mean ± SD</th>
<th>FAT Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days 1-7</td>
<td>Negative energy balance</td>
<td>1,000 kcal HC Diet</td>
<td>142 ± 24 †</td>
<td>67 ± 27 †</td>
<td>35 ± 7</td>
</tr>
<tr>
<td>Days 8-14</td>
<td>Positive energy balance</td>
<td>2,600 kcal HC Diet</td>
<td>288 ± 42</td>
<td>76 ± 8</td>
<td>67 ± 10</td>
</tr>
<tr>
<td>Overall average</td>
<td>HC Diet</td>
<td>215 ± 25</td>
<td>71 ± 15</td>
<td>51 ± 7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HP Diet</td>
<td>187 ± 33</td>
<td>94 ± 10</td>
<td>46 ± 9</td>
<td></td>
</tr>
</tbody>
</table>

Significant differences in macronutrient intake (protein and carbohydrate) occurred and were expected based on prescribed diet protocols.

† = p<0.001 difference between HC and HP groups.

Body Weight

Body weight was analyzed at each of the 11 time points over the study. Table 5 indicates the means ± SD of body weight (kg) of each diet group over the course of the study. Significant weight loss occurred in both diet groups over time (p < 0.001); however, body weight changes did not differ between groups (p > 0.05).
Figure 1 illustrates the change in body weight over the course of the study. Regardless of diet assignment, participants lost approximately 2 kg over the 14 day study period. Even though no significant body weight differences were seen between diet groups, participants continued to lose body weight, granted at a much slower rate, during the positive energy balance phase of the diet (days 8-14). Changes in body weight were seen over time, but not between types of diet. Therefore, the hypothesis H0: There will be no difference in changes in body weight over time will be rejected, and H1: There will be no difference between types of diets and changes in body weight will be accepted.

![Graph showing body weight changes](image)

**Figure 1.** Changes in body weight expressed in kg. Weight loss was significant over time (p < 0.001) and continued during positive energy balance (Days 8-14).

**Body Composition**

At the same time points in which body weight was assessed, body composition was assessed via DEXA. Table 5 indicates the mean ± SD of scanned body fat mass, lean body mass, and body fat percentage for each diet group over the course of the 14 day
Table 5

**Body Weight, Body Fat, Lean Body Mass and Body Fat Percentage**

<table>
<thead>
<tr>
<th>Testing Session</th>
<th>Diet Assignment</th>
<th>Body Weight (kg) Mean ± SD</th>
<th>Fat Mass (kg) Mean ± SD</th>
<th>Lean Mass (kg) Mean ± SD</th>
<th>Body Fat Percentage Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>HC Diet</td>
<td>100.0 ± 13.4</td>
<td>44.4 ± 8.6</td>
<td>48.9 ± 5.9</td>
<td>46.3 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>HP Diet</td>
<td>101.1 ± 24.2</td>
<td>44.5 ± 14.2</td>
<td>49.7 ± 10.1</td>
<td>45.7 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>Avg. both diets</td>
<td>100.5 ± 19.6</td>
<td>44.4 ± 11.7</td>
<td>49.3 ± 8.2</td>
<td>46.0 ± 4.0</td>
</tr>
<tr>
<td>Day 1</td>
<td>HC Diet</td>
<td>99.3 ± 13.2</td>
<td>44.6 ± 8.9</td>
<td>48.2 ± 5.7</td>
<td>46.8 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>HP Diet</td>
<td>100.0 ± 24.1</td>
<td>44.8 ± 14.8</td>
<td>48.8 ± 9.0</td>
<td>46.2 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>Avg. both diets</td>
<td>99.7 ± 19.4</td>
<td>44.7 ± 12.2</td>
<td>48.5 ± 7.5</td>
<td>46.5 ± 4.4</td>
</tr>
<tr>
<td>Day 2</td>
<td>HC Diet</td>
<td>98.9 ± 13.1</td>
<td>44.8 ± 8.6</td>
<td>47.6 ± 5.7</td>
<td>47.2 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>HP Diet</td>
<td>99.7 ± 24.2</td>
<td>44.9 ± 14.6</td>
<td>48.2 ± 9.4</td>
<td>46.6 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>Avg. both diets</td>
<td>99.4 ± 19.5</td>
<td>44.9 ± 12.0</td>
<td>47.9 ± 7.8</td>
<td>46.9 ± 4.2</td>
</tr>
<tr>
<td>Day 3</td>
<td>HC Diet</td>
<td>98.6 ± 13.3</td>
<td>44.2 ± 8.4</td>
<td>47.9 ± 5.9</td>
<td>46.7 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>HP Diet</td>
<td>99.3 ± 24.2</td>
<td>44.7 ± 14.4</td>
<td>47.8 ± 9.9</td>
<td>46.7 ± 4.8</td>
</tr>
<tr>
<td></td>
<td>Avg. both diets</td>
<td>99.0 ± 19.6</td>
<td>44.5 ± 11.8</td>
<td>47.8 ± 8.1</td>
<td>46.7 ± 4.7</td>
</tr>
<tr>
<td>Day 4</td>
<td>HC Diet</td>
<td>98.2 ± 13.5</td>
<td>43.2 ± 8.5†</td>
<td>48.7 ± 5.8</td>
<td>45.9 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>HP Diet</td>
<td>99.2 ± 24.1</td>
<td>44.4 ± 14.7†</td>
<td>47.9 ± 9.0</td>
<td>46.4 ± 4.7</td>
</tr>
<tr>
<td></td>
<td>Avg. both diets</td>
<td>98.7 ± 19.6</td>
<td>43.9 ± 12.0</td>
<td>48.1 ± 7.6</td>
<td>46.2 ± 4.3</td>
</tr>
<tr>
<td>Day 7</td>
<td>HC Diet</td>
<td>97.7 ± 13.5</td>
<td>43.4 ± 8.3†</td>
<td>48.0 ± 6.2</td>
<td>46.2 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>HP Diet</td>
<td>99.0 ± 24.2</td>
<td>44.5 ± 14.3†</td>
<td>47.8 ± 9.8</td>
<td>46.6 ± 4.4</td>
</tr>
<tr>
<td></td>
<td>Avg. both diets</td>
<td>98.4 ± 19.6</td>
<td>44.0 ± 11.7</td>
<td>47.9 ± 8.2</td>
<td>46.4 ± 4.0</td>
</tr>
<tr>
<td>Day 8</td>
<td>HC Diet</td>
<td>97.8 ± 13.5</td>
<td>42.8 ± 8.7†</td>
<td>48.5 ± 5.7</td>
<td>46.6 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>HP Diet</td>
<td>99.1 ± 24.3</td>
<td>43.3 ± 14.3†</td>
<td>48.2 ± 9.8</td>
<td>46.3 ± 4.1</td>
</tr>
<tr>
<td></td>
<td>Avg. both diets</td>
<td>98.5 ± 19.7</td>
<td>43.6 ± 11.8</td>
<td>48.3 ± 8.0</td>
<td>46.0 ± 4.0</td>
</tr>
<tr>
<td>Day 9</td>
<td>HC Diet</td>
<td>98.0 ± 13.5</td>
<td>43.1 ± 7.7†</td>
<td>48.6 ± 6.2</td>
<td>45.9 ± 3.2</td>
</tr>
<tr>
<td></td>
<td>HP Diet</td>
<td>99.0 ± 24.2</td>
<td>43.8 ± 14.8</td>
<td>48.5 ± 8.9</td>
<td>45.7 ± 4.6</td>
</tr>
<tr>
<td></td>
<td>Avg. both diets</td>
<td>98.5 ± 19.6</td>
<td>43.5 ± 11.9</td>
<td>48.5 ± 7.6</td>
<td>45.8 ± 3.9</td>
</tr>
<tr>
<td>Day 10</td>
<td>HC Diet</td>
<td>97.8 ± 13.5</td>
<td>42.8 ± 8.1†</td>
<td>48.5 ± 6.2</td>
<td>45.6 ± 3.8</td>
</tr>
<tr>
<td></td>
<td>HP Diet</td>
<td>99.0 ± 24.3</td>
<td>44.2 ± 14.8†</td>
<td>48.2 ± 9.3</td>
<td>46.1 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>Avg. both diets</td>
<td>98.4 ± 19.7</td>
<td>43.5 ± 12.0</td>
<td>48.4 ± 7.9</td>
<td>45.9 ± 4.0</td>
</tr>
<tr>
<td>Day 11</td>
<td>HC Diet</td>
<td>97.8 ± 13.5</td>
<td>42.7 ± 8.0†</td>
<td>48.9 ± 6.1</td>
<td>45.4 ± 3.5</td>
</tr>
<tr>
<td></td>
<td>HP Diet</td>
<td>99.0 ± 24.3</td>
<td>44.3 ± 13.7†</td>
<td>48.1 ± 10.4</td>
<td>46.4 ± 4.0</td>
</tr>
<tr>
<td></td>
<td>Avg. both diets</td>
<td>98.4 ± 19.7</td>
<td>43.6 ± 11.2</td>
<td>48.5 ± 8.5</td>
<td>46.0 ± 3.8</td>
</tr>
<tr>
<td>Day 14</td>
<td>HC Diet</td>
<td>97.7 ± 13.7</td>
<td>42.4 ± 8.2†</td>
<td>48.9 ± 6.2</td>
<td>45.2 ± 3.6</td>
</tr>
<tr>
<td></td>
<td>HP Diet</td>
<td>98.9 ± 24.3</td>
<td>43.6 ± 14.6†</td>
<td>48.7 ± 9.4</td>
<td>45.5 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>Avg. both diets</td>
<td>98.4 ± 19.8</td>
<td>43.1 ± 11.9</td>
<td>48.8 ± 7.9</td>
<td>45.4 ± 3.9</td>
</tr>
</tbody>
</table>

Significance

| Diet       | p = 0.89 | p = 0.84 | p = 0.48 | p = 0.93 |
| Time       | p < 0.001 | p < 0.001 | p = 0.28 | p = 0.002 |
| Diet x Time| p = 0.48 | p = 0.02 | p = 0.19 | p = 0.023 |

† = p<0.05 difference between HC and HP groups; * = p<0.05 difference from day 0 values
study protocol. Scanned mass from the DEXA analysis does not include the head, whereas body weight discussed in the previous section included the head.

Figure 2 illustrates the changes in fat mass throughout the study. Significance was found regarding changes in body fat mass in relation to time (p < 0.001) and time x diet (p = 0.02). Post hoc analysis revealed significant differences (p < 0.05) in fat mass from day 1 among the higher carbohydrate diet (HC Diet) at days 4, 7, 8, 9, 10, 11, and 14 and among the HP diet at day 14 of the study. Significant differences between diet groups (p < 0.05) were seen at days 4, 7, 8, 10, 11, and 14.

![Figure 2](image-url)

*Figure 2.* Changes in DEXA fat mass (kg) over the course of the study. † represents p<0.05 between HC and HP groups. * represents p<0.05 difference from day 0 values for HC diet. ‡ represents p<0.05 difference from day 0 values for HP diet.

Figure 3 illustrates the change in lean body mass throughout the study. No significance was found regarding the changes in lean body mass over the 14 day study protocol.
Figure 3. Changes in DEXA fat free mass (kg) over the course of the study. No significant changes in fat free mass were found.

Figure 4 illustrates the changes in body fat percentage throughout the study. Significance was found regarding changes in body fat percentage in relation to time ($p < 0.002$) and time x diet ($p = 0.023$). Unlike body fat mass which saw significant differences from baseline (day 0), post hoc analysis revealed significant differences ($p < 0.05$) in body fat percentage among the HC diet from days 1 and 2 at days 4, 8, 9, 10, 11, and 14. Post hoc analysis revealed significant differences ($p < 0.05$) among the HP diet from day 3 at day 14.

