ABSTRACT

Effects of Acute Dietary Changes on Estimates of Body Composition

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The purpose of this investigation was to examine the effects of acute pre-assessment diets on body composition estimates obtained by dual-energy x-ray absorptiometry (DXA) and bioelectrical impedance analysis (BIA) in active adults. In a counterbalanced design, 48 male and female participants completed two one-day dietary conditions: a very low-carbohydrate diet (1 – 1.5 g CHO/kg) and a high-carbohydrate diet (9 g CHO/kg). For each condition, measures of body composition were taken in the morning after an overnight fast, in the afternoon after feeding, and the following morning after an overnight fast. Three-factor (time, gender, and dietary condition) repeated measures analysis of variance was performed for measures of total and regional body composition, and appropriate post-hoc procedures were followed. Acute food ingestion, regardless of macronutrient content, altered body composition estimates obtained from DXA and BIA, and both genders responded similarly. DXA total and regional lean soft tissue estimates increased up to 1.7% and 3% on average in response to feeding, with some participants demonstrating an increase of over 4.5% and 9%, respectively. DXA total and trunk fat mass estimates decreased by up to 3% on average in response to

feeding, with individuals demonstrating both decreases and increases of greater magnitude. All DXA-derived measures of body composition returned to baseline values after a second overnight fast. Impedance measured by tetrapolar BIA decreased by 4.4% in response to feeding, and this subsequently increased measures of total body water and fat-free mass by up to 2% on average, with individuals exhibiting increases as large as 4.5%. BIA fat mass estimates decreased 1.4 to 2.4%, with individuals exhibiting decreases of as much as 10%. Unlike DXA, the BIA-derived measures of body composition did not return to baseline values after a second overnight fast. The magnitude of changes seen in present study is deemed sufficient to potentially obscure true changes in body composition or produce artificial changes. It is recommended that body composition assessment take place after an overnight fast in order to minimize error due to acute food and fluid ingestion, although additional dietary control may be necessary when BIA is utilized.

Effects of Acute Dietary Changes on Estimates of Body Composition

by

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A Dissertation

Approved by the Department of Health, Human Performance, and Recreation

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DEDICATION

To Jesus Christ, the Lord of Lords and King of Kings

CHAPTER ONE

Introduction

Brief Review of Related Literature

Body composition assessment is a critical part of medical, health, and fitness research. Studies of obesity, weight loss, aging, growth and development, exercise, and athletics all utilize methods of body composition assessment. Since each method of body composition assessment provides only an estimate of an individual's actual body composition, it is important to minimize the errors inherent to estimation. Error in measurement, whether due to technical error or biological variation, can compromise the impact of studies utilizing body composition assessment. Furthermore, inadequate reporting of pre-assessment procedures in reports of research leaves the reader to question whether relevant potential errors were accounted for or simply ignored.

Dual-energy x-ray absorptiometry (DXA) and bioelectrical impedance analysis (BIA) are two methods of body composition assessment that are frequently used in research settings. DXA has gained popularity over recent years and provides a large amount of body composition information (e.g. total and regional lean soft tissue, body fat, and bone density). However, the quality of the estimates obtained from DXA can be compromised due to a number of factors, including hydration, activities of daily living, and food intake. Ferrari et al. (10) recently conducted research examining the impact of removing 2.8% of body mass via hemodialysis on DXA measurements of lean soft tissue. They reported that non-bone lean mass estimates decreased by approximately 5% in a

matter of hours as a result of the fluid removal. While this may be an extreme example, it demonstrates that DXA measurements are not immune to the effects of environmental alterations to the body.

The International Society for Clinical Densitometry's Official Positions warn that information derived from scans are dependent on a number of factors, including hydration status and prior food intake (14). They state that, "Body composition is influenced by body hydration as well as stomach and intestinal content. To keep variability as low as possible, standardized measurement conditions, including time of day, pre-measurement diet, and activities need to be defined." While some, but not all, researchers conduct body composition assessment after an overnight fast, the diet consumed for the days prior to assessment is not typically standardized. This may be particularly concerning when studies are conducted that intentionally alter the dietary pattern between groups (41), since this essentially ensures different pre-assessment dietary intake.

Nana et al. (30) investigated the effects of daily activities, including food intake, on DXA measurements. Subjects in this study were assessed by DXA five times throughout a two day period. The intake of food and fluid influenced the DXA estimates of total and regional body composition and also increased the errors of measurement. When a standardized protocol (consisting of an overnight fast and consistent presentation and positioning of the subject) was followed, the errors in total body composition were low on two consecutive mornings. However, there were substantial errors in regional body composition. The authors recommend that if enhanced precision is desired, as it

should be, subjects in body composition research should be assessed after an overnight fast.

Not all researchers adhere to the recommendation to assess body composition after an overnight fast, and others do not report when and under what conditions body composition assessment was conducted. While estimating body composition after an overnight fast is preferable to estimating it later in the day after activities of daily living have occurred (30), this recommendation makes the assumption that an overnight period without food is sufficient to negate nutritional differences from the previous day or days. However, the habitual diet as well as the diet followed on the day(s) immediately prior to body assessment could potentially impact the values obtained, even after an overnight fast. For DXA, stores of muscle glycogen and the associated 3 to 4 grams of water per gram of glycogen (35) are included in the lean soft tissue compartment. Therefore, it is reasonable to assume that an individual may appear to have a different amount of lean mass depending on the state of the glycogen stores (e.g. glycogen depleted, normal levels, or glycogen loaded) and total body water content at the time of assessment. If nutritional intake during the day(s) before assessment does affect total body water and lean soft tissue values, then a vast array of body composition research may not have utilized sufficient control in order to obtain the most accurate measurements. Intra- and inter-individual differences in glycogen stores and body water could lead to inappropriate conclusions regarding changes in body composition over the course of time.

Studies that specifically manipulate carbohydrate intake may be particularly affected by the aforementioned concerns. For example, it has been reported that ketogenic diets often lead to a decrease in lean body mass with weight loss, but it is

possible that the methods of body composition assessment may be artificially inflating this loss due to chronic low levels of glycogen present during post-baseline assessments (52). The impact of altered glycogen concentrations and the associated water stores may affect the integrity of body composition comparisons between low-carbohydrate and normal carbohydrate groups.

A recent study by Rouillier et al. (41) examined the effect of following a non-standardized high-carbohydrate diet for three days on measures of body composition using DXA. Participants switched from their habitual diet to a self-selected high-carbohydrate diet, in which carbohydrate supplied approximately 84% of total caloric intake. After three days on this diet, total body weight and lean body mass increased (0.8% and 1.5%, respectively) and percent body fat decreased. A 4.5% increase in appendicular lean mass was also reported, indicating that the apparent alterations in lean body mass were not distributed proportionally throughout the body. Since these very short-term changes do not likely represent a true change in body composition, it seems apparent that the acute diet led to the observed alterations. Rouillier and colleagues (41) recommend that future studies consider using a standard preparatory diet for participants prior to undergoing body composition assessment by DXA.

Due to the potentially prohibitive cost and of a DXA machine and regulations concerning its usage, both researchers and health practitioners frequently use BIA to evaluate the body composition of patients and clients. There are a variety of BIA devices, ranging from inexpensive bipolar hand-to-hand units to more advanced units (e.g. tetrapolar and octapolar units). One beneficial aspect of BIA is that it provides information concerning the amount of total body water (TBW) contained within an

individual. This information is typically reliable in euhydrated individuals, but BIA may not necessarily be appropriate to measure total body water when hydration status is altered (34).

As with DXA, BIA can be influenced by hydration and variation in body fluids (23, 50). Several researchers have reported that bioelectrical impedance values are decreased by acute food and fluid intake, which leads to underestimations of body fat (11, 42). Slinde and Rossander-Hulthén (42) conducted BIA 18 times in a 24-hour period in young healthy men and women, while providing standardized meals. The ingestion of the meals led to decreases in impedance and calculated body fat percentage. Similarly, Gallagher et al. (11) reported a decrease in impedance, relative to fasting values, in response to 550 calorie standardized meals. This led to an underestimation of body fat mass by an average of 5%, and there was no difference in impedance changes between isocaloric meals containing either 61% or 83% of the energy from carbohydrate (11). The decrease in impedance was present beginning 2 hours after the meal and persisted until the end of the measurement period, 5 hours after the meal. As with DXA, the recommendation to conduct BIA after an overnight fast has been presented, even though some BIA manufacturers state that BIA can be conducted several hours after food ingestion (11). Although BIA can clearly be affected by food and fluid intake, the extent to which manipulation of food intake of varying macronutrient content, as well as the timing of food intake, affects measurements of impedance, total body water, and body composition is not entirely clear.

Purpose and Objectives

The purpose of this study was to examine the effects of acute pre-assessment dietary changes on body composition estimates in recreationally active and active male and female adults. Specifically, we examined the effects of a very low-carbohydrate diet and a high-carbohydrate diet on body composition estimates obtained by DXA, tetrapolar foot-to-foot BIA, and bipolar hand-to-hand BIA. Subjects completed both 2-day conditions with a washout period between conditions.

The primary study objectives were to address the following questions: (1) As measured by DXA and BIA, are changes in lean body mass (including regional measurements by DXA) induced after several hours (~8 h) of consuming a diet containing high or very low amounts of carbohydrate?; (2) As measured by BIA, are changes in total body water induced after several hours (~8 h) of consuming a diet containing high or very low amounts of carbohydrate?; (3) After one entire day of consuming a diet containing high or very low amounts of carbohydrate, followed by an overnight fast, are changes in lean body mass (including regional measurements by DXA) evident, as measured by DXA and BIA?; (4) After one entire day of consuming a diet containing high or very low amounts of carbohydrate, followed by an overnight fast, are changes in total body water evident, as measured by BIA?; and (5) For the previous four questions, are there differences between males and females in these responses?

Study Hypotheses

We hypothesized there would not be an appreciable change in lean soft tissue estimates and no change in fat mass estimates between the beginning and end of the very low-carbohydrate condition, as measured by DXA. The majority of glycogen is stored in

the muscle, and these glycogen stores are primarily depleted by physical activity, not absence of dietary carbohydrate (22). Therefore, we expected that muscle glycogen would decrease minimally during the 36-hour period with low amounts of dietary carbohydrate. While hepatic glycogen stores may become depleted during this period without dietary carbohydrate (45), this represents a small amount of the total glycogen stores and would not likely play a major role in changing lean soft tissue measurements. However, due to the intestinal content of food items and fluids consumed throughout the day, we expected there would be an increase in total body mass and lean soft tissue measurements between the morning and evening of the day of the consumption of the low-carbohydrate diet.

We hypothesized there would be an increase in lean soft tissue estimates and no change in fat mass estimates between the beginning and end of the high-carbohydrate condition, as measured by DXA. We believed that the acute high-carbohydrate diet would be sufficient to increase intramuscular glycogen stores beyond their normal levels. This would lead to an increase in both carbohydrate and water within the muscle, which we believed would lead to a higher lean soft tissue measurement. We also believed that the apparent increase in lean soft tissue would be evident between the morning and evening of the day of the consumption of the high-carbohydrate diet.

We hypothesized that within the day of diet consumption, both diets would lead to an increase in lean mass and a decrease in fat mass as estimated by BIA. However, we believed there would be no differences in body composition estimates between the morning of the first and second days in the low-carbohydrate condition.

We hypothesized there would be an increase in fat-free mass estimates and no change in fat mass estimates between the beginning and end of the high-carbohydrate condition, as measured by BIA. The increase in water stored in association with carbohydrate could potentially decrease the measured impedance and thus increase the estimate of fat-free mass.

We hypothesized that alterations in body composition estimates, particularly lean soft tissue estimates, would be greater in male participants than in female participants. There are generally recognized differences in average body size and body composition between males and females, such as greater average total mass and lean soft tissue in males (55). There is also some evidence that females may not respond to nutritional strategies such as carbohydrate loading in the same way as males (56). Specifically, it has been reported that muscle glycogen concentrations in females do not increase to the same extent as males in response to carbohydrate loading (28, 49). Based on this information, as well as the greater average lean soft tissue content in males, we believed that males would potentially have a greater ability to accommodate the ingested food items, particularly as intramuscular glycogen during the high-carbohydrate condition.

Assumptions, Limitations, and Delimitations

Assumptions

There are several assumptions we made in this study: (1) The equipment used (stadiometer, scale, BIA devices, DXA machine, thermometer, and associated equipment) functioned properly and produced accurate data (keeping in mind the inherent errors associated with each device used); (2) The methods used for body composition

assessment were appropriate; (3) Participants followed dietary protocols, as well as accurately and honestly reported food and fluid intake on dietary record forms; (4) Nutritional content listed on nutrition labels was accurate; (5) Participants complied with overnight fasts and restrictions on physical activity and ingested substances (e.g. nicotine, caffeine, and alcohol) imposed during the study; (6) Female participants accurately reported the schedule of their menstrual bleeding, allowing for appropriate scheduling of visits relative to the menstrual cycle.

Limitations

The limitations of this study were: (1) The implemented acute changes in diet may have elicited different effects than if these diets were followed habitually; (2) Differences in the activities of individual participants prior to each condition may have added variability that is not fully controlled for in this design (e.g. physical activity and fluid intake); (3) This study relied on participant reports of food consumption rather than direct observation, which could have affected the overnight fasting periods and consumption of experimental diets; (4) Based on the population selected for this study, we may not be able to generalize results to individuals who are younger than 18, older than 30, or have a known disease; (5) This study relied on self-report of menstrual bleeding in female participants and no additional laboratory measures were utilized to confirm the phase of the menstrual cycle; (6) Muscle glycogen content was not measured; (7) Urinary volumes and specific gravity of urine were not assessed.

Delimitations

The delimitations of this study were: (1) Male and female participants age 18 to 30 were recruited for the study; (2) Only premenopausal female participants with regularly occurring menstrual cycles were eligible, and female participants were assessed at the same point of the menstrual cycle for both conditions, based on self-report; (3) Healthy recreationally active (defined as engaging in regular leisure-time or work-related physical activity) or active individuals were utilized; (4) One researcher analyzed all DXA scans to promote consistent analysis; (5) Pre-packaged food items were provided to all participants; (6) Participants had not started or ceased creatine supplementation within the past month; (7) Participants were asked to not consume any dietary supplements beginning one day prior to first research visit and continuing until the conclusion of their final research visit; (8) The consumption of alcohol, nicotine, and caffeine was limited beginning 12 hours prior to the first experimental visit of each condition; (9) Physical activity was restricted during the experimental conditions; (10) All participants were assessed using the same calibrated equipment.

Significance of Study

This study helps clarify the impact of acute alterations in pre-assessment nutrition for two popular methods of body composition assessment. This is critically important since a wide variety of medical and health research relies on body composition assessment. Additionally, since all subjects typically consume food in the days leading up to body composition assessment, it is important to continue to elucidate the impact of the content of these diets on estimates of body composition. This study demonstrates what size of effect can be expected due to severe reductions and large increases in dietary

carbohydrate intake, both within a single day and after an overnight fast. If these effects are deemed large, this serves as a call for better standardization of pre-assessment dietary practices prior to body composition estimation. If these effects are deemed trivial or nonexistent, this research provides evidence regarding the robustness of these methods against acute manipulations of dietary carbohydrate.

CHAPTER TWO

Literature Review

Introduction

The study of human body composition has been part of modern science for almost 200 years (54). Today, the assessment of human body composition is an integral part of medical, health, and fitness research. Body composition is an important consideration in growth and aging, as well as in impaired physiological conditions such as obesity and sarcopenia. Additionally, body composition can play an important role in physical fitness and athletic performance. Thus, it is imperative that researchers and health practitioners are able to accurately assess the makeup of the human body and quantify changes in its component parts over time in order to appropriately understand the impact of body composition on health, disease, athletic performance, and wellbeing.

Indirect methods of body composition assessment, those that do not involve analysis of the human body by dissection or certain chemical measures, are widely used in research today (19), and provide only an estimation of an individual's true body composition. Due to the inherent errors associated with estimating body composition, it is critical that researchers exert the highest level of control possible when preparing individuals for body composition assessment, thereby minimizing errors due to controllable factors. Furthermore, complete reporting of pre-assessment procedures and analysis methods should be included in research reports in order to notify the reader of steps taken to ensure accurate body composition assessment. When these details are

lacking, as is often the case, the reader is left to question whether potential errors were addressed in the research design or simply disregarded.

Two methods of body composition estimation frequently used in research settings are dual-energy x-ray absorptiometry (DXA) and bioelectrical impedance analysis (BIA). The focus of the present study is the potential impact of food consumption of differing macronutrient content on the body composition estimates obtained by these two methods. This review chapter will focus on research methodology to minimize error during body composition assessment, with particular emphasis on food and fluid intake.