Regarding hypotheses, $H_2$: There will be no difference in changes in body composition over time will be rejected, and $H_3$: There will be no difference between types of diet and changes in body composition will be rejected.
Resting energy expenditure (REE) measurements were obtained over the course of the study. Table 6 demonstrates means ± SD for REE (kcals/day) and relative REE (kcal/kg/day) over the 14 day study protocol. A time effect was seen with both diets (p = 0.03); however, no significance was seen in REE between diet groups. Figure 5 shows the change in relative REE over the course of the study. REE declined during the negative energy balance phase of the diet (days 1-7), and rebounded during the positive energy balance phase of the diet (days 8-14). Thus, H₄: There will be no difference in changes in resting energy expenditure over time will be rejected, and H₅: There will be no difference between types of diet and changes in resting energy expenditure will be accepted.
Table 6

*REE and Relative REE*

<table>
<thead>
<tr>
<th>Testing Session</th>
<th>Diet Assignment</th>
<th>REE kcal/day Mean ± SD</th>
<th>REE kcal/kg/day Mean ± SD</th>
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<tbody>
<tr>
<td>Day 0</td>
<td>HC Diet</td>
<td>1,681 ± 227</td>
<td>16.9 ± 1.9</td>
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<tr>
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<td>HP Diet</td>
<td>1,694 ± 254</td>
<td>17.1 ± 2.0</td>
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<tr>
<td></td>
<td>Avg. both diets</td>
<td>1,688 ± 238</td>
<td>17.0 ± 1.9</td>
</tr>
<tr>
<td>Day 1</td>
<td>HC Diet</td>
<td>1,681 ± 272</td>
<td>17.0 ± 2.1</td>
</tr>
<tr>
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<td>HP Diet</td>
<td>1,673 ± 275</td>
<td>17.0 ± 1.9</td>
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<td>Avg. both diets</td>
<td>1,677 ± 269</td>
<td>17.0 ± 1.9</td>
</tr>
<tr>
<td>Day 2</td>
<td>HC Diet</td>
<td>1,601 ± 225</td>
<td>16.3 ± 1.9</td>
</tr>
<tr>
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<td>HP Diet</td>
<td>1,641 ± 285</td>
<td>16.8 ± 1.9</td>
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<tr>
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<td>Avg. both diets</td>
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<td>16.5 ± 1.9</td>
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<tr>
<td>Day 3</td>
<td>HC Diet</td>
<td>1,634 ± 229</td>
<td>16.7 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>HP Diet</td>
<td>1,636 ± 253</td>
<td>16.8 ± 1.8</td>
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<tr>
<td></td>
<td>Avg. both diets</td>
<td>1,635 ± 238</td>
<td>16.7 ± 1.8</td>
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<td>1,616 ± 322</td>
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<td>1,535 ± 186</td>
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<tr>
<td>Day 14</td>
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<tr>
<td></td>
<td>Avg. both diets</td>
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Significance

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<th>Diet x Time</th>
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<td>Diet x Time</td>
<td>p = 0.41</td>
<td>p = 0.35</td>
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</table>
Figure 5. Changes in REE (kcal/kg/day). REE decreased during negative energy balance (Days 1-7), but rebounded during positive energy balance (Days 8-14).

**Total Body Water**

Bioelectrical impedance analysis was used to assess changes in body water. Total body water changed over the course of the study for both diet groups. Significance was seen over time ($p = 0.009$); however there was no difference between diet groups. Approximately a 2 liter change in body water was seen in both diet groups over the 14-day intervention. Figure 6 illustrates the change in percentage of total body water over the course of the study.

**Thyroid Hormones**

Thyroxine (T4), triiodothyronine uptake (T3U), free thyroxine index (FTI) and thyroid stimulating hormone (TSH) were tracked over the course of the study. Table 7 shows the means ± SD for the thyroid panel. A significant time effect was discovered regarding the changes in T4 ($p = 0.035$), T3U ($p = 0.003$), and TSH levels ($p = 0.031$).
### Table 7

**Thyroid Hormones**

<table>
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<tr>
<th>Testing Session</th>
<th>Diet Assignment</th>
<th>T4 Mean ± SD</th>
<th>T3U Mean ± SD</th>
<th>FTI Mean ± SD</th>
<th>TSH Mean ± SD</th>
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<td>HC Diet</td>
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<td>8.5 ± 2.2</td>
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<td>Avg. both diets</td>
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<td>1.6 ± 0.87</td>
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<td>1.7 ± 0.87</td>
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<tr>
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</tr>
<tr>
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<td>HC Diet</td>
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<td>1.9 ± 0.86</td>
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<tr>
<td>Day 9</td>
<td>HC Diet</td>
<td>8.9 ± 1.9</td>
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**Significance**

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<th>Diet x Time</th>
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<td>Diet x Time</td>
<td>p = 0.10</td>
<td>p = 0.56</td>
<td>p = 0.27</td>
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Figure 6. Change in percentage total body water. A significant time effect ($p = 0.009$) was seen over the course of the study.

T4 and TSH trended downward over the 14 day study period, while T3U trended upward. A significant diet effect was seen in T3U ($p = 0.049$). No significant changes were found in FTI levels.

Correlations were calculated for REE and T4, T3U, and TSH. Calculated correlations between REE and T4 data found significant relationships at days 9 ($r = 0.40, p = 0.03$), 10 ($r = 0.40, p = 0.04$), and 11 ($r = 0.53, p = 0.004$). No significant correlations were found between REE and T3U or REE and TSH. In regards to hypotheses, significant differences in blood markers of metabolic rate were seen over time (T4, T3U, TSH) as well as between diets (T3U). Therefore, $H_6$: There will be no difference in changes in blood markers of metabolic rate over time will be rejected, and $H_7$: There will be no difference between types of diet and changes in blood markers of metabolic rate will be rejected.
CHAPTER FIVE

Discussion

This study examined the acute effects of caloric intake and macronutrient type on body weight, body composition, resting energy expenditure, and blood markers of metabolic rate. The main findings of this study were overall caloric intake as opposed to macronutrient type displayed a greater effect on REE and weight loss. REE decreased during the negative energy balance phase of the diet and rebounded during the positive energy balance phase of the diet. Interestingly, weight loss continued during positive energy balance. The following discusses the results of this study in greater detail and in light of available research.

Diet Compliance

Prior to the dietary intervention, participants were consuming on average 2,239 ± 598 calories, 295 ± 130 grams of carbohydrates, 78 ± 24 grams of protein, and 98 ± 35 grams of fat a day. Given the average weight and height of participants, they consumed approximately 22 ± 6 kcal/kg/day, 2.9 ± 1.3 g/kg/d of carbohydrate, 0.78 ± 0.24 g/kg/d of protein, and 0.98 ± 0.35 g/kg/d of fat. Protein intake was slightly below the RDA of 0.8 g/kg/d prior to starting the study.

During days 1-7 of the study (negative energy balance phase), the HC diet consumed 67 ± 27 grams of protein a day (~0.67 g/kg/d), and the HP diet consumed 109 ± 13 grams of protein a day (~1.08 g/kg/d). These values, although different from each other, are not drastically different than the RDA; thus the macronutrient differences are
really relative to one another and not absolute. The HP diet would not be considered a true high protein diet; in fact, the typical US adult consumes approximately 1.2 g/kg/d (Eisenstein et al., 2002). The protein intake of the HP diet was merely higher than the HC diet, yet still somewhat typical when compared to the average US adult protein intake. Even though the average consumption during days 8-14 (positive energy balance phase) did not reach 2,600 calories, participants did increase caloric intake above 1,000 calories a day and calorie and nutrient intake were quite similar between groups (average calorie intake 2,015 and 2,026). Another factor to take into consideration is underreporting on dietary recalls. Research has indicated that underreporting occurs with all types of dietary recall techniques (Scagliusi et al., 2003; Schoeller, 1990). Studies have also shown that underreporting of total energy intake, high fat foods, and carbohydrate foods is directly related to a person’s BMI (Johansson et al., 2001). Other factors contributing to underreporting include: socially desirable foods versus socially undesirable foods; gender (females underreport more than males); education levels; and error in portion size estimation (Johansson et al., 2001; Scagliusi et al., 2003). Therefore, the fact that subjects only reported an average intake of ~2,020 calories during days 8-14 may be a reflection of this known underreporting phenomenon. The results indicate the main differences in food consumption in this study occurred during days 1-7 of the study protocol.

**Body Weight**

Body weight decreased in both groups over time, but no differences were seen between diets. Greater weight loss occurred during days 1-7 when participants were in negative energy balance. Based on the results of this study, it appears that energy
balance has a greater effect on overall weight loss rather than macronutrient content of the diet. A couple of recent studies found that low-fat, energy reduced diets, whether low protein (~15% total energy) or high protein (30% total energy) were equally effective at reducing body weight (Farnsworth et al., 2003; Johnston et al., 2004). Other studies have examined the effects of low carbohydrate, energy restricted diets with varying amounts of fat and protein. Results from these trials have indicated no differences in overall weight loss; thus supporting the idea that energy balance/energy intake as a greater overall effect on weight loss (Luscombe-Marsh et al., 2005; Luscombe et al., 2002). On the other hand, several studies indicate that macronutrient type has an affect on weight loss resulting in greater weight loss as well positive effects on body composition, blood lipids, blood glucose control, and satiety (Buchholz & Schoeller, 2004; Eisenstein et al., 2002; Layman et al., 2003). The majority of the studies reviewed which support the positive effects of varied macronutrients, particularly higher protein and lower carbohydrate, in weight loss diets are much longer in length (≥ 10 weeks) (Buchholz & Schoeller, 2004; Eisenstein et al., 2002; Layman et al., 2003). The results of the current study support energy balance as having a greater effect on weight loss compared to macronutrient type.

Interestingly, despite a significant loss in body weight during the negative energy intake period, subjects continued to lose weight despite increasing caloric intake. In the past, refeeding research has indicated weight gain will occur with the introduction of more calories following a period of restricted caloric intake (Cleary, 1986; Norgan & Durnin, 1980). However, these findings suggest that there may be a delay in how the body adapts to increases in caloric intake after dieting.
Body Composition

Over the 14 day study period, both diet groups saw decreases in body fat mass and body fat percentage. Significance was found regarding changes in body fat mass and body fat percentage in relation to time (p < 0.001; p < 0.002 respectively) and time x diet (p = 0.02; p = 0.023 respectively). Post hoc analysis revealed significant differences (p < 0.05) in fat mass among the higher carbohydrate diet (HC Diet) at Days 4, 7, 8, 9, 10, 11, and 14 of the study. Regarding body fat percentage, post hoc analysis revealed significant differences (p < 0.05) in body fat percentage among the higher carbohydrate diet (HC) at days 4, 8, 9, 10, 11, and 14 and among the higher protein diet (HP) at days 0, 9, and 14. No significant differences were found in lean tissue mass between diet groups. However, overall results indicated that the HC diet lost more fat mass (-2.0 kg vs. -0.8 kg) and less lean tissue (-0.03 kg vs. -2.18 kg) than the HP diet. This contradicts the literature supporting the metabolic advantage of higher protein diets for greater weight loss (Layman et al., 2003; Schoeller & Buchholz, 2005). Schoeller & Buchholz (2005) reference two studies in which a higher protein intake protected REE and TEF during weight loss by either increasing overall REE and TEF or stunting the decline of REE and TEF when compared to low fat diets. Other studies report higher protein meals slow the decrease in TEF associated with weight loss and increase satiety thus affecting the amount of food eaten at the next meal (Eisenstein et al., 2002; Luscombe-Marsh et al., 2005). Protein diets are also typically touted for their nitrogen-sparing effect and ultimate protection of lean tissue (Farnsworth et al., 2003; Hoffer et al., 1984); yet, the findings of this study somewhat support the protein-sparing nature of carbohydrates. Vasquez et al. (1995) studied similar protein intakes (0.8 g/kg ideal body weight (IBW)
vs. 1.1 g/kg IBW) while varying carbohydrate amounts to assess the interaction between protein and ketosis. The nitrogen-sparing effect of increased carbohydrates far out weighed that of protein (Vazquez et al., 1995). Another study completed under similar conditions also supports the protein-sparing benefits of carbohydrates versus protein (Howard et al., 1978). Perhaps the prescribed diet regardless of diet group was so drastically different than usual intakes that the negative energy balance allowed participants to tap into fat stores for energy, thus contributing to changes in body composition. Also, the difference in protein intake between the two diet groups was only 30 grams/day. This difference in protein intake may not have been large enough to make a difference in the overall results between the two diet groups. With current research supporting higher protein diets for greater weight loss, one would have expected the HP diet to have affected nitrogen balance, glycogen stores, overall REE and TEF more drastically than the HC diet and thus greater losses in fat mass and greater changes in body fat percentage would have resulted. However, the acute nature of the study protocol and the amount of protein consumed could be limiting factors. Most studies supporting a higher protein diet for greater fat loss have participants consuming anywhere from 30-100% of calories from protein and are much longer in length (3-10+ weeks) (Buchholz & Schoeller, 2004; Eisenstein et al., 2002).

**Resting Energy Expenditure**

Resting energy expenditure changed over time in both diet groups, but no significance between diet groups occurred. REE decreased during the negative energy balance phase (days 1-7). REE continued to decrease until the positive energy phase of the diet (days 8-14), where REE began to rebound. The HC diet actually experienced a
rebound in REE back to pre-dietary intervention levels. These events suggest that caloric consumption affects REE; caloric restriction decreases REE and refeeding increases REE. This is consistent with studies that have found decreases in 24-hour energy expenditure during energy restriction (Whitehead et al., 1996). Decreases in energy expenditure are undesirable for individuals trying to lose weight since it will reduce the energy deficit. The decreased 24-hour energy expenditure then leads to a decrease in the rate of weight loss over time (Whitehead et al., 1996). On the other hand, refeeding studies tend to examine the effects of restricted feeding/refeeding cycles and/or overfeeding on body weight, body composition, and energy metabolism. One study done in men determined that overfeeding led to weight gain and subsequent increases in metabolic rate were associated with increases in body size and tissue gain (Norgan & Durnin, 1980). The remarkable event in the findings of this study is the continued weight loss during positive energy balance. The rebounding of metabolism, yet not body weight is an important factor that could prove useful in the future formulation of diet strategies for overweight and obese individuals. These findings should be replicated and looked at in a long term setting.

**Total Body Water**

Total body water changed over time; however, there were no differences between diet groups. Total body water showed a downward trend during the negative energy balance phase of the diet, but stabilized during the positive energy balance phase of the diet. The declining trend during the negative energy balance phase of the diet could possibly be related to the change in overall food intake. Food contributes to water intake, and decreasing the amount of food consumed could affect total body water. Typically,
water losses are associated with higher protein diets. The loss of liver and muscle glycogen related to changes in carbohydrate intake below that required to sustain glycogen stores results in water loss of 1.9 kg in the first 10 days of a very low carbohydrate diet (<25 g carbohydrates/day) (Schoeller & Buchholz, 2005). However, the results from the current study did not indicate any differences between diet groups. The carbohydrate intake >25 g/day of the current study more than likely did not affect glycogen stores and thus makes it difficult to compare with the findings of Scholler & Buchholz (2005). Also, variable and transient diuresis during the initial period of negative energy balance in a weight loss diet makes weight loss results hard to interpret particularly for shorter periods of time (studies lasting 2 weeks or less) (Eisenstein et al., 2002; Feinman & Fine, 2003); therefore, the acute nature of the study could also be a limiting factor.

Of interesting note, if a gram of glycogen is associated with 2.7 grams of water (McArdle, 2001), then a 200 g decrease in stored glycogen—the body stores approximately 500g total in muscle and liver glycogen (McArdle, 2001)—would be associated with a 540 ml decrease in body water. A 540 ml decrease in body water is roughly equal to 0.5 kg. In the current study, subjects lost an average of 2 kg over the 14-day intervention. Given the above information regarding water weight and glycogen and considering the short duration of negative energy balance combined with a carbohydrate intake that likely sustained stored glycogen, the results of the current study suggest the 2 kg weight loss was mostly body weight rather than just water weight. This is somewhat contradictory to the literature indicating that water makes up approximately 70% of the weight lost during the first week of negative energy balance (McArdle, 2001;
Schoeller & Buchholz, 2005) and does not support the common thought that weight loss on energy-restricted, higher protein diets is simply water (Schoeller & Buchholz, 2005).

**Thyroid Hormones**

Thyroxine (T4), triiodothyronine uptake (T3U), free thyroxine index (FTI) and thyroid stimulating hormone (TSH) were monitored over the course of the study. A significant time effect was discovered regarding the changes in T4 (p = 0.035), T3U (p = 0.003), and TSH levels (p = 0.031). Overall, T4 trended downward over the 14 day study period. During caloric restriction and starvation, the total concentration of free and bound T4 typically remains constant (Jung et al., 1980). If a decrease in T4 is seen, then it generally recovers as the fasting or calorie restricted state continues (Jung et al., 1980). The acute nature of the negative energy balance phase of this study makes it difficult to compare T4 values with results from Jung et al (1980). Also, there is no general scientific agreement regarding the mechanisms in which thyroid hormones stimulate heat production and thus affect REE (*Handbook of obesity: Etiology and pathophysiology*, 2004). However, Stenlof et al. (1993) determined that BMR is negatively related to free T4 concentrations. Given this information, then the overall downward trend seen in T4 over time should be associated with a rise in REE. Calculated correlations between REE and T4 data found significant relationships at days 9, 10, and 11. Correlated values indicated a slightly positive relationship between REE and T4 rather than a negative relationship.

A significant diet effect was seen in T3U (p = 0.049) as well as a significant time effect (p=0.003). The HP diet group had a greater T3U than the HC diet group. Jung et al. (1980) summarized several studies in which investigators observed a rapid decline in
T3 during starvation; the decline in T3 varied anywhere from 1-4 days and depended on the carbohydrate content of the diet pre-intervention. These studies also observed the fall in T3 to be much less among obese women. Spaulding et al. (1976) reported that serum concentrations of T3 are reduced in caloric deprivation, yet increase during refeeding. Carbohydrate deprivation decreased plasma T3 levels and increased protein catabolism. Starvation decreased plasma T3 levels, resting energy expenditure, and nitrogen excretion (Bisschop et al., 2001). The acute nature of this study may have been too short in duration to actually see a decline in T3; however, an upward trend was apparent during days 8-14 (refeeding).

TSH also experienced a significant time effect (p = 0.031) over the course of the study. TSH trended downward but eventually reached a plateau during days 8-14. Jung et al. (1980) discusses the possibility of food intake regulating TSH. The results of the current study would support this idea since the decline in TSH occurred mainly during days 1-7 (negative energy balance). Elevated TSH in obese women is significantly reduced by diet induced weight loss (Kok et al., 2005), but subjects in the current study had TSH within normal limits prior to dietary intervention. Decreases in TSH may blunt energy expenditure in response to long-term caloric restriction; thus, lowering TEE and frustrating weight loss efforts (Kok et al., 2005). Unfortunately, the acute nature of this study makes it difficult to interpret the changes in thyroid hormones. The relationships between thyroid hormones and macronutrient type both in ad libitum and energy restricted diets as well as thyroid hormone levels and energy balance deserve to be further explored in future research studies.
Conclusion

The results of the current study suggest that calorie intake and energy balance have a greater overall effect on REE and weight loss than macronutrient type. The rebounding of metabolism during refeeding, yet not body weight is an important factor that could prove useful in the future formulation of diet strategies for overweight and obese individuals. The fact that weight loss over the 14 day intervention was more than just water weight is another interesting result. These findings should be replicated and looked at in a long term setting. Hormonal changes were not as evident; a longer study would more than likely be needed to see any hormonal effects.
APPENDICES
APPENDIX A

Curves Metabolism IRB Application

Application to the Baylor IRB
For Review of Research/Activity Proposal

Part 1: Signature Page

1. Name ____________________________ Richard B. Kreider, PhD, FACSM

2. Email Address (optional) __________ Richard_Kreider@baylor.edu

3. Complete Mailing Address __________ P.O. Box 97313

4. Position _________________________ Professor & Chair

5. Faculty Advisor (if researcher is Graduate Student) __________________________

6. Department/School ________________ HEIPR

7. Telephone # ______________________ x4003 FAX # __________ x3527

8. Are you using subjects in research (Y or N) Y or in teaching exercises (Y or N)?

9. Title of the research project/teaching exercise:
   Effects of Changes in Caloric and Macronutrient Intake on Resting Energy
   Expenditure and Body Composition

10. Please return this signed form along with all the other parts of the application and
    other documentation to the University Committee for Protection of Human Subjects in
    Research; Dr. Matt Stanford, P.O. Box 97334, Waco, TX 76798-7334. If you have
    questions, or if you would like to see a copy of the OHRP Report on protection of human
    subjects in research, contact Dr. Stanford at 254-710-2286.

_______________________________ 1/14/95 ______________________________
Signature of Principal Investigator Date

_______________________________
Signature of Faculty Advisor (required if researcher is a Graduate Student)

Departmental Review: ____________________________ Department Chair or the Chair's Designate
Part 2: Introduction & Rationale

The Curves International fitness and weight loss program has become a very popular means of promoting health and fitness among women. Over 3 million women belong to some 7,000 Curves centers in the United States. The program involves a 30-minute circuit training program involving use of twelve bidirectional hydraulic exercise machines that train all major muscle groups interspersed with calisthenic-type exercises designed to maintain an elevated heart rate and increase energy expenditure. For members wishing to lose weight, the program recommends following a short caloric restricted diet (1,200 kcal/day) designed to promote weight loss followed by a moderately caloric restricted diet (1,600 kcal/day) that is designed to promote a gradual reduction in body fat. The diet recommends one of two types of macronutrient manipulations based on initial dietary practices and response to a carbohydrate tolerance questionnaire. Since resting energy expenditure (REE) decreases during periods of caloric restriction and the reduction of REE has been implicated as a contributor to weight regain, the program recommends intermittent periods of increased caloric intake designed to normalize hormone levels and REE. This program is designed to promote fat loss and improve fitness without maintaining a very low calorie diet (i.e., ≤ 800 kcal/day) that is often used in weight loss trials. Additionally, it is designed to decrease the incidence of subjects experiencing a weight regain once their weight goals have been achieved. The Exercise & Sport Nutrition Lab has been conducting extensive studies on the efficacy of the Curves for Women program over the last two years. Results have shown that the Curves program is highly effective in promoting weight loss, improving markers of health, and improving fitness. While these results are promising, we feel that this program could be even more successful with some minor revisions in the diet protocol and/or implementation of various nutritional strategies designed to promote weight loss.

Resting energy expenditure (REE) accounts for approximately 75% of daily energy expenditure. Caloric restriction and/or rapid weight loss typically decreases REE which makes it easier to regain weight. Conversely, exercise and increased caloric intake raises REE. Our prior research has shown that participating in the Curves fitness and weight loss program serves to increase REE by 150 - 400 kcal/day (1-2 kcal/kg/day). Moreover, REE has been maintained and/or increased during maintenance periods which have enabled women participating in this program to achieve impressive weight loss and maintenance up to a year. This has been accomplished by only observing a short-term moderate caloric restriction (1,200 kcal/day) for 7-14 days followed by a possible 1,500 kcal/day diet during the remainder of the weight loss period. Consequently, subjects maintained a normal caloric intake (2,600 kcal/day) followed by intermittent dieting (1,500 kcal/day for 1-day). These caloric intakes were based on a best guess regarding the amount of calories women engaged in weight loss and/or maintenance may need to achieve weight loss while preserving and/or increasing metabolic rate. However, we need to conduct additional research to assess the short-term effects of maintaining a hypocaloric diet as well as understand the effects of resuming a normal diet in order to assess whether these estimates are metabolically efficient. The purpose of this study is to examine the acute effects of reducing and increasing caloric intake on REE, hormonal markers of metabolic rate, body water, and body composition in women. These data will be helpful in determining how long people can optimally restrict dietary intake before REE and hormonal regulation of metabolic rate is impacted. Additionally, these data will help determine the amount of calories and length of time needed to promote increases in REE.

Part 3: Methodology

Subjects

Approximately 100 healthy, trained, and moderately overweight female subjects between the ages 18 to 65 will participate in this study. Subjects will not be allowed to participate in this study if they have any metabolic disorder including known electrolyte abnormalities, heart disease, anemia, diabetes, or thyroid disease, uncontrolled hypertension, hypertriglyceridemia, or neurological disease, and/or, if they have taken weight loss dietary supplement (e.g., thermogenic, etc) or medications within three months prior to the start of the study. The only exception will be if the prospective subject has a medical condition or history that the subject's personal physician feels is controlled and therefore would not be a limitation for them to participate in the study. Subjects meeting eligibility criteria will be informed of the requirements of the study and sign informed consent statements in compliance with the Human Subjects Guidelines of Baylor University and the American College of Sports Medicine.
Study Site

All testing will be conducted in the Exercise & Sport Nutrition Laboratory (ESNL) in the Department of Health, Human Performance, and Recreation at Baylor University.

Experimental Design

Table 1 shows the general research design and time course for assessments. The independent variable will be dietary intake. Dependent variables will include: estimated dietary energy intake, REE, body weight, body water, body composition, hip and waist anthropometric measurements, and fasting clinical blood profiles (substrates, electrolytes, muscle and liver enzymes, red cells, white cells, thyroid panel, insulin, leptin, ketones bodies, and lipoprotein enzymes).

Entry and Familiarization Session

Subjects expressing interest in participating in this study will be interviewed on the phone to determine whether they appear to qualify to participate in this study. Subjects believed to meet eligibility criteria will then be invited to attend an entry/familiarization session. During this session, subjects will sign Informed Consent Statements and complete personal and medical histories. Any subject who does not meet entry criteria will be required to obtain medical clearance from his or her personal physician prior to participating in baseline assessments. Subjects eligible to participate in the study will be familiarized with the study protocol via a verbal and written explanation outlining the study design. This will include describing the dietary program and familiarizing the subjects to the tests to be performed. Subjects will then be given an appointment time to perform baseline assessments.

Baseline Testing

Following the familiarization session, the subjects will record all food intake on dietary record forms for four days (4–7). Subjects will be instructed to refrain from exercise for 48 hours and fast for 8 hours prior to baseline testing. Subjects will then report to the ESBL for clinical assessment. Once reporting to the lab, subjects will be weighed, have total body water determined by bioelectrical impedance (BIA), and have body composition determined using a Hologic Discovery W Dual Energy X-ray Absorptiometer (DEXA). Subjects will then have REE, blood pressure, and resting heart rate, and hip and waist measurements determined using standard procedures. Subjects will donate approximately 30 ml of fasting blood using venipuncture techniques of an antecubital vein in the forearm according to standard procedures. Blood samples will be analyzed in the Exercise & Biochemical Nutrition lab for standard clinical chemistry profiles (glucose, ammonia, total protein, blood urea nitrogen, creatinine, BUN/creatinine ratio, uric acid, AST, ALT, CK, LDH, GGT, albumin, globulin, sodium, chloride, calcium, carbon dioxide, total bilirubin, alkaline phosphatase, triglycerides, cholesterol, HDL, LDL, and whole blood cell counts (including hemoglobin, hematocrit, red blood cell counts, MCV, MCH, MCHC, RDW, white blood cell counts, neutrophils, lymphocytes, monocytes, eosinophils, basophils). In addition, serum samples will be assayed for a thyroid panel (e.g., TSH, T3, T4), beta-hydroxybutyric acid, insulin, leptin, and lipoprotein enzymes.