Dual-energy X-ray Absorptiometry

DXA is a commonly used criterion method in body composition assessment (9). DXA machines produce x-rays of two different low-energy levels and allow for the estimation of fat mass, lean soft tissue, and bone mineral content due to the differences in attenuation as the rays pass through different body tissues. DXA combines two different two-compartment comparisons, soft tissue vs. bone mineral and fat-free mass vs. fat mass, in order to obtain these three compartments (38). The amount of radiation exposure from a DXA scan is low, and the National Health and Nutrition Examination Survey (NHANES) Body Composition Procedures Manual states that the amount received falls within the range of background radiation (32). DXA is a popular method of body composition assessment among researchers due to the large amount of information it provides, as well as its accuracy and reproducibility; however, DXA is susceptible to errors when the associated assumptions are not met (26).

Principles of Body Composition Estimation by DXA

DXA utilizes two different x-ray energies in order to obtain measures of body composition. The proportion of fat and lean content in each pixel of the image produced by a DXA machine is dictated by ratio of x-ray attenuation at the lower energy level compared to the higher energy level (39). Assuming a constant attenuation for fat and bone-free lean tissue, DXA calculates the proportion of fat and lean content within each pixel. Pixels containing bone are not included in the calculations for soft tissues, and an assumption of DXA is that the excluded bone-containing pixels are similar in their content of fat and lean soft tissue to the adjacent non-bone pixels. Since DXA yields a 2-dimensional scan, this assumption is necessary to estimate the fat and lean tissue above and below bony structures within the body.

Effects of Food and Fluid Consumption on DXA Body Composition Results

While DXA is viewed as an accurate and appropriate method of body composition assessment for many populations, DXA scan results can be influenced by several factors, including positioning of the body during scans, daily activities, and food and fluid consumption. The limited number of studies that examine the potential impact of food and fluid consumption on DXA results will be discussed in this section.

Studies of acute fluid removal from the human body provide examples of the error that can be introduced into DXA estimates of body composition when body mass and body water are altered. An assumption in these particularly short-term studies, including the present study, is that changes in muscle, adipose, and bone tissue are very unlikely to occur over these short periods of time (i.e. hours). By extension, any changes that are seen in body composition estimates due to experimental procedures do not likely

represent “real” changes to body tissues, but rather indicate transient alterations of body fluids and/or gastrointestinal content.

Ferrari et al. (10) performed DXA scans before and after removing 2.8% of body mass from patients via hemodialysis. This amount of fluid removal led to an apparent decrease in total non-bone lean mass by approximately 5%, a phenomenon that was particularly pronounced in the legs (- 6.5%). Similarly, Horber et al. (15) reported that lean body mass measurements decreased by approximately 3 kilograms in individuals after a single hemodialysis treatment. While studies of hemodialysis patients may not directly apply to most individuals undergoing body composition assessment, these studies provide basic support for the idea that acute manipulation of total body water content translates to alterations in lean mass as measured by DXA. The results of Ferrari et al. and Horber et al. cannot be attributed to changes in muscle, adipose, or bone tissue due to the extremely short duration of the study. Therefore, it is likely that the results represent an artificial change in lean tissue evoked by manipulations of body fluids.

Not only can fluid removal by hemodialysis decrease estimates of lean soft tissue, hypohydration also appears to produce this effect. Rodriguez-Sanchez and Galloway (40) reported that lean soft tissue decreased by 2.5% in response to exercise in the heat which elicited a 2% decrease in body mass. This change was present in males and females and was almost exclusively due to alterations in the trunk region. There was no change in fat mass, and therefore the decreased estimate of lean soft tissue led to an increase in calculated body fat percentage.

The intake of fluid and food items will introduce added mass to an individual relative to the fasting state. Due to the water content of food items, food items can also

potentially affect total body water. Acute food and fluid consumption can alter body composition assessment by DXA, primarily by increasing lean mass measurements in proportion to the amount of food and fluid ingested. Nana et al. (30) performed an informative study in which individuals were scanned by DXA five times over the course of a two-day period. When an unstandardized diet was consumed by participants, the errors of measurement associated with total and regional body composition were increased and average total and regional lean mass measurements increased up to 1.3% and 1.9%, respectively. In response to a single standardized test meal, average total and regional lean mass increased up to 1.5% and 3.2%, with the trunk region showing the largest increase.

Horber et al. (15) performed a study in which healthy volunteers were weighed and scanned by DXA before and 1 hour after 3 meals (breakfast, lunch, and dinner). In response to a breakfast containing 632 kcal of energy and 400 mL water, no changes in body composition estimates were observed. However, in response to a lunch of 530 kcal and 1950 mL water on average and a dinner of 530 kcal and 1200 mL on average, lean body mass increased between 1 and 2 kilograms, exclusively within the trunk. The increase in lean mass was strongly correlated to the amount of food and water consumption minus urine output ($r = 0.93$). Fat mass and bone mineral content were unaffected by any of the feedings.

Thomsen et al. (51) reported increases of approximately 1 kilogram in lean soft tissue 30 to 60 minutes after a standard meal of approximately 1.3 kilograms and increases of approximately 800 grams 10 to 30 minutes after the ingestion of 1 kilogram of water.

Taken together, these findings indicate that alterations in the ingestion or abstinence from food and fluid may particularly impact the lean soft tissue compartment when body composition is assessed by DXA. Specifically, the impact on lean soft tissue directly corresponds to the amount of fluid removed from the body via hemodialysis or dehydration or added to the body via food and fluid intake. Therefore, the magnitude of the changes in lean soft tissue estimates could be small in cases of minor dehydration or small amounts of intake or could be rather large (i.e. several kilograms or more) in cases of large amounts of fluid removal, extreme dehydration, or large quantities of food and fluid intake. Based on the limited research studies discussed, in the absence of hemodialysis, alterations of body fluids seem to impact lean soft tissue by up to ± 2 kilograms. Lean soft tissue is the compartment that is almost exclusively affected by these fluid alterations due to DXA's inability to differentiate between body water and lean soft tissue, as well as the much higher fluid content of lean soft tissue as compared to adipose tissue and bone compartments. In fact, when pure water has been measured by DXA, it is categorized as 91.4% lean tissue and only 8.6% fat tissue (33).

Bioelectrical Impedance Analysis

BIA is a simple and safe method of assessing total body water (TBW), fat-free mass, and fat mass. Due to the high cost and lack of portability of DXA machines, BIA devices are often used for body composition assessment in both research and clinical settings. BIA devices function by applying a small alternating current to the body via source electrodes placed in contact with the skin and measuring the impedance of body tissues based on the electrical signal received by detection electrodes (24). This allows for the estimation of total body water via regression equations, which utilize information

about biological relationships between impedance and body water for a given reference population (9).

BIA devices either utilize a single frequency, which allows the determination of TBW, or multiple frequencies, which allow the additional differentiation of TBW into intracellular water (ICW) and extracellular water (ECW) (26). Most single frequency devices, including both of the devices in the present study, utilize a frequency of 50 kHz. This frequency represents the average optimal measurement frequency of muscle tissue, although an individual's optimal frequency may range from 30 to over 100 kHz (39). Multi-frequency BIA units often utilize frequencies ranging from 0 to 500 kHz, although the higher (>200 kHz) and lower (<5 kHz) ends of the spectrum may lead to poor reproducibility of measurements (24). Higher frequencies within this range are thought to better penetrate the cell membrane, enabling estimation of ICW, whereas low frequencies which are unable to penetrate the cell membrane provide information regarding ECW (7). Each BIA device can utilize a variable number of electrodes (e.g. two, four, and eight electrodes for bipolar, tetrapolar, and octopolar devices, respectively), and many different equations have been used for the prediction of TBW, fat-free mass, fat mass, ECW, and ICW from impedance measurements (24).

Principles of Body Composition Estimation by BIA

Body composition estimation by BIA is based on the assumption that fat-free mass contains approximately 73% water, although the estimated range spans from 69 to 77% (39). When an assumption is made about the percentage of fat-free mass that is water, fat-free mass can be estimated from TBW (26). When the amount of fat-free mass is subtracted from total body mass, the amount of fat mass and body fat percentage can

be predicted. The estimates of body composition obtained by BIA are dependent on the particular equation used, whether it is applied automatically by the device or by researchers and health practitioners. A variety of equations have been utilized in healthy and diseased adults of varying ethnicity, age, and body weight (24). These equations often make the assumption that the entire body is a single cylinder, although this is clearly not the case. The body can be thought of as five cylinders – two arms, two legs, and a trunk – which are not uniform. The different cross-sectional area of each cylinder plays a large role in the amount of resistance measured in that part of the body. For example, due to the relatively small cross-sectional area of the arms relative to the trunk and legs, the arms account for a disproportionate amount of resistance when whole-body resistance is measured due to the relative difficulty of current flow (39).

Effect of Food and Fluid Consumption on BIA Body Composition Results

The effects of food and fluid consumption on BIA estimates of body composition have previously been investigated. Gallagher et al. (11) sought to clarify the impact of a breakfast meal on body composition estimates by tetrapolar BIA. Following a 12-hour overnight fast, baseline BIA measurements were taken in non-obese adult males and females. Test meals containing 550 kilocalories, but differing in macronutrient content (61% of energy from carbohydrate vs. 83%), were provided after baseline measurements. BIA was conducted after the meal and hourly until 5 hours after the baseline measurement, for a total of 7 measurements. Regardless of the macronutrient content of the test meal, bioelectrical impedance was significantly lower than baseline beginning at 2 hours after baseline. The impedance values reached their lowest point at 4 hours post-baseline (5 to 8% lower than baseline on average), but remained significantly lower than

baseline for the whole duration of measurement (up to 5 hours after baseline). The authors state that the changes in impedance are likely due to alterations in fluid and electrolyte distribution accompanying the ingestion of the meal. The lower impedance values following food ingestion led to a decrease in calculated body fat mass by 5 ± 5% relative to fasting. Although changes in the estimates of fat-free mass were not explicitly reported, it can be inferred that fat-free mass values were higher as a result of the lower estimation of fat mass.

The delayed decrease in impedance described by Gallagher et al. (11) has potentially impactful implications for the usage of BIA technology. Most BIA devices recommend fasting for a period of several hours prior to assessment. However, these recommendations typically fall into the period of time when a decrease in impedance was reported by Gallagher et al (11).

The BIA devices employed in the present study serve as examples: the Tanita SC 331S product manual recommends that assessment should be conducted at least three hours after eating, and the Omron HBF 306 product manual recommends not using the device within 1 to 2 hours after eating a meal or drinking a large amount of water. Interestingly, following these recommendations could potentially be more detrimental to the accuracy of body composition assessment than using the BIA device immediately after eating the first meal of the day (or after a longer fast), based on the information presented by Gallagher et al. (11). It should be noted that different BIA devices could potentially affect the relationship between the time course of impedance changes in response to food ingestion. In particular, as ingested fluid is taken up and assimilated into various body fluid compartments and body tissues, the measurements made by multi-

frequency BIA devices could vary based on the frequencies used. As mentioned previously, lower frequencies tend to capture information regarding ECW, while higher frequencies can penetrate cell membranes and allow for the estimation of ICW.

Slinde and Rossander-Hulthén (42) investigated the effects of the repeated ingestion of an identical meal throughout the day. Healthy male and female adults were assessed by BIA 18 times throughout a 24-hour period. Baseline assessment was conducted after an overnight fast, and subsequent assessments were conducted throughout the day as the participants consumed 3 identical test meals containing 652 ± 77 kilocalories and 49 ± 1% of energy from carbohydrate. Impedance values decreased in response to food ingestion, which led to decreases in calculated body fat percentage. The largest difference in body fat percentage between fasting (the highest value) and a post-prandial value was 8.5% for females and 9.9% for males, which corresponded to reductions of 2.3 and 1.7 percentage points, respectively. After an overnight fast, impedance returned to baseline values.

In 2004, the European Society for Clinical Nutrition and Metabolism published a two-part set of guidelines for BIA (23, 24) which describe background principles, methods, and clinical applications of BIA. They recommend that subjects should not consume food or alcohol for 8 hours prior to assessment, although they state that a shorter period of fasting may be acceptable for non-research purposes. They also conclude that BIA is appropriate for healthy subjects with stable body water, but that BIA may not be a recommended practice for use in individuals with disease or abnormal hydration. Based on the evidence put forth in these guidelines, as well as the aforementioned studies, it appears that food and fluid consumption impact BIA by reducing impedance

measurements. While the guidelines report relatively small impacts of feeding on impedance (~3% reduction) over the 2 to 4 hours after food consumption, others have reported declines of impedance of up to 8% on average 4 hours after eating (11). Greater individual changes have also been reported; for example, a reduction of 11% was reported 2 hours after eating (42). These reductions in impedance can potentially lead to higher estimations of TBW and fat-free mass and lower estimations of fat mass and body fat percentage relative to a fasting state.

Dietary Impact on Glycogen Concentrations

The intake of food and fluid can potentially impact body composition estimates in at least two temporally different ways. First, as previously mentioned, introducing food and fluid into a body adds mass to that body immediately upon ingestion. Measurements taken after foods and fluids have been introduced may be affected simply by the mass added to the body (e.g. gastrointestinal content). An area of concern is determining where the added mass is assimilated within the body, as this could affect body composition estimates when assessment occurs after ingestion. That is, does the added mass appear as lean body mass, fat mass, or a combination of both, and how does the time between feeding and assessment influence this? If it is a combination of both, is the addition of mass to each compartment proportional to the size of the compartment or is one compartment disproportionately impacted by the additional mass? The present study will not fully answer all of these questions, but it will provide some information that will be useful in future investigations of these questions. Second, specific dietary patterns over the course of day(s), rather than hours, could lead to changes within the body that could affect body composition estimates. One potentially important example of this

occurrence relates to the concentrations of glycogen and associated water in the body. The potential impact of glycogen concentrations in the body has not been extensively described as it relates to current methods of body composition assessment.

In theory, both DXA and BIA could be affected by altered glycogen concentrations. DXA estimates of lean soft tissue include glycogen stores and the associated 3 to 4 grams of water stored with each gram of glycogen (35). While most acute fluid changes within the body occur in the extracellular compartment (i.e. the plasma and interstitium), altering glycogen stores could potentially impact the intracellular fluid, which is known to typically stay constant. Alterations in glycogen concentration could also impact the ratio of extracellular water to intracellular water, which is known to potentially affect BIA measurements (24). Therefore, it is possible that prior carbohydrate content of the diet and current glycogen stores should be a factor that is considered when assessing body composition. This is potentially important not only for research settings, but also in clinical application, where failure to evaluate the diet prior to assessment could compromise the accurate quantification of patients' body composition changes over time. In research, studies comparing high and low-carbohydrate diets could be promoting different liver and muscle glycogen concentrations, which could potentially impact estimates of body composition. However, long-term dietary manipulations are not necessary to induce these changes as glycogen stores can be acutely depleted, restored, or supercompensated. Additionally, other acute factors, such as prior physical activity, body temperature, and overall hydration, can alter glycogen and/or water content within the body and thus potentially play a role in this issue.

While stores of glycogen vary with body size, dietary intake and fitness status, it can be estimated that a 70-kg man stores approximately 500 grams of glycogen in his body, although much greater amounts can be stored in response to carbohydrate overfeeding and classic and modified glycogen loading regimens (1). When the additional 3 to 4 grams of water per gram of glycogen are considered, there are several kilograms of body weight that can be acutely modified (i.e. depleted or bolstered), in addition to alterations of body weight and body water that can occur via dehydration or hyperhydration. Approximately 80% of glycogen is stored in skeletal muscle, while the majority of the remaining glycogen is located in the liver (18). It is believed that there are different causes of glycogen depletion in muscle and liver tissue. Hepatic glycogen stores are rapidly depleted during the first day without dietary carbohydrate (45), whereas muscle glycogen is likely primarily depleted by physical activity, not absence of dietary carbohydrate (22). Since hepatic glycogen represents a relatively small amount of the total glycogen stores, alterations of hepatic glycogen and associated water may not impact whole body stores to the extent muscle glycogen would.