Randomization and Dietary Intervention

Approximately 10 subjects will be randomized into one of the following groups:

1. **Normal Control Diet** (no dietary intervention)

2. **Diet I** 1,000 kcal/day for 7 days [115 g/d (46%) carbohydrate, 60 g/d (24%) protein, 33 g/d (30%) fat] 1,600 kcal/day for 7 days [220 g/d (55%) carbohydrate, 60 g/d (15%) protein, 53 g/d (30%) fat]

3. **Diet II** 1,000 kcal/day for 7 days [60 g/d (24%) carbohydrate, 115 g/d (46%) protein, 33 g/d (30%) fat] 1,600 kcal/day for 7 days [220 g/d (55%) carbohydrate, 60 g/d (15%) protein, 53 g/d (30%) fat]
4. **Diet III** 1,000 kcal/day for 7 days: [115 g/d (46%) carbohydrate, 60 g/d (24%) protein, 33 g/d (30%) fat]
   2,100 kcal/day for 7 days: [185 g/d (55%) carbohydrate, 80 g/d (15%) protein, 70 g/d (30%) fat]

5. **Diet IV** 1,000 kcal/day for 7 days: [160 g/d (24%) carbohydrate, 115 g/d (46%) protein, 33 g/d (30%) fat]
   2,100 kcal/day for 7 days: [185 g/d (55%) carbohydrate, 80 g/d (15%) protein, 70 g/d (30%) fat]

6. **Diet V** 1,000 kcal/day for 7 days: [115 g/d (46%) carbohydrate, 60 g/d (24%) protein, 33 g/d (30%) fat]
   2,600 kcal/day for 7 days: [335 g/d (55%) carbohydrate, 100 g/d (15%) protein, 85 g/d (30%) fat]

7. **Diet VI** 1,000 kcal/day for 7 days: [160 g/d (24%) carbohydrate, 115 g/d (46%) protein, 33 g/d (30%) fat]
   2,600 kcal/day for 7 days: [335 g/d (55%) carbohydrate, 100 g/d (15%) protein, 85 g/d (30%) fat]

8. **Diet VII** 1,000 kcal/day for 7 days: [115 g/d (46%) carbohydrate, 60 g/d (24%) protein, 33 g/d (30%) fat]
   3,100 kcal/day for 7 days: [425 g/d (55%) carbohydrate, 115 g/d (15%) protein, 105 g/d (30%) fat]

9. **Diet VIII** 1,000 kcal/day for 7 days: [160 g/d (24%) carbohydrate, 115 g/d (46%) protein, 33 g/d (30%) fat]
   3,100 kcal/day for 7 days: [425 g/d (55%) carbohydrate, 115 g/d (15%) protein, 105 g/d (30%) fat]

Subjects will consume 1,000 kcal/day for seven days consisting of either a high carbohydrate or high protein diet (in percentage but not relative terms). Once completed, the subject will consume a high carbohydrate diet (55% carbohydrate, 15% protein, and 30% fat) for seven days consisting of 1,600, 2,100, 2,600, or 3,100 kcal/day. Diets will be prepared by a registered dietitian and will not consist of less than 60 g/day of carbohydrate or more than 115 g/day of protein.

**Assessment Schedule**

Table 1 describes the assessment schedule to be used in the study. Subjects will return to the lab following an 8-hour fast on days 1, 2, 3, 4, 7, 8, 9, 10, 11, and 14 of the study. Subjects will be weighed, have total body water and DEXA body composition measurements determined, have REE, resting heart rate and blood pressure measurements obtained, and donate approximately 20 ml of venous blood. These tests will help determine the acute effects of changes in caloric and macronutrient intake on metabolism and body composition.

**Data Analysis**

Analysis of variance (ANOVA) for repeated measures univariate tests will be used to analyze data. Data will be considered statistically significant when the probability of type I error is 0.05 or less. If a significant group, treatment and/or interaction alpha level is observed, least significant differences (LSD) post-hoc analyses will be performed to determine where significance was obtained. Power analysis of the design indicates that an n-size of 15 per group and 45 per supplement intervention yields high power (>0.8) for delta values of 0.75 to 1.5.

**Research Team**

*Richard B. Kraemer, PhD, MX, FACSM.* Dr. Kraemer has recently been named Professor and Chair of the Department of Health, Human Performance, & Recreation at Baylor University. Dr. Kraemer is an internationally recognized exercise scientist and is currently President of the American Society of Exercise Physiologists. He has conducted a vast amount of research primarily focusing on the role of exercise and nutrition and health and performance. Dr. Kraemer will serve as the supervising investigator in coordinating the conduct of the study.

*Chris Rasmussen, MS, CSCS, EPC, MX.* Mr. Rasmussen serves as research coordinator in the ESRL. He is responsible for day to day scheduling and testing for this study.

*Ronald Wilson, MD.* Dr. Wilson serves as medical supervisor for the ESRL and Center for Exercise, Nutrition & Preventive Health Research (CENPHR).
Research Assistants. Research assistants and a research nurse will be assigned to assist in data collection and analysis. Additional research assistants will supervise training sessions.

Registered Dietitian. The registered dietitian (RD) will develop meal plans according to the macronutrient intake goals described in Table 2. The RD will also supervise analysis of nutritional records and assist in conducting resting energy expenditure assessments.

Research Nurse. Subjects will complete a symptoms and side effects inventory during each assessment. The research nurse will monitor reported side effects and report any unusual findings to our research physician.

Procedures

Medical Monitoring. Interested subjects will be invited to familiarization sessions. During this time, subjects will sign consent forms and complete medical history information. Subjects will then undergo a general exam to determine whether the subject meets entry criteria to participate in the study. A trained researcher will evaluate the medical and training history questionnaires to determine whether the subject meets entry criteria and may therefore participate in the study. Trained, non-physician exercise specialists certified in CPR will supervise subjects undergoing exercise assessment. A telephone and an automated electronic defibrillator is located in the laboratory in case of any emergencies and there will be no less than two researchers working with each subject during testing sessions. In the event of any unlikely emergency, one researcher will check for vital signs and begin any necessary interventions while the other researcher contacts Baylor’s campus police at extension 2222. Instructions for emergencies are posted above the phone in the event that any other research investigators are available for assistance. Subjects will be informed to report any unexpected problems or adverse events they may encounter during the course of the study to Richard B. Kadoski, PhD, FACP or Carri Krumm, MS, MSc, EPIC, CSCS. If clinically significant side effects are reported, the subjects will be referred to discuss the problem with the research nurse (currently McLean Guileth, FNP) or Lori Greenwood, PhD, ATC who is an Associate Professor of Athletic Training at Baylor University. If deemed necessary, Dr. Greenwood will refer the subject to Ben Wilson, MD for medical follow-up. Dr. Wilson is one of the Sports Medicine physicians for Baylor University and is an adjunct Professor in the Department of FHPR. He has agreed to provide medical support and consultation for this study and to our lab. Dr. Wilson will evaluate the complaint and make a recommendation whether any medical treatment is needed and/or whether the subject can continue in the study. If Dr. Wilson feels medical follow-up is necessary, the subject will be referred to obtain medical treatment from their personal physician. This is a similar referral/medical follow-up system that Baylor athletes are provided with the exception that subjects in this study will not be provided medical care. New findings and/or medical referrals of unexpected problems and/or adverse events will be documented, placed in the subjects research file, and reported to the Baylor IRB committee.

Dietary Invoenties. Subjects will record all food and fluid intake on dietary record forms for 4 days prior to baseline testing. Dietary intake will be assessed using the Food Processor III Nutrition Software.

Body Composition Assesments. Subjects will undergo body composition tests in the ESNL. Prior to each assessment, height will be measured using standard stadiometer and total body weight will be measured using a calibrated electronic scale with a precision of ± 0.02 kg. Total body water will then be estimated using a Xitron 4200 Bioelectrical Impedance Analyzer (San Diego, CA) which measures bio-resistance of water and body tissues based on a 5 minute low energy, high frequency current (500 micro-amps at a frequency of 50 kHz) transmitted through the body. This analyzer is commercially available and has been used in the health care/fitness industry in a manner to assess body composition and body water for over 20 years. The use of this device has been approved by the Food and Drug Administration (FDA) to assess total body water and the current to be used has been deemed safe [1]. This is measured through from electrodes placed on the body: one electrode will be placed on the posterior surface of the right wrist, in between the radial and ulna styloid processes (wrist bone), another electrode will be placed on the posterior surface of the right hand at the distal base of the second metacarpal; the third electrode will be placed on the anterior surface of the right foot at the distal end of the first metatarsal. Subjects will lie on a table in the supine position and electrodes will be connected to the analyzer. After the subject is connected, age, gender, weight, height, and activity level are entered into the unit by the technologist. After the unit has measured the resistance, which takes approximately 30 seconds, the unit then calculates total body water and body water percent.
Body composition/bone density will then be determined using a calibrated Hologic 4500W dual-energy x-ray absorptiometry (DEXA) by qualified personnel with limited x-ray technology training under the supervision of Richard B. Kreider, PhD, MDX. The DEXA body composition test will involve having the subject lie down on their back in a standardized position in a pair of shorts/underwear. A low dose of radiation will then scan the entire body for approximately six (6) minutes. The DEXA segments regions of the body (right arm, left arm, trunk, right leg, and left leg) into three compartments for determination of fat, soft tissue (muscle), and bone mass. Radiation exposure from DEXA for the whole body scan is approximately 1.5 mR per scan. This is similar to the amount of natural background radiation a person would receive in one month while living in Texas. The maximal permissible x-ray dose for non-occupational exposure is 500 mR per year. Total radiation dose will be approximately 15 mR for the entire study. Since women of child bearing age will serve as subjects in this study, each subject will complete a questionnaire related to their menstrual cycle timing, sexual activity, use of birth control pills, and desire to become pregnant (see attached). DEXA tests will be performed within 14-days of the onset of their menstrual period to menstruating women of child bearing age who do not use oral contraceptives according to NCRP and AARP radiology standards in order to reduce the possibility of exposure of an unknown fetus to radiation.

**Resting Heart Rate & Blood Pressure.** Heart rate will be determined by palpitation of the radial artery using standard procedures [2]. Blood pressure will be assessed in the supine position after resting for 5-min using a mercury sphygmomanometer using standard procedures [2].

**Blood Samples.** Subjects will fast overnight for twelve (12) hours and then donate approximately 4 teaspoons of fasting venous blood (20 milliliters). Blood samples will be obtained using standard phlebotomy procedures using standard sterile venipuncture of an antecubital vein by laboratory technician’s trained in phlebotomy in compliance with guidelines established by the Texas Department of Health and Human Services. The phlebotomists and lab technicians will wear personal protective clothing (gloves, lab coats, etc.) when handling blood samples. Subjects will be seated in a phlebotomy chair. Their arm will be cleaned with a sterile alcohol wipe and sterile gauze. A standard rubber tourniquet will then be placed on the brachium. An antecubital vein will be palpated and then a 23 gauge sterile needle attached to a plastic vacutainer holder will be inserted into the vein using standard procedures. Three serum separation vacutainer tubes (red tops) and one EDTA vacutainer tubes (purple top) will be inserted into the vacutainer holder for blood collection in succession using multiple sample phlebotomy techniques. Once samples are obtained, the vacutainers holder and needle will be removed. The needle will be discarded as hazardous waste in a plastic sharps container. The site of the blood draw will then be cleaned with a sterile alcohol wipe and gauze and a sterile Band-Aid will be placed on the site. The blood collection tubes will be labeled and placed in a test tube rack. Laboratory technicians (who have received blood borne pathogen training and will be wearing personal protective clothing) will centrifuge the serum samples, transfer serum into labeled serum storage containers, and prepare samples for shipment or storage into a refrigerator or freezer for subsequent analysis. Serum and whole blood samples will be analyzed in the Exercise Biomedical and Nutrition Laboratory (EBNL) at Baylor University for assay of a standard clinical chemistry profile (glucose, total protein, blood urea nitrogen, creatinine, BUN/creatinine ratio, urine acid, AST, ALT, CK, LDH, GGT, albumin, globulin, sodium, chloride, calcium, carbon dioxide, total bilirubin, alkaline phosphatase, triglycerides, cholesterol, HDL, LDL) and whole blood cell counts (including hemoglobin, hematocrit, red blood cell count, MCV, MCH, MCHC, RDW, white blood cell counts, neutrophils, lymphocytes, monocytes, eosinophils, basophils) in order to evaluate markers of catabolism and clinical safety of the supplementation protocol. Serum macronutrient samples will be assayed for insulin, leptin, and lipogenic enzymes in the EBNL at Baylor University. Analysis of these blood parameters will determine the effects of the dietary interventions on general markers of clinical health status and metabolism.

**Resting Energy Expenditure Assessment.** Resting energy expenditure assessment will be made according to standard protocol using the Parvo Medics TrueMet-2400 Metabolic Measurement System (Sandy, UT). This will involve the subjects lying down on an exam table, having a light blanket placed over them to keep warm, and inserting ear plugs in their ears to reduce distractions. A see through metabolic canopy will then be placed over the subject's neck and head so that metabolic measurements can be obtained. The subject will lie motionless without going to sleep for 15-minute. Metabolic measurements will then be obtained to determine resting oxygen uptake and energy expenditure.
Equipment

**Digital Scale.** Total body weight will be determined using a digital scale accurate to ±0.02 kg. The scale is calibrated by placing certified 25-kg weights and balancing the scale. Other than general instructions, special skills are not required to measure body weight.

**Bioelectrical Impedance Analyzer (BIA).** The Xitron 4200 Bioelectrical Impedance Analyzer (San Diego, CA) which measures bio-resistance and body composition based on a minute low energy, high frequency current transmitted through the body from surface electrodes attached to standardized anatomical locations on the dorsal surface of the right hand and foot while the subject lies supine in a supine position. The analyzer is calibrated internally to a standard electrical current by passing the calibration key located on the unit. A trained research assistant will perform this procedure.

**Dual-Energy X-Ray Absorptiometer (DEXA).** Body composition measurements will be determined by qualified personnel (in compliance with State Regulations) using a Hologic 4500W dual energy x-ray absorptiometer (Waltham, MA). This system segments regions of the body (right arm, left arm, trunk, right leg, and left leg) into three compartments (i.e., bone mass, fat mass, and fat-free/soft tissue mass). Quality control (QC) calibration procedures will be performed on a spine phantom (Hologic X-CALIBER Model DPAQDE-1 anthropometric spine phantom) prior to each testing session. In addition, weekly calibration procedures will be performed on a density step calibration phantom.

**Anthropometric Measurements.** Anthropometric hip and waist measurements will be determined using standard procedures with a tension-regulated tape measure. The hip to waist ratio will be examined to assess the impact of this fitness and weight loss program on cardiovascular risk.

**Mercurial Sphygmomanometer.** Blood pressure will be assessed by auscultation of the brachial artery using a mercury sphygmomanometer using standard clinical procedures.

**Metabolic Measurements.** Resting energy expenditure measurements will be obtained using Parvo Medics TrueMax metabolic measurement system (Sandy, UT).

Subjects

**Recruitment**

Approximately 100 female subjects between the ages 18 - 65 will participate in this study. A recruitment flyer that will be posted on campus, at area fitness centers, and on the Internet ([www.1-baylor.edu/HHPR/FNSN](http://www.1-baylor.edu/HHPR/FNSN)) is attached.

**Selection Criteria**

Subjects will not be allowed to participate in this study if they:

1. have any metabolic disorder including known electrolyte abnormalities; heart disease, arrhythmias, diabetes, thyroid disease, or hypogonadism; a history of hypertension, nephrolithiasis, musculoskeletal, autoimmune, or neurological disease.
2. are taking thyroid, hyperlipidemic, hypoglycemic, anti-hypertensive, or androgenic medications;
3. have taken ergogenic doses of nutritional supplements that may affect muscle mass (e.g., creatine, HMB), anabolic/catabolic hormone levels (androstenedione, DHEA, etc.), or weight loss (e.g., sibutramine, thermogenic, etc.) within three months prior to the start of the study;
4. report any unusual adverse events associated with this study that in consultation with the supervising physician recommends removal from the study.

The only exception to these selection criteria will be if the prospective subject has a medical condition or history that the subject's personal physician feels is controlled and therefore would not be a limitation for them to participate in
the study. In this case, the subject must obtain a letter from their physician indicating that they approve them participating in this study.

Compensation or Incentives

Subjects completing all familiarization and testing sessions and turning in all required materials (i.e., food and training logs) will be paid $250. Subjects will also be given free fitness assessments during the course of the study as described above and may receive information regarding results of these tests if they desire. If subjects are Baylor students, they will not receive any academic credit for participating in this study.

Potential Risks

Subjects who meet eligibility criteria will be exposed to a low level of radiation during the DEXA body composition tests, which is similar to the amount of natural background radiation a person would receive in one month while living in Waco. In addition, a very low level of electrical current will be passed through each subject’s body using a bioelectrical impedance analyzer. This analyzer is commercially available and has been used in the health care/fitness industry as a means to assess body composition and body water for over 20 years. The use of the BIA and DEXA analyzers has been shown to be safe methods of assessing body composition and total body water and is approved by the FDA. Subjects will donate about 4 teaspoons (20 milliliters) of venous blood eleven (11) times during the study over a 14-day period using standard phlebotomy procedures. This procedure may cause a small amount of pain when the needle is inserted into the vein as well as some bleeding and bruising. The subject may also experience some dizziness, nausea, and/or faint if they are unaccustomed to having blood drawn. Subjects will also have a see through plastic canopy placed over their heads during the BIA assessment. This may cause some women to experience symptoms of claustrophobia such as anxiety. Subjects will follow a prescribed dietary regimen involving consuming 1,000, 1,600, 2,100, 2,600, or 3,100 calories per day during various phases of the program. In addition, subjects may ingest a higher than normal amount of calories from protein. Although the total amount of total protein is not excessive (80 – 115 g/day or 10 – 15 g/kg/day for a 70 kg female) it may be higher than the subject is accustomed to ingesting. As a result, subject may experience weight loss or gain, feelings of hunger or fullness, and/or changes in appetite and/or mood during various phases of the dietary intervention.

Researchers involved in collecting data represent trained, non-physician, certified exercise specialists (American Society of Exercise Physiologists, Certified Exercise Physiologist, Certified Strength & Conditioning Specialist, Certified Athletic Trainers, and/or American College of Sports Medicine Health Fitness Instructor, Exercise Technology, Exercise Specialist, or Program Director for Preventive and Rehabilitative Exercise Program). All personnel involved in collecting data will be certified in CPR, which is also a condition to holding these professional certifications. A telephone and automated electronic defibrillator is in the laboratory in case of any emergencies, and there will be no less than two researchers working with subject during testing. In the event of any unlikely emergency one researcher will check for vital signs and begin any necessary interventions while the other researcher contacts Baylor’s campus police at extension 2222. Instructions for emergencies are posted above the phone in the event that any other research investigators are available for assistance.

Potential Benefits

The main benefit that the subject may obtain from this study is that they will learn more about how their resting metabolism is influenced by changes in caloric and macronutrient intake. However, even if no individual benefit is obtained, participating in this study will help to determine the metabolic responses to dieting.

Assessment of Risk

This study will help identify the impact of changing caloric and macronutrient intake on resting metabolism. During the course of the study, subjects will have a number of assessments performed to examine how the body responds to changes in caloric intake and macronutrient intake. These tests are commonly performed in clinical settings. The greatest risk of discomfort will come from taking daily blood samples. This may cause bruising of the arms and or trauma to antecubital veins. However, it is our view that the potential benefits of subjects participating in this study outweigh the potential risks.
Confidentiality

Information obtained from this research (including questionnaires, medical history, laboratory findings, or physical examination) will be kept confidential to the extent permitted by law. However, according FDA regulations, records will be open to FDA representatives to review if necessary. This may include questionnaires, medical history, laboratory findings/reports, statistical data, and/or notes taken throughout this study. Records of the research may also be subpoenaed by court order or may be inspected by federal regulatory authorities. Data derived from this study may be used in reports, presentations and publications. However, subjects will not be individually identified unless they give their written consent.

Data Analysis, Presentation, & Publication

Data will be analyzed using group x time repeated measures analysis of variance (ANOVA) with SPSS for Windows Version 11 software (SPSS Inc., Chicago, IL). Data will be considered significantly different when the probability of error was 0.05 or less. Least significant differences (LSD) post-hoc procedures will be performed when a significant interaction was observed. Data will be presented at an appropriate scientific conference (e.g., American College of Sports Medicine, Experimental Biology, etc) and published in a peer-reviewed scientific journal (e.g., Medicine & Science in Sport and Exercise, Nutrition, International Journal of Sport Nutrition and Exercise Metabolism, etc).

Statement on Conflict of Interest

Funding for this study will be obtained Curves International (Waco, TX) through a grant awarded to Baylor University. Researchers involved in collecting data in this study have no financial or personal interest in the outcome of results or sponsors.

References

<table>
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<th>Familiarization and Entry</th>
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<td>Body Mass Body Water DEXA Body Composition Fasting Blood Collection Resting BP/ECG Resting Energy Expenditure Anthropometric Measurements Subjects matched according to BMI and age for random assignment into diet regimen.</td>
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<td>Assessment of medical history to determine qualifications to participate in study</td>
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<td>Dietary History (=Days)</td>
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</table>

Table 1. Overview of Research Design and Testing Schedule
Table 2. Overview of Dietary Interventions:

1. Normal Control Diet (no dietary intervention)

2. Diet I 1,000 kcal/day for 7-days [115 g/d (46%) carbohydrate, 60 g/d (24%) protein, 33 g/d (30%) fat]
   1,600 kcal/day for 7-days [220 g/d (55%) carbohydrate, 60 g/d (15%) protein, 55 g/d (30%) fat]

3. Diet II 1,000 kcal/day for 7-days [60 g/d (24%) carbohydrate, 115 g/d (46%) protein, 33 g/d (30%) fat]
   1,600 kcal/day for 7-days [120 g/d (55%) carbohydrate, 60 g/d (15%) protein, 55 g/d (30%) fat]

4. Diet III 1,000 kcal/day for 7-days [115 g/d (46%) carbohydrate, 60 g/d (24%) protein, 33 g/d (30%) fat]
   2,100 kcal/day for 7-days [185 g/d (55%) carbohydrate, 80 g/d (15%) protein, 70 g/d (30%) fat]

5. Diet IV 1,000 kcal/day for 7-days [60 g/d (24%) carbohydrate, 115 g/d (46%) protein, 33 g/d (30%) fat]
   2,100 kcal/day for 7-days [185 g/d (55%) carbohydrate, 80 g/d (15%) protein, 70 g/d (30%) fat]

6. Diet V 1,000 kcal/day for 7-days [115 g/d (46%) carbohydrate, 60 g/d (24%) protein, 33 g/d (30%) fat]
   2,600 kcal/day for 7-days [350 g/d (55%) carbohydrate, 100 g/d (15%) protein, 85 g/d (30%) fat]

7. Diet VI 1,000 kcal/day for 7-days [60 g/d (24%) carbohydrate, 115 g/d (46%) protein, 33 g/d (30%) fat]
   2,600 kcal/day for 7-days [350 g/d (55%) carbohydrate, 100 g/d (15%) protein, 85 g/d (30%) fat]

8. Diet VII 1,000 kcal/day for 7-days [115 g/d (46%) carbohydrate, 60 g/d (24%) protein, 33 g/d (30%) fat]
   3,100 kcal/day for 7-days [425 g/d (55%) carbohydrate, 115 g/d (15%) protein, 105 g/d (30%) fat]

9. Diet VIII 1,000 kcal/day for 7-days [60 g/d (24%) carbohydrate, 115 g/d (46%) protein, 33 g/d (30%) fat]
   3,100 kcal/day for 7-days [425 g/d (55%) carbohydrate, 115 g/d (15%) protein, 105 g/d (30%) fat]
BAYLOR UNIVERSITY
Department of Health, Human Performance, & Recreation
Informed Consent Form

Title of Investigation: Effects of Changes in Caloric and Macronutrient Intake on Resting Energy Expenditure and Body Composition

Principal Investigator: Richard B. Kreider, PhD, FACSM, MX
Chair, Department of HHPR, Baylor University

Co-investigators: Chris Rasmussen, MS, MX, CSSS, FPC
Coordinator, Exercise and Sport Nutrition Lab, Baylor University
Ashli Thomas, BS, RD
Dietetics Coordinator, Exercise and Sport Nutrition Lab, Baylor University
Ron Wilson, MD
Department of HHPR, Baylor University

Sponsor: Curves International (Waco, TX)

Rationale:

Resting energy expenditure (REE) accounts for approximately 75% of daily energy expenditure. Caloric restriction and/or rapid weight loss typically decreases REE which makes it easier to regain weight. Conversely, exercise and increased caloric intake raises REE. The purpose of this study is to examine the acute effects of reducing and increasing caloric intake on REE, hormonal markers of metabolic rate, body water, and body composition. These data will be helpful in determining how long people can optimally restrict dietary intake before REE and hormonal regulation of metabolic rate is impacted. Additionally, these data will help determine the amount of calories and length of time needed to promote increases in REE.