It is questionable whether the absence of dietary carbohydrate (i.e. fasting or a low-carbohydrate diet) makes an appreciable impact on muscle glycogen concentrations. Knapik et al. reported no statistically significant difference between muscle glycogen concentrations when a 14-hour overnight fast was compared to a 3.5 day fast in young healthy men (22). However, no power analysis was conducted, and the small sample size (n=8) may have compromised the ability to detect differences. In fact, the average quantity of muscle glycogen was approximately 11% lower after the 3.5 day fast (~82 μm/g wet weight) as compared to after the overnight fast (~92 μm/g wet weight). It has

previously been reported by Hultman and Bergström that there is a slow and steady decline in muscle glycogen stores during 7 days of a very low-carbohydrate diet (which is often viewed as being metabolically similar to fasting), even in the absence of exercise or vigorous physical activity (16). They reported that muscle glycogen concentrations fell from 1.44 to 0.99 g per 100g wet muscle during this time, representing a 37% decrease. Additionally, Hultman and Bergström reported that, in non-exercised limbs, muscle glycogen concentrations fell 35% after 2 days of fasting or 3 days of a very low-carbohydrate diet. Subsequently, when participants were placed on a high-carbohydrate diet after this period, muscle glycogen concentrations increased approximately 90% on average (16). While the depletion and repletion of glycogen was much more pronounced in exercised limbs, the same pattern was present in muscle tissue that had not been exercised. In contrast to these results, Vendelbo et al. reported that 72 hours of fasting led to an increase in skeletal muscle glycogen content by 10%, likely due to decreased glucose oxidation during fasting (53).

Although there are mixed reports as to whether the absence of dietary carbohydrate may lead to a significant alteration in muscle glycogen content (22, 53), acute increases in the intake of dietary carbohydrate, even in the absence of previous glycogen depletion, appear to increase muscle glycogen stores (16, 41). Olsson and Saltin demonstrated that increasing muscle glycogen content through the diet can further translate to alterations in total body water: During a 3-day period of increased water and carbohydrate intake following glycogen-depleting exercise, it was reported that body water increased 2.2 kg as muscle glycogen stores increased approximately 4-fold (35). As mentioned previously, Hultman and Bergström reported that this increase in glycogen

occurs to a lesser extent in muscle tissue that has not been previously depleted of glycogen.

From the perspective of body composition assessment, a recent study conducted by Rouillier et al. (41) provides some preliminary information regarding the potential of increasing glycogen and associated water stores through dietary means, as well as how these changes impact body composition estimates. Participants switched from their habitual diet to a non-standardized high-carbohydrate diet for three days (in which participant-reported carbohydrate intake accounted for approximately 84% of energy intake), and body composition was assessed via DXA before and after the dietary alteration. After three days of the high-carbohydrate diet, total body weight and lean body mass increased (0.8% and 1.5%, respectively) and percent body fat decreased. The reported 4.5% increase in appendicular lean mass indicated that the apparent alterations in lean body mass were not necessarily distributed equivalently throughout the body. Rather than representing a "real" alteration in body composition, these reported changes are likely due to the increased carbohydrate content of the diet and specific, preferential distribution of ingested carbohydrates.

Conclusions

The accurate assessment of human body composition is a crucial part of ensuring high-quality research in variety of health-related research areas. Dual-energy x-ray absorptiometry and bioelectrical impedance analysis are two common methods of body composition estimation, and both can be impacted by alterations in the pre-assessment state of an individual, including the diet in the hours and days leading up to assessment. Food and fluid ingestion prior to body composition estimation present a potential concern

due to uncertainty in how different measurement tools categorize the additional mass added to the body relative to the fasting state.

Alterations of body glycogen and water content over the day(s) prior to assessment could impact total body mass and may specifically induce variability in the lean mass compartment when body composition is assessed by DXA or BIA. In response to large fluid shifts caused by hemodialysis or exercise, DXA lean soft tissue measurements may be affected by up to ±5% (10, 40), while a single standardized meal may increase average total and regional lean soft tissue estimates by up to 1.5% and 3.2%, respectively. Additionally, fluid ingestion appears to preferentially increase lean soft tissue estimates in proportion to the water ingested. In response to food and flood ingestion, BIA measurements of impedance decrease by up to 8% on average (11, 42). Lower impedance values can impact fat mass, and likely fat-free mass, by up to 5% on average (11). Additionally, the cumulative effect of multiple meals likely leads to a greater impact on BIA estimates than a single meal (42), which may potentially impact optimal timing of assessment during the course of a day.

The existing research concerning the impact of food and fluid intake on DXA and BIA body composition estimates is limited, and little information is available concerning the potential impact of the macronutrient content of the diet prior to body composition assessment. In particular, examinations of how carbohydrate content of the diet could alter glycogen concentrations and body water, and thus affect lean mass estimates, are warranted. The recent study by Rouillier et al. (41) provides some preliminary evidence that high-carbohydrate diets may artificially inflate lean soft tissue measurements by

DXA, but the lack of standardized diets and questions concerning inconsistent timing of research visits relative to feeding prevent definitive conclusions from being drawn (4).

In order to address some of unanswered questions raised in this review of literature, the present study will seek to employ a design that will allow for comparisons of diets of differing macronutrient content, different methods of body composition assessment and gender comparisons, while exerting greater dietary control than previous investigations. The side-by-side comparison of the influence of our dietary manipulations on DXA and BIA estimates of body composition, particularly lean mass, will potentially allow for a more complete view of the acute changes induced in body composition estimates. Measurements of lean soft tissue provided by DXA can be compared with measurements of total body water and fat-free mass obtained by BIA in order to enhance our understanding of the transient changes that may occur. It is important for researchers to clarify the actual impact of these dietary manipulations in the hours and days prior to assessment in order to minimize error and promote accurate estimations of an individual's body composition. Addressing these concerns will promote accurate body composition research and benefit numerous health-related research areas, as well as clinical practice.

CHAPTER THREE

Methods

Overview

This experiment utilized a counterbalanced design consisting of two two-day conditions in order to examine the effects of acute dietary manipulations on body composition estimates provided by DXA and BIA. Participants underwent two dietary conditions: a very low-carbohydrate (VLC) diet and high-carbohydrate (HC) diet. The first day of each condition consisted of baseline measurements for that condition in the morning and the consumption of a standardized diet throughout the day. In the afternoon of the first day, participants returned to the lab to repeat the assessment procedures. The second day of each condition consisted of identical experimental procedures as the previous morning, with the exception of the provision of food items. After the morning measurements were collected on the second day, the condition was complete. For male subjects, a washout period of five days was utilized. For female subjects, a one menstrual cycle washout period (approximately one month) was used in order to ensure that both conditions were completed at the same point in the menstrual cycle. This design allowed an examination of the effects of two drastically different diets, both soon after consumption of food items and after an overnight fast, on body composition estimates. An overview of the study design is depicted in Figure 3.1.

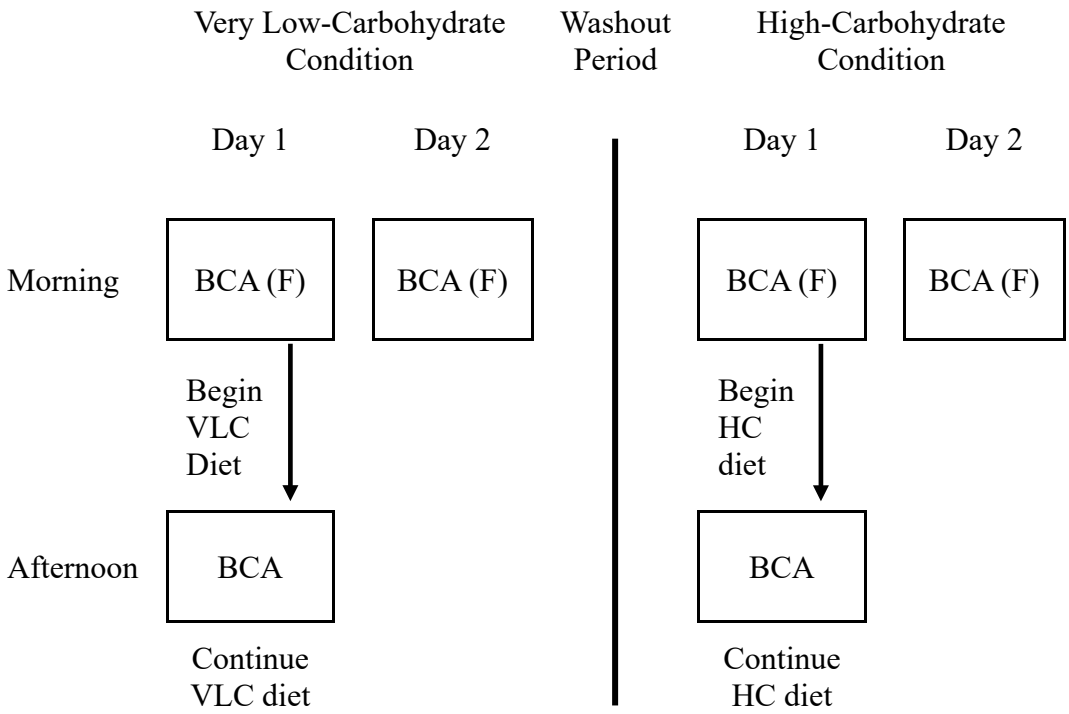

Figure 3.1. Study Design. Each participant underwent body composition assessment (BCA) a total of 6 times. BCA assessment consisted of DXA, tetrapolar BIA, and bipolar BIA. For all morning visits (4 total), each volunteer reported to the lab after a 12-hour overnight fast (F). The two dietary conditions were very low-carbohydrate (VLC – 1 to 1.5 g/kg carbohydrate) and high-carbohydrate (HC – 9 g/kg carbohydrate). Half of the subjects began the study with the HC diet, while half began with the VLC diet. A washout period of 5 days for males and approximately 1 month for females was placed between dietary conditions.

Participants

The target population of this study was recreationally active and active adult males and females ages 18 to 30. Participants were required to be engaged in regular leisure-time or work-related physical activity of a moderate or vigorous intensity for a minimum of 120 minutes per week, but the modality of activity was unrestricted. Female participants were premenopausal with regular menstrual cycles over the previous 6 months.

Inclusion criteria for the study were that participants must be between the ages of 18 and 30, generally healthy, premenopausal with regularly occurring menstrual cycles over the past 6 months for female participants, and recreationally active or active (i.e. >

120 minutes per week of moderate or vigorous physical activity). Potential participants were excluded from the study if they failed to meet any of the inclusion criteria, were currently pregnant or may have become pregnant during the study, or had started or stopped taking the dietary supplement creatine within the past month. Due to the provision of food items as part of the study, potential participants were also excluded from participation in the study if they had a known or suspected food allergy to any of the food items utilized. Additionally, individuals were not allowed to participate if they had any medical condition that could reasonably be negatively affected by participation in the study (e.g. diabetes, hyperlipidemia, etc.).

Preliminary Experimental Procedures

Prior to any experimental procedures, participants were screened for potential eligibility and read and signed a university-approved informed consent document. A medical history form was also completed in order to screen for additional potential contraindications to participation in the study.

Formulation of Diets

Both dietary conditions were formulated based on carbohydrate content relative to body weight. The high-carbohydrate condition consisted of approximately 9 g/kg body weight of carbohydrate. Similar high levels of carbohydrate consumption are frequently used in carbohydrate loading programs (2, 6, 12, 13). The very low-carbohydrate condition consisted of approximately 1 – 1.5 g/kg body weight of carbohydrate. Although this represents a very low intake of carbohydrate, it has frequently been used safely in research (e.g. studies of ketogenic diets (36, 47, 52)). Numerous studies have examined

complete fasting for this period of time or longer without appreciable adverse effects (21, 27, 29, 44, 53), meaning that individuals have safely consumed zero carbohydrate for the duration of time we will be providing a very low-carbohydrate diet. Therefore, based on previous research studies utilizing both high-carbohydrate and very low-carbohydrate (or no carbohydrate) diets, we believe that both dietary conditions were safe for healthy individuals to follow for a one-day period of time.

While dietary carbohydrate was the focus of the diet formulation, the total caloric content of each diet condition was kept within 4% of the caloric content of the other condition. Due to the nature of the carbohydrate manipulation, fat and protein content were not equivalent between conditions.

Each participant was categorized by baseline body weight in order to prescribe the appropriate carbohydrate and energy content of the diet. The following weight categories were utilized (values in kg): <45, 45 – 55, 55 – 65, 65 – 75, 75 – 85, 85 – 95, 95 – 105, 105 – 115, and >115.

Order of Dietary Conditions

Prior to the commencement of the first experimental condition, the initial participants were randomly assigned by coin flip to undergo either the VLC or HC condition first. Once an equal number of participants had been randomly assigned to undergo the VLC or HC condition first, a permuted block method was used on the remaining participants in order to ensure an equal number of participants began each condition first.

Scheduling of Visits Relative to Menstrual Cycle

Female subjects were interviewed concerning their menstrual cycle in order to schedule research visits within the mid-follicular phase. Both experimental conditions were completed within 5 days after cessation of menstrual bleeding. One condition was completed per month in two consecutive months, and both conditions were completed during the mid-follicular phase of the female's monthly cycle.

Dietary Considerations

A one-day diet and fluid intake record was collected prior to the first day of each experimental condition. Prior to the second condition, participants were provided with their diet and fluid intake record from prior to the first experimental condition and asked to follow this pattern of intake as closely as possible. Participants were asked to fast (i.e. consume no food or fluids) for 12 hours prior to each morning research visit, as well as refrain from consuming alcohol, nicotine, or caffeine for 12 hours prior to the first visit of a particular condition until the completion of the final visit of that same condition. These items were allowed to be consumed during the washout period, but were restricted again beginning 12 hours prior to the first visit of the second condition. Participants were asked to not consume any dietary supplements during the experimental conditions (beginning one day prior to the first research visit).

Physical Activity

Participants were asked to refrain from moderate to vigorous activity starting the evening prior to the first day of a given condition. Participants were also asked to not engage in any exercise (i.e. minimize physical activity beyond activities of daily living,

such as walking) throughout the first day and the morning of the second day until after the research visit has been completed. Participants were encouraged to continue normal physical activity during the washout period, provided that they refrain from moderate to vigorous activity beginning the evening prior to the second condition.

Experimental Procedures

There were six total experimental visits for each participant. Each condition consisted of three visits, with two visits taking place on the first day of the condition and one visit taking place on the second day of the condition. The procedures for each visit were identical with the exception of the provision of the food items, which only occurred on the first day of each condition. The participant was in the fasted state for the first and third visits of each condition (i.e. the morning visits), but was in the postprandial state for the second visit (i.e. the afternoon visit).

Reporting to the Lab

For each visit, participants reported to the Baylor Laboratory for Exercise Science and Technology (BLEST) between 6 a.m. and 10 a.m. after a 12-hour overnight food and fluid fast. Participants provided researchers with their diet record from the previous day upon reporting to the lab. Each participant was required to report to the lab at approximately the same time for each morning visit of a particular condition, and efforts were made to conduct all morning visits throughout both conditions at approximately the same time of day. Participants were asked to wear minimal clothing (e.g. athletic shorts and a t-shirt) and to wear equivalent clothing items during all visits. Participants were asked to refrain from wearing any items that contained metal.

For the second visit of each condition, each participant reported to the lab in the afternoon between 2 and 5 p.m. after consuming the midafternoon snack (see *Provision of Food Items* section). The time since the most recent consumption of the provided food items was recorded. The experimental procedures were conducted as in the morning session, with the exception that the participants were not reporting to the lab in the fasted state.

Body Weight, Temperature, and Composition Assessment

Upon arrival, each participant was asked to void his or her bladder prior to obtaining body weight. Body weight and height were determined on an electronic scale and stadiometer (Seca 703). Body temperature was obtained bilaterally using a Braun ThermoScan PRO 4000 infrared ear thermometer, and the values for the left and right ear were averaged.

The participant underwent a DXA scan on a calibrated Hologic Discovery W machine. The same researcher analyzed all DXA scans using the Hologic APEX software (version 13.3) in order to promote consistency between measurements. The effective dose of radiation reported for a whole body scan on the Discovery W system is 8.4 μSv in adults (3). According to the National Health and Nutrition Examination Survey (NHANES) Body Composition Procedures Manual, the average effective dose of background radiation that an individual in the United States receives each year is 3600 μSv, and the dose of radiation an individual receives from a DXA scan falls within the range of background radiation (32). Additionally, an individual would receive approximately 1100 μSv from a standard diagnostic x-ray of the spine (32). The NHANES Body Composition manual reasons that the risk from DXA scans is low.

Therefore, even when 6 DXA scans are conducted over the course of a week to a month, the amount of effective radiation added to the individual participant is low.

BIA estimates of body composition were collected using tetrapolar foot-to-foot (Tanita SC 331S) and bipolar hand-to-hand (Omron HBF-306) BIA devices. Measurements were collected in triplicate for each BIA device, and the values were averaged.