Description of the Study:

I will be one of approximately 100 apparently healthy, untrained, moderately overweight females between the ages 18 - 65 who will participate in this study. During an initial familiarization session, I will be informed of the requirements of the study and sign an informed consent statement in compliance with the Human Subjects Guidelines of Baylor University and the American College of Sports Medicine. A trained individual will examine my medical history to determine if I am qualified to participate in this study. I understand that if I qualify medically to participate in this study, I will be familiarized to the diet program and tests to be conducted during the study. This session will take approximately 60 minutes to complete. Once I complete the familiarization sessions, I will be scheduled for baseline testing.

Prior to reporting to the lab for baseline testing, I will record all food that I eat on dietary record forms for four days (including one weekend day). I will not exercise for 48 hours nor eat for 8 hours prior to reporting to the lab for baseline testing. I will then undergo a number of tests as described in Table 1. I will be weighed and have my total body water determined using a bioelectrical impedance analyzer (BIA). The BIA analysis will involve lying down on my back on a table and having two small electrodes placed on my right hand and my right foot. The analyzer wires will be attached and a small and safe current (100 micro-amps at a frequency of 5 kHz) will pass through my body so that the amount of water can be measured. This analyzer is commercially available and has been used in the health care industry as a means to assess body composition and body water for over 20 years. The use of this device has been approved by the Food and Drug Administration (FDA) to assess total body water and the current to be used has been deemed safe.

My body composition and bone density will then be determined by using a Hologic Discovery dual-energy x-ray absorptiometer (DEXA). This will involve lying down on my back on the DEXA exam table in a pair of shorts or a gown for about 8 minutes. I understand that a low dose of radiation will scan my entire body to determine the amount of fat weight, muscle weight, and bone weight. I understand that I will be exposed to an x-ray dose that is similar to the amount of natural background radiation a person would receive in one month while living in Waco. After this test, I will have resting blood pressure determined using a blood pressure cuff and stethoscope, heart rate determined by taking my pulse, and hip and waist measurements taken using a measuring tape.

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I will then have my resting energy expenditure determined. This will involve lying down on an exam table and having a light blanket placed over me to keep me warm and placing ear plugs in my ears to reduce distractions. I understand that a see through plastic canopy will then be placed over my neck and head so that the air that I am breathing can be measured for oxygen and carbon dioxide. I understand that I should stay motionless without going to sleep for 15-20 minutes so that my resting energy expenditure can be calculated.

I understand that I will then donate about 20 milliliters (4 teaspoons) of venous blood from a vein in my arm. Blood samples will be obtained using standard sterile procedures using a needle inserted into a vein in my arm. I understand that personnel who will be taking my blood are experienced in phlebotomy (procedures to take blood samples) and are qualified to do so under guidelines established by the Texas Department of Health and Human Services. This will take about 5 minutes.

I understand that after baseline testing, I will be randomized into one of nine different types of diets described in Table 2. The diets will basically involve consuming a 1,000 calories per day high carbohydrate or high protein diet for 7 days followed by ingesting a high carbohydrate diet of 1,600, 2,000, 2,400, or 3,000 calories per day for 7 days. I understand that a registered diettitian will provide menus for me to follow and that I must follow the diet exactly during the study.

I understand that I must report back to the lab after 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 14 days to be tested again in a similar fashion as described above. In addition, I will complete a health status report daily which will monitor any side effects. I understand that if clinically significant side effects are reported, I will be referred to discuss the problem with a research nurse or Lori Greenwood, PhD, ATC who is a Professor and athletic trainer at Baylor University. Upon discretion, I may be referred to discuss the matter with Dr. Ron Wilson to determine whether any medical treatment is needed and/or whether I can continue in the study. I understand that if I fail to report my progress and health status to the research assistant I may be removed from the study.

I agree to do my best to: 1) follow the instructions outlined by the investigators; 2) show up to all scheduled testing and training sessions on time; and 3) follow the diet prescribed. I agree not to take weight loss aids or dietary supplements during this study. In addition, I agree not to take any non-medically prescribed medications and to report any medication that is prescribed for me to take during this study. I understand that if I take any other dietary supplements or medications during the course of the study that may affect vitamin/mineral status, body composition, or strength that I may be removed from the study.

Exclusionary Criteria

I understand that in order to participate in the study, a trained individual will examine me to determine whether I qualify to participate. I understand that I will not be allowed to participate in this study if: 1) I have any metabolic disorder including known electrolyte abnormalities; heart disease, arrhythmias, diabetes, thyroid disease, or hypogonadism; 2) I have a history of hypertension, hepatotoxic, musculoskeletal, autoimmune, or neurologic disease; 3) I am taking thyroid, hypoglycemic, hypoglycemic, anti-hypertensive, or anabolic medications; 4) I have taken nutritional supplements that may affect muscle mass (e.g., creatine, HMB), anabolic or anabolic hormones levels (androstenedione, DHEA, etc.), or weight loss (e.g., ephedra, thermogenic, etc.) within three months prior to the start of the study; and/or 5) I report any unusual adverse events associated with this study that in consultation with the supervising physician recommends removal from the study. I understand that the only exception will be if my personal physician does not feel that any condition that I have or medication that I am currently taking would prohibit me from participating in this study. In this case, I must obtain a letter signed by my physician approving my participation in this study.

I have reported all nutritional supplements, medically prescribed drugs, and non-medically prescribed drugs that I am presently taking. I have completed medical history questionnaires and am not aware of any additional medical problems that would prevent me from participating in this study. I agree to report all changes in medical status, nutritional and/or pharmaceutical agents (drugs) that I take during the course of the investigations to Richard Kreider, PhD (254/710-4003). I understand that if I experience any unexpected problems or adverse events from participating in this study I may be referred to discuss the problem with Lori Greenwood, PhD, ATC who is a professor and athletic trainer at Baylor University. Upon her discretion, I may be referred to discuss the matter with Dr. Ron Wilson to determine whether any medical treatment is needed and/or whether I can continue in the study.

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Risks and Benefits

I understand that numerous studies have evaluated the effects of changes in diet on markers of health and weight loss, and that I will follow a diet program involving consuming 1,000 for seven days and either 1,600, 2,100, 2,600, or 3,100 calories for seven days. I understand that I may ingest a high percentage of calories in the form of protein during the first week of the study and higher than the normal amount of calories than I consume in my normal diet during the second week of the study. Although the total amount of total protein is not excessive (60-115 grams/day or 1.0 – 2.0 grams/kg/day) it may be higher than I am accustomed to ingesting. As a result, I may experience weight loss or gain, feelings of hunger or fullness, and/or changes in appetite and/or mood during various phases of the dietary intervention.

I understand that I will be exposed to a low level of radiation during the DEXA body composition test, which is similar to the amount of natural background radiation a person would receive in one month while living in Waco, TX. In addition, a very low level of electrical current will be passed through my body using a triboelectrical impedance analysis. This analyzer is commercially available and has been used in the health care assessment industry as a means to assess body composition and body water for over 20 years. The use of the BIA and DEXA analyzers has been shown to be safe methods of assessing body composition and total body water and is approved by the FDA. I also understand that I will have about 4 teaspoons (20 milliliters) of blood drawn from a vein in my forearm using a sterile needle and blood tubes by an experienced phlebotomist eleven (11) times during the study. This procedure may cause a small amount of pain when the needle is inserted into my vein as well as some bleeding and bruising. I may also experience some dizziness, nausea, and/or faint if I am my head during the REE assessment. This may cause me to experience symptoms of claustrophobia such as anxiety. I understand that personnel in the lab are trained in CPR, emergency equipment such as an automated defibrillator, and emergency procedures are posted in the lab in the unlikely event that any emergency may arise.

I understand that the researchers conducting this study have extensive experience conducting studies investigating the effects of exercise and dietary interventions on health and performance.

I understand that the main benefit that I may obtain from this study is that I may learn more about how my energy metabolism is influenced by changes in diet and diet composition. However, even if I don't receive individual benefit, I understand that participating in this study will help to determine the metabolic responses to dieting.

Alternative Treatments

This is not a medical treatment. Therefore, if medical treatment is needed, I must continue to obtain treatment for any medical problem I might have from my personal physician.

Costs and Payments

If I am a Baylor University student, I will not receive any academic credit for participating in this study. Eligible subjects will be paid $250 for completing all familiarization and experimental testing sessions as well as turning in all required materials (i.e., food logs). I also understand that I will be given free assessments during the course of the study as described above and may receive information regarding results of these tests if I desire.

New Information

Any new information obtained during the course of this research that may affect my willingness to continue participation in this study will be provided to me. In addition, I will be informed of any unusual/abnormal clinical findings in which medical referral to my personal physician may be warranted. If I desire, I may request that this information be provided to my physician.

Confidentiality

I understand that any information obtained about me in this research, including questionnaires, medical history, laboratory findings, or physical examination will be kept confidential to the extent permitted by law. However, I understand in order to ensure that FDA regulations are being followed, it may be necessary for a representative of the FDA to review my records from this study which may include questionnaires, medical history, laboratory findings/reports, statistical data, and/or notes taken about my participation in this study. In addition, I understand that my records of this research may be subpoenaed by court order or may be inspected by federal regulatory authorities. I understand that data derived may be used in reports, presentations, and publications. However, I will not be individually identified unless my consent is granted in writing. Additionally, that confidentiality will be
maintained by assigning code numbers to my files, limiting access to data to research assistants, looking cabinets that store data, and providing passwords to limit access to computer files to authorized personnel only. I understand that once blood samples are analyzed that they will be discarded.

Right to Withdrawal

I understand that I am not required to participate in this study and I am free to refuse to participate or to withdraw from the study at any time. Further, that my decision to withdraw from the study will not affect my care at this institution or cause a loss of benefits to which I might be otherwise entitled. If there is concern about my medical safety, I may be referred to seek medical attention.

Compensation for Illness or Injury

I understand that if I am injured as a direct result of taking part in this study, I should consult my personal physician to obtain treatment. I understand that the cost associated with the care and treatment of such injury will be the responsibility of me or my insurance carrier. In some cases, insurers may not reimburse claims submitted for a research-related injury resulting from medical procedures or treatments performed as part of a research study. I understand that Baylor University, the investigator’s institutions, and the grant sponsor have not budgeted funds to compensate me for injury or illness that may result from my participation in this study and thus will not be accountable for illness or injury acquired during the course of this study. However, I may be referred to my personal physician if any clinically significant medical/psychological findings are observed during the course of this study.

I AGREE TO INDEMNIFY AND HOLD HARMLESS BAYLOR UNIVERSITY, ITS OFFICERS, DIRECTORS, FACULTY, EMPLOYEES, AND STUDENTS FOR ANY AND ALL CLAIMS FOR ANY INJURY, DAMAGE OR LOSS I SUFFER AS A RESULT OF MY PARTICIPATION IN THIS STUDY REGARDLESS OF THE CAUSE OF MY INJURY, DAMAGE OR LOSS.

Statement on Conflict of Interest

I understand that funding for this study will be obtained from Curves International (Waco, TX) through a grant awarded to Baylor University in collaboration with Imaginution (Laguna Niguel, CA). I understand that researchers involved in collecting data for this study have no financial or personal interest in the outcome of results or sponsors.

Voluntary Consent

I certify that I have read this consent form or it has been read to me and that I understand the contents and that any questions that I have pertaining to the research have been, or will be answered by Richard Kreider, PhD (Professor & Chair, Department of Health, Human Performance & Recreation, 106 Marx McLean Gymnasium, Baylor University, phone: 254/710-4205) or one of the research associates in the ESNL (254/710-7195 or 254/710-7277). My signature below means that I am at least 18 years of age and that I freely agree to participate in this investigation. I understand that I will be given a copy of this consent form for my records. If I have any questions regarding my rights as a research subject in this study, I may contact Baylor’s University Committee for Protection of Human Subjects in Research. The chairman is Dr. Misti Stanford, Professor of Psychology and Neurosciences, RSS A120, P.O. Drawer 7734, Waco, TX 76798-7734, phone number 254-710-2296.

Date ________  Subject’s Signature ____________________________

I certify that I have explained to the above individual the nature and purpose of the potential benefits and possible risks associated with participation in this study. I have answered any questions that have been raised and have witnessed the above signature. I have explained the above to the volunteer on the date stated on this consent form.

Date ________  Investigator’s Signature ____________________________

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Table 2. Overview of Dietary Interventions

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<td>I</td>
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<td>1,000 kcal/day for 7-days [220 g/d (37%) carbohydrate, 69 g/d (15%) protein, 33 g/d (30%) fat]</td>
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<td>II</td>
<td>1,000 kcal/day for 7-days [60 g/d (24%) carbohydrate, 115 g/d (46%) protein, 33 g/d (50%) fat]</td>
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<td>1,000 kcal/day for 7-days [220 g/d (37%) carbohydrate, 69 g/d (15%) protein, 33 g/d (30%) fat]</td>
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<td>1,000 kcal/day for 7-days [115 g/d (44%) carbohydrate, 69 g/d (24%) protein, 33 g/d (30%) fat]</td>
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<td>2,000 kcal/day for 7-days [185 g/d (55%) carbohydrate, 89 g/d (15%) protein, 70 g/d (30%) fat]</td>
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<td>1,000 kcal/day for 7-days [60 g/d (24%) carbohydrate, 115 g/d (46%) protein, 33 g/d (50%) fat]</td>
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<td>2,000 kcal/day for 7-days [185 g/d (55%) carbohydrate, 89 g/d (15%) protein, 70 g/d (30%) fat]</td>
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<td>V</td>
<td>1,000 kcal/day for 7-days [115 g/d (44%) carbohydrate, 69 g/d (24%) protein, 33 g/d (30%) fat]</td>
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<td>2,000 kcal/day for 7-days [355 g/d (55%) carbohydrate, 100 g/d (15%) protein, 65 g/d (30%) fat]</td>
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<td>3,000 kcal/day for 7-days [425 g/d (55%) carbohydrate, 115 g/d (15%) protein, 195 g/d (30%) fat]</td>
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<tr>
<td>VIII</td>
<td>1,000 kcal/day for 7-days [60 g/d (24%) carbohydrate, 115 g/d (46%) protein, 33 g/d (50%) fat]</td>
</tr>
<tr>
<td></td>
<td>3,000 kcal/day for 7-days [425 g/d (55%) carbohydrate, 115 g/d (15%) protein, 195 g/d (30%) fat]</td>
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</table>
Part 5: Informed Consent Form Checklist

When using humans as subjects in research you must obtain their informed consent. Check each of the following items as they appear on your Informed Consent Form and include this checklist with your protocol:

__X__ (a) A statement explaining the purpose of the research.

__X__ (b) A statement of the expected duration of the subject's participation.

__X__ (c) A description of the procedures to be followed.

__X__ (d) A description of any reasonable foreseeable risks or discomforts to the subject, including invasion of privacy.

__X__ (e) A description of any benefits resulting from the research, either to the subject or to others.

__X__ (f) A statement that informs subject of his/her right not to be a subject in a research project that is also a teaching exercise.

__X__ (g) A statement informing subject about how his/her anonymity will be guarded; i.e., that their confidentiality will be protected by assigned code numbers, by limitations of who has access to data, by data storage in locked cabinets, by locked computer files, etc.

__X__ (h) A statement that the subject's participation is voluntary, and that his/her refusal to participate will involve no penalty or loss of benefits to which the subject is otherwise entitled, and that the subject may discontinue participation at any time without penalty or loss of benefits to which the subject is otherwise entitled.

__X__ (i) A disclaimer, if applicable, regarding the use of the Internet to collect data.

__X__ (j) For research involving more than minimal risk, an explanation regarding the availability of any compensation or any medical treatments if injury occurs (if applicable, see OHRP Reports).

__X__ (k) If written informed consent is required, a place for the subject to sign and date the form and a statement that a copy of the signed consent form will be given to the subject for his/her records.

__X__ (l) If the subject is a minor, a statement of parental responsibility in consenting to the child's participation in the study with a place for the parent to sign and date the form in addition to the participant's signature.

__X__ (m) Include a short summary of your expertise related to this research proposal.

__X__ (n) The name, address, and telephone number of the principal investigator of the research project, and his/her affiliation with Baylor University. If the principal investigator is a graduate student, the name and telephone number of the faculty advisor is also required.

__X__ (o) A statement informing subject that inquiries regarding his/her rights as a subject, or any other aspect of the research as it relates to his/her participation as a subject, can be directed to Baylor's University Committee for Protection of Human Subjects in Research. The chairman is Dr. Ken Wilkins, Associate Dean Graduate Studies and Research, Graduate School, PO Box 97264, Waco, Texas 76798, phone number 254-710-3361.
Medical History Inventory

**Directions:** The purpose of this questionnaire is to enable the staff of the Exercise and Sport Sciences Laboratory to evaluate your health and fitness status. Please answer the following questions to the best of your knowledge. All information given is **CONFIDENTIAL** as described in the Informed Consent Statement.

Name: ___________________________ Age: ______ Date of Birth: __________

Name and Address of Your Physician: ____________________________________________

**MEDICAL HISTORY**

Do you have or have you ever had any of the following conditions? (Please write the date when you had the condition in blank).

___ Heart murmur, clicks, or other cardiac findings?
___ Frequent extra, skipped, or rapid heartbeats?
___ Chest Pain or Angina (with or without exercise)?
___ High cholesterol?
___ Diagnosed high blood pressure?
___ Heart attack or any cardiac surgery?
___ Leg cramps (during exercise)?
___ Chronic swelling ankles?
___ Varicose veins?
___ Frequent dizziness/fainting?
___ Muscle or joint problems?
___ High blood sugar/diabetes?
___ Thyroid Disease?
___ Low testosterone/hypergondism?
___ Glaucoma?
___ Asthma/breathing difficulty?
___ Bronchitis/Chest Cold?
___ Cancer, Malignancy, or Suspected Skin Lesions?
___ Stroke or Blood Clots?
___ Emphysema/lung disease?
___ Epilepsy/seizure?
___ Rheumatic fever?
___ Scarlet fever?
___ Ulcers?
___ Paroxysms?
___ Anoxia?
___ Liver or kidney disease?
___ Arthritis/arthrosis?
___ Nerve disease?
___ Psychological Disorders?

Do you have or have you been diagnosed with any other medical condition not listed?

__________________________________________________________________________

Please provide any additional comments/explanations of your current or past medical history.

__________________________________________________________________________

Please list any recent surgery (i.e., type, date, etc.).

__________________________________________________________________________

List all prescribed non-prescription medications and nutritional supplements you have taken in the last 3 months.__________________________________________________________________________

What was the date of your last complete medical exam?

__________________________________________________________________________

Do you know of any medical problem that might make it dangerous or unsafe for you to participate in this study (including strength and maximal exercise tests)? ____ If yes, please explain: ____________________________________________

**Recommendation for Participation**

___ No exclusion criteria presented. Subject is cleared to participate in the study.
___ Exclusion criteria is/are present. Subject is not cleared to participate in the study.

Signed: ___________________________ Date: ____________
Baylor University
Exercise & Sport Nutrition Laboratory

Personal Information

Name:

Address:

City: _______________ State: _____ Zip Code: _____ SS#: ____________________

Home Phone: (___) _______ Work Phone: (___)

Beep: (___) _______ Cellular: (___) ________

Fax: (___) _______ Email address: __________________

Birth date: _____ / _____ / _____ Age: _____ Height: _____ Weight: _____

Exercise History/Activity Questionnaire

1. Describe your typical occupational activities.

2. Describe your typical recreational activities.

3. Describe any exercise training that you routinely participate.
Baylor University
Exercise & Sport Nutrition Laboratory

Radiation Exposure Questionnaire for Women of Child Bearing Age

Radiation exposure may affect fetal development. Although the DEXA test will only expose you to a small amount of radiation (1.5 mR per scan), you should be aware that there is a possibility that if you become pregnant during the course of the study that the x-ray exposure may be harmful to the fetus. Therefore, it is important to conduct x-ray tests within 10-14 days of the start of a female’s menstrual cycle if she is of child bearing age, sexually active, and/or is not taking birth control pills. The following questionnaire must be completed so that we know when it is an appropriate time to conduct the DEXA body composition tests. Please be assured that this information will be kept confidential within the limits permitted by law.

Current Age: 
Age of first period: 
Date of last period: 
Normal length of menstrual cycle: 
Have you been sexually active within the last month? 
Do you use birth control pills? 
Are you pregnant or have a desire for pregnancy?

Note: If you happen to get pregnant during the course of this study, you must notify research assistants so that appropriate precautions can be made.

I confirm that I have completed this questionnaire honestly and agree to notify researchers within the ESNL of any change in the length of my menstrual cycle and/or pregnancy status.

Name __________________________  Date __________________________
NAME _________________________ Date ________________________

INSTRUCTIONS

1. Record everything you eat for 4 days (including one weekend day). If you eat pretzels, record how many. If you eat a bag of chips, record the number of ounces. For drinks, record the number of cups or ounces. Record everything you drink except water.

2. Record the Food, Amount, Brand Name, and Preparation Method. For example: baked vs. fried chicken; 1 cup of rice; 2 teaspoons of margarine; 1 cup of 2% milk; McDonald’s, Healthy Choice, or Frosted Flakes.

3. Record immediately after eating. Waiting until that night may make it difficult to remember all foods and quantities.

<table>
<thead>
<tr>
<th>Food (include brand)</th>
<th>Method of Preparation</th>
<th>Quantity (cups, oz., etc.)</th>
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<td><strong>BREAKFAST:</strong></td>
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<td><strong>SNACKS:</strong></td>
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INSTRUCTIONS

Circle the number or dot between numbers that best indicates the degree you have felt the following symptoms during the last week:

**Appetite**

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<thead>
<tr>
<th>None</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
<th>Severe</th>
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<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
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</table>

**Hunger**

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<tr>
<th>None</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
<th>Severe</th>
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</thead>
<tbody>
<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
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</table>

**Satisfaction from Food**

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<tr>
<th>None</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
<th>Severe</th>
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<tr>
<td>0</td>
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<td>3</td>
<td>4</td>
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</table>

**Feeling of Fullness**

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<th>None</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
<th>Severe</th>
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<tr>
<td>0</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
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**Amount of Energy**

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<th>None</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
<th>Severe</th>
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<tr>
<td>0</td>
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**Overall Quality of Diet**

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<th>None</th>
<th>Low</th>
<th>Moderate</th>
<th>High</th>
<th>Severe</th>
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<tr>
<td>0</td>
<td>1</td>
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</table>
Baylor University  
Exercise & Sport Nutrition Laboratory  
Post Study Questionnaire

NAME ____________________________ Date ____________________________

INSTRUCTIONS

Circle the number or dot between numbers that best indicates the degree you have felt the following symptoms during the last week:

Rate the Difficulty in Adhering to the Diet

None Low Moderate High Severe
0 . . . . 1 . . . . 2 . . . . 3 . . . . 4 . . . . 5 . . . . 6 . . . . 7 . . . . 8 . . . . 9 . . . . 10

Rate the Satisfaction in the Changes in Body Composition that You Made

None Low Moderate High Severe
0 . . . . 1 . . . . 2 . . . . 3 . . . . 4 . . . . 5 . . . . 6 . . . . 7 . . . . 8 . . . . 9 . . . . 10

Comments/Suggestions About the Curves Fitness & Weight Loss Program
Want to Get in Shape and Lose Weight?

Women Needed for a Fitness & Weight Loss Study

Researchers in the Exercise & Sport Nutrition Lab at Baylor University are recruiting 100 healthy, untrained, and moderately overweight female subjects between the ages of 18 and 65 to participate in a study to evaluate the effects of dietary changes on resting metabolism. Subjects will be required to follow a diet for 14 days and participate in 11 testing sessions during a two-week period. Eligible subjects will receive free resting energy expenditure and body composition/bone density screening, nutritional counseling, and $250 for completing the study.

For more information call:

Exercise & Sport Nutrition Lab
Department of HHPR
Repa Mims McLean Gymnasium, Room 122

For more information call

254-710-7860 or 710-7856

www3.baylor.edu/HHPR/Curves
**Weekly Follow-up Assessment**

Effects of Changes in Caloric and Macronutrient Intake on Resting Energy Expenditure and Body Composition

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<tr>
<th>Subject Name: ____________________</th>
<th>Subject #: _____</th>
<th>Date: __________</th>
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<th>Week</th>
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<td><strong>Are you following the diet plan?</strong></td>
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<td><strong>Are you exercising regularly?</strong></td>
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<td>1 = minimal (1-2 per wk)</td>
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<td>3 = occasional (5-6 per wk)</td>
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<td>4 = frequent (7-8 per wk)</td>
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<td>5 = severe (9 or more per wk)</td>
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<td><strong>Nervousness?</strong></td>
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APPENDIX B

Diet Booklets

Menu booklets were provided to participants. Two menus along with substitution options were given for each diet group for each phase of the diet.