Provision of Food Items

At the end of the morning research visit on the first day of either condition, participants were given commercially packaged food items for consumption that day. For the VLC condition, food items consisted primarily of individually packaged nuts, cheeses, hard-boiled eggs, dried meats, and low-carb shakes (note: this shake is considered a food item, not a dietary supplement). For the HC condition, food items included individually packaged fruit juices, pasta, fruit snacks, granola bars, and fruit bars. The provision of commercially prepackaged food items minimized the risk of contamination and allowed for precise measurements of nutrient contents within each dietary condition.

The participants were asked to not consume any other food items other than those provided. They were also asked to consume all of the food items provided. The food items were assigned to be consumed in five occasions: morning meal (7 – 10 a.m.; immediately after research visit is complete), midday meal (11 a.m. – 1 p.m.), midafternoon snack (2 – 4 p.m.), evening meal (5 – 7 p.m.), and nighttime snack (7 – 9 p.m.). The timing of the nighttime snack was altered as needed in order to allow for a 12-hour fast before the participant returned to the lab for assessment the following morning.

Tracking Adherence to Dietary Protocol

Participants were given a dietary checklist in order to record each food item they consumed on a given day. Participants were instructed not to consume any food in addition to the food items provided to them, but were asked to record any additional items they consumed if this did occur. Additionally, although participants were allowed *ad libitum* non-caloric, non-caffeinated fluid intake, they were asked to record all fluid intake on a provided form. This form included images to serve as a guide in estimating quantities of fluids consumed.

Analysis of Diet Records

Diet records for the day prior to each experimental condition were analyzed using the freely available United States Department of Agriculture SuperTracker tool (48). The total amounts of calories, carbohydrate, fat, protein, sodium, and fluid for each diet record were obtained. One trained researcher performed all dietary analysis to promote consistency of results.

Washout Period

It has been demonstrated that glycogen levels can remain elevated for at least five days after carbohydrate loading (2). Therefore, the washout period in male subjects was 5 days. In order to ensure that both conditions were completed at the same time of the menstrual cycle, female participants completed one condition immediately after the cessation of menstrual bleeding in two consecutive months (i.e. two consecutive menstrual cycles). Both male and female washout periods were as short as possible in an attempt to minimize real changes in body composition. Participants were instructed to

attempt to continue their regular dietary intake and physical activity habits in order to remain weight stable in the washout period.

Statistical Procedures

Appropriate descriptive characteristics of study participants were generated. Paired samples t-tests were performed to compare anthropometric characteristics of participants at the baseline visit for each condition, dietary intake in participants the day prior to beginning each condition, total fluid intake during each experimental condition, and the time between last food consumption and experimental visits. Independent samples t-tests were used to compare baseline characteristics of male and female participants.

Three-factor repeated measures analysis of variance was performed for dependent variables using time and condition as within-subjects factors and sex as the between-subjects factor. An alpha level of 0.05 was used in order to determine statistical significance. Significant two-way interactions were followed up via simple main effects. For two-way interactions between two within-subjects factors (i.e. time and condition), repeated measures analysis of variance was utilized to compare each time point in each condition and to compare each condition at each time point. The resultant pairwise comparisons were examined for significant differences. For two-way interactions with one within-subjects factor and one between-subjects factor (i.e. gender), the simple main effect for the within-subjects factor was determined via repeated measures analysis of variance and examination of the resultant pairwise comparisons. For the between-subjects factor, independent samples t-tests were used to compare genders. Significant time main effects were examined using post-hoc pairwise comparisons.

Partial η^2 effect sizes and 90% confidence intervals for effect sizes were calculated using scripts generated by Smithson (43) in IBM SPSS Statistics (version 20.0.0). Partial η^2 represents the proportion of the effect plus error variance that is attributable to the effect for a given dependent variable. In order to ensure that the confidence interval for a proportion of variance effect size, such as partial η^2, does not include zero when there is a significant difference in an F-test using an alpha level of .05, it is necessary to use a 90% confidence interval (25, 46). Statistical analysis was conducted using IBM SPSS Statistics (version 20.0.0).

CHAPTER FOUR

Results

Participants

Fifty-four individuals (27 males and 27 females) were screened for eligibility. Two individuals did not meet entry criteria due to food allergies or lack of menstrual cycle. Four individuals were eligible, but never commenced the study. Forty-eight individuals (24 males and 24 females) initiated and completed all aspects of the study. The completion rate was 100%. Baseline characteristics of study participants are presented by gender in Table 4.1. There were no differences in baseline body weight, lean soft tissue, fat mass, body fat percentage measured by DXA, or total body water between the HC and VLC conditions, as shown in Table 4.2.

Table 4.1. Participant Characteristics.

Characteristic	All (n = 48)	Males (n = 24)	Females (n = 24)	p-value
Age (y)	21.8 ± 2.9	22.2 ± 3.5	21.4 ± 2.1	.350
Height (cm)	172.1 ± 9.9	179.5 ± 6.2	164.8 ± 6.8	<.001
Weight (kg)	69.3 ± 15.2	77.7 ± 16.0	60.9 ± 8.2	<.001
BMI (kg/m^2)	23.2 ± 3.4	24.0 ± 3.7	22.5 ± 3.0	.129
PA (min/week)	288.2 ± 158.6	319.2 ± 179.6	257.1 ± 130.8	.177

Note: Data presented as mean ± SD. BMI: body mass index; PA: physical activity.

Table 4.2. Baseline Visit Comparison.

Characteristic	HC Baseline	VLC Baseline	p-value
Body Weight (kg)	69.3 ± 15.2	69.2 ± 15.1	.565
LST (kg)	47.4 ± 12.1	47.3 ± 12.1	.420
FM (kg)	18.2 ± 5.7	18.2 ± 5.4	.713
DXA Body Fat %	27.0 ± 6.7	27.0 ± 6.6	.938
Total Body Water (kg)	37.5 ± 8.1	37.4 ± 8.1	.233

Note: Data presented as mean ± SD. HC: high-carbohydrate; VLC: very low-carbohydrate; LST: lean soft tissue; FM: fat mass.

Dietary Intake

Dietary intakes on the day prior to each experimental condition were not significantly different ($p>0.05$, Table 4.3). As planned, the high-carbohydrate diet consisted of approximately 9 g/kg body weight of carbohydrate, and the very low-carbohydrate diet consisted of approximately 1 – 1.5 g/kg body weight of carbohydrate. Total caloric content of the HC and VLC diets for each weight class were kept within 4% of each other. The total energy, macronutrient, and sodium content of each of the diets are displayed in Table 4.4.

Table 4.3. Dietary Comparison of Day Prior to Experimental Conditions.

Variable	Pre HC	Pre VLC	p-value	Mean Difference	95% CI for Difference
Energy (kcal)	2047 ± 802	1838 ± 865	.067	209	-15.26 – 432.51
Carbohydrate (g)	227 ± 112	198 ± 113	.152	29	-11.24 – 69.77
Fat (g)	81 ± 40	73 ± 39	.150	8	-2.75 – 17.48
Protein (g)	103 ± 54	97 ± 56	.335	6	-6.77 – 19.44
Sodium (mg)	3301 ± 1524	3041 ± 1399	.141	260	-89.07 – 608.63
Fluid (oz)	51 ± 32	46 ± 32	.464	5	-9.94 – 21.165

Note: Data presented as mean ± SD. HC: high-carbohydrate; VLC: very low-carbohydrate; CI: confidence interval.

There was a 94.8% adherence rate for following the food intake plan exactly as prescribed. Noncompliance included consuming very small amounts of additional food (n=1) and failing to finish a very small amount of the provided food items (n=4). Uneaten food items were returned to study investigators. Deviations from the protocol were deemed small by study investigators, and the decision was made not to exclude these individuals from the analysis. There was no difference between conditions for the duration between last food or fluid consumption and experimental visits (Table 4.5).

Table 4.4. Nutritional Content of Experimental Diets.

Variable		Weight class (kg)								
		<45	45–55	55–65	65–75	75–85	85–95	95–105	105–115	>115
Energy (kcal)	*HC*	1900	2100	2560	3000	3440	3810	4210	4650	4850
	VLC	1860	2140	2500	2970	3370	3680	4050	4530	4780
Carbohydrate (g)	*HC*	404	452	542	634	730	803	899	991	1041
	VLC	61	66	85	96	114	116	140	162	171
Fat (g)	*HC*	21	21	31	36	42	49	49	54	54
	VLC	145	166	195	232	264	288	307	345	367
Protein (g)	*HC*	38	41	49	62	69	74	80	93	94
	VLC	99	115	127	154	170	192	223	241	250
Sodium (mg)	*HC*	2103	2115	3028	3280	3528	4380	4405	4898	4898
	VLC	2900	3788	4115	4275	4835	5240	5690	6430	6600

Table 4.5. Comparison of Duration since Last Food or Fluid Consumption Prior to Each Study Visit.

Research Visit	HC	VLC	p-value
Morning 1 (min)	804 ± 180	790 ± 82	.558
Afternoon 1 (min)	55 ± 38	47 ± 35	.213
Morning 2 (min)	733 ± 50	763 ± 112	.053

Note: Data presented as mean ± SD. HC: high-carbohydrate; VLC: very low-carbohydrate.

Body Weight and Body Composition

No three-way interactions (i.e. gender by condition by time) were present for any dependent variables. A time by condition interaction was present for body weight as measured by electronic scale (Table 4.6), with the variation in body weight due to the interaction accounting for 13.6% of the variation due to the interaction plus error. Body weight increased in both conditions in response to feeding. In the HC condition, body weight on the second morning returned to baseline values, but in the VLC condition, body weight fell below baseline values.

Table 4.6. Body Weight

Condition	Morning 1	Afternoon 1*	Morning 2*	p-value†	η_p^2	η_p^2 90% CI
HC	69.3 ± 2.2^a	70.0 ± 2.2^b	69.3 ± 2.2^a	.031	.136	.006 - .268
VLC	69.2 ± 2.2^a	69.7 ± 2.2^b	69.0 ± 2.2^c			

Note: Data presented as mean ± SE. Means within conditions are equal if superscript letter is the same. * denotes a difference between conditions at a given research visit ($p < .05$). †p-value for condition by time simple two-way interaction. CI: confidence interval; HC: high-carbohydrate; VLC: very low-carbohydrate.

Time main effects were present for body mass, total lean soft tissue, and total fat mass as measured by DXA (Table 4.7). Estimates of total mass, total lean soft tissue mass, and trunk lean soft tissue mass increased in response to feeding by 0.6 kg, 0.8 kg, and 0.6 kg, respectively, while total fat mass decreased by 0.2 kg. Each of these parameters returned to baseline values by the final research visit within each condition. Based on η_p^2 values, variation in total mass, total lean soft tissue, and trunk lean soft tissue due to time accounted for 79%, 76%, and 74% of the variation due to time plus error. While time main effects were also present for leg lean soft tissue and trunk fat mass, no effects were observed for arm lean soft tissue, nor leg or arm fat mass (Table

4.8). As a result of feeding, estimates of leg lean soft tissue increased by 0.2 kg and trunk fat mass decreased by 0.1 kg.

Table 4.7. Whole-body Body Composition Results (DXA).

Variable	Morning 1	Afternoon 1	Morning 2	p-value†	η_p^2	η_p^2 90% CI
Total Mass (kg)	68.0 ± 1.8^a	68.6 ± 1.8^b	67.9 ± 1.8^a	<.0001	.788	.680 - .836
Total LST (kg)	47.3 ± 1.2^a	48.1 ± 1.2^b	47.2 ± 1.2^a	<.0001	.764	.645 - .817
Total FM (kg)	18.2 ± 0.8^a	18.0 ± 0.8^b	18.2 ± 0.8^a	.005	.193	.034 - .331

Note: Data presented as mean ± SE. †p-value for time main effect. Means for a variable are equal if superscript letters are the same. CI: confidence interval; LST: lean soft tissue; FM: fat mass.

Table 4.8. Regional Body Composition Results (DXA).

Variable	Morning 1	Afternoon 1	Morning 2	p-value†	η_p^2	η_p^2 90% CI
Trunk LST (kg)	23.0 ± 0.6^a	23.6 ± 0.6^b	22.9 ± 0.6^a	<.0001	.742	.615 - .800
Legs LST (kg)	16.2 ± 0.5^a	16.4 ± 0.5^b	16.2 ± 0.5^a	<.0001	.371	.171 - .498
Arms LST (kg)	5.1 ± 0.2^a	5.1 ± 0.2^a	5.1 ± 0.2^a	.719	.014	0 - .077
Trunk FM (kg)	8.0 ± 0.4^a	7.9 ± 0.4^b	8.0 ± 0.5^a	<.001	.266	.082 - .402
Legs FM (kg)	7.3 ± 0.3^a	7.4 ± 0.3^a	7.4 ± 0.3^a	.331	.046	0 - .147
Arms FM (kg)	1.9 ± 0.1^a	1.9 ± 0.1^a	1.9 ± 0.1^a	.417	.018	0 - .088

Note: Data presented as mean ± SE. Means for a variable are equal if superscript letters are the same. †p-value for time main effect. CI: confidence interval; LST: lean soft tissue; FM: fat mass.

A time by gender interaction was present for body fat percentage measured by DXA (Table 4.9). In males, body fat percentage decreased by 0.3 percentage points in response to feeding, but returned to baseline values after the second overnight fast. In females, body fat percentage decreased by 0.6 percentage points in response to feeding, and then increased 0.9 percentage points after the second overnight fast so that body fat percentage was higher after the second overnight fast as compared to after the first. However, the variation in body fat percentage due to the gender by time interaction only accounted for 14% of the variation in the interaction plus error.

Table 4.9. DXA Body Fat Percentage.

Gender	Morning 1*	Afternoon 1*	Morning 2*	p-value†	Partial η^2	Partial η^2 90% CI
Male	22.6 ± 1.0^a	22.2 ± 1.0^b	22.5 ± 1.0^a	.03	.138	.006 - .270
Female	31.5 ± 1.0^a	30.9 ± 1.0^b	31.8 ± 1.0^c			

Note: Data presented as mean ± SE. Means for a variable are equal if superscript letters are the same. * denotes a difference between conditions at a given research visit ($p < .001$). †p-value for time by gender interaction. CI: confidence interval.

Time main effects were present for impedance, total body water mass, fat-free mass, fat mass, and body fat percentage as measured by tetrapolar BIA (Table 4.10). Impedance dropped by 25 Ω in response to feeding, but increased 35 Ω after the second overnight fast so that impedance was higher on the second morning of the study than on the first. Total body water increased in response to feeding, but returned to baseline values after the second overnight fast in each condition. In accordance with the impedance changes, fat-free mass estimates increased in response to feeding, but fell below baseline values at the second morning visit in each condition. Additionally, fat mass and body fat percentage decreased in response to feeding but were increased after the second overnight fast so that values were higher on the second morning of the study as compared to the first. Body fat percentage as measured by bipolar BIA increased in response to feeding and fell after the second overnight fast, but remained higher than the baseline visit. η_p^2 values indicate that the variation in BIA parameters due to time accounts for 37 to 79% of the variation due to time plus error.

Average percent changes for dependent variables, representing changes at the afternoon visit relative to the baseline morning visit, are displayed in Figures 4.1 – 4.3. Table 4.11 depicts the numerical values for the percent change between the baseline visit and the afternoon visit, as well as the range of individual responses, which indicates the most extreme individual responders for each dependent variable.

Table 4.10. BIA Results.

Variable	Morning 1	Afternoon 1	Morning 2	p-value†	η_p^2	η_p^2 90% CI
Impedance (Ω)	560.4 ± 8.8^a	535.3 ± 8.9^b	570.2 ± 8.9^c	<.0001	.794	.688 - .840
TBW (kg)	37.4 ± 0.6^a	38.1 ± 0.6^b	37.3 ± 0.6^a	<.0001	.596	.420 - .685
FFM (kg)	53.6 ± 0.9^a	54.5 ± 1.0^b	53.3 ± 0.9^c	<.0001	.749	.625 - .805
FM (kg)	14.9 ± 1.0^a	14.7 ± 1.0^b	15.2 ± 1.1^c	<.0001	.369	.169 - .496
Tetrapolar BF%	21.5 ± 0.9^a	20.9 ± 0.9^b	21.8 ± 0.9^c	<.0001	.588	.411 - .679
Bipolar BF%	17.4 ± 0.9^a	18.3 ± 0.9^b	17.7 ± 0.9^c	<.0001	.579	.399 - .671

Note: Data presented as mean ± SE. †p-value for time main effect. Means with different superscripts are statistically different.