Menu booklet for HC Diet:

<table>
<thead>
<tr>
<th>WEEK 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>1,000 calories (46% CHO, 24% PRO, 30% FAT)</td>
</tr>
</tbody>
</table>

**Breakfast**
- ½ cup shredded wheat
- 1 cup skim milk
- 1 small banana

**Substitutions**
- 1 – 2oz bagel
- ¼ cup applesauce

**Lunch**
- 2 slices reduced calorie bread
- 2 oz lunchmeat with 0-3 g fat or less
- 4 lettuce leaves
- 2 tomato slices
- 1 tbsp reduced fat mayo
- 1 cup raw carrots, cucumbers or vegetable of preference
- 1 tbsp reduced fat Ranch dressing

**Substitutions**
- 1 – 6 in tortilla
- 2 oz cheese 0-3g fat or less
- 1 tbsp reduced fat Ranch dressing
- ¾ cup green beans

**Dinner**
- 3 oz ground beef
- 1 cup cooked pasta noodles
- ½ cup spaghetti sauce
- Italian spices – garlic, basil, oregano
- ½ cup green beans

**Substitutions**
- 3 oz ground turkey or chicken
- ½ cup cooked carrots

**Snack**
- 1 ¼ cup strawberries
- 2 tbsp light whipped topping

**Substitutions**
- 1 small apple
### WEEK 1
1,000 calories (46% CHO, 24% PRO, 30% FAT)

<table>
<thead>
<tr>
<th>Breakfast</th>
<th>Substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ 1 ½ cups puffed cereal</td>
<td>□ 3 tbsp Grape Nuts</td>
</tr>
<tr>
<td>□ 1 cup skim milk</td>
<td>□ 1 cup low fat fruit yogurt</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lunch (Mexican Potato)</th>
<th>Substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ 1 medium baked potato (~6 oz)</td>
<td>□ 6 chicken nuggets***</td>
</tr>
<tr>
<td>□ 3 tbsp reduced fat sour cream</td>
<td>(**Nuggets replace potato, sour cream, and cheese)</td>
</tr>
<tr>
<td>□ 1 oz regular grated cheese</td>
<td>□ 3 tbsp honey mustard or BBQ sauce</td>
</tr>
<tr>
<td>□ ¼ cup salsa</td>
<td></td>
</tr>
<tr>
<td>□ 1 cup sugar free gelatin</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dinner (Pizza and Salad)</th>
<th>(Soup and Salad)</th>
</tr>
</thead>
<tbody>
<tr>
<td>□ 1 slice thin crust cheese pizza (1/4th of 10 in pizza)</td>
<td>□ 1 – 10 ¾ oz can Chunky soup</td>
</tr>
<tr>
<td>□ 2-3 cups of salad greens</td>
<td></td>
</tr>
<tr>
<td>□ 1 cup chopped raw</td>
<td></td>
</tr>
<tr>
<td>vegetables – carrots,</td>
<td></td>
</tr>
<tr>
<td>tomatoes, etc.</td>
<td></td>
</tr>
<tr>
<td>□ 2 tbsp reduced fat salad dressing</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Snack</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>□ 17 grapes</td>
<td>□ 1 small banana</td>
</tr>
<tr>
<td>□ 1 Mozzarella String Cheese</td>
<td></td>
</tr>
</tbody>
</table>
## WEEK 2

2,600 calories (55% CHO, 15% PRO, 30% FAT)

### Breakfast
- 1 cup sugar frosted cereal
- 1 cup 2% milk
- 1 large apple

### Substitutions
- 1 bagel (2oz)
- 1 – 6-8 oz fruited yogurt 1 fruited yogurt
- 1 large banana

### Snack
- 1 large orange

### Lunch (Sandwich Wrap)
- 1 – 12" tortilla
- 3 oz lunchmeat with 3g fat or less
- 3-4 lettuce leaves (optional)
- 2 tomato slices (optional)
- 2 tbsp reduced fat salad dressing
- 1 cup cooked green beans
- 2 cups apple juice

### Tuna Salad Sandwich
- 2 slices bread
- 3 oz tuna
- 1 tbsp reduced fat mayonnaise
- 1 cup cooked carrots
- 2 cups orange juice

### Dinner (Pizza and Salad)
- 2 slice thin crust meat topping pizza (1/2 of 10 in pizza)
- 2-3 cups of salad greens
- 1 cup chopped raw vegetables – carrots, tomatoes, etc.
- 2 tbsp reduced fat salad dressing
- 1 cup canned fruit

### Tacos or Taco Salad
- 2 Hard Shell Beef Tacos (e.g. taco bell – meat, cheese, lettuce)
- 2-3 cups of salad greens – (can crush tacos into salad greens)
- 1 cup chopped raw vegetables – carrots, tomatoes, etc.
- 2 tbsp reduced fat salad dressing
- 1 large pear or orange or apple

### Snack
- 8 Animal crackers, unfrosted
- ½ cup applesauce, unsweetened

- 1/2 cup fat free, no sugar added ice cream
- 1 ¼ cup strawberries
### WEEK 2

**2,600 calories (55% CHO, 15% PRO, 30% FAT)**

<table>
<thead>
<tr>
<th>Breakfast</th>
<th>Substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cup cooked oatmeal</td>
<td>1 cup shredded wheat</td>
</tr>
<tr>
<td>1 cup 2% milk</td>
<td>½ English Muffin</td>
</tr>
<tr>
<td>1 slice toast</td>
<td>2 tsp margarine***</td>
</tr>
<tr>
<td>1 tsp margarine</td>
<td>***Replaces 1 tsp margarine and bacon</td>
</tr>
<tr>
<td>1 slice bacon</td>
<td></td>
</tr>
<tr>
<td>1 cup orange juice</td>
<td>1 cup apple juice</td>
</tr>
<tr>
<td>1 small banana</td>
<td>1 tbsp 100% fruit spread</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lunch</th>
<th>Tuna or Chicken Salad</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 slices bread</td>
<td>1 bagel</td>
</tr>
<tr>
<td>2 oz lunchmeat with 3g fat or less</td>
<td>2 oz tuna or chicken</td>
</tr>
<tr>
<td>1 oz American Cheese</td>
<td></td>
</tr>
<tr>
<td>¾ oz pretzels</td>
<td></td>
</tr>
<tr>
<td>2-3 cup salad greens</td>
<td>15-20 fat free tortilla chips</td>
</tr>
<tr>
<td>1 cup tomato, carrots, cucumbers or mix of vegetable preference – must = 1 cup</td>
<td></td>
</tr>
<tr>
<td>2 tbsp reduced fat salad dressing</td>
<td>1 tbsp reduced fat mayonnaise</td>
</tr>
<tr>
<td>1 large orange</td>
<td>1 large apple or banana</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Snack</th>
<th>Trail Mix</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Graham Cracker squares</td>
<td>3 cups low fat popcorn</td>
</tr>
<tr>
<td>4 tsp peanut butter</td>
<td>20 peanuts</td>
</tr>
<tr>
<td>1 cup apple juice</td>
<td>4 tbsp raisins</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dinner</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>4 oz fried chicken</td>
<td>4 oz hamburger patty</td>
</tr>
<tr>
<td>1 cup mashed potatoes</td>
<td>1 hamburger bun</td>
</tr>
<tr>
<td>2 tbsp gravy</td>
<td>12-18 potato chips****</td>
</tr>
<tr>
<td>1 small, plain dinner roll</td>
<td>***Replaces gravy, dinner roll, and margarine</td>
</tr>
<tr>
<td>1 tsp margarine</td>
<td></td>
</tr>
<tr>
<td>1 cup green beans</td>
<td>1 cup cooked carrots</td>
</tr>
<tr>
<td>1 cup canned peaches</td>
<td>1 cup cantaloupe cubes</td>
</tr>
</tbody>
</table>
Menu booklet for HP diet:

**WEEK 1**

1,000 calories (24% CHO, 46% PRO, 30% FAT)

<table>
<thead>
<tr>
<th>Breakfast</th>
<th>Substitutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ 1.5 cups puffed cereal</td>
<td>☐ ¼ cup Grape Nuts</td>
</tr>
<tr>
<td>☐ 1 cup skim milk</td>
<td>☐ 1- 6-8 oz low fat yogurt</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Snack</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ 1 boiled egg</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Lunch – (Chicken Salad)</th>
<th>(Seafood Salad)</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ 2 slices reduced calorie bread</td>
<td>☐ ½ - 6” pita</td>
</tr>
<tr>
<td>☐ 4 oz skinless chicken breast</td>
<td>☐ 4 oz crab meat</td>
</tr>
<tr>
<td>☐ 1 tbsp fat free mayonnaise</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Snack (Pig in Cheese Blanket)</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ 1 oz fat free cheese</td>
</tr>
<tr>
<td>☐ 2 oz hot dog with 1g fat or less</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Dinner</th>
</tr>
</thead>
<tbody>
<tr>
<td>☐ 6 oz sirloin steak</td>
</tr>
<tr>
<td>☐ 1.5 cups broccoli</td>
</tr>
<tr>
<td>WEEK 1</td>
</tr>
<tr>
<td>--------</td>
</tr>
<tr>
<td><strong>Breakfast</strong></td>
</tr>
<tr>
<td>2 slices reduced calorie bread</td>
</tr>
<tr>
<td>3 oz Canadian Bacon</td>
</tr>
</tbody>
</table>

| **Lunch (Salad)** | | |
| 3 oz fat free lunchmeat | | |
| 2 oz fat free cheese | | |
| 3 cups salad greens | | |
| 1 tbsp ranch dressing or other salad dressing | | |

| **Snack (Chicken salad)** | | |
| 2 oz grilled chicken breast | | |
| 1 tbsp fat free mayonnaise | | |
| 1 cup raw carrots | | |

| **Dinner (Stir-fry)** | **Chicken Spaghetti** |
| 2/3 cup rice | 1 cup pasta |
| 3 oz lean beef | 3 oz grilled chicken |
| ½ cup stir fry vegetables | ½ cup tomato sauce |
| 1 tbsp soy sauce | Italian spices |
## WEEK 2

### 2,600 calories (55% CHO, 15% PRO, 30% FAT)

<table>
<thead>
<tr>
<th>Time</th>
<th>Meal Description</th>
<th>Calories</th>
<th>Protein</th>
<th>Fat</th>
<th>Carbs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breakfast</td>
<td>1 cup sugar frosted cereal 1 cup 2% milk 1 large apple</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snack</td>
<td>1 large orange</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lunch</td>
<td>1 – 12” tortilla 3 oz lunchmeat with 3g fat or less 3-4 lettuce leaves (optional) 2 tomato slices (optional) 2 tbsp reduced fat salad dressing 1 cup cooked green beans 2 cups apple juice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinner</td>
<td>2 slice thin crust meat topping pizza (1/2 of 10 in pizza) 2-3 cups of salad greens 1 cup chopped raw vegetables – carrots, tomatoes, etc. 2 tbsp reduced fat salad dressing 1 cup canned fruit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Snack</td>
<td>8 Animal crackers, unfrosted 1/2 cup applesauce, unsweetened</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Substitutions

- 1 bagel (2oz)
- 1 – 6-8 oz fruited yogurt 1 fruited yogurt
- 1 large banana
- 1 large orange
- 1 large grapefruit
- 2 slices bread
- 3 oz tuna
- 1 tbsp reduced fat mayonnaise
- 1 cup cooked carrots
- 2 cups orange juice
- 2 Hard Shell Beef Tacos (e.g. taco bell – meat, cheese, lettuce)
- 2-3 cups of salad greens – (can crush tacos into salad greens)
- 1 cup chopped raw vegetables – carrots, tomatoes, etc.
- 2 tbsp reduced fat salad dressing
- 1 large pear or orange or apple
- 1/2 cup fat free, no sugar added ice cream
- 1 1/4 cup strawberries
## WEEK 2

**2,600 calories (55% CHO, 15% PRO, 30% FAT)**

### Breakfast
- 1 cup cooked oatmeal
- 1 cup 2% milk
- 1 slice toast
- 1 tsp margarine
- 1 slice bacon
- 1 cup orange juice
- 1 small banana

### Lunch
- 2 slices bread
- 2 oz lunchmeat with 3g fat or less
- 1 oz American Cheese
- ¾ oz pretzels
- 2-3 cup salad greens
- 1 cup tomato, carrots, cucumbers or mix of vegetable preference – must = 1 cup
- 2 tbsp reduced fat salad dressing
- 1 large orange

### Snack
- 3 Graham Cracker squares
- 4 tsp peanut butter
- 1 cup apple juice

### Dinner
- 4 oz fried chicken
- 1 cup mashed potatoes
- 2 tbsp gravy
- 1 small, plain dinner roll
- 1 tsp margarine
- 1 cup green beans
- 1 cup canned peaches

### Substitutions
- 1 cup shredded wheat
- ½ English Muffin
- 2 tsp margarine***
  ***Replaces 1 tsp margarine and bacon
- 1 cup apple juice
- 1 tbsp 100% fruit spread

### Tuna or Chicken Salad
- 1 bagel
- 2 oz tuna or chicken
- 15-20 fat free tortilla chips
- 1 tbsp reduced fat mayonnaise
- 1 large apple or banana

### Trail Mix
- 3 cups low fat popcorn
- 20 peanuts
- 4 tbsp raisins
- 4 oz hamburger patty
- 1 hamburger bun
- 12-18 potato chips***
  ***Replaces gravy, dinner roll, and margarine
- 1 cup cooked carrots
- 1 cup cantaloupe cubes