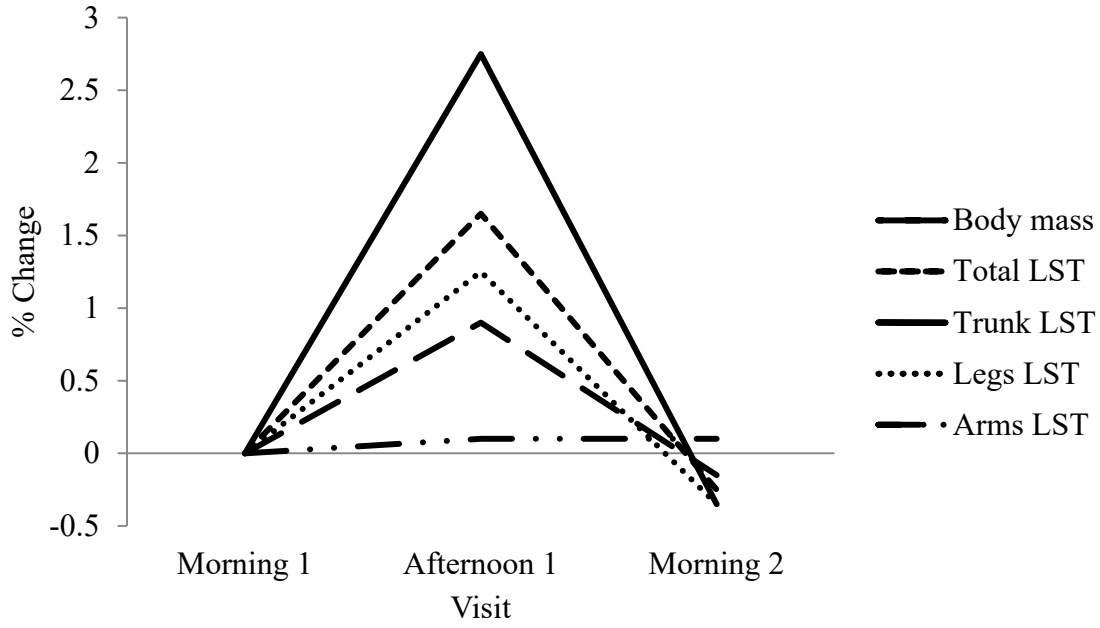

Figure 4.1. DXA Lean Soft Tissue Changes. Average percent changes in DXA measurements relative to baseline visit demonstrate an increase in most regions at visit 2 (first afternoon; fed), but a return to baseline values at visit 3 (second morning; fasted).

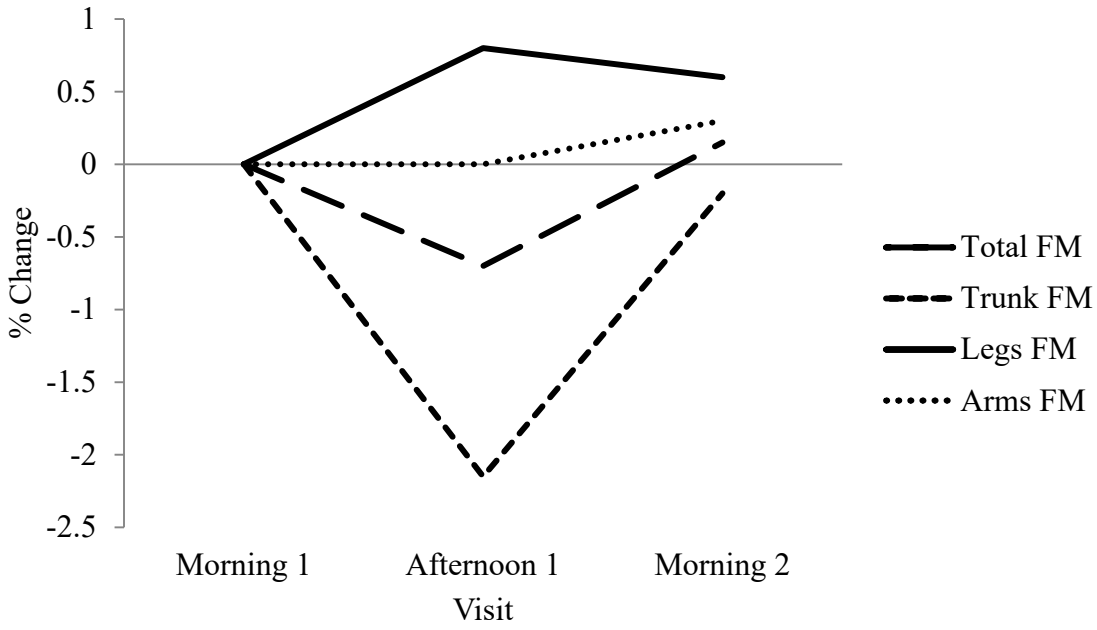

Figure 4.2. DXA Fat Mass Changes. Average percent changes in DXA measurements relative to baseline visit demonstrates a decrease in total fat mass due to the decline in estimates of trunk fat mass. These changes were evident at visit 2 (first afternoon; fed), but returned to baseline values at visit 3 (second morning; fasted).

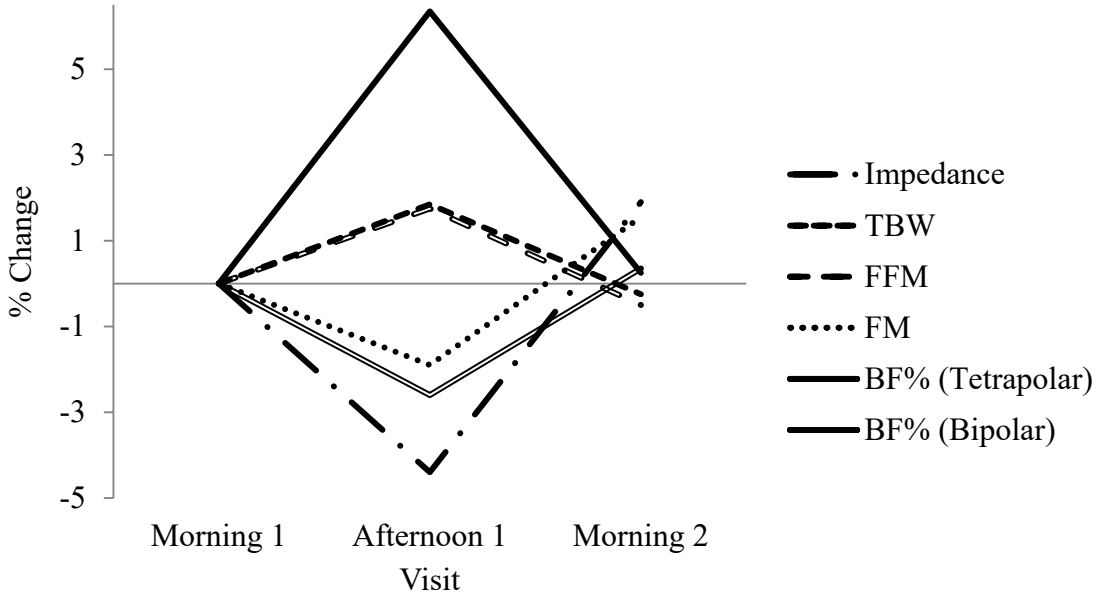

Figure 4.3. BIA Changes. Average percent changes in BIA measurements relative to baseline visit demonstrate a decrease in impedance, fat mass, and body fat percentage assessed by tetrapolar BIA, as well as increases in total body water, fat free mass, and body fat percentage assessed by bipolar BIA. Differences relative to baseline were still present at visit 3, although the direction of these changes varied.

Table 4.11. Percent Changes for Dependent Variables.

Variable	Condition	Males		Females	
		Average % Change†	Range of % Change	Average % Change†	Range of % Change
Body weight	*HC*	1.1 ± 0.7	-0.2 to 2.7	0.9 ± 0.5	0.2 to 1.8
	VLC	0.7 ± 0.7	-0.4 to 2.3	0.7 ± 0.8	-2.1 to 2.0
Body mass	*HC*	1.0 ± 0.8	-0.6 to 2.4	1.0 ± 0.5	-0.1 to 1.6
	VLC	0.7 ± 0.8	-0.4 to 2.9	0.9 ± 0.6	0 to 2.4
Total LST	*HC*	1.6 ± 1.4	-1.1 to 5.0	1.9 ± 1.3	-0.8 to 4.1
	VLC	1.2 ± 1.5	-1.4 to 4.0	2.1 ± 1.3	-0.3 to 4.6
Trunk LST	*HC*	3.1 ± 2.8	-2.6 to 9.3	2.8 ± 2.9	-4.5 to 7.4
	VLC	1.6 ± 2.9	-3.3 to 6.8	3.5 ± 2.6	-3.3 to 7.3
Legs LST	*HC*	0.8 ± 2.7	-4.4 to 7.8	1.8 ± 2.4	-2.3 to 7.1
	VLC	1.0 ± 2.9	-5.3 to 5.9	1.3 ± 2.7	-3.3 to 9.6
Arms LST	*HC*	-0.4 ± 4.0	-6.0 to 6.5	0.4 ± 3.7	-6.0 to 6.3
	VLC	0.4 ± 2.7	-4.3 to 7.9	-0.1 ± 4.4	-9.3 to 8.2
Total FM	*HC*	-0.2 ± 4.0	-6.4 to 7.0	-0.7 ± 2.6	-4.6 to 5.4
	VLC	-0.6 ± 3.5	-7.6 to 6.0	-1.4 ± 2.6	-7.8 to 5.1
Trunk FM	*HC*	-0.3 ± 5.8	-13.7 to 12.1	-2.0 ± 5.3	-11.1 to 6.6
	VLC	-2.8 ± 5.9	-12.4 to 7.4	-3.4 ± 5.1	-14.2 to 8.6
Legs FM	*HC*	0.6 ± 5.2	-8.3 to 10.3	0.4 ± 3.9	-6.8 to 9.7
	VLC	2.4 ± 5.2	-4.0 to 17.5	-0.2 ± 2.7	-3.9 to 7.0
Arms FM	*HC*	-0.8 ± 6.3	-7.5 to 10.3	0.4 ± 6.4	-12.6 to 19.0
	VLC	-0.7 ± 3.7	-8.0 to 7.2	1.0 ± 6.1	-14.0 to 12.4
Impedance	*HC*	-3.9 ± 4.0	-14.6 to 3.7	-4.9 ± 3.0	-11.0 to 2.1
	VLC	-3.7 ± 4.3	-10.1 to 7.5	-5.2 ± 3.7	-10.8 to 3.3
TBW	*HC*	1.7 ± 1.5	-1.0 to 6.5	2.1 ± 1.2	-0.4 to 4.5
	VLC	1.5 ± 1.4	-1.9 to 3.9	2.1 ± 1.4	-1.0 to 4.5
FFM	*HC*	1.7 ± 1.6	-1.0 to 6.2	2.0 ± 1.1	-0.5 to 4.2
	VLC	1.1 ± 2.0	-4.9 to 5.9	2.1 ± 1.4	-0.9 to 4.4
BIA FM	*HC*	-0.8 ± 4.8	-12.5 to 7.1	-2.1 ± 3.3	-10.0 to 2.0
	VLC	-2.3 ± 7.2	-15.1 to 17.2	-2.5 ± 4.0	-9.2 to 6.8
DXA BF%	*HC*	-1.5 ± 4.2[a]	-10.7 to 6.3[b]	-1.6 ± 2.5[c]	-5.9 to 4.7[d]
	VLC	-1.2 ± 3.5[a]	-8.0 to 5.0[b]	-1.9 ± 2.6[c]	-8.0 to 3.1[d]
Tetrapolar BF%	*HC*	-1.8 ± 4.7[e]	-14.3 to 6.0[f]	-3.1 ± 3.2[g]	-11.0 to 1.8[h]
	VLC	-2.3 ± 6.3[e]	-10.9 to 14.0[f]	-3.4 ± 3.8[g]	-10.5 to 4.7[h]
Bipolar BF%	*HC*	10.5 ± 11.6[i]	-2.6 to 45.2[j]	4.4 ± 4.5[k]	-4.2 to 13.0[l]
	VLC	6.2 ± 11.6[i]	-28.5 to 26.6[j]	4.4 ± 5.8[k]	-1.9 to 20.0[l]

Note: Data presented as mean ± SD. †calculated as the average of $\frac{(\text{afternoon visit measurement}) - (\text{baseline visit measurement})}{\text{baseline visit measurement}} * 100$ for each participant.

a: This corresponds to -0.3 ± 0.9 and -0.3 ± 0.8 percentage points for HC and VLC, respectively
b: This corresponds to -1.9 to 1.1 and -2.1 to 1.1 percentage points for HC and VLC, respectively
c: This corresponds to -0.5 ± 0.8 and -0.6 ± 0.8 percentage points for HC and VLC, respectively
d: This corresponds to -1.8 to 1.1 and -2.6 to 1.0 percentage points for HC and VLC, respectively
e: This corresponds to -0.3 ± 0.8 and -0.4 ± 1.1 percentage points for HC and VLC, respectively
f: This corresponds to -2.0 to 1.0 and -3.4 to 2.1 percentage points for HC and VLC, respectively
g: This corresponds to -0.8 ± 0.8 and -0.8 ± 1.0 percentage points for HC and VLC, respectively
h: This corresponds to -2.7 to 0.6 and -2.4 to 1.2 percentage points for HC and VLC, respectively
i: This corresponds to 1.1 ± 1.0 and 0.7 ± 1.3 percentage points for HC and VLC, respectively
j: This corresponds to -0.4 to 4.2 and -3.6 to 2.9 percentage points for HC and VLC, respectively
k: This corresponds to 0.9 ± 1.1 and 0.7 ± 0.7 percentage points for HC and VLC, respectively
l: This corresponds to -1.4 to 3.8 and -0.4 to 2.2 percentage points for HC and VLC, respectively

CHAPTER FIVE

Discussion

Purpose and Major Results of Study

The purpose of this study was to examine the effects of acute pre-assessment dietary changes on body composition estimates in recreationally active and active male and female adults. Specifically, we examined the effects of a one-day very low-carbohydrate diet and a one-day high-carbohydrate diet on body composition estimates obtained by DXA, tetrapolar foot-to-foot BIA, and bipolar hand-to-hand BIA.

The major result of our study was that acute food ingestion, regardless of macronutrient content, altered a number of body composition estimates obtained from DXA, tetrapolar BIA, and bipolar BIA. For DXA, total mass, total lean soft tissue, trunk lean soft tissue, and leg lean soft tissue estimates were higher by approximately 1%, 1.7%, 2.8%, and 1.3% on average in response to feeding, while total fat mass and trunk fat mass were lower by 0.7% and 2.2% on average. All parameters assessed by tetrapolar BIA were impacted by feeding. Impedance, fat mass, and body fat percentage decreased by approximately 4.4%, 1.8%, and 2.5% on average. Total body water and fat-free mass were both increased by approximately 2% on average. Statistical interactions were also present that indicated that body fat percentage measured by DXA responded differently to the nutritional intervention in males and females and that body weight measurements were altered differently by the high-carbohydrate and low-carbohydrate diets. The variability in individual responses to feeding indicate that some individuals may be more

resistant to alterations in body composition estimates due to acute feeding, while others exhibited noticeably large changes that could easily compromise the accurate assessment of their body composition over time.

Study Hypotheses

We hypothesized there would not be a change in lean soft tissue estimates or fat mass estimates between the beginning and end of the VLC condition, as measured by DXA. Since the majority of glycogen is stored in the muscle, and it is not clear the extent to which these stores are depleted, if they are depleted at all, by absence of dietary carbohydrate, we expected that muscle glycogen would decrease minimally during the low-carbohydrate condition (22). However, due to the intestinal content of food items and fluids consumed throughout the day, we expected an increase in total body mass and lean soft tissue measurements between the morning and afternoon of the first day of the VLC condition. The results of the present study support these hypotheses. Total lean soft tissue increased by 1.6% from the morning to afternoon measurements in the VLC condition, but lean soft tissue mass on the second morning was not different from the baseline visit. There were also regional differences present, as lean soft tissue of the trunk and legs increased significantly by 2.5% and 1.2%, but did not change for the arms. The relatively large amount of muscle mass located in the trunk and legs may have been most responsible for accommodating the ingested fluids and food items. Additionally, intestinal content is located within the trunk region on a DXA scan and was likely classified as lean soft tissue. As expected, total fat mass did not change between the beginning and end of the VLC condition. However, total fat mass and trunk fat mass were lower in the afternoon visit than both morning visits, although this difference was

minor. The trunk region has been previously reported to be particularly impacted by changes in fluid intake and feeding (15, 30, 40), and the changes seen in the trunk region in the present study support the contention that this compartment may experience the largest changes in response to acute food and fluid ingestion.

We hypothesized there would be an increase in lean soft tissue estimates and no change in fat mass estimates between the beginning and end of the HC condition, as measured by DXA. We believed that the acute high-carbohydrate diet would be sufficient to increase intramuscular glycogen stores beyond their normal levels based on previous research of a similar level of carbohydrate intake (6). We believed that this level of intake would lead to an increase in both carbohydrate and water stored within skeletal muscle tissue. We also believed that the apparent increase in lean soft tissue would be evident between the morning and afternoon of the first day of the HC condition. Contrary to our hypothesis, lean soft tissue estimates were not higher at the end of the HC condition as compared to the baseline visit. There were no group differences for lean soft tissue between the VLC and HC conditions. In agreement with our hypothesis, estimates of total lean soft tissue increased by 1.7% from the morning baseline visit to the afternoon visit, and total fat mass was not different between the beginning and end of the condition. The increase in lean soft tissue was driven primarily by the trunk and secondarily by the legs, as they increased by 3.0% and 1.2% respectively.

We hypothesized that within the day of diet consumption, both diets would lead to an increase in fat-free mass and a decrease in fat mass as estimated by tetrapolar BIA. This hypothesis was correct as feeding in both conditions led to higher measures of fat-free mass (1.6 to 1.9%) and lower measures of fat mass (-1.4 to 2.4%). Feeding produced

a 4.4% decrease in impedance and subsequent increase in total body water estimates by approximately 2%.

We hypothesized there would be no difference in body composition estimates between the first and second mornings in the VLC condition, but that differences would be present in the HC condition, as measured by tetrapolar BIA. We believed the increase in water stored in association with carbohydrate could potentially decrease the measured impedance and thus increase the estimate of fat-free mass. Interestingly, although there were no differences between the HC and VLC conditions, measurements at all three time points were different from each other for each dependent variable measured by BIA, except for total body water. Fat-free mass estimates increased in response to feeding, but fell to below initial values on the second morning of each condition, even though the same overnight fast was employed prior to each morning visit. This was contrary to our hypothesis for both conditions. Fat mass estimates decreased in response to feeding, but were higher the second morning of each condition than on the first morning. This was also contrary to our hypothesis, as we projected no changes in fat mass between the first and second morning visits. Impedance and body fat percentage declined in response to feeding, but rebounded and were higher the second morning than the first morning. Clearly, the results obtained for each of these parameters are related. The alterations in measured impedance played a role in all other BIA variables, as they are all derived from equations using the impedance measurement. Contrary to the tetrapolar BIA device, the bipolar BIA device produced higher measurements of body fat percentage in the fed state: +7.4% in the HC condition and +5.3% in the VLC condition, which corresponds to 1.0 and 0.7 percentage points.

Comparison to Previous Investigations

The altered estimations of DXA lean soft tissue seen in the present study were similar in type and magnitude to those of Nana et al. (31), who reported an increase in average total and regional lean mass measurements increased up to 1.3% and 1.9% in response to an unstandardized diet and an increase in average total and regional lean soft tissue of up to 1.5% and 3.2% in response to a single standardized test meal, with the largest change present in the trunk region. The increases in total lean and trunk lean soft tissue in the present study were 1.7% and 2.8%. Nana et al. performed the DXA scan following the standardized test meal an average of 36 minutes after the commencement of the meal, whereas in the present study, the afternoon DXA scan took place 51 minutes on average after the last recorded food consumption. The findings of Horber et al. (15) provide additional support that the trunk region is most responsible for the increase in lean soft tissue in response to feeding.

The BIA changes observed in the present study are also largely consistent with previous findings, although potentially important differences were also apparent. We found that impedance decreased by 4.4% within an hour of food ingestion, which follows a similar pattern to the changes reported by Gallagher et al. (11) and Slinde and Rossander-Hulthén (42), although the magnitude of the decline within the first hour after food consumption appears greater in the present study. In accordance with the decrease in impedance, Gallagher et al. (11) and Slinde and Rossander- Hulthén (42) reported declines in fat mass and body fat percentage in response to feeding, as we observed in the present study. However, while Slinde and Rossander-Hulthén (42) reported that both impedance and body fat percentage returned to baseline fasting values after an additional

overnight fast, in the present study impedance, fat-free mass, fat mass, and body fat percentage were all significantly different on two consecutive mornings following an overnight fast. Regardless of condition, impedance was higher on the second morning, leading to lower fat-free mass estimates and great fat mass and body fat percentage. Although the two dietary conditions were drastically different, they both seem to have contributed to this phenomenon. While the magnitude of the changes from one morning to the next were relatively small, it is possible that an overnight fast is not sufficient dietary control prior to body composition assessment by single frequency tetrapolar BIA.

Additional Considerations

Gender Differences

The present study supports that males and females are impacted similarly by food and fluid ingestion prior to body composition assessment. The sole gender difference observed was in body fat percentage as measured by DXA. Body fat percentage in males decreased by 1.3% (0.3 percentage points) on average in response to feeding, but was identical on the first and second morning of the study. Body fat percentage in females decreased by 1.9% (0.6 percentage points) on average in response to feeding, but then increased to above baseline levels by 1.0% (0.3 percentage points) after the second overnight fast. This may indicate that, as measured by DXA, body fat percentage is more irregular in females in response to nutritional interventions, even after overnight fasts.

Differences Due to Macronutrient Profile

Contrary to our expectations, the macronutrient profile of the diets had little impact on the estimates of body composition obtained. Similar caloric and fluid intake

led to similar responses, regardless of the drastically different macronutrient content of the experimental diets. The sole statistically significant difference was in regards to body weight. In the HC condition, body weight increased by 1.0% on average in response to feeding, but was identical on the first and second morning of the study. In the VLC condition, body weight increased by 0.7% in response to feeding, but was 0.3% lower than baseline on the second morning.

Practical Importance

The practical importance of the present study may depend on the context in question. In research settings, when all known sources of error should be minimized, it is clear that it would be ideal to perform body composition after a standardized set of procedures, such as an overnight food and fluid fast and voiding of the bladder. In the clinical setting, if a one-time body composition assessment is being conducted to provide the patient, client, or health care professional with a general estimate of an individual's body composition, it may not be necessary to employ meticulous preparatory procedures. However, if serial body composition measurements will be taken over the course of time, it is likely very important to ensure that the patient or client follows a standard pre-assessment procedure. Errors due to food and fluid intake, hydration, and physical activity could either produce artificial changes in body composition estimates or mask real changes. Regardless of which occurs, if either occurs, unnecessary error should be eliminated in order to obtain the highest quality body composition information possible.

An additional consideration is the potential importance of the observed heterogeneous alterations of different body regions. The distribution of lean soft tissue has been reported to play a role in resting energy expenditure (17), adipose tissue volume

(5), and mortality (20). It is also well known that body fat distribution plays a role in the risk for developing cardiovascular and metabolic disease (8, 37). Based on the results of the present study, different body regions may be affected to different degrees by acute food ingestion. For example, the trunk region exhibited a concomitant increase in lean soft tissue and decrease in fat mass, but other body regions did not experience the same degree of change. If the food and fluid intake of a research participant or clinical patient is not standardized prior to assessment, it could compromise the accuracy of measurements related to regional body composition and disease risk.

The recommendation to perform body composition assessment after an overnight food and fluid fast potentially presents a problem for researchers examining both body composition and athletic performance within the same study. Often, exercise performance is not measured in the fasted state. This is reasonable, as many active individuals and athletes do not perform most of their training in the fasted state. The external validity of performance studies may be enhanced by allowing participants to eat a standard meal or self-select a normal pre-exercise meal prior to testing. However, in light of the results of the present study and the overall body of literature, this is potentially problematic if body composition assessment and exercise performance are assessed at the same research visit. Even if standardized meals are utilized prior to assessment, individual variation in the response of body composition parameters could introduce unwanted error into measurements. The ideal scenario may be performing body composition assessment after a standardized overnight fast and then obtaining performance measures later in the day when the participant is in the fed state. However, this may be difficult practically as it could effectively double the number of research visits necessary in a study examining

both body composition and performance. The most feasible route may be to prioritize which outcome is most important in the context of the study and arrange the timing of the study visits accordingly. Regardless, the complete reporting of pre-assessment procedures is strongly encouraged so that the consumer of research articles may understand which relevant errors have and have not been accounted for.

Significance of Research

This study helps clarify the impact of acute alterations in pre-assessment nutrition on estimates of body composition by DXA and single frequency BIA. As body composition assessment is regularly performed in numerous health-related settings, an understanding of the factors that may artificially alter body composition results is critical in order to promote accurate assessments. This study provided preliminary evidence for similar responses in males and females, as well as similar responses for diets of similar energy content, regardless of macronutrient content. This study demonstrated the size of effects seen in response to acute food ingestion, as well as displayed the wide range in individual responses. For DXA, this study provided additional evidence that an overnight food and fluid fast may be sufficient dietary control prior to body composition assessment. For BIA, it appears that body composition estimates may be different on adjacent mornings, even when an identical overnight fast was performed prior to each morning. The results of the present study can help inform researchers and clinicians regarding the optimal assessment schedule in order to promote accurate tracking of an individual's body composition changes over time.

Future Research

In regards to body composition assessment by BIA, future studies should examine the impact of acute food and fluid intake on the intracellular and extracellular fluid compartments. While it is reasonable to believe that the increase in total body water seen in response to acute food and fluid ingestion is primarily due to an expansion of the extracellular compartment, certain dietary strategies such as high-carbohydrate diets and glycogen loading may potentially also impact the intracellular space. Multi-frequency BIA technology is necessary to examine this phenomenon.

While DXA and BIA are two common methods of body composition assessment that will likely be utilized for years to come, new methods of assessment will also emerge. It is important that these new methods are scrutinized in order to determine their validity as well as the impact of pre-assessment procedures on their accuracy.

Lastly, it is strongly recommended that researchers in future studies examining body composition changes over time employ standardization procedures prior to body composition assessment. Based on the present study, non-standardized pre-assessment procedures can artificially increase total lean soft tissue in some participants by over 4.5%, and can also induce artificial increases or decreases in total fat mass by over 5%. Regional measures of body composition can be subject to even greater changes. The pre-assessment procedures utilized by researchers should be carefully adhered to and reported in research publications in order to allow for greater confidence in the accuracy of body composition measurements.

Conclusions

Body composition assessment by DXA and single frequency BIA is affected by acute food and fluid ingestion on the day of assessment. The magnitude of these changes varies between individuals, but is sufficient to potentially obscure true changes in body composition or produce artificial changes. Total lean soft tissue measurements made by DXA are increased approximately 1.7% on average in response to feeding, with some participants demonstrating an increase of over 4.5%. Regional lean soft tissue measurements are increased up to 3% on average by feeding, and individual participants exhibited changes up to ± 9%. DXA total and trunk fat mass estimates are decreased by up to 3% on average in response to feeding. Impedance measured by tetrapolar BIA decreased by 4.4% in response to feeding, and this subsequently affected measures of total body water, fat-free mass, and fat mass by approximately 1.5 to 2%.

Based on the results of the present study, it is recommended that body composition assessment should take place after an overnight food and fluid fast in order to minimize error due to acute food and fluid ingestion. It appears that when body composition is being assessed by DXA, an overnight fast is sufficient dietary control even when acute diets of drastically different macronutrient content are being consumed on the day prior. However, when body composition is being assessed by BIA, acute diets of high or low carbohydrate content may influence body composition estimates, even after an overnight fast. Obtaining diet records or prescribing a standardized diet the day prior to body composition assessment may help combat influences of different diets, particularly when BIA is being used as the method of assessment. The use and reporting of standardized procedures prior to body composition assessment will enhance the

accuracy of measurements and allow researchers and clinicians to minimize errors that could be detrimental to accurate tracking of body composition over time.

APPENDICES

APPENDIX A

Recruitment Flyer

RESEARCH STUDY FOR MEN AND WOMEN

BAYLOR UNIVERSITY

Be part of an important study that will help us determine the impact of following a 1-day high carbohydrate diet and 1-day low carbohydrate diet on measurements of body composition (i.e. muscle and fat tissue).

You may be eligible if you:

- ✓ **Are a male or female between 18 and 30 years**
- ✓ **Are generally healthy**
- ✓ **Are engaged in regular physical activity**

Participants will be required to:

1. Follow a 1-day high carbohydrate diet and a 1-day low carbohydrate diet (pre-packaged store-bought food items will be provided)
2. Come to our lab for a total of 6 visits (~20-30 minutes each) for noninvasive body composition assessment

All participants will receive:

1. Detailed body composition analysis by DXA (dual-energy x-ray absorptiometry) and BIA (bioelectrical impedance analysis)
2. $40 for completion of the study
3. Food items for two days

For more information contact:

Grant Tinsley
Grant_Tinsley@baylor.edu

The study is being conducted at the Baylor Laboratories for Exercise Science and Technology in the Department of Health, Human Performance, and Recreation at Baylor University

APPENDIX B

Initial Screening Survey

Initial Screening Survey

Instructions: Progress through questions until an exclusionary response is given. If an exclusionary factor is indicated, thank the volunteer for their time and shred this document at the conclusion of your conversation.

1. What is your age? _____

2. Are you male or female? _____

3. Do you have any diagnosed disease or other medical condition that could be impacted by alterations in your diet?

_____
_____
_____

4. (If female) Are you pre-menopausal with regularly occurring menstrual cycles over the past 6 months? _____

5. (If female) Are you pregnant or is there a possibility that you could become pregnant during the next 3 months? _____

6. Are you engaged in regular physical activity? What type, frequency, intensity, and duration?

_____
_____
_____

7. Have you taken creatine (via supplementation) regularly within the last month?

_____

8. Do you have any known or suspected food allergies or digestive issues? If so, what are they?

_____
_____
_____

9. Name: _____

10. Contact Information

Best Phone Number: _____

e-mail: _____

APPENDIX C

Informed Consent Document

Baylor University
Department of Health, Human Performance, and Recreation

Consent Form for Research

PROTOCOL TITLE:	Effects of Acute Pre-assessment Dietary Changes on Estimates of Body Composition
PRINCIPAL INVESTIGATORS:	Grant M. Tinsley, M.S. and Peter W. Grandjean, Ph.D.
SUPPORTED BY:	Baylor University

Introduction

Please read this form carefully. The purpose of this form is to provide you with important information about taking part in a research study. If any of the statements or words in this form is unclear, please let us know. We would be happy to answer any questions. You have the right to discuss this study with another person who is not part of the research team before making your decision whether or not to be in the study.

Taking part in this research study is up to you. If you decide to take part in this research study we will ask you to sign this form. We will give you a copy of the signed form.

The person in charge of this study is Grant Tinsley**.** We will refer to this person as the "researcher" throughout this form.

Why is this study being done?

The purpose of this study is to see if following different short-term 1-day diets leads to a change in body composition (the amount of fat and muscle in your body) measurements using two common forms of assessment. We want to see if these methods of body composition assessment are affected by following a high carbohydrate diet and a low carbohydrate diet (for 1 day each). These diets are designed to potentially change the amount of water and carbohydrate stored in your body, and we are going to look at how lean body mass and total body water are affected by these dietary changes. The two methods of body composition measurement are called DXA (dual-energy x-ray absorptiometry) and BIA (bioelectrical impedance analysis). Both of these methods are non-invasive and take less than 10 minutes each.

We are asking you to take part in this study because you are between the ages of 18 and 30, and you have expressed interest in this research study.
Approximately 36 subjects will take part in this research study at Baylor University.

How long will I take part in this research study?

If you take part in this study, you will be involved in two different 2-day conditions. Each condition will consist of 1day of eating the pre-packaged food items we provide you and 3 visits, each lasting no more than 30 minutes, to the lab. Between each condition, there will be a break (called the "washout period") of at least 5 days for males and about 1 month for females. In total, you will make 6 visits to the lab.

What will happen if I take part in this research study?

If you agree to take part in this study, we will ask you to sign this consent form before we begin any study procedures. If you sign the consent form, we will proceed with the study.

On the day before each research visit, we will ask you to record all the food you eat and fluids you drink on a form that we will give you. We will also ask you to not participate in any intense/vigorous exercise. Additionally, we will ask you not to consume any food, alcohol, caffeine, or nicotine for 12 hours prior to your research visit the next morning (You will undergo this same 12-hour overnight fast between all four of the morning research visits).

The overview of the research visits that will take place during the study is presented in Figure 1. During the study, you will eat a high carbohydrate diet for one day (food items will include fruit juices, pre-packaged macaroni and cheese, bars (i.e. Clif bars and Nutri-grain bars), and fruit snacks) and a low carbohydrate diet for one day (food items will include cashews, hardboiled eggs, chocolate shakes, string cheese, almonds, and low-carb bars). All food items for both 1-day diets will be pre-packaged by the manufacturer and will not be expired. We will flip a coin to determine whether you go through the high carbohydrate or low carbohydrate condition first.

For the day before each condition, as well as during the condition, we will ask you to refrain from consuming dietary supplements. You will be able to consume dietary supplements during the washout period, but will have to stop again before your second condition. The one exception is creatine; you won't be eligible to participate in this study if you have regularly taken creatine in the past month, and we ask you not to consume creatine during your involvement in the study.

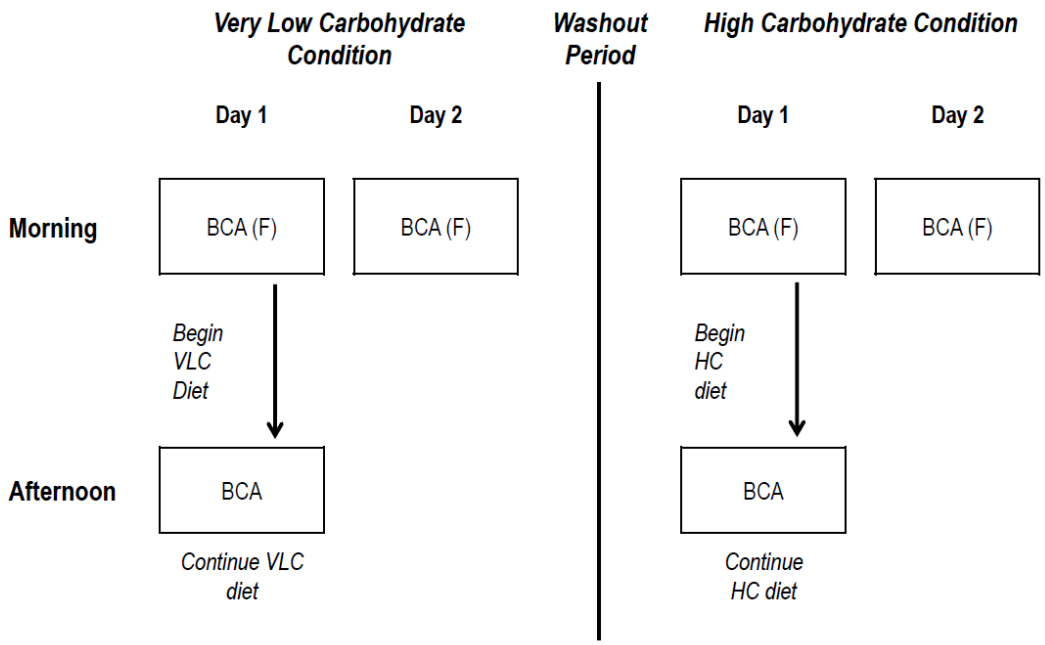

Figure 1. Overview of Study Design. Each volunteer with undergo body composition assessment (BCA) a total of 6 times. For each morning visit (there are 4 total), each volunteer will come to lab after a 12-hour overnight fast (F). The two different short-term diets each volunteer will follow, in random order, are the very low carbohydrate (VLC) diet and the high carbohydrate (HC) diet. A period of time called the washout period will take place between each diet condition. This period of time will be at least 5 days for males and approximately 1 month for females.

Study Visit 1

Visit 1 will take about **20-30 minutes** to complete. At this visit, we will ask you to perform the following procedures:

- Report to the lab wearing athletic shorts and a t-shirt (for females, we will ask you to wear a sports bra or other type of bra that does not contain a wire) after a 12-hour overnight fast. We will also ask that you don't drink any fluids in the morning before you come to the lab. We would like you to come to the lab sometime between 6 AM and 10 AM, based on your availability and preference. However, we will ask you to return at the same time for all of your morning visits (there are 4 total for the whole study).
- Turn in your food and fluid record from the previous day.
- Use the bathroom to empty your bladder
- Get your height, weight, and body temperature measured. Body temperature will be measured by an infrared ear thermometer (similar to the ones used at the doctor's office).
- Get your body composition measured by DXA scanner. A DXA is a type of x-ray used to measure body composition or bone strength. During this test, X-ray pictures of your body will measure how much fat and muscle are present. You will lie flat on a table and a machine will take pictures of different areas of the body. This test will last about 6 minutes. The amount of radiation is similar to

what you would receive by living in Waco for one month, and the National Health and Nutrition Examination Survey (NHANES) states that "the risk from DXA scans is low" and that the dose of radiation received is "within the range of background radiation."

- Get your body composition measured by the Tanita Body Composition Analyzer, which will use small electrical currents that pass through your feet to estimate your body composition (the small currents are undetectable). This takes less than one minute and will be repeated 3 times to get a more accurate measure.
- Get your body composition measured by the Omron HBF-306 handheld body composition analyzer. Just like the previous item, this device uses small undetectable currents to estimate your body composition. However, these currents pass through your hands rather than your feet. This takes less than one minute and will be repeated 3 times to get a more accurate measure.

After these procedures, we will provide you with all your pre-packaged food items for the day and a schedule of when to consume those items. We will not restrict how much fluid you drink, but we ask you not to consume any fluids that contain calories other than the ones we give you. We will also ask you to record how much fluid you drink and mark all the food items you eat on a checklist we provide you. We will ask you to eat all the food items we give you and not to eat any food items that we did not give you.

We will ask you to refrain from any physical activity other than normal daily activities (e.g. walking to class or work) for this day and the morning of the next day.

Study Visit 2
Visit 2 will take place in the afternoon of the same day you came in for your first research visit. It will take up to **30 minutes** to complete. At this visit, we will measure your weight, body temperature, and body composition just like we did at Visit 1 in the morning.

Study Visit 3
Visit 3 will take place the morning after Visits 1 and 2. We will ask you to return to the lab at the same time as you did the previous day at Visit 1. We will also ask you to undergo the same 12-hour overnight fast before coming into the lab and wear equivalent clothing items. After you finish this visit in the morning, you can return to your regular diet.

Between Study Visits 3 and 4
There will be a short break between visits 3 and 4 (a.k.a. the "washout period"). During this time, you can resume all normal activities (diet, exercise, etc.). However, we will ask you to try to maintain your normal diet and exercise patterns and try not to let your body weight change during this period of time.

Study Visit 4
Visit 4 will be identical to Visit 1, except that we will provide you with the other diet. For example, if you got assigned to eat the high carbohydrate diet at Visit 1, you will now be given the low carbohydrate food items.

Study Visit 5
Visit 5 will take place in the afternoon of the same day you came in for Visit 5. It will be identical to Visit 2 (the same weight, body temperature, and body composition procedures will be followed).

Study Visit 6
Visit 6 will be identical to Visit 3. After you complete Visit 6, you will be done with the study.

What are the risks of taking part in this research study?

Risks from Radiation
Procedures such as DXA scans, CT scans, and/or X-rays will be used during this research study. The cumulative radiation exposure from these tests is considered small and is not likely to adversely affect you. However, the effects of radiation add up over a lifetime. It is possible that having several of these tests may add to your risk of injury or disease. When deciding to enter this study, think about your past and future contact with radiation. Examples of contact with radiation include x-rays taken for any reason or radiation therapy for cancer treatment. As mentioned previously, the amount of radiation is similar to what you would receive by living in Waco for one month, and the National Health and Nutrition Examination Survey (NHANES) states that "the risk from DXA scans is low" and that the dose of radiation received is "within the range of background radiation." You should not take part in this study if you are pregnant or may become pregnant during the course of the study.

Loss of Confidentiality
A risk of taking part in this study is the possibility of a loss of confidentiality. Loss of confidentiality includes having your personal information shared with someone who is not on the study team and was not supposed to see or know about your information. The researcher plans to protect your confidentiality. Their plans for keeping your information private are described later in this consent form.

Incidental Findings

Although the procedures/tests you will have in this study are being undertaken for research purposes only, it is possible that researchers may notice something that could be important to your health. If so, we will contact you to explain what was noticed. If you so desire, we will also talk with your private physician. If you do not have a private physician, we will refer you to an appropriate clinic for follow-up. It will be your choice whether to proceed with additional tests and/or treatments to evaluate what we observed, and you or your insurer will be responsible for these costs.

Are there any benefits from being in this research study?
You may or may not benefit from taking part in this study. Possible benefits include improved knowledge of your body composition through the body composition analysis we provide you.

What alternatives are available?
You may choose not to take part in this research study.

Storing Study Information for Future Use
We would like to store your study information for future research related to body composition assessment. We will label all your study information with a code instead of your name. The key to the code connects your name to your study information. The researcher will keep the code in a locked file.

Future use of study information is required for this study**.** If you do not want your information to be used for future research, you should not be in this study.

How Will You Keep My Study Records Confidential?
We will keep the records of this study confidential by using a coding system and keeping your files locked in a file cabinet (separate from the coding key). We will make every effort to keep your records confidential. However, there are times when federal or state law requires the disclosure of your records.

Reporting risk of harm to self or others: If, during your participation in this study, we have reason to believe that you are at risk for harming yourself or others, we are required to take the necessary actions. This may include notifying your doctor, your therapist, or other individuals. If this were to occur, we would not be able to assure confidentiality.

The following people or groups may review your study records for purposes such as quality control or safety:

- The Researcher and any member of his research team
- Authorized members of Baylor University who may need to see your information, such as administrative staff members from the Office of the Vice Provost for Research and members of the Institutional Review Board (a committee which is responsible for the ethical oversight of the study)
- Federal and state agencies that oversee or review research (such as the HHS Office of Human Research Protection or the Food and Drug Administration)

The study data will be stored in a locked cabinet on Baylor University's campus**.**

The results of this study may also be used for teaching, publications, or presentations at professional meetings. If your individual results are discussed, your identity will be protected by using a code number or pseudonym rather than your name or other identifying information.

Study Participation and Early Withdrawal

Taking part in this study is your choice. You are free not to take part or to withdraw at any time for any reason. No matter what you decide, there will be no penalty or loss of benefit to which you are entitled. If you decide to withdraw from this study, the information that you have already provided will be kept confidential. You cannot withdraw information collected prior to your withdrawal.

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or your grades at Baylor University. You will not be offered or receive any special consideration if you take part in this research study.

You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your job status at Baylor University. You will not be offered or receive any special consideration if you take part in this research study.

If the researcher can withdraw the subject: The researcher may take you out of this study without your permission. This may happen because:

- The researcher thinks it is in your best interest
- You can't make the required study visits
- Other administrative reasons

Will I get paid for taking part in this research study?

Potentially, yes. If you complete one of the diets (3 visits), you will receive $20. If you complete both diets (all 6 visits), you will receive $40.

What will it cost me to take part in this research study?

There are no anticipated costs to you for taking part in this research study other than the time and effort required to complete the study procedures.

What happens if I am injured as a result of participating in this research study?

If you become ill or injured as a result of your participation in the study, you should seek medical treatment from your doctor or treatment center of choice. You should promptly tell the researcher about any illness or injury.

There are no plans for Baylor University to pay you or give you other compensation for your injury or illness. You do not give up any of your legal rights to seek compensation by signing this form.

What if I have any questions or concerns about this research study?

You can call us with any concerns or questions about the research.

- The study's primary investigator, Grant Tinsley, can be contacted at (913) 449-0306 or by email at grant_tinsley@baylor.edu.

- Additional investigators can be contacted via email:
    - Peter Grandjean: Peter_grandjean@baylor.edu

If you want to speak with someone **not** directly involved in this research study, you may contact the Baylor University IRB through the Office of the Vice Provost for Research at 254-710-1438. You can talk to them about:

- Your rights as a research subject
- Your concerns about the research
- A complaint about the research

Future Contact

We may like to contact you in the future either to follow-up to this study or to see if you are interested in other studies taking place at Baylor University.

Do you agree to let us contact you in the future?

_____YES _____NO _____INITIALS

Statement of Consent

SIGNATURE OF SUBJECT:

I have read the information in this consent form including risks and possible benefits. I have been given the chance to ask questions. My questions have been answered to my satisfaction, and I agree to participate in the study.

_____ _____

Signature of Subject Date

Signature of Person Obtaining Consent:

I have explained the research to the subject and answered all his/her questions. I will give a copy of the signed consent form to the subject.

_____ _____

Signature of Person Obtaining Consent Date

APPENDIX D

Daily Food and Physical Activity Record

Daily Food & Physical Activity Record

This record will be used to determine the composition of your diet and record your physical activity. Your accuracy and attention to detail are important.

*Please record your diet beginning **1 day before** reporting for your scheduled lab visit.*

DIET RECORD

- RECORD EVERYTHING YOU EAT AND DRINK INCLUDING SNACKS AND BEVERAGES.
- RECORD IMMEDIATELY AFTER FOOD IS CONSUMED
- INDICATE PORTION SIZES. MEASURE AMOUNTS OF EACH FOOD USING MEASURING CUPS OR SPOONS WHEN IT IS PRACTICAL. RECORD PORTION SIZES IN GRAMS, OUNCES, CUPS, TABLESPOONS, TEASPOONS, OR PIECES. (example: 8 oz. orange juice, 1 piece wheat bread, 1 tbsp. butter) YOU MAY ALSO USE OBJECTS OF KNOWN SIZE TO HELP QUANTIFY PORTION SIZES (your hand, a deck of cards, a tennis ball, etc.) YOU MAY USE YOUR CELL PHONE CAMERA TO HELP WITH THIS ASPECT OF YOUR RECORDKEEPING.
- INDICATE THE BRAND NAME. (3 oz. Ruffles BBQ Potato Chips, 1 cup Uncle Ben's Long Grain Rice, McDonald's Large French Fries) AND FORM OF PURCHASE. (fresh, frozen, canned, etc.)
- RECORD TIME OF DAY MEAL WAS EATEN
- PLEASE INCLUDE THE FOOD PACKAGE LABEL WHENEVER POSSIBLE AND/OR INFORMATION FROM THE RESTAURANT'S WEBSITE. COPY RECIPIES FOR HOME-COOKED MEALS.

PHYSICAL ACTIVITY RECORD

- REMEMBER TO NOT PERFORM ANY VIGOROUS/INTENSE PHYSICAL ACTIVITY ON THE 1 DAY PRIOR TO REPORTING TO THE LAB FOR YOUR FIRST VISIT
- RECORD EACH LOW TO MODERATE INTENSITY ACTIVITY ON THE TABLE ON THE FOLLOWING PAGE (E.G. WALKING TO CLASS, BIKING TO CAMPUS, ETC.). INCLUDE AS MUCH DETAIL AS POSSIBLE.

If you have any questions, please contact us:

Mr. Grant Tinsley
(913) 449-0306

Dr. Peter Grandjean
Office: 710-3909

Grant_Tinsley@baylor.edu **Peter_Grandjean@baylor.edu**

Return your completed record to our lab in one of 2 ways:

1) hand-deliver it to our lab (MMG 127)

2) send it as an e-mail attachment to grant_tinsley@baylor.edu

Name: _____ **Phone:** _____ **e-mail:** _____

Day of the Week: _____ **Date:** _____

DIETARY RECORD

Time	Food or Drink Description	Portion Size	Quantity	Preparation (fried, grilled, baked, broiled)

PHYSICAL ACTIVITY RECORD

Time of Day	Description of Activity	Duration of activity	How intense was the activity?

APPENDIX E

Diet Checklists for Each Weight Class

Low Carbohydrate Food Consumption Checklist/Fluid Intake Log (<45 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Frigo Stringhead String Cheese		
		Atkins Chocolate Shake		
Lunch	12 - 2 PM	Slim Jim Smoked Snack (2)		
		Wonderful Almonds		
		Frigo Stringhead String Cheese		
Afternoon Snack	2 - 4 PM	Planter's Salted Cashews (2)		
		Slim Jim Smoked Snack (2)		
Dinner	5 - 7 PM	Planter's Salted Cashews		
		Frigo Stringhead String Cheese (2)		
		Atkins Chocolate Shake		
Evening Snack	7 - 9 PM	Slim Jim Smoked Snack (2)		
		Wonderful Almonds		

Please remember to only consume the food items listed on this table. Please consume all the food items given to you. If you accidentally consume other food items, write them down and let the researchers know as soon as possible.

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	**Time of Day**	**Total amount of fluid (in ounces or measuring cups).** Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

Low Carbohydrate Food Consumption Checklist/Fluid Intake Log (45 - 55 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Frigo Stringhead String Cheese (2)		
		Atkins Chocolate Shake		
Lunch	12 - 2 PM	Slim Jim Smoked Snack (4)		
		Wonderful Almonds		
		Frigo Stringhead String Cheese		
Afternoon Snack	2 - 4 PM	Planter's Salted Cashews (2)		
		Slim Jim Smoked Snack (3)		
Dinner	5 - 7 PM	Planter's Salted Cashews		
		Frigo Stringhead String Cheese (2)		
		Atkins Chocolate Shake		
Evening Snack	7 - 9 PM	Slim Jim Smoked Snack (4)		
		Wonderful Almonds		

Please remember to only consume the food items listed on this table. Please consume all the food items given to you. If you accidentally consume other food items, write them down and let the researchers know as soon as possible.

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	**Time of Day**	**Total amount of fluid (in ounces or measuring cups).** Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

Low Carbohydrate Food Consumption Checklist/Fluid Intake Log (55 - 65 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Frigo Stringhead String Cheese (2)		
		Atkins Chocolate Shake		
Lunch	12 - 2 PM	Slim Jim Smoked Snack (4)		
		Wonderful Almonds		
		Frigo Stringhead String Cheese		
Afternoon Snack	2 - 4 PM	Planter's Salted Cashews (2)		
		Slim Jim Smoked Snack (4)		
Dinner	5 - 7 PM	Planter's Salted Cashews (3)		
		Frigo Stringhead String Cheese (2)		
		Atkins Chocolate Shake		
Evening Snack	7 - 9 PM	Slim Jim Smoked Snack (4)		
		Wonderful Almonds		

Please remember to only consume the food items listed on this table. Please consume all the food items given to you. If you accidentally consume other food items, write them down and let the researchers know as soon as possible.

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	Time of Day	Total amount of fluid (in ounces or measuring cups). Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

Low Carbohydrate Food Consumption Checklist/Fluid Intake Log (65 - 75 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Frigo Stringhead String Cheese (2)		
		Atkins Chocolate Shake		
		Hardboiled Eggs (2)		
Lunch	12 - 2 PM	Slim Jim Smoked Snack (4)		
		Wonderful Almonds (2)		
		Frigo Stringhead String Cheese (2)		
Afternoon Snack	2 - 4 PM	Planter's Salted Cashews (2)		
		Slim Jim Smoked Snack (4)		
Dinner	5 - 7 PM	Planter's Salted Cashews (3)		
		Frigo Stringhead String Cheese (2)		
		Atkins Chocolate Shake		
Evening Snack	7 - 9 PM	Slim Jim Smoked Snack (4)		
		Wonderful Almonds		

Please remember to only consume the food items listed on this table. Please consume all the food items given to you. If you accidentally consume other food items, write them down and let the researchers know as soon as possible.

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	Time of Day	Total amount of fluid (in ounces or measuring cups). Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

Low Carbohydrate Food Consumption Checklist/Fluid Intake Log (75 - 85 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Frigo Stringhead String Cheese (3)		
		Atkins Chocolate Shake		
		Hardboiled Eggs (2)		
Lunch	12 - 2 PM	Slim Jim Smoked Snack (4)		
		Wonderful Almonds (2)		
		Frigo Stringhead String Cheese (2)		
Afternoon Snack	2 - 4 PM	Planter's Salted Cashews (3)		
		Slim Jim Smoked Snack (4)		
Dinner	5 - 7 PM	Planter's Salted Cashews (4)		
		Frigo Stringhead String Cheese (2)		
		Atkins Chocolate Shake		
Evening Snack	7 - 9 PM	Slim Jim Smoked Snack (4)		
		Wonderful Almonds		

Please remember to only consume the food items listed on this table. Please consume all the food items given to you. If you accidentally consume other food items, write them down and let the researchers know as soon as possible.

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	Time of Day	Total amount of fluid (in ounces or measuring cups). Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

Low Carbohydrate Food Consumption Checklist/Fluid Intake Log (85 - 95 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Frigo Stringhead String Cheese (3)		
		Atkins Chocolate Shake		
		Hardboiled Eggs (2)		
Lunch	12 - 2 PM	Slim Jim Smoked Snack (4)		
		Wonderful Almonds (2)		
		Frigo Stringhead String Cheese (2)		
Afternoon Snack	2 - 4 PM	Planter's Salted Cashews (2)		
		Slim Jim Smoked Snack (4)		
		Wonderful Almonds (2)		
Dinner	5 - 7 PM	Hardboiled Eggs (2)		
		Frigo Stringhead String Cheese (3)		
		Atkins Chocolate Shake		
Evening Snack	7 - 9 PM	Slim Jim Smoked Snack (4)		
		Planter's Salted Cashews (4)		

Please remember to only consume the food items listed on this table. Please consume all the food items given to you. If you accidentally consume other food items, write them down and let the researchers know as soon as possible.

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	Time of Day	Total amount of fluid (in ounces or measuring cups). Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

Low Carbohydrate Food Consumption Checklist/Fluid Intake Log (95 - 105 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Frigo Stringhead String Cheese (3)		
		Atkins Chocolate Shake		
		Hardboiled Eggs (2)		
Lunch	12 - 2 PM	Slim Jim Smoked Snack (4)		
		Wonderful Almonds (2)		
		Frigo Stringhead String Cheese (2)		
Afternoon Snack	2 - 4 PM	Planter's Salted Cashews (2)		
		Slim Jim Smoked Snack (4)		
		Wonderful Almonds (2)		
Dinner	5 - 7 PM	Hardboiled Eggs (2)		
		Frigo Stringhead String Cheese (3)		
		Atkins Chocolate Shake (2)		
Evening Snack	7 - 9 PM	Slim Jim Smoked Snack (4)		
		Planter's Salted Cashews (4)		
		Atkins Choc Granola Pretzel Bar		

Please remember to only consume the food items listed on this table. Please consume all the food items given to you. If you accidentally consume other food items, write them down and let the researchers know as soon as possible.

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	Time of Day	Total amount of fluid (in ounces or measuring cups). Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

Low Carbohydrate Food Consumption Checklist/Fluid Intake Log (105 - 115 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Frigo Stringhead String Cheese (3)		
		Atkins Chocolate Shake		
		Hardboiled Eggs (2)		
Lunch	12 - 2 PM	Slim Jim Smoked Snack (4)		
		Wonderful Almonds (2)		
		Frigo Stringhead String Cheese (2)		
Afternoon Snack	2 - 4 PM	Planter's Salted Cashews (4)		
		Slim Jim Smoked Snack (8)		
		Wonderful Almonds (2)		
Dinner	5 - 7 PM	Hardboiled Eggs (2)		
		Frigo Stringhead String Cheese (3)		
		Atkins Chocolate Shake (2)		
Evening Snack	7 - 9 PM	Slim Jim Smoked Snack (4)		
		Planter's Salted Cashews (4)		
		Atkins Choc Granola Pretzel Bar		

Please remember to only consume the food items listed on this table. Please consume all the food items given to you. If you accidentally consume other food items, write them down and let the researchers know as soon as possible.

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	**Time of Day**	**Total amount of fluid (in ounces or measuring cups).** Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

Low Carbohydrate Food Consumption Checklist/Fluid Intake Log (>115 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Frigo Stringhead String Cheese (3)		
		Atkins Chocolate Shake		
		Hardboiled Eggs (2)		
Lunch	12 - 2 PM	Slim Jim Smoked Snack (4)		
		Wonderful Almonds (2)		
		Frigo Stringhead String Cheese (2)		
Afternoon Snack	2 - 4 PM	Planter's Salted Cashews (4)		
		Slim Jim Smoked Snack (8)		
		Wonderful Almonds		
Dinner	5 - 7 PM	Planter's Salted Cashews (4)		
		Frigo Stringhead String Cheese (3)		
		Atkins Chocolate Shake (2)		
Evening Snack	7 - 9 PM	Hardboiled Eggs (2)		
		Slim Jim Smoked Snack (4)		
		Wonderful Almonds (2) Atkins Choc Granola Pretzel		

Please remember to only consume the food items listed on this table. Please consume all the food items given to you. If you accidentally consume other food items, write them down and let the researchers know as soon as possible.

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	Time of Day	Total amount of fluid (in ounces or measuring cups). Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

High Carbohydrate Food Consumption Checklist/Fluid Intake Log (<45 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Ocean Spray Cranberry		
		Clif Bar		
Lunch	12 - 2 PM	Kraft Mac and Cheese		
		Kellogg's Fruity Snacks		
Afternoon Snack	2 - 4 PM	Kellogg's Nutri-grain Bar		
Dinner	5 - 7 PM	Kraft Mac and Cheese		
		Clif Bar		
		Ocean Spray Cranberry		
Evening Snack	7 - 9 PM	Welches Grape Juice (10 oz)		

Please remember to only consume the food items listed on this table. Please consume all the food items given to you. If you accidentally consume other food items, write them down and let the researchers know as soon as possible.

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	Time of Day	Total amount of fluid (in ounces or measuring cups). Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

High Carbohydrate Food Consumption Checklist/Fluid Intake Log (45 - 55 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Ocean Spray Cranberry		
		Clif Bar		
Lunch	12 - 2 PM	Kraft Mac and Cheese		
		Kellogg's Fruity Snacks		
Afternoon Snack	2 - 4 PM	Kellogg's Nutri-grain Bar		
Dinner	5 - 7 PM	Kraft Mac and Cheese		
		Clif Bar		
		Ocean Spray Cranberry		
		Kellogg's Fruity Snacks		
Evening Snack	7 - 9 PM	Welches Grape Juice (10 oz)		

Please remember to only consume the food items listed on this table. Please consume all the food items given to you. If you accidentally consume other food items, write them down and let the researchers know as soon as possible.

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	Time of Day	Total amount of fluid (in ounces or measuring cups). Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

High Carbohydrate Food Consumption Checklist/Fluid Intake Log (55 - 65 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Ocean Spray Cranberry		
		Clif Bar		
Lunch	12 - 2 PM	Kraft Mac and Cheese (2)		
		Kellogg's Fruity Snacks		
Afternoon Snack	2 - 4 PM	Kellogg's Nutri-grain Bar		
		Welches Grape Juice (10 oz)		
Dinner	5 - 7 PM	Kraft Mac and Cheese		
		Clif Bar		
		Ocean Spray Cranberry		
Evening Snack	7 - 9 PM	Welches Grape Juice (10 oz)		
		Kellogg's Nutri-grain Bar (2)		

Please remember to only consume the food items listed on this table. Please consume all the food items given to you. If you accidentally consume other food items, write them down and let the researchers know as soon as possible.

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	Time of Day	Total amount of fluid (in ounces or measuring cups). Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

High Carbohydrate Food Consumption Checklist/Fluid Intake Log (65 - 75 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Ocean Spray Cranberry		
		Clif Bar (2)		
Lunch	12 - 2 PM	Kraft Mac and Cheese (2)		
		Kellogg's Fruity Snacks		
Afternoon Snack	2 - 4 PM	Kellogg's Nutri-grain Bar (2)		
		Welches Grape Juice (10 oz)		
Dinner	5 - 7 PM	Kraft Mac and Cheese		
		Clif Bar		
		Ocean Spray Cranberry		
		Kellogg's Fruity Snacks		
Evening Snack	7 - 9 PM	Welches Grape Juice (10 oz)		
		Kellogg's Nutri-grain Bar		

Please remember to only consume the food items listed on this table. Please consume all the food items given to you. If you accidentally consume other food items, write them down and let the researchers know as soon as possible.

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	**Time of Day**	**Total amount of fluid (in ounces or measuring cups).** Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

High Carbohydrate Food Consumption Checklist/Fluid Intake Log (75 - 85 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Ocean Spray Cranberry		
		Clif Bar (2)		
Lunch	12 - 2 PM	Kraft Mac and Cheese (2)		
		Kellogg's Fruity Snacks (2)		
		Kellogg's Nutri-grain Bar		
Afternoon Snack	2 - 4 PM	Kellogg's Nutri-grain Bar (2)		
		Welches Grape Juice (10 oz)		
Dinner	5 - 7 PM	Kraft Mac and Cheese		
		Clif Bar		
		Ocean Spray Cranberry		
		Kellogg's Fruity Snacks		
Evening Snack	7 - 9 PM	Welches Grape Juice (10 oz)		
		Kellogg's Nutri-grain Bar (2)		

Please remember to only consume the food items listed on this table. Please consume all the food items given to you. If you accidentally consume other food items, write them down and let the researchers know as soon as possible.

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	Time of Day	**Total amount of fluid (in ounces or measuring cups).** Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

High Carbohydrate Food Consumption Checklist/Fluid Intake Log (85 - 95 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Ocean Spray Cranberry		
		Clif Bar (2)		
Lunch	12 - 2 PM	Kraft Mac and Cheese (2)		
		Kellogg's Fruity Snacks		
		Kellogg's Nutri-grain Bar (2)		
		Ocean Spray Cranberry		
Afternoon Snack	2 - 4 PM	Kellogg's Nutri-grain Bar (2)		
		Welches Grape Juice (10 oz)		
Dinner	5 - 7 PM	Kraft Mac and Cheese (2)		
		Clif Bar		
		Ocean Spray Cranberry		
		Kellogg's Fruity Snacks		
Evening Snack	7 - 9 PM	Welches Grape Juice (10 oz)		
		Kellogg's Nutri-grain Bar (2)		

Please remember to only consume the food items listed on this table. Please consume all the food items given to you. If you accidentally consume other food items, write them down and let the researchers know as soon as possible.

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	**Time of Day**	**Total amount of fluid (in ounces or measuring cups).** Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

High Carbohydrate Food Consumption Checklist/Fluid Intake Log (95 - 105 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Ocean Spray Cranberry		
		Clif Bar (2)		
Lunch	12 - 2 PM	Kraft Mac and Cheese (2)		
		Kellogg's Fruity Snacks (2)		
		Kellogg's Nutri-grain Bar (2)		
		Ocean Spray Cranberry		
Afternoon Snack	2 - 4 PM	Kellogg's Nutri-grain Bar (2)		
		Welches Grape Juice (10 oz)		
Dinner	5 - 7 PM	Kraft Mac and Cheese (2)		
		Clif Bar		
		Ocean Spray Cranberry		
		Kellogg's Fruity Snacks (2)		
Evening Snack	7 - 9 PM	Welches Grape Juice (10 oz)		
		Kellogg's Nutri-grain Bar (2)		

Please remember to only consume the food items listed on this table. Please consume all the food items given to you. If you accidentally consume other food items, write them down and let the researchers know as soon as possible.

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	Time of Day	**Total amount of fluid (in ounces or measuring cups).** Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

High Carbohydrate Food Consumption Checklist/Fluid Intake Log (105 - 115 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Ocean Spray Cranberry		
		Clif Bar (2)		
		Kellogg's Fruity Snacks (1)		
Lunch	12 - 2 PM	Kraft Mac and Cheese (2)		
		Kellogg's Fruity Snacks (2)		
		Kellogg's Nutri-grain Bar (2)		
		Ocean Spray Cranberry		
Afternoon Snack	2 - 4 PM	Kellogg's Nutri-grain Bar (2)		
		Welches Grape Juice (10 oz)		
		Clif Bar		
Dinner	5 - 7 PM	Kraft Mac and Cheese (2)		
		Clif Bar		
		Ocean Spray Cranberry		
		Kellogg's Fruity Snacks (2)		
Evening Snack	7 - 9 PM	Welches Grape Juice (10 oz)		
		Kellogg's Nutri-grain Bar (2)		

Please remember to only consume the food items listed on this table. Please consume all the food

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	**Time of Day**	**Total amount of fluid (in ounces or measuring cups).** Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

High Carbohydrate Food Consumption Checklist/Fluid Intake Log (>115 kg)

Meal	Time	Food item	Place check mark when item is consumed	Actual time of consumption
Breakfast	7 - 10 AM	Ocean Spray Cranberry		
		Clif Bar (2)		
		Kellogg's Fruity Snacks (1)		
Lunch	12 - 2 PM	Kraft Mac and Cheese (2)		
		Kellogg's Fruity Snacks		
		Kellogg's Nutri-grain Bar (2)		
		Ocean Spray Cranberry		
Afternoon Snack	2 - 4 PM	Kellogg's Nutri-grain Bar (2)		
		Welches Grape Juice (10 oz)		
		Clif Bar		
Dinner	5 - 7 PM	Kraft Mac and Cheese (2)		
		Clif Bar		
		Ocean Spray Cranberry		
		Kellogg's Fruity Snacks (2)		
Evening Snack	7 - 9 PM	Welches Grape Juice (10 oz) (2)		
		Kellogg's Nutri-grain Bar (2)		
		Kellogg's Fruity Snacks		

Please remember to only consume the food items listed on this table. Please consume all the food

Please record any beverages consumed (other than those listed in the table above).
Remember to only consume beverages that don't have any calories (e.g. water).
Don't consume any caffeine-containing beverages or alcohol.

Type of fluid consumed (water, diet Coke, etc.)	**Time of Day**	**Total amount of fluid (in ounces or measuring cups).** Be sure to record which unit of measurement you use.

One normal-sized water bottle is around 16 fluid ounces. One measuring cup is 8 fluid ounces.

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